PROTOCOL FOR CLINICAL STUDIES

TITLE

THE EFFECT OF VITAMIN E AND DOCOSAHEXAENOIC ACID ETHYL ESTER ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) - A