Vitamin E Dosing Study (VEDS): A dose finding clinical trial of vitamin E for the treatment of adult NAFLD

Protocol Number: 10

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.3, 8.1.4, 8.1.4.1, 8.2	Allow the focused physical exam to be completed via telemedicine, lab tests to be collected at outside/local labs at select follow-up visits, and questionnaires to be completed via correspondence (electronic or mail).	Decrease patient contact when necessary due to local restrictions and patient safety related to pandemic/endemic diseases.
1.1, 5.2	Exclusion criteria regarding hepatitis C was clarified	Updates needed to address potential participants that may have had HCV in the past.
8.1.2, 8.1.5	References to liver biopsy documentation were removed. Visits at which the Bev-Q questionnaire will be completed were corrected.	Erroneously included in the text of the previous version. These were made to be consistent with the schedule of activities.

1.3, 8.1.4, 10.2.1	**C-reactive protein (CRP) added to the data collection schedule and whole blood draw schedule.**	**Erroneously omitted from data collection schedule. CRP is needed for a secondary objective.**
8.1.2, 8.1.3	**Clarified screening/randomization check procedures and visit window generation/display in the data system.**	**New language is consistent with the mechanics of the data system developed for this trial.**

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

United States (US) Code of Federal Regulations (CFR) applicable to clinical studies and the NIDDK Terms of Award. The Principal Investigators will assure that no deviation from, or changes to the protocol will take place without prior agreement from the IND sponsor, funding agency and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the trial participants.

All National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the single Institutional Review Board (IRB) of record for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol, after DSMB and NIDDK approval, will require review and approval by the sIRB before the changes are implemented to the study. In addition, all changes to the consent form will be sIRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Vitamin E Dosing Study (VEDS): A dose-finding clinical trial of vitamin E for the treatment of adult NAFLD
Study Description:	This is a 200 patient, multicenter, randomized, double masked, placebo-controlled, parallel treatment groups dosing trial of vitamin E (d-alpha-tocopherol) in adult nonalcoholic fatty liver disease (NAFLD). Adults age 18 years or older will be enrolled for 48 weeks and treated with 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) of vitamin E or matching placebo for 24 weeks. The primary objective of the study is to determine the minimum effective dose of vitamin E based upon relative change in alanine aminotransferase (ALT) from baseline to 24 weeks.
Objectives:	Primary Objective: To determine the minimum effective dose of vitamin E based upon relative change in alanine aminotransferase (ALT) from baseline to 24 weeks. Secondary Objectives: To determine whether 24 weeks of treatment with vitamin E at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) compared to treatment with placebo improves measures of nonalcoholic fatty liver disease (NAFLD) such as change in liver fat (controlled attenuation parameter: CAP) evaluated by ultrasound (FibroScan® Echosens) and other biomarkers. To explore post-treatment (24-48 weeks) patterns of liver enzymes, CAP, and other biomarkers by vitamin E dose group compared to placebo group.

Study Population:	The study population will be 200 adults age 18 years or older with NAFLD located in the United States.
Phase:	Phase 2
Description of Sites/Facilities Enrolling Participants:	Participants will be enrolled at 9 NASH CRN adult sites located throughout the United States.
Description of Study Intervention:	Participants will be given 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) of vitamin E or a matching placebo. All participants will be instructed to take assigned capsules with water by mouth once per day with breakfast.
Study Duration:	126 weeks • Recruitment phase: 72 weeks • Follow-up phase: 48 weeks • Expected enrollment rate: 1.2 participants per month per clinical center (22 participants each at 9 centers)
Participant Duration:	13 months total • Screening phase is 60 days • 24-week treatment period; 24-week post-treatment follow-up
Inclusion Criteria:	• 18 years of age or older as of the initial screening interview and provision of consent • FibroScan CAP>280 dB/m within 60 days prior to randomization. • Serum alanine aminotransferase (ALT) ≥ 60 U/L within 30 days of randomization
Exclusion Criteria:	• Concurrent or prior use (within 90 days) of vitamin E supplements in excess of 40 IU/day • 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 (~1.5 drinks/day) (> 10.5 drinks per week) in females and more than 30 g/day (~2 drinks/day) (>14 drinks per week) in males, respectively. One "standard" drink (or one alcoholic drink equivalent) contains roughly 14 grams of pure alcohol, which is found in: 12 ounces of regular beer, 5 ounces of wine, or 1.5 ounces of distilled spirits. • Inability to reliably quantify alcohol consumption based upon local study physician judgment • Continued 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 6 months prior to randomization • Current use of anticoagulation therapy (not including antiplatelet agents such as aspirin or clopidogrel) • Platelet count below 150,000 /mm^3 within 90 days of randomization • History of condition(s) that cause increased risk of bleeding, including hemophilia A, hemophilia B, von Willebrand disease, or other clotting factor deficiencies. • Prior or planned (during the study period) bariatric surgery (eg,

gastroplasty, Roux-en-Y gastric bypass)

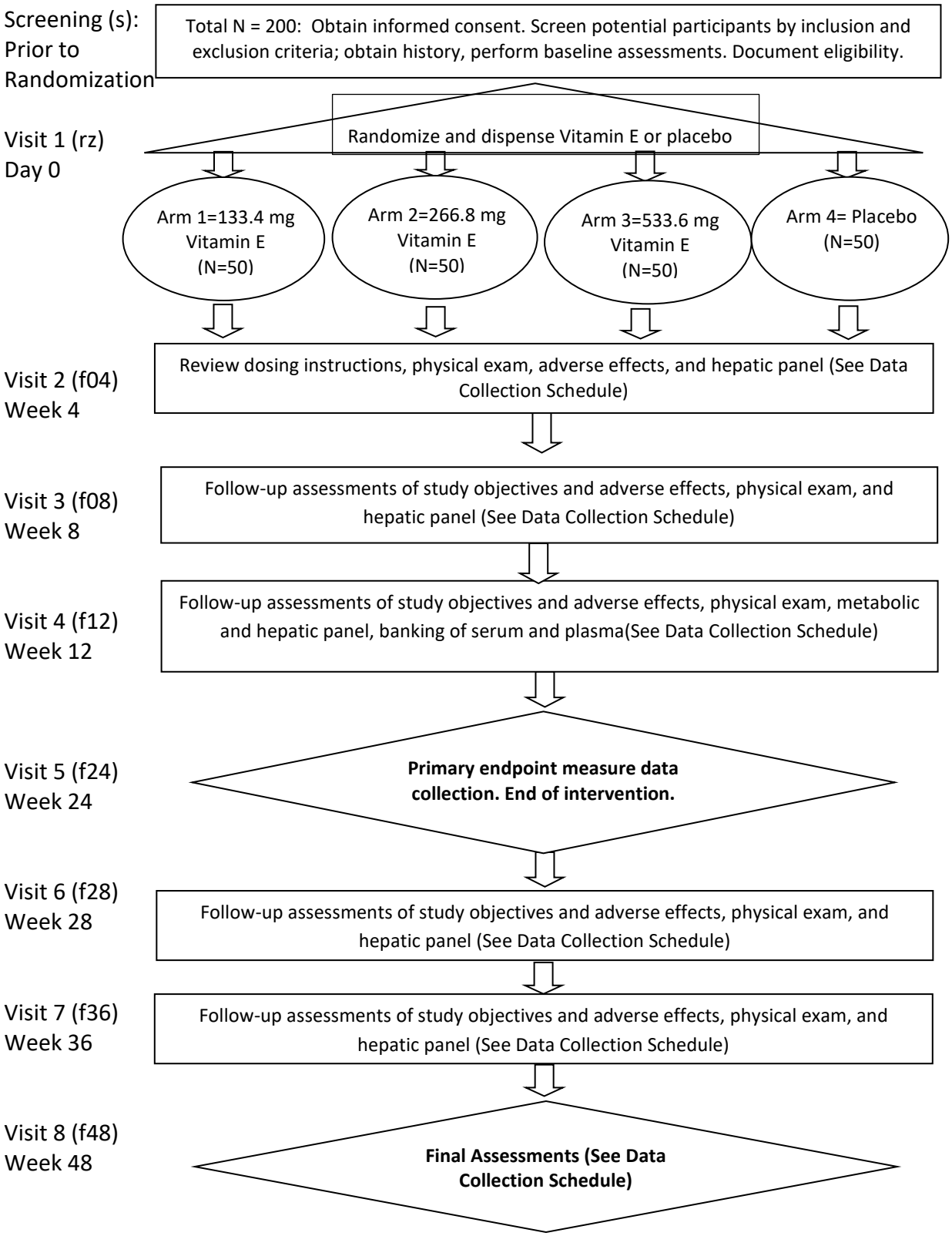
- Uncontrolled diabetes defined as HbA1c 9.5% or higher within 60 days prior to randomization
- Clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities:
    - Serum albumin less than 3.2 g/dL
    - International Normalized Ratio (INR) greater than 1.3
    - Direct bilirubin greater than 1.0 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)
    - Evidence of recent or current hepatitis C as marked by the presence of anti HCV antibody with detectable HCV RNA in serum within 1 year prior to enrollment. Participants with anti HCV antibody positivity who have undetectable HCV RNA at least 1 year prior to enrollment (either due to spontaneous clearance or clearance with treatment) will be eligible to participate if HCV RNA at entry remains undetected.
    - 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
        - Biochemical evidence of cholestasis based mainly on alkaline phosphatase elevation
        - Presence of anti-mitochondrial antibody (AMA)
        - Histologic evidence of nonsuppurative destructive cholangitis and destruction of interlobular bile ducts[1]
    - Primary sclerosing cholangitis
    - Known history of Wilson disease, alpha-1-antitrypsin liver disease or hemochromatosis. Any other type of liver disease that is currently active other than NASH such as drug-induced liver disease, autoimmune liver disease, liver cancer or bile duct obstruction.
- Serum alanine aminotransferase (ALT) greater than 400 U/L within 90 days of randomization
- Moderate or severe renal impairment (serum creatinine ≥ 2.0 mg/dL or eGFR < 60 mg/mL/1.73m^2)
- History of biliary diversion or evidence of current biliary obstruction
- 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 ≥ 1 effective form(s) of birth control during the trial, breast feeding
- Current use of medications that may impact the absorption of fat-soluble vitamins (i.e. orlistat or cholestyramine)
- Pre-existing history of fat malabsorption

- Males at high risk of prostate cancer, including:
  - PSA >ULN at baseline
  - History of prostate cancer
  - Age 45 or older with a first-degree relative (father or brother) diagnosed with prostate cancer at an early age (younger than age 65).
  - Age 40 or older with more than one first-degree relative who had prostate cancer at an early age (younger than age 65)
- 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, including inability to swallow treatment capsules
- Failure or inability to give informed consent

Sample size / Power:

- Total sample size = 200; 50 per group
- Power = 90%
- Type I error (2-sided) = 5%
- Increase in sample size due to missing data = 10%
- Mean (SD) difference (Vitamin E – Placebo) in relative change [(BL-FU)/BL] in ALT at 24 weeks = 20% (31%)
  - Mean relative change (%) in ALT at 24 weeks in placebo = 20% (based on data from PIVENS trial)
  - Mean relative change (%) in ALT at 24 weeks in Vitamin E = 40% (based on data from PIVENS trial)
- Method of analysis = Williams' test for minimum effective dose

1.2 SCHEMA


Screening (s): Prior to Randomization
Total N = 200: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, perform baseline assessments. Document eligibility.
Visit 1 (rz) Day 0
Randomize and dispense Vitamin E or placebo
Arm 1=133.4 mg Vitamin E (N=50)
Arm 2=266.8 mg Vitamin E (N=50)
Arm 3=533.6 mg Vitamin E (N=50)
Arm 4= Placebo (N=50)
Visit 2 (f04) Week 4
Review dosing instructions, physical exam, adverse effects, and hepatic panel (See Data Collection Schedule)
Visit 3 (f08) Week 8
Follow-up assessments of study objectives and adverse effects, physical exam, and hepatic panel (See Data Collection Schedule)
Visit 4 (f12) Week 12
Follow-up assessments of study objectives and adverse effects, physical exam, metabolic and hepatic panel, banking of serum and plasma(See Data Collection Schedule)
Visit 5 (f24) Week 24
Primary endpoint measure data collection. End of intervention.
Visit 6 (f28) Week 28
Follow-up assessments of study objectives and adverse effects, physical exam, and hepatic panel (See Data Collection Schedule)
Visit 7 (f36) Week 36
Follow-up assessments of study objectives and adverse effects, physical exam, and hepatic panel (See Data Collection Schedule)
Visit 8 (f48) Week 48
Final Assessments (See Data Collection Schedule)

1.3 DATA COLLECTION SCHEDULE (DCS)

	Screening Visits		Follow-up visits Weeks from Randomization						
Assessment/Procedure	S	RZ	F04	F08	F12	F24	F28	F36	F48
Consent and reaffirmation	X	X	.	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.	.	.
Follow-up medical history (with AUDIT-C)	.	.	X	X	X	X	X	X	X
Alcohol Use Disorders Identification Test (AUDIT)	X	.	.	.	.	.	.	.	.
Lifetime Drinking History (Skinner)	X	.	.	.	.	.	.	.	.
Review for adverse effects	.	.	X	X	X	X	X	X	X
Review for concomitant medications	X	X	X	X	X	X	X	X	X
Drug dispensing	.	X	.	.	.	.	.	.	.
Review of study drug adherence	.	.	X	X	X	X	.	.	.
Detailed* (D) or Focused† (F) physical exam	D	.	F	F	D	D	F	F	D
NASH CRN Liver Symptom Questionnaire	X	.	X	X	X	X	X	X	X
Cardiovascular risk factor assessment	X	.	.	.	.	X	.	.	X
Beverage Intake Questionnaire (BEVQ-15)	X					X			X
FibroScan®: liver stiffness and steatosis (CAP)	X	.	.	.	.	X	.	.	X
Complete blood count (CBC) ‡	X	.	.	.	.	X	.	.	X
Basic metabolic panel (BMP) **§**	X	.	.	.	.	X	.	.	X
Fasting lipid profile¶	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
Prothrombin time (PT), activated Partial Thromboplastin Time (aPTT), and INR	X	.	.	.	.	X	.	.	X
HbA1c	X	.	.	.	.	X	.	.	X
C-reactive protein (CRP)	X	.	.	.	X	X	.	.	X
Pregnancy test	X	.	.	.	.	X	.	.	.
Prostate Specific Antigen (PSA) Test††	X	.	.	.	.	.	.	.	X
Banking‡‡: Fasting serum, plasma	X	.	.	.	X	X	.	.	X
Etiologic tests§§	X	.	.	.	.	.	.	.	.
Closeout form	.	.	.	.	.	.	.	.	X

*** Detailed physical exam:** anthropometric assessments (body weight [kg], body height [cm], waist circumference [cm], and hip circumference [cm]); vital signs (temperature, heart rate, respiratory rate, blood pressure), organ systems (skin, chest, lungs, heart, abdomen, nervous); liver signs and changes in bleeding (e.g. bleeding gums).

†**Focused physical exam:** anthropometric assessments (body weight [kg], body height [cm], waist circumference [cm], and hip circumference [cm]); vital signs (temperature, heart rate, respiratory rate, blood pressure); liver signs and **changes in bleeding (e.g. bleeding gums).** The focused physical exam may be completed remotely via telemedicine video call per institutional guidelines.

‡**Complete blood count:** hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), red blood cell count (RBC), platelet count

§Basic metabolic panel: glucose, calcium, sodium, potassium, CO_2 (carbon dioxide, bicarbonate), chloride, blood urea nitrogen (BUN), creatinine. eGFR will be calculated using the CKD-EPI formula.

¶Fasting Lipid profile: total cholesterol, triglyceride, LDL, HDL.

****Hepatic panel**: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, albumin, total protein

††**Prostate Specific Antigen (PSA) Test:** males (sex assigned at birth) only

‡‡**Banking:** Fasting plasma and serum will be collected for planned analysis of measures of vitamin E (alpha-tocopherol, gamma-tocopherol, and carotenoids) and future ancillary analyses.

§§Etiologic tests as needed: Hepatitis B surface antigen, hepatitis C antibody, alpha-1-antitrypsin level, ceruloplasmin. Autoantibodies: (ANA, AMA ASMA), serum iron, ferritin, total iron binding capacity (TIBC), and Thyroid Stimulating Hormone (TSH)

2 INTRODUCTION

2.1 STUDY RATIONALE

NAFLD 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 unattainable for most individuals. Pharmacologic treatments have been sought but none has proved sufficiently efficacious. This may be related to the fact that the histopathological changes seen on liver biopsy currently described as NAFLD may be the result of multiple pathogenetic mechanisms acting in concert to varying degrees. Based on the prevalence and risk of progression of NAFLD to NASH, cirrhosis, and cancer, the burden of significant disease is large, and drug therapy to prevent or treat NAFLD is needed.

2.1.1 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 (primarily triglyceride) in more than 5% of hepatocytes. In the largest population study of adults using MR spectroscopy, the threshold value for abnormal liver fat fraction was similar to these other assessments.[2]

Nonalcoholic steatohepatitis (NASH) is the name applied to a constellation of biopsy abnormalities occurring in the presence of NAFLD that typically include 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 zone 3-predominant perisinusoidal fibrosis.[3]

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.[4]

2.2 BACKGROUND

Oxidative stress and free radicals in NAFLD

Oxidative stress, resulting from an imbalance between pro-oxidant and antioxidant mechanisms in favor of pro-oxidation, has been recognized as a key mechanism in the progression of steatosis to steatohepatitis[5, 6]. Several studies have shown an association between NAFLD and biomarkers of oxidative stress or lipid oxidation[7-10]. Oxidative stress is mediated largely by reactive oxygen species (ROS). Molecular oxygen readily accepts electrons from exogenous substances and during intracellular metabolic processes to form various free radicals including hydroxyl radical, nitric oxide radical and superoxide anion. These free radicals react with proteins, free fatty acids (FFA), and DNA to mediate oxidative injury[11].

The production of ROS occurs mainly in mitochondria, endoplasmic reticulum (ER), and peroxisomes, and to a lesser extent through various enzymatic processes including xanthine oxidase and cytochrome P450[9, 12]. Models of NAFLD have been shown to have altered mitochondrial morphology, impaired mitochondrial bioenergetics, increased mitochondrial lipid peroxides and increased mRNA levels of peroxisome proliferator activated receptor-α (PPAR-α), involved in regulating the expression of mitochondrial and peroxisomal β-oxidation enzymes [9, 13][14, 15].

Oxidative stress and organelle dysfunction in NAFLD

Mitochondrial dysfunction

In mitochondria, the catabolic process of β-oxidation which breaks down FFA occurs through three main steps[6]. During the first step, long-chain FFA enter into the mitochondria. In the second step, the long-chain FFA are oxidized into medium and short chain fatty acids, a process that involves conversion of oxidized nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) into their de-oxidized version, NADH and FADH2. In the third step, NADH and FADH2 are re-oxidized into NAD and FAD through the transfer of electrons along the mitochondrial respiratory complex (MRC) enzymatic chain. Most of these electrons combine with oxygen and protons to form water, however a few electrons react with oxygen to form ROS. Impairment of the MRC complex results in disruption of β-oxygenation and increased production of ROS, including superoxide, hydrogen peroxide, and hydroxyl radicals [9, 16-20] that increase steatosis and resultant inflammation.

Mitochondrial dysfunction facilitates NASH pathogenesis at several levels, including impaired lipid metabolism, increased ROS and cytokine production, triggering cell death and promoting inflammation[6, 9]. Accumulation of lipid in the mitochondria leads to influx of water and calcium, causing release of cytochrome C and cell death[21]. Lysosomal permeabilization, associated with caspase activation and ROS generation via cytochrome P450 2E1 (CYP2E1) [22], can also mediate mitochondrial injury. Mitochondrial injury can subsequently lead to apoptosis via mechanisms involving Sab, SH3 Domain Binding Protein 5 (SH3BP5), a substrate of Janus kinase (JNK) located in the mitochondrial outer membrane [23]. Continuous mitochondrial injury further results in hepatocyte injury and progression to NASH.

Other organelles

Although the mitochondria are the main source of hepatocyte ROS, other organelles have also been shown to participate in this process [6]. Peroxisomes are able to rapidly oxidize long-chain FFA through β-oxidation, resulting in hydrogen peroxide that is converted into the highly reactive hydroxyl (OH) radical [21]. The generation of ROS in a lipid-rich environment induces lipid peroxidation of polyunsaturated fatty acids (PUFAs), leading to the formation of 4- hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) that have longer half-lives than ROS and amplify the effects of ROS via diffusion into the extracellular space to affect distant cells. The increase in lipid peroxidation and protein oxidation impair the hepatocyte mitochondrial respiratory chain, increasing ROS production with resultant detrimental effects on fat metabolism in the liver. Moreover, chronic ER stress may contribute to oxidative stress by promoting toxic accumulation of ROS which triggers other signaling pathways within the cell; ROS generated through inflammation of mitochondria and other organelles may also accelerate ER dysfunction [24, 25]. In addition, lipo-oxygenation of long chain fatty acids via microsomal cytochromes, CYP2E1 and cytochrome P450 4A (CYP4A), leads to release of ROS.

Role of inflammation and oxidative stress in NAFLD

The generation of intracellular ROS via mitochondria, ER, peroxisomes, or enzymatic pathways all contribute to disease progression in NAFLD. In addition to excessive production of ROS within organelles, oxidative stress also occurs as a result of reduced antioxidant defense. NASH is associated with decreased levels of various antioxidant pathways and enzymes, including hepatic glutathione (GSH), glutathione transferase activity, SOD, glutathione peroxidase (GPx), and catalase[9, 26]. The process of oxidative injury is augmented by obesity and insulin resistance (IR), which lead to increased production of ROS in the liver, increased lipid peroxidation, and decreased plasma antioxidant capacity[27, 28].

Mitochondrial dysfunction facilitates the production of ROS and contributes to NAFLD progression through induction of hepatic inflammatory cytokines. It has been proposed that obesity, IR, and adipokine/cytokine

pathways mediate liver fat accumulation and NASH development[6, 29]. This results in additional lipid peroxidation of mitochondrial membranes, augmenting mitochondrial dysfunction and ROS generation [30]. The resultant damage to nuclear and mitochondrial DNA results in necro-inflammation especially in the hepatocyte nucleus and cytoplasm and in sinusoidal cells [31, 32]. Hepatic necro-inflammation is evidenced by increased levels of 8- hydroxy-2' deoxyguanosine (8-OHdG), a modified DNA base product generated by ROS [31]. Hepatic inflammation and fibrosis progression are key features in the pathogenesis of NASH.

Vitamin E mechanisms in NASH

Given the crucial role of oxidative stress and free radical injury in the pathogenesis of NASH, it is not surprising that free radical-scavenging agents have shown ameliorating effects on disease progression[33, 34]. Vitamin E is an antioxidant that has been studied in several animal models as well as in clinic trials of NAFLD. There are 8 naturally occurring forms of vitamin E, including 4 tocopherol homologs (α-, β-, γ-, δ-) with a phytyl side chain, and 4 tocotrienols (α-, β-, γ-, δ-) that have 3 double bonds on the side chain[35-38]. The term vitamin E describes the antioxidant activity of any combination of tocopherol and tocotrienols. However, α-tocopherol is the only form recognized to meet human vitamin E requirements.[39]

The hepatic α-tocopherol transfer protein (α-TTP) functions to select alpha-tocopherol for secretion from the liver in very low density lipoproteins; the non-alpha-tocopherol forms are not selected and are actively metabolized and excreted in urine.[35] Persons with a genetic defect in the α-TTP develop vitamin E deficiency early in life.[40]

Vitamin E is efficient in scavenging peroxyl radicals in vivo and preventing lipid peroxidation [41, 42]. It also inhibits the production of isoprostanes which are biomarkers of lipid peroxidation [43, 44]. Vitamin E is rapidly consumed and converted back to its reduced form by the cytoplasmic antioxidant cycling network including vitamin C and glutathione [45]. The antioxidant role of vitamin E is important in maintaining membrane stability.

Vitamin E in animal models of NAFLD

Vitamin E has been studied in several experimental models of NAFLD. Vitamin E supplementation reduced the levels of liver enzymes, hepatic steatosis and necro-inflammation in a methionine-choline-deficient (MCD) diet-induced animal model of NASH[46]. Superoxide dismutase activity was enhanced in these animals, and levels of MDA and genes related to inflammation, apoptosis, and fibrosis were reduced. In another MCD diet-induced model of NASH, hepatic glutathione stores were restored, while oxidative stress markers, hepatic stellate cell activation, and histologic fibrosis were reduced in mice on vitamin E[47].

In a diet-induced obesity model in rats, vitamin E ameliorated oxidative stress[48]. In obese (ob/ob) mice, α- as well as γ-tocopherol protected against lipopolysaccharide-induced liver injury, decreasing hepatic MDA and tumor necrosis factor (TNF)-α levels[49]. In leptin-receptor deficient rats, vitamin E protected against bile acid-induced hepatocyte injury, improving portal inflammation, lobular inflammation and hepatocellular necrosis[50]. These animal studies laid the groundwork for clinical trials in NAFLD.

Clinical trials of vitamin E in NAFLD

Plasma levels of α-tocopherol are decreased in NASH patients compared to healthy controls, providing a rationale for vitamin E supplementation[51]. Among the many agents that have been tested for NASH, vitamin E has had the most substantial effect on disease activity[52]. The impact of vitamin E on clinically meaningful outcomes and progression to cirrhosis are yet to be demonstrated. However, current recommendations for therapeutics focus on decreasing disease activity. Vitamin E is recommended by both European and North

American practice guidelines for the treatment of NASH [53, 54]. Although some studies have raised concerns about the long-term safety of Vitamin E[55, 56] other studies have challenged these concerns[57, 58]. Earlier studies assessing the efficacy of Vitamin E in NAFLD as monotherapy and combination therapy gave conflicting results likely due to small sample sizes, differences in primary end points, and differences in vitamin E formulations.

Two separate multicenter randomized-control trials conducted by the NASH Clinical Research Network (CRN) showed that vitamin E was effective in improving steatohepatitis. In Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, 247 non-diabetic and non-cirrhotic adults with NASH received vitamin E (800 IU/day), pioglitazone (30 mg/day), or placebo for a period of 96 weeks[41]. The primary outcome measured was improvement in histological features, based on standardized scores for steatosis, inflammation, and hepatocellular ballooning. A p-value less than 0.025 between the treatment group (vitamin E or pioglitazone) versus the placebo group was considered significant. Vitamin E therapy demonstrated significant histologic improvement in NASH (43% vs 19%, P= 0.001) while pioglitazone did not reach statistical significance (34% vs 19%, P= 0.04). Significant reduction in hepatic steatosis, lobular inflammation, and hepatocellular ballooning was observed, however, no significant improvement in fibrosis was seen in both treatment groups compared to placebo. It is noteworthy that in a prior small pilot study, combination therapy with vitamin E and pioglitazone was shown to be superior to vitamin E alone in reducing the histological features of NASH and improving pericellular fibrosis [59].

In the Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC) trial, 173 children received vitamin E (400 IU twice daily), metformin (500 mg twice daily), or placebo for a 96-week period[60]. Resolution of NASH, attributed mainly to improved hepatocellular ballooning, was significantly greater for vitamin E treatment group compared to placebo (p=0.006); however, neither vitamin E nor metformin was superior to placebo in attaining the primary outcome of reduction in alanine aminotransferase (ALT) level by 50% or more compared to baseline, or a level of 40 U/L or less from 48 to 96 weeks.

Two meta-analyses reported significant improvement in liver histology in NASH patients treated with vitamin E[61, 62]. A meta-analysis of five studies showed significant improvement in liver enzymes as well as the individual histological features of NASH, steatosis, lobular inflammation, and hepatocellular ballooning. Among adult patients, there was also an improvement in fibrosis[61]. Another meta-analysis performed by a different group showed improvement in all the histological parameters of NASH, including fibrosis[62].

Vitamin E in other forms of chronic liver disease

Oxidative injury and decreased levels of vitamin E have been implicated in various etiologies of chronic liver diseases other than NAFLD, including alcoholic liver disease and viral hepatitis. However, the role of vitamin E in these conditions remains to be defined. In a randomized, placebo-controlled study of alcoholic hepatitis, vitamin E did not confer any benefit on liver function or survival during the 1-year study period[63]. In chronic hepatitis C patients, high-dose vitamin E significantly reduced oxidative stress with no effect on liver enzymes or the degree of hepatocellular inflammation or fibrosis[64]. In a small pilot study of 32 patients with chronic hepatitis B, treatment with vitamin E was associated with improved ALT levels and complete response (normal ALT and negative hepatitis B virus DNA) in 47% of the patients treated[65]. These findings are yet to be replicated in larger studies. At present, vitamin E supplementation can be proposed only for patients with NASH, with the role of vitamin E supplementation in other etiologies of chronic liver disease remaining controversial.

Safety of vitamin E

Some studies have raised concerns about the long-term safety of vitamin E [55, 56], while other studies have challenged these concerns[57, 58]. A meta-analysis published in 2005 suggested that high-dose vitamin E increased all-cause mortality in a dose-dependent manner, and cautioned against the use of any high-dose vitamin supplementation until appropriately designed trials were available [55]. However, this meta-analysis has been criticized because it excluded several studies with low mortality. Moreover, concomitant use of vitamin A and other drugs, as well as factors such as smoking, were not taken into consideration during the analyses. Subsequently, a meta-analysis of those trials, together with more studies investigating vitamin E suggested that the observed differences were due to a disproportionately higher number of male patients in the trials, compared to other trials, thus casting doubt on the causal relationship of vitamin E supplementation and increased mortality[66].

A large meta-analysis of 57 studies involving 246,371 subjects studied for 1 to 10 years did not demonstrate an association between vitamin E supplementation and all-cause mortality[57]. More recently, another large meta-analysis involving 123,001 participants confirmed the lack of association between vitamin E on both all-cause mortality (Risk Ratio=1.00, 95% CI=0.97-1.03) and cardiovascular mortality (Risk Ratio=0.95, 95% CI=0.88-1.03)[67]. A RCT in 2011, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) trial, showed modestly increased incidence of prostate cancer in healthy men on vitamin E over a 7-year period, translating to an absolute increase of 1.6 per 1,000 person-years of vitamin E 400 IU/day [56]. However, based on subsequent analysis, it appears that this risk may be modified by baseline selenium concentration[68] or genetic variants involved in the metabolism of vitamins[69].

Based on the efficacy of vitamin E in improving the histological features of NASH, it is recommended as first line off-label therapy for NASH. Current practice guidance from the American Association for the Study of Liver Disease (AASLD) recommends consideration of vitamin E in treating non-diabetic, non-cirrhotic NASH patients with biopsy-proven NASH[53, 54, 70]. It is suggested that risks and benefits should be discussed with the patient prior to initiating therapy. Guidance from a joint task force including the European Association for the Study of the Liver (EASL)/ European Association for the Study of Diabetes (EASD)/ European Association for the Study of Obesity (EASO) suggests the use of vitamin E for NASH, with a plan to stop treatment if there is no significant improvement in liver enzymes after 6 months of therapy[54].

Conclusion

Vitamin E is beneficial in improving the biochemical and histological features of NAFLD, a major cause of chronic liver disease which increases the risk of cirrhosis and liver cancer. Although the mechanisms by which vitamin E improves NAFLD, and more specifically NASH, are unclear, vitamin E is known to restore antioxidant activity in NASH patients. The beneficial effects of vitamin E on NAFLD and NASH have been demonstrated both in animal studies as well as in human clinical trials. This trial aims to determine the optimal dose of vitamin E. Larger studies and longer follow-up RCTs are crucial in defining long-term beneficial effects of vitamin E as well as potential side effects. Nonetheless, current findings substantiate an important role of vitamin E in ameliorating chronic liver disease including NASH.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risks of vitamin E: Vitamin E at 533.6 mg (800 IU) daily dose (the maximum dose for this study) has no significant toxicity in adults [71, 72]. Side effects of long-term use of vitamin E in excess of 400 to 800 IU/day may include: diarrhea, nausea, flatulence, stomach cramps, weakness, dizziness, headache, fatigue, and blurred vision [73]. Long-term use of supplemental vitamin E has also been associated with an increased risk of bleeding, specifically gingival bleeding [74]. While some studies have shown no effect of vitamin E on

biomarkers of bleeding (eg. prothrombin time)[75, 76], vitamin E use has been associated with increased bleeding tendencies in vitamin K deficient patients, which is very rare in adults without specific underlying conditions (e.g. cystic fibrosis, biliary obstruction) [73]. In one study, vitamin E supplementation was associated with prostate cancer in men [56]. Additionally, one meta-analysis suggested that doses of vitamin E >400 IU/day were associated with an increase in all-cause mortality[55]. However, this analysis has been criticized, as explained in section 2.2, and at least one subsequent meta-analysis has failed to demonstrate a relationship between vitamin E supplementation and all-cause mortality [57].

Blood drawing: Blood draw may cause mild discomfort, such as swelling, temporary sensation of pain, burning, or a bruise that may develop and last for a few days. Less common risks include a blood clot at the site of puncture, swelling of the vein and surrounding tissues, and possible bleeding from the puncture site. Very rarely, fainting, blood clots, or an infection at the site can occur. During the entire study period (up to 56 weeks), subjects will have approximately 20 tablespoons of blood drawn (300 mL).

Study visits: This study requires approximately 9 visits to the study center (screening visit, randomization visit, and 7 post randomization visits) which may cause some inconvenience to some individuals.

Safety issues related to participant privacy: For this study, data are being recorded about each participant's medical condition via medical records and case report forms. All data will be kept confidential. Case report forms on which a participant's data or results from specified procedures have been recorded and will be stored in locked drawers of file cabinets. Study data are keyed to the NASH CRN web-based study database with encrypted (https:) transmission of data and via a secure network using an encrypted, password-protected computer accessible only by the study coordinator, the site Principal Investigator (PI), and study-certified personnel. It is the investigator's responsibility to conduct the protocol under the current version of the Declaration of Helsinki, Good Clinical Practice, and the rules of local IRBs. Each investigator must ensure that the participant's anonymity is maintained in their data submissions to the DCC. Participants will be identified by an arbitrary unique identification code and not by their name, social security number, address, or hospital medical record number. Under no circumstances will any identifiable data be transmitted and stored in the NASH CRN database or be sent in email, scans, or paper-based transmissions to the DCC. Investigators will locally maintain a separate confidential enrollment log, which links identification codes with the participants' names and addresses (i.e., available only to local clinic staff). All study material will be maintained in strict confidence.

2.3.2 KNOWN POTENTIAL BENEFITS

Individual participants in the trial will receive several potential benefits. First, vitamin E has been shown in several studies to improve markers of inflammation and fibrosis in NASH. For example, the PIVENS trial demonstrated that 43% of patients treated for two years met the histological endpoint compared to 19% in the placebo group (P<0.01) [77]. Improvement in fibrosis was not observed. Similar to the pioglitazone-treated patients, the ALT improved over a period of 3-6 months. Second, all participants will receive lifestyle counseling at baseline, which is standard of care for NAFLD. Lifestyle counseling will be standardized and based upon the established protocols of the NASH CRN.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Rationale for necessity of exposing participants to risk and minimization of risks: Risks to patients in this study include that vitamin E (d-alpha-tocopherol) may cause increased bleeding, diarrhea, nausea, flatulence, stomach cramps, weakness, dizziness, headache, fatigue, or blurred vision with prolonged use. Risks are minimized by exclusion of patients with higher than normal risk of bleeding and by close monitoring. Risks are

also minimized in this trial by using ALT as a surrogate marker of histologic response, thus avoiding the need for repeat liver biopsies in this phase 2 study.

Justification of the risks: All participants will receive standard of care treatment as part of the trial and will potentially benefit if they are in a treatment group (133.4 mg, 266.8 mg, or 533.6 mg of vitamin E) by improvement in ALT. The value of identifying a minimal effective dose for vitamin E in NAFLD is high because vitamin E has been shown to be effective in NAFLD at high doses (533.6 mg per day), but if a lower dose can be used effectively, potential long term adverse effects may be avoided. By conducting the trial and furthering the understanding of vitamin E for adult NAFLD, all of the participants in the study stand to benefit in the future if the minimum effective dose of vitamin E is identified.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective is to determine the minimum effective dose of vitamin E (133.4 mg, 266.8 mg, or 533.6 mg) at which measures of NAFLD are improved	Relative change in ALT at week 24 from baseline [i.e., (ALT at baseline – ALT at week 24)/ ALT at baseline]	ALT is the most accepted short term (<12 months) surrogate marker for histologic improvement in NAFLD.
Secondary		
The secondary objectives have been selected to provide further information on the minimum effective dose of vitamin E needed to improve measures of NAFLD progression, and to provide information on the post-treatment effects of vitamin E on the liver.	Proportion of patients achieving normalization* of ALT at 24 weeks Mean change in ALT from baseline to 24 weeks Mean change in AST and GGT from baseline to 24 weeks Mean change in hepatic fat determined by FibroScan® CAP score from baseline to 24 weeks Mean change in liver stiffness as assessed by FibroScan® from baseline to 24 weeks Time course and end of treatment improvement in ALT, AST, GGT, and alkaline phosphatase Time course and end of treatment improvement in C-reactive protein (serum marker of inflammation)	These endpoints were selected because they measure important co-morbidities of NAFLD and are useful for determining if vitamin E improves other features commonly associated with NAFLD. Safety and quality of life outcomes are also included.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Time course and end of treatment improvement in change in total cholesterol, LDL cholesterol, HDL cholesterol, and serum triglycerides Changes in fasting glucose levels from baseline to 24 weeks Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference) from baseline to 24 weeks Change in symptom assessment scores from baseline to 24 weeks Change in frequency of CTCAE defined adverse events from baseline to 24 weeks Mean change in ALT from 24 to 48 weeks (post-treatment). Mean change in AST and GGT from 24 to 48 weeks (post-treatment). Mean change in hepatic fat determined by FibroScan® CAP score from 24 to 48 weeks (post-treatment). Mean change in liver stiffness as assessed by FibroScan® from 24 to 48 weeks (post-treatment).	
Tertiary/Exploratory		
Tertiary/exploratory objectives have been selected to serve as a basis for explaining or supporting findings of primary analyses and for suggesting further hypotheses for later research.	Change in measures of vitamin E (including alpha-tocopherol, gamma-tocopherol, and carotenoids) from baseline to 12 weeks and 24 weeks	The pharmacokinetics of vitamin E are not well known in NAFLD

*Normalization of ALT is defined as a decrease in ALT to less than or equal to the ULN at the 24 week visit among participants who had an ALT value greater than ULN at baseline. Values for ULN are defined at each clinical center per institutional guidelines.

4 STUDY DESIGN

4.1 OVERALL DESIGN

- The hypothesis of the trial is that 24 weeks of treatment with vitamin E (at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) daily) in adults with NAFLD will find the minimum effective dose to improve ALT compared to placebo.
- Phase 2 trial
- A 200 patient, multicenter, randomized, double-masked, placebo-controlled, parallel treatment groups trial with relative change (%) in serum ALT from baseline to 24 weeks as the primary endpoint.
- Methods to decrease bias will include placebo control, double masking, and randomization.
- Four study arms
- Nine centers
- Study intervention is vitamin E at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) daily or matching placebo.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study design is a multicenter, randomized, double-masked, placebo-controlled, parallel treatment groups, phase 2 trial.

Rationale for a Placebo Group: In order to assess the efficacy of an agent in NAFLD, a placebo-arm is needed to determine its relative efficacy in improving NAFLD histology beyond that achieved with a placebo.[78] Currently, there are no FDA approved therapies for NAFLD. The 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).[77] 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.[79, 80] In order to have the highest quality of evidence to test our hypothesis, this phase 2 pilot study utilizes a randomized, double-masked, placebo-controlled study design. As there is no proven pharmacologic therapy for NAFLD/NASH, using a placebo for comparative purposes is justified. The trial is double masked to prevent bias by investigators and participants; participants will be randomly assigned to either the 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) vitamin E dose or matching placebo (neither the study staff nor the participants will know if they have active or placebo capsules).

Rationale for treatment duration of 24 weeks: An ideal duration of a treatment should only expose participants to a study drug long enough to show meaningful improvement in ALT if it is going to occur. This enables any positive findings to be reported as soon as possible. The duration of treatment in the PIVENS and TONIC trials demonstrated significant changes relative to placebo in serum ALT at 24 weeks.

4.3 JUSTIFICATION FOR DOSE

The purpose of this study is to find the minimum effective dose of vitamin E as a therapy from NAFLD. For this reason, three separate doses of vitamin E will be tested alongside a placebo group. The maximum daily dose being tested in this trial (533.6 mg (800 IU)) has been show to successfully lower ALT levels in previous studies.

The additional doses of 133.4 mg (200 IU) and 266.8 mg (400 IU) are to be included in this study to explore the ability of vitamin E to improve ALT at lower doses than previously studied.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Data Collection Schedule (DCS), Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the DCS in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

- 18 years of age or older as of the initial screening interview and provision of consent
- FibroScan CAP>280 dB/m within 60 days prior to randomization.
- ALT ≥ 60 U/L within 30 days of randomization

5.2 EXCLUSION CRITERIA

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

- Concurrent or prior use (within 90 days) of vitamin E supplements in excess of 40 IU/day
- 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 (~1.5 drinks/day) (> 10.5 drinks per week) in females and more than 30 g/day (~2 drinks/day) (>14 drinks per week) in males, respectively. One "standard" drink (or one alcoholic drink equivalent) contains roughly 14 grams of pure alcohol, which is found in: 12 ounces of regular beer, 5 ounces of wine, or 1.5 ounces of distilled spirits).
- Inability to reliably quantify alcohol consumption based upon local study physician judgment
- Continued 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 6 months prior to randomization
- Current use of anticoagulation therapy (not including antiplatelet agents such as aspirin or clopidogrel)
- Platelet count below 150,000 /mm^3 within 90 days of randomization
- History of condition(s) that cause increased risk of bleeding, including hemophilia A, hemophilia B, von Willebrand disease, or other clotting factor deficiencies.
- 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 randomization
- Clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities:
  - Serum albumin less than 3.2 g/dL
  - International Normalized Ratio (INR) greater than 1.3
  - Direct bilirubin greater than 1.0 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)
  - Evidence of recent or current hepatitis C as marked by the presence of anti HCV antibody with detectable HCV RNA in serum within 1 year prior to enrollment. Participants with anti HCV antibody positivity who have undetectable HCV RNA at least 1 year prior to enrollment (either due to spontaneous clearance or clearance with treatment) will be eligible to participate if HCV RNA at entry remains undetected
  - 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[1]
  - Primary sclerosing cholangitis
  - Known history of Wilson disease, alpha-1-antitrypsin liver disease, or hemochromatosis. Any other type of liver disease that is currently active other than NASH such as drug-induced liver disease, liver cancer, or bile duct obstruction.
- Serum alanine aminotransferase (ALT) greater than 400 U/L within 90 days of randomization
- Moderate or severe renal impairment (serum creatinine ≥ 2.0 mg/dL or eGFR < 60 mg/mL/1.73m^2)
- History of biliary diversion or evidence of current biliary obstruction
- 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 ≥ 1 effective form(s) of birth control during the trial, breast feeding
- Current use of medications that may impact the absorption of fat-soluble vitamins (i.e. orlistat or cholestyramine)
- Pre-existing history of fat malabsorption
- Males at high risk of prostate cancer, including:
  - PSA >ULN at baseline
  - History of prostate cancer
  - Age 45 or older with a first-degree relative (father or brother) diagnosed with prostate cancer at an early age (younger than age 65).
  - Age 40 or older with more than one first-degree relative who had prostate cancer at an early age (younger than age 65)
- 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, including inability to swallow treatment capsules
- Failure or inability to give informed consent

5.3 LIFESTYLE CONSIDERATIONS

In addition to the study medication, participants will receive a standardized 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 participants in both groups receive the same standard of care treatment for NAFLD/NASH.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a low ALT, low CAP, or other acute issue may be rescreened once. Participants may also be rescreened once in the case of unexpected delay between registration and randomization (more than 60 days). Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

5.5.1 RECRUITMENT

Approximately 200 adult participants in 4 groups of equal size (50 per group) will be recruited at the 9 participating centers of the NASH CRN (averaging 22 patients per center) over a 72-week (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 females 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.

6 STUDY INTERVENTION

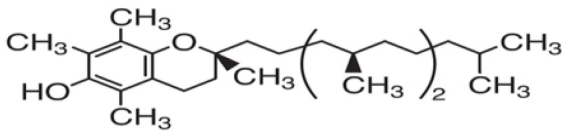
6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Natural vitamin E, d-α-tocopherol (*RRR*-alpha-tocopherol), will be the active ingredient used in the study drug for the VEDS trial. The total chemical composition of the raw Vit E DA tocopherol blend to be used in the manufactured capsules will be: d-alpha tocopherol (66.80%), mixed tocopherols (0.40%), soybean oil (32.80%).

Active ingredient (d-α-tocopherol)

Structural formula:



Chemical formula: $C_{29}H_{50}O_2$

Vitamin E will be supplied to participants in identical softgel capsules. Each capsule will contain 133.4 mg or 266.8 mg of vitamin E, d-alpha tocopherol, and inactive ingredients (eg. soybean oil). The 133.4 mg and 266.8 mg capsules will contain 200 IU and 400 IU of the d-alpha tocopherol, respectively. The matching placebo will contain high oleic safflower oil. All capsules will be identical in size and color with an oval shape containing a straw to amber colored, viscous liquid.

The softgel shell will be made in situ using 43.0% gelatin (bovine), 38.4% water, 18.0% glycerin (vegetable-based), and 0.6% natural colorant.

6.1.2 DOSING AND ADMINISTRATION

Participants will be treated with 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6mg (800 IU) of vitamin E or matching placebo for 24 weeks. These doses will be delivered to patients as follows:

- **133.4 mg (200 IU):** one capsule of 133.4 mg (200 IU) vitamin E AND one matching placebo capsule.
- **266.8 mg (400 IU):** two capsules of 133.4 mg (200 IU) vitamin E.
- **533.6 mg (800 IU):** two capsules of 266.8 mg (400 IU).
- **Placebo:** two matching placebo capsules.

Participants will be instructed to take the two capsules contained in one blister packaging compartment with water by mouth each morning with breakfast.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

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. All drug and placebo will be distributed to the individual sites by the Drug Distribution Center (DDC) at the direction of the Data Coordinating Center (DCC) and received by the research pharmacist at each site. Expired or unused drug at the end of the study will be disposed of by each site.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

For this study, each of the 133.4 mg (200 IU) and 266.8 mg (400 IU) vitamin E and placebo capsules will be specially formulated to be identical in size and coloring. Capsules will be provided by Pharmavite.

Study drug will be supplied to participants in blister packaging labeled as “Vitamin E or placebo for investigational use only”. Each compartment or ‘blister’ in the blister packaging will contain the intended two capsule dose (ie, 200 IU + matching placebo).

6.2.3 PRODUCT STORAGE AND STABILITY

Capsules should be stored at 59 °F to 75 °F in tightly closed containers and relative humidity not to exceed 50%.

6.2.4 PREPARATION

No preparation is required because capsules will be provided to sites in patient ready packaging.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization: The randomization scheme will assign patients into four groups to receive vitamin E at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) or placebo daily. The randomization design will be stratified by clinical center with assignments in permuted blocks of random length within each clinic. This scheme will ensure that the four groups will be balanced by calendar time of randomization (to minimize secular effects) and by clinic (to minimize clinic-specific effects of differences in patient populations and management).

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.

Treatment assignments are double masked throughout the study until all data collection for the VEDS trial has been completed (i.e., after completion of the post-trial follow-up for all participants). Every effort will be made to maintain the masking throughout the study except in emergencies. The code of specific pharmacological treatment will not be broken without the knowledge of the clinical center's principal investigator and the study leadership.

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), and the investigator may be unmasked.
- **Pregnancy during the study**: Study medication will be stopped indefinitely, and the coded medication may be unmasked. The patient, PCP, and investigator will be notified of the associated risks of teratogenicity.
- **Development of hepatotoxicity:** The drug induced liver injury (DILI) criteria described in section 7.1 will be used to define hepatotoxicity for this study. Study medication will be discontinued; however, there will be no unmasking until study conclusion.
- **Development of serious bleeding**: Serious bleeding will be defined as bleeding associated with a serious adverse event (SAE) (see section 8.3.2). The patient, primary care provider (PCP), and the investigator may be unmasked.

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

6.4 STUDY INTERVENTION COMPLIANCE

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 drug will provide clinic staff a means to identify participants having problems with adherence. Adherence will be assessed during the trial by conducting a brief, structured interview, in which the study coordinator will assist the patients in identifying problems in taking the study drug and in estimating adherence to the prescribed medicine since their previous visit. These assessments will guide the consideration of strategies to improve adherence. Resources may be provided by clinics to remove barriers to participation such as child care,

transportation, and parking expenses. These resources can be provided as cash, transportation vouchers, or parking passes. An honorarium may be paid to participants in recognition of their time and effort when scheduled visits and procedures are completed successfully. Certificates of appreciation may be given at randomization and at conclusion of the VEDS trial as an incentive. Counts of capsules in the patient's returned blister packaging will be used as a measure of adherence at the end of the treatment period.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and supplements.

6.5.1 RESCUE MEDICINE

N/A

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

If the participant is in the treatment phase of the trial, and he/she develops a treatment related CTCAE v5.0-defined adverse event grade 3 or above, the medication will be stopped and the participant assessed by the site study investigator who is a licensed physician/hepatologist. If the adverse event was severe, prolonged, or in the view of the investigator the participant is intolerant of the medication, the study drug will be stopped and the participant will no longer receive the study medication, but will continue to be followed in the study according to the protocol, in keeping with the "intention-to-treat" paradigm.

Participants will be monitored for drug induced liver injury and will use the scale below to grade the adverse effects*:

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
ALT	BSL elevated: 3x BSL or >400 U/L	BSL elevated: >3-5x BSL	BSL elevated: >5 -20x BSL	BSL elevated: >20x BSL
AST	BSL nl:3 x ULN BSL elevated: 3x BSL	BSL nl:>3-5 x ULN BSL elevated: >3-5x BSL	BSL nl:>5-20 x ULN BSL elevated: >5-20x BSL	BSL nl:>20 x ULN BSL elevated: >20x BSL
Total Bilirubin	1.5 x ULN	>1.5-3.0 x ULN	>3.0-10.0 x ULN	>10.0 x ULN

Any participant found to meet the above criteria for grade 2 or higher drug induced liver injury will have the study medication stopped pending further study investigator evaluation.

In addition, participants will have the study medication stopped if **ANY** of the following occur[81]:

If normal baseline AST:

- AST value >5 x ULN in two repeat tests (within 14 days); OR
- AST value is >8 x ULN; **OR**
- AST value >5 x ULN AND total bilirubin >2x ULN‡ OR presence of symptoms suggesting liver injury (severe fatigue, nausea, vomiting or right upper quadrant pain),

If elevated baseline AST or ALT:

- ALT or AST value >3 x BSL or 300 U/L in two repeat tests (within 14 days); OR
- ALT or AST value >5 x BSL or >500 U/L (whichever occurs first); OR
- ALT or AST value >3 x BSL or >300 U/L (whichever occurs first) AND total bilirubin >2x ULN‡ OR presence of symptoms suggesting liver injury (severe fatigue, nausea, vomiting, or right upper quadrant pain)

Follow-up visits will continue, but the study medication will not be restarted. The participant will be included in intention-to-treat analyses.

Additionally, if a participant is found to have signs of increased bleeding (i.e. petechiae) on physician assessment or a participant reports any signs of increased bleeding (i.e. bleeding gums), the study medication will be discontinued until the participant can be further examined by a study physician. The study medication may be restarted after further examination if the bleeding was not associated with a serious adverse event (CTCAE grade 3 or higher) and if the study physician deems a re-challenge appropriate.

* Values for ULN are defined at each clinical center per laboratory guidelines.
‡ For participants with suspected Gilbert's Syndrome at baseline: doubling of direct bilirubin

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance (as determined by primary investigator)
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive study drug for > 4 weeks.

Discontinuation from the study medication (vitamin E or matching placebo) does not mean discontinuation from the study and remaining study procedures should be completed as indicated by the study protocol. The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Participants who sign the informed consent form and are registered but do not receive the study intervention may be replaced. Participants who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant, reschedule the missed visit within 4 weeks, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable after two visit windows have passed, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

8.1.1 VISIT SCHEDULE OVERVIEW

- Screening for eligibility for randomization (60 days)
- Randomization to treatment (1 visit)
- Treatment phase (4 visits over 24 weeks)
- Post-treatment phase (3 visits over 24 weeks)

8.1.2 SCREENING AND BASELINE DATA COLLECTION

Patients who appear to be eligible after chart review 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 detailed below will include questionnaires, baseline medical history, cardiovascular risk factor assessment, physical examination, measurement of fasting serum glucose and insulin, lipid and metabolic tests, etiologic tests, FibroScan® VCTE exam, and blood collection for serum and plasma banking. Prior therapy for NAFLD will be reviewed as outlined in the inclusion and exclusion criteria. Patient charts will be reviewed for historical information.

All participants who sign the consent documents will be registered in the trial database. Each participant who starts screening will be accounted for at the end of screening, as either a screening success (enrolling in the trial) or a screening failure. A screening failure is defined as a participant who signed the consent form, but is found to be ineligible prior to randomization. Screening failures include patients who meet medical eligibility criteria but who refuse randomization in the trial. Reasons for screening failure will be recorded in the trial database. Screening 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. The procedures completed during screening are described below.

Screening visits

Determination of eligibility will be based mostly on chart review of standard of care tests and procedures that were completed before the first screening visit. The participant will sign the consent at the first screening visit (or before) to obtain any tests and procedures needed to finalize eligibility after chart review. Participants will undergo a medical history and detailed physical examination including anthropometric assessments (body weight [kg], body height [m], body mass index [BMI], waist circumference [cm], and hip circumference [cm]) to identify other illnesses and contraindications for participation, including signs of bleeding (gum, petechiae), hepatosplenomegaly, peripheral manifestations of liver disease, and ascites. Laboratory test results that need to be recorded from chart review or obtained as part of the screening visits include: tests for hepatitis B (HBsAg) and hepatitis C, anti-nuclear antibody (ANA), anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA), ceruloplasmin, A1AT concentration, iron overload (iron, TIBC, and ferritin) and thyroid stimulating hormone (TSH). The ALT measurement must be completed within 30 days of randomization. The additional lab tests for screening must be completed within 60 days of randomization: fasting serum glucose, insulin, HgbA1C, complete blood count (CBC) with white blood cell differential, hepatic panel (total and direct bilirubin, AST, alkaline phosphatase, albumin, total protein, GGT), coagulation tests (aPTT, PT, INR), metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, uric acid), eGFR (using the CKD-EPI equation), C-reactive protein, and lipid panel (total cholesterol, triglyceride, LDL, HDL), PSA (males only). Use of medications that could potentially interact with vitamin E (e.g. anticoagulants, those altering fat absorption) will be queried and documented.

Females of childbearing potential must have a negative pregnancy test and will be assessed for willingness to use ≥ 1 form(s) of effective birth control for the duration of the study. Frequency and amount of alcohol intake will be obtained using the Skinner Questionnaire and the Alcohol Use Disorders Identification Test (AUDIT). Patients will complete the BEV-Q questionnaire to document habitual beverage intake (grams, energy) and a liver symptoms questionnaire developed by the NASH CRN to identify symptoms associated with liver disease, severity of symptoms, and impact on lifestyle. A blood sample will be drawn to obtain serum/plasma for banking for future analysis of markers of exploratory endpoints including measures of vitamin E.

Baseline FibroScan®

A FibroScan® procedure will be performed within 60 days of randomization for baseline data collection purposes. Before the procedure, the patient will verify that he/she has fasted for 3 hours prior to the exam. The FibroScan® exam will be performed by a FibroScan®-certified technician. Duration of the exam will be no longer than 30 minutes.

The NASH CRN clinic data system will include coding to check patient eligibility based on keyed data forms. The randomization visit will only become available in the data system when all forms are keyed, and any issues will be flagged as red until resolved or verified, so clinic staff can use the data system to visually identify the items that still need to be completed, keyed, or verified after data from the screening visit are keyed. The randomization visit should not take place until the data system indicates that the patient is eligible except for the items that can be completed only at the randomization visit.

8.1.3 RANDOMIZATION

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 the screening visits have been keyed to the trial database. Since these processes take time, randomization cannot be done at a screening visit, 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 the screening visit is necessary. Since this will be a visit on a different calendar day and the study medication will be prescribed 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: verification that the patient is feeling well; affirmation of consent; review of concomitant drugs and vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature). All patients will be given information on a healthy life style and diet appropriate for their weight and other factors.

Generation of the random treatment assignment will occur at this visit. The randomization 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 'kit' numbers; these numbers will be unique and will be specific to the particular patient it was generated for. They will correspond to numbered cartons ('kit') of medications which have been sent to the clinical center's research pharmacy (or clinical coordinator if not using a pharmacy) by the Drug Distribution Center. The research pharmacy (or clinical coordinator) will issue the specific numbered kit 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 drug and instructed about starting the drug and monitoring for adverse effects. The date of randomization is the 0 time for reckoning all follow-up visits (i.e., all follow-up visits are scheduled at specific times measured from the date of randomization). The data system will display 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. This will ensure that the data collected at the follow-up visit may be used in the trial.

8.1.4 FOLLOW-UP VISITS

Patients will return to the clinical center (see section 8.1.4.1 for optional remote visit allowances) for treatment phase follow-up visits at 4, 8, 12, and 24 weeks after randomization. Post-treatment phase visits will occur at week 28, week 36, and week 48 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 date for a visit is the exact anniversary date from randomization. Visit windows will be constructed to be contiguous, so that at any point in time, some visit window is open, subject to a check on the minimum separation of at least two weeks is required between consecutive visits. The specific procedures to be completed at each of the follow-up visits are:

- **Week 4 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; review of study drug adherence; blood draw for fasting glucose and insulin, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein); questionnaire for symptoms of NAFLD; focused physical examination including height, weight, waist and hip measurements, vital signs, oropharynx, and skin examination for petechiae or other signs of increased bleeding.
- **Week 8 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; review of study drug adherence; blood draw for fasting glucose and insulin, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein); questionnaire for symptoms of NAFLD; focused physical examination including height, weight, waist and hip measurements, vital signs, oropharynx, and skin examination for petechiae or other signs of increased bleeding.
- **Week 12 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; review of study drug adherence; blood draw for fasting glucose and insulin, fasting lipid profile, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), and C-reactive protein; blood draw for plasma and serum banking at a central repository; questionnaire for symptoms of NAFLD; detailed physical examination including height, weight, waist and hip measurements, vital signs, oropharynx, and skin examination for petechiae or other signs of increased bleeding.
- **Week 24 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; detailed physical examination, including height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), organ systems and liver signs; FibroScan®; blood draw for CBC, comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, eGFR), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), GGT, PT, aPTT, INR, C-reactive protein, fasting glucose, insulin, HbA1c, and lipid profile; blood draw for plasma and serum banking at a central repository; pregnancy test for women of childbearing potential, questionnaire for symptoms of NAFLD; BEVQ; standardized nutrition and exercise prescription and counseling. Review study drug adherence with patient. Collect all study drug blister packaging, including empty packaging. END OF TREATMENT PHASE
- **Week 28 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; blood draw for fasting glucose and insulin, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein); questionnaire for symptoms of NAFLD; focused physical examination including height, weight, waist and hip measurements, vital signs, oropharynx, and skin examination for petechiae or other signs of increased bleeding.
- **Week 36 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; blood draw for fasting glucose and insulin, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein); questionnaire for symptoms of NAFLD; focused physical examination including height, weight, waist and hip measurements, vital signs, oropharynx, and skin examination for petechiae or other signs of increased bleeding.
- **Week 48 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; detailed physical examination, including height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), organ systems and liver signs; FibroScan®; blood draw for CBC, comprehensive metabolic panel (sodium, potassium, chloride, carbon

dioxide, calcium, BUN, creatinine, eGFR), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), GGT, PT, aPTT, INR, C-reactive protein, fasting glucose, insulin, HbA1c, lipid profile, and PSA (males only); blood draw for plasma and serum banking at a central repository; BEVQ questionnaire for symptoms of NAFLD; Closeout form

8.1.4.1 REMOTE VISITS

Investigators and staff may elect to perform the week 4, 8, 28 and/or 36 visit(s) either in part or completely remotely, via telemedicine. Remote visits will adhere to the Data Collection Schedule (section 1.3) with the following modifications allowable for visit procedure completion:

- Focused physical exam may be completed via the local institutional telemedicine portal. Video calls should be utilized.
- Laboratory test results may be obtained through an outside lab, as needed.
- Questionnaires may be completed via correspondence.

8.1.5 STANDARDIZED QUESTIONNAIRES

Alcohol Use Disorders Identification Test (AUDIT) is a 10-item questionnaire with a simple scoring scale that will be administered during screening. A 3-item interim drinking history (AUDIT-C) measuring consumption since the last visit will be obtained during follow-up visits as part of the follow-up medical history. The purpose of these questionnaires is to ascertain that there is no significant alcohol consumption prior to randomization or during the study period.

Skinner Lifetime Drinking History: is an interview based questionnaire that will be administered during screening. The purpose of this tool is to obtain quantitative indices of the patient's alcohol consumption patterns from the onset of regular drinking.

Bev-Q: The Beverage questionnaire will be administered during screening and at the 24 week and 48 week follow-up visits. The purpose of this questionnaire is to determine the patient's regular beverage intake, including nonalcoholic beverages.

8.1.6 SPECIMEN REPOSITORIES

Specimens will be collected and stored in the NIDDK central repositories for use as approved by the Steering Committee of the NASH CRN. Specimens include serum and plasma. The blood collected during screening, and at the 12, 24, and 48 week follow-up visits will be separated into plasma and serum, and divided into 0.5 mL aliquots. Aliquots will be kept in a storage facility at -70 degrees C until they are shipped to the NIDDK Central Repository on dry ice.

8.2 SAFETY AND OTHER ASSESSMENTS

Assessments for Safety

- **Physical examination** (e.g., height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), liver symptoms, gingival/orocutaneous bleeding; petechiae.
- **Laboratory results –** All labs including creatinine (and calculated GFR) potassium levels and bicarbonate will be monitored at sites and in the central database and all abnormal values will be managed by the site investigator.
- **Bleeding risk** – Participants will be evaluated for bleeding risk throughout the study using physician examination, laboratory results of prothrombin time, activated Partial Thromboplastin Time (aPTT), and INR, and patient self-report.
- **Concurrent use of anticoagulant** – Participants will be questioned about use of any anticoagulants, including aspirin, throughout the study.

Biological specimen collection and laboratory evaluations – There will be consistent methods throughout the study to ensure comparison. ALT will be measured at each site and the same lab at each site will be used for the measurement at baseline, 24 week and 48 week visits. Other local labs may be used, if necessary, when completing an allowable remote visit (see section 8.1.4.1), and will be noted in the data system. **Follow up of Ongoing Adverse Events**

Provisions for follow up of adverse events include phone calls by the coordinators to assess symptoms, in person follow up exams when needed to assess vital signs and physical findings and referral to local ongoing care by the patient's primary care physician or hospital when needed.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is **not required** to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

Site PIs and investigators will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Site coordinators will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

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.

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 5.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE).[82]

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 sIRB. Analyses or listings of adverse events will not be provided to the sIRB; however, adverse events involving unanticipated problems involving risks to patients, or breaches of protocol which might entail risk to patients must be reported to the sIRB and, as required, to the local IRB as soon as possible after they are discovered. Each participating center is responsible for ensuring that all sIRB and local IRB requirements for reporting adverse events are met.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Serious adverse events (SAE) must be reported upon discovery at the clinical center. This will involve completing a data form describing the severity and details of the event, which must be submitted to the Data Coordinating Center within one business day for review by the Safety Officer.

If the SAE is **unexpected AND there is a reasonable possibility that the study drug caused the SAE,** then the clinical center must complete a data form for an IND Safety Report and submit it along with a narrative and a copy of the IRB report to the DCC. The DCC will submit a preliminary report to the NIDDK Program Official, Project Scientist and the NIDDK Regulatory Affairs Specialist for review within three business days of receiving the SAE data form. The study drug manufacturer will also be notified within 7 days of the serious adverse event, if applicable. If NIDDK determines that the SAE requires an expedited IND Safety Report, 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 by the DCC (no later than 7 calendar days if the SAE is fatal or life threatening). The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form and additional information for a follow-up SAE report as information becomes available. If the FDA determines that a change to the Investigator's Brochure (IB), IND, or protocol is needed, the Data Coordinating Center will send a copy of the IND Safety Report to all clinical centers, with instructions to forward the report to their IRB.

The DCC will maintain a list of all SAEs for reporting and review at Steering Committee meetings and DSMB meetings. The DSMB will review each SAE report. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the SAE and recommend any actions to the NIDDK sponsor. The clinical center must submit to the NIDDK and to the Data Coordinating Center a follow-up memo within one month of the SAE (and periodic updates if needed) to report the details of the disposition of the SAE.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

N/A

8.3.8 EVENTS OF SPECIAL INTEREST

N/A

8.3.9 REPORTING OF PREGNANCY

Participants will be monitored for pregnancy and will be required to take precautions to prevent pregnancy. However, if a participant is found to be pregnant, the study medication will be stopped immediately and the coded medication may be unmasked. The participant, primary care physician, and investigator will be notified of the associated risks of teratogenicity.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to patients or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the patient population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Other events meeting prompt reporting criteria as detailed by the Johns Hopkins Medicine, Institutional Review Board (JHM IRB) "Organizational Policy on Prompt Reporting of Reportable Events" are:

- **Potential serious or continuing non-compliance**. Non-compliance is defined as "the failure to follow the research protocol, federal, state, or local laws or regulations governing human subjects research, institutional policies, or the requirements or determinations of the IRB". Incidents of non-compliance are reportable when they are instances of either:
    - Serious Non-compliance that either "(a) significantly harms or poses an increased risk of significant harm to subjects or others, or (b) significantly compromises the rights and welfare of research subjects."
    - Continuing Non-compliance, where "a pattern of non-compliance significantly compromises the scientific integrity of the study or the rights and welfare of the subjects or the integrity of the Organization's human research protection program"
- **Potential Breaches of Confidentiality**, which are defined as "any unauthorized disclosure of subject's personally identifiable information".
- **Incarceration.** If a patient becomes incarcerated during their participation in the study, and the local clinical team intends to continue any data collection while the subject is incarcerated, it is immediately reportable.

Unresolved Subject Complaints. "Complaints of subjects when the complaint indicates unexpected risks or cannot be resolved by the research team.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing single Institutional Review Board (sIRB), the local relying IRB), and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 10 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 10 days of the investigator becoming aware of the problem.

- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 10 days of the IRB's receipt of the report of the problem from the investigator.
- Reports should be sent to: *IRPT.OS@hhs.gov*

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

AEs will be reported to participants if they are unexpected, related or possibly related, and suggest greater risk of harm to participants.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s): That one of the doses of vitamin E at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) daily is the minimum effective dose for improving relative change in alanine aminotransferase (ALT) from baseline to 24 weeks.

- Secondary Efficacy Endpoints(s): That one or more of vitamin E at 133.4 mg (200 IU), 266.8 mg (400 IU), or 533.6 mg (800 IU) daily is better than placebo in improving:
    - Proportion of patients achieving normalization* of ALT at 24 weeks
    - Mean change in ALT from baseline to 24 weeks
    - Mean change in AST and GGT from baseline to 24 weeks
    - Mean change in hepatic fat determined by FibroScan® CAP score from baseline to 24 weeks
    - Mean change in liver stiffness as assessed by FibroScan® from baseline to 24 weeks
    - Time course and end of treatment improvement in ALT, AST, GGT, and alkaline phosphatase
    - Time course and end of treatment improvement in C-reactive protein (serum marker of inflammation)
    - Time course and end of treatment improvement in change in total cholesterol, LDL cholesterol, HDL cholesterol, and serum triglycerides
    - Changes in fasting glucose levels from baseline to 24 weeks
    - Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference) from baseline to 24 weeks
    - Change in symptom assessment scores from baseline to 24 weeks
    - Change in frequency of CTCAE defined adverse events from baseline to 24 weeks
    - Mean change in ALT from 24 to 48 weeks (post-treatment).

- Mean change in AST and GGT from 24 to 48 weeks (post-treatment).
- Mean change in hepatic fat determined by FibroScan® CAP score from 24 to 48 weeks (post-treatment).
- Mean change in liver stiffness as assessed by FibroScan® from 24 to 48 weeks (post-treatment).

*Normalization of ALT is defined as a decrease in ALT to less than or equal to the ULN at the 24 week visit among participants who had an ALT value greater than ULN at baseline. Values for ULN are defined at each clinical center per institutional guidelines.

9.2 SAMPLE SIZE DETERMINATION

The primary endpoint is the relative change in ALT at week 24 from baseline [i.e., (ALT at baseline – ALT at week 24)/ALT at baseline]. There are three vitamin E treatment groups: 133.4 mg/day, 266.8 mg/day, 533.6 mg/day and one placebo group. The primary analysis is intention-to-treat analysis of minimum effective dose using Williams' test of mean 24-week relative change in ALT. If the percentage of patients with missing follow-up data on the primary endpoint is greater than 10%, multiple imputation using chained equations method with 50 imputations modeling will be used to impute missing values; otherwise complete-case analysis will be used.

Among patients in PIVENS with baseline ALT ≥ 60 U/L, the mean 24-week relative change in ALT was 40% in the vitamin E (800 IU/day) group and 20% in the Placebo group. The Mean (SD) of the vitamin E – placebo difference in relative change (%) was -20% (31%). Using this estimate as the clinically meaningful difference, the trial has 90% power to detect a significant minimum effective dose given a sample size of 200 patients (50 per group with equal allocation) given a 2-sided Type I error of 5%, missing data rate of 10% and using Williams' test for minimum effective dose (MED) (Williams 1971, Williams 1972) obtained from PASS 2019 software (PASS 2019 Power Analysis and Sample Size Software (2019). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass).

The testing for minimum effective dose starts by comparing the 24-week relative change in ALT in each of the 3 dose groups with the placebo group using critical values obtained from Williams' test for MED. (Critical values will be obtained using SAS PROBMC function.) Williams' test assumes the same or increasing efficacy with increasing dose; therefore, if the mean change at a dose level is less in absolute value than the mean change in a lower adjacent dose category, the groups are combined for statistical comparison. The lowest dose that is significantly different from placebo is declared the MED. By definition, all doses greater than the MED are also significantly different from placebo.

Sample size estimates for varying sizes of the treatment effect are given below given the same assumptions as above.

24-week relative change in ALT			**Sample size**	
Vitamin E	**Placebo**	**Vitamin E – Plbo**	**No. / group**	**Total**
48%	20%	28%	25	100
44%	20%	24%	34	136
40%	**20%**	**20%**	**50**	**200**
36%	20%	16%	76	304

32%	20%	12%	135	540
28%	20%	8%	303	1212

9.3 POPULATIONS FOR ANALYSES

The analysis dataset will include:

- Intention-to-Treat (ITT) Analysis Dataset (i.e., all randomized participants)
- Safety Analysis Dataset: defines the subset of participants for whom safety analyses will be conducted (e.g., participants who took at least one dose of study intervention)
- Other Datasets that may be used for sensitivity analyses (e.g. subgroups by dose, sex, age, baseline labs, adherence)

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The primary intention-to-treat analysis uses Williams' ANOVA-based test to find, with high statistical power (90%), the minimum effective dose of vitamin E that improves the per-person primary endpoint, defined as the percentage relative change in ALT from baseline over 24 weeks of treatment with either vitamin E or matching placebo. The sample size justification allows for 10% missing data using a complete-case Williams' test on the non-missing data; however, if the percentage of patients with missing data on the primary endpoint is greater than 10%, multiple imputation modeling at each step in the Williams' test procedure will be used to include all randomized patients in the determination of the step-by-step p-values needed for the Williams' test dose-finding procedure.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

- Intention-to-Treat (ITT) Analysis Dataset

The primary endpoint is 24-week relative change in serum ALT, defined as the ALT obtained at the 24-week visit minus ALT at the baseline visit divided by ALT at baseline [i.e., (ALT at baseline – ALT at week 24)/ALT at baseline]. The change in serum ALT is measured on a continuous scale and is a single endpoint. The Williams' ANOVA-based test for minimum effective dose of vitamin E starts by comparing the primary endpoint between the lowest vitamin E dose of 133.4 mg (200IU) vs. placebo. If significantly better ($p<0.05$) using the 5th percentile from the Williams' test, then 133.4 mg is declared the minimum effective dose and no other comparisons are made; If the previous step is not significantly better, then the 266.8 mg (400 IU) dose is compared vs placebo. If significantly better ($p<0.05$) using the 5th percentile from of the Williams' test, then 266.8 mg (400IU) is declared the minimum effective dose and no other comparisons are made. If the previous step is not significantly better, the 533.6 (800IU) mg dose is compared vs placebo. If significantly better ($p<0.05$) using the 5th percentile from the Williams' test, the 533.6 (800IU) mg is declared the minimum effective dose and no other comparisons are made. If the previous step is not significantly better, then declare that no minimum effective dose for vitamin E was found. The primary analysis will present the (up to) 3 p-values from the Williams' test steps above and, only if the William's

test finds a minimum effective dose, the primary analysis will also include the estimated difference and 95% confidence interval for 24-week percentage relative change vs. baseline, comparing the minimum effective 24-week dose group vs placebo. As sensitivity analyses, the 3 separate comparisons of each vitamin E dose group vs placebo with estimates and 95% confidence limits be presented. Sensitivity analyses will include use of robust regression to down-weight the effect on the Williams' test minimum effective dose selection of influential outliers in the relative ALT change from baseline measures. Other sensitivity analyses may be used to balance potential effects on results of confounders not balanced out by randomization, as judged by the trial Data Safety and Monitoring Board.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

For each secondary endpoint:

- Analyses of secondary endpoints are not dependent on findings of the primary endpoint or on the results of the Williams' test performed on the primary endpoint.
- Analyses will use complete case subjects of the Intention-to-Treat (ITT) Analysis Dataset (i.e., all randomized participants with complete data on each specific secondary endpoint).

The analysis of the 16 secondary endpoints listed in Section 3, Objectives and Endpoints, endpoints will use ANCOVA for continuous endpoints, logistic regression for binary endpoints, and Poisson regression for counts. Complete case analyses will be used for each secondary endpoint, if missing data on that endpoint < 10% and multiple imputation will be used if missing data on the secondary endpoint is 10% or greater. The covariate in the continuous endpoint ANCOVA models will be the baseline value of the continuous endpoint. Sensitivity analyses include use of non-parametric and influence-reducing regression for continuous endpoints (lowess plots and robust regression) and generalized linear models with scale factor estimation to account for under- or over-dispersion for binary endpoint data (dispersion-corrected logistic regression) and count endpoint data (dispersion-corrected negative binomial regression). Each secondary endpoint in the planned table of 16 secondary endpoints will be compared across the 3 vitamin E doses vs Placebo using treatment effect estimates, 95% confidence limits, and p-values. The Benjamini-Hochberg Method will be use to adjust the presented p-values for the false discovery rate (FDR) from the 16x3=48 pairwise p-values.

9.4.4 SAFETY ANALYSES

Safety data will be presented by treatment group to the Data and Safety Monitoring Board during the course of the trial. Each adverse event will be displayed showing AE name per the CTCAE v5.0 when applicable, class, severity, patient ID, clinic, treatment group, time since randomization, resolution status and physician's assessment of relatedness to treatment. Patient-specific summary tables including any adverse event (yes vs no), frequency of multiple adverse events (i.e., 0, 1, 2, 3+) and highest severity of adverse event (none, mild, moderate, severe, life-threatening) will be displayed by treatment group along with p-values from Fisher's exact tests.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline data will be presented by treatment group and total. Data include stratification variables (clinic) demographics, liver enzymes, lipids, hematology, chemistries, metabolic factors, concomitant medications, comorbidities, and liver histology findings. P-values will be presented as descriptive statistics noting that any treatment group differences are due to chance.

9.4.6 PLANNED INTERIM ANALYSES

There are no planned interim analyses.

9.4.7 SUB-GROUP ANALYSES

There are no formal planned sub-group analyses. Exploratory sub-group analyses will include treatment effects of primary and selected secondary endpoints by stratum variable (clinic), demographics (age, race, sex), severity of NAFLD, and study drug adherence.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

For monitoring of adverse effects, individual data may be presented to the Data and Safety Monitoring Board. In that case, study patients will be identified only by study ID. Names and other unique individual identifiers such as Social Security Number are not collected by the study and therefore will not be presented to the DSMB. Data on individual patients will not be presented in any manuscript or presentation.

9.4.9 EXPLORATORY ANALYSES

In parallel to the ANCOVA statistical methods used to compare continuous endpoints between the treatment groups for both the primary and secondary endpoints, we will compare results arising from ANOVA analysis (linear regression of 24-week change among treatment groups without adjusting for the baseline value of the continuous endpoint) will be used for exploratory analyses. Since ANCOVA and ANOVA are alternative models for “difference in difference” data as in the VEDS trial, we expect, given the randomized design, that the two methods will give similar results or the primary and secondary endpoints.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the patient and written documentation of informed consent is required prior to participation in the study.

Master consent documents will be approved by the single Institutional Review Board of reliance (sIRB) for the study for screening to determine eligibility with an affirmation of consent for enrollment in the study. Relying sites may not delete material from the master consent. Relying sites will add a site-specific addendum containing locally required language to the master consent. The consent document will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Copies of the signed consent forms will be provided to the patient by paper or electronically, and this fact will be documented in the patient’s record.

At relying sites in which Spanish-speaking patients will be recruited, certified translations of the consent document will be available in Spanish.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. This process may be completed in-person or remotely.

If the consent process is to be completed remotely WITHOUT an electronic signature, the following procedures must be followed:

- A copy of the sIRB approved Informed Consent document must be provided to the participant prior to the teleconsent meeting either via email, fax or mail or must have been previously provided during an in person visit. The person conducting the consent process may conduct the process via telephone or video conference.

- After the consent designee and participant reviews the consent form, the participant is offered the opportunity to ask any questions and have those questions answered.

- The participant will sign and date/time the informed consent document.

- The consent designee must verify the participant physically signed the consent document either by viewing via video conference, obtaining a photo of the complete signed consent document, or obtaining verbal confirmation from the participant that he/she signed the consent form and agreed to participate electronically.

- The signed document is then mailed, emailed or faxed to the consent designee.

- If the signed consent is emailed or faxed or returned via photo, the participant will be asked to return the original signed document on their first in person visit. Once received, the sIRB-approved consent designee signs, dates/times the original informed consent document. Both the original and emailed or faxed copy should be retained in the patient record and a copy of the original signed by both parties should be provided to the patient.

- If the informed consent form is mailed to the consent designee by the participant, the sIRB-approved consent designee will sign the copy which they possess after the participant has acknowledged signature on their copy. Once the original is received by the consent designee the copies will be attached to make a single document.

If electronic signatures are permitted per local institutional guidelines, an electronic documentation of the consent process is permissible.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the reviewing single Institutional Review Board (sIRB), local IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility
- Cessation of in-person clinical activities due to a pandemic or other catastrophe

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, sIRB and/or Food and Drug Administration (FDA). The DCC will ensure that sites follow local, state, and institutional requirements regarding cessation and restart of Human Subjects research during the COVID19 pandemic or other natural disaster and collect IRB approvals, as indicated.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the single Institutional Review Board (sIRB), regulatory agencies or company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing sIRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NASH CRN Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a

unique study identification number. The study data entry and study management systems used by clinical sites and by the NASH CRN Data Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NASH CRN Data Coordinating Center and NIDDK Central Repository.

To further protect the privacy of study participants, the research is covered under a Certificate of Confidentiality by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the NASH CRN Data Coordinating Center. After the study is completed, the de-identified, archived data will be transmitted to and stored at the NIDDK Central Repository, for use by other researchers including those outside of the study. Permission to transmit data to the NIDDK Central Repository will be included in the informed consent.

With the participant's approval and as approved by the single Institutional Review Boards (sIRB), de-identified biological samples will be stored at the NIDDK Central Biorepository with the same goal as the sharing of data with the NIDDK Central Repository. These samples could be used to research the causes of NASH, its complications and other conditions for which individuals with NASH are at increased risk, and to improve treatment. The NIDDK Central Repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, the NIDDK Central Repository will provide oversight for the access to and use of study data and/or samples.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Independent Medical Monitor**
Arun J. Sanyal, MD	Victor Chen, MD
Virginia Commonwealth University Medical Center	Johns Hopkins University
West Hospital, 14th Floor 1200 East Broad Street Box 980341 Richmond, VA 23298	1830 E Monument St Baltimore, MD 21205
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NASH CRN Executive Committee (EC)- Consists of NASH CRN chairperson, SC representatives, principal investigator of the Data Coordinating Center, safety officer, NIDDK program official, and NIDDK project scientist. The NASH CRN EC discusses directions and strategic issues related to the scientific aims of the NASH CRN, organizes and sets agenda for Steering Committee meetings, and provides oversight of the study.

NASH CRN Steering Committee (SC) – Consists of principal investigators of each of the clinical centers, the principal investigator of the Data Coordinating Center, the co-chairs of the Pathology Committee (who share one vote), the chair of the Radiology Committee, and the NIDDK project officer, each of whom has one vote in any decision requiring formal vote. Ex officio members include NIDDK scientific staff. The NASH CRN SC is the major decision making body for NASH CRN, which provides oversight in planning and conduct of the study. The SC votes on all important decisions and approves the final protocol and any amendments or modifications of the protocol.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including gastroenterology, hepatology, cardiology and statistics. Members of the DSMB are independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest.

An Independent Medical Monitor and an independent Data and Safety Monitoring Board (DSMB), appointed by the NIDDK, will review the protocol for the VEDS trial and monitor the 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 communicate DSMB recommendations to the investigators, as appropriate. The DSMB will hold a meeting to approve the protocol. The DSMB will review performance and safety data. The DSMB may request 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. 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 2 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 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.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the NASH CRN DCC.
- To ensure data quality, the DCC will incorporate into the data entry programs a series of edit checks, including range checks, logic checks (e.g., skip patterns), and consistency checks, both within a data form and across forms. These integral checks are designed to detect deficiencies in the data before they are saved into the study database, so that the deficiencies may be easily and accurately corrected as they are being entered. In addition, the DCC will perform frequent computerized edit checks on entered data. The DCC will also perform ongoing comparisons of copies of source documents with the databases to ensure that data entered into the data system reflect those recorded on the source documents. Such database validations will help to pinpoint problems that cannot be detected by computer editing and may be used to guide increased scrutiny when needed. Any data queries identified will be flagged for action by the clinical site until resolved.
- The data system will maintain a clear and complete audit trail of all changes to the study databases. All changes to data forms will be documented appropriately on the source document and entered into NASH CRN data system. All forms entered and/or edited in the data system will be identified by the PIN of the operator, the network address (the "IP" address) of the computer being used, and the date and time of the operation. Records will not be deleted but rather marked as having been superseded.
- Performance monitoring: The web-based data system will allow for real-time reporting of most recruitment and data management activities. However, additional performance reports will be generated and circulated monthly and typically reviewed at each Steering Committee (SC) meeting. These reports include several key indicators of study performance, including counts of patients screened and randomized, of completed visits and of missing key data, of missed visits, and statistics summarizing performance with respect to timeliness of data entry and response to data queries.
- The SC and the DSMB will conduct a formal review of the study by webinar twice a year and will address quality assurance as part of their agenda. These committees will have responsibility for recommending corrective actions based on the performance data. It is anticipated that the primary responsibility for formulating and implementing these actions will reside in the SC. Potential actions might include specific recommendations for training, redistribution of study resources, or possibly termination of support for a center.
- Independent audits outside the DCC will not be conducted.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the NASH CRN monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the NASH CRN, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hard copies of the study visit worksheets will be printed by clinics for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into the 21 CFR Part 11-compliant electronic data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Under the HIPAA Privacy rule, investigators must maintain study records for 7 years after the study ends or 7 years after the last disclosure of identifiable health information from study records, whichever is later.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Standard Operating Procedures (SOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents and reported to NIDDK Program Official and the Data Coordinating Center. Protocol deviations must be sent to the reviewing single Institutional Review Board (sIRB) and the local Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing sIRB and local IRB requirements. Further details about the handling of protocol deviations will be included in the SOP.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations and the policies of the NASH CRN:

National Institutes of Health (NIH) Public Access Policy ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and the results of this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from the NIDDK Central Repository (*https://www.niddkrepository.org/search/study/*) two years after the completion of the primary endpoint.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The NASH CRN leadership in conjunction with the NIDDK has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

10.2.1 WHOLE BLOOD DRAW SCHEDULE (AMOUNTS IN ML)

	Study visit									
Procedure	**S**	**RZ**	**F04**	**F08**	**F12**	**F24**	**F28**	**F36**	**F48**	**Total**
Fasting glucose and insulin	5	-	5	5	5	5	5	5	5	40
Fasting lipid	5	-	-	-	5	5	-	-	5	20
Complete blood count	5	-	-	-	-	5	-	-	5	15
Clinical chemistry	5	-	-	-	-	5	-	-	5	15
Hepatic panel	5	-	5	5	5	5	5	5	5	40
PT, aPTT, INR	5	-	-	-	-	5	-	-	5	15
HbA1c	5	-	-	-	-	5	-	-	5	15
C-Reactive Protein	1	-	-	-	1	1	-	-	1	4
Plasma	10	-	-	-	10	10	-	-	10	40
Serum	20	-	-	-	20	20	-	-	20	80
Other screening*	20	-	-	-	-	-	-	-	-	20
PSA (males only)	*1*	-	-	-	-	-	-	-	*1*	*2*
Total	**86** *(87)*	**0**	**10**	**10**	**46**	**66**	**10**	**10**	**66** *(67)*	**304** *(362)*

VEDS study visits are fasting visits and need to be scheduled for early morning. Fasting is defined as nothing by mouth except water in the 12 hours prior to blood draw.

* Etiologic tests as needed

10.3 ABBREVIATIONS

A1AT	Alpha-1-antitrypsin
AE	Adverse Event
ALT	Alanine aminotransferase
AMA	Antimitochondrial antibody
ANA	Antinuclear antibody
ANCOVA	Analysis of Covariance
aPTT	Activated partial thromboplastin time
ASMA	Anti-smooth muscle antibody
AST	Aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
BMI	Body mass index (kg/m^2)
BUN	Blood urea nitrogen
CC	Clinical center
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRN	Clinical Research Network
CRP	C-reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DCC	Data Coordinating Center
DCS	Data Collection Schedule
DSMB	Data and Safety Monitoring Board
EC	Executive Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IMM	Independent Medical Monitor
IND	Investigational New Drug Application
INR	International normalized ratio
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
MCV	Mean corpuscular volume
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NAS	Nonalcoholic fatty liver disease activity score
NASH	Nonalcoholic steatohepatitis

NCT	National Clinical Trial
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator
PT	Prothrombin time
QC	Quality Control
SAE	Serious Adverse Event
sIRB	Single Institutional Review Board
SOA	Schedule of Activities
SOP	Standard Operating Procedure
ULN	Upper limit of normal
UP	Unanticipated Problem
US	United States
WBC	White blood cell count

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.1	08 May 20	Add pregnancy test at 24 weeks and delete z-score	Changes requested by DSMB
1.2	07 Aug 20	Change study drug information throughout	New study drug supplier: Pharmavite
1.3	25 May 21	Corrections made throughout for additional study drug information provided, adjusted dosing scheme, and language compliant with sIRB requirements.	Updated information available
2.0	28 Jul 21	Corrections and additions made throughout in response to FDA comments	Requested by FDA
2.1	01 Apr 22	Allowances for remote visits/procedures. Updated exclusion criteria regarding HCV. Remove baseline data collection listed that is inconsistent with the data collection schedule. Add CRP to data collection schedule. Updated language pertaining to the data system mechanics.	Need for limitations to in-person contact. Corrections throughout for inconsistencies or clarification.

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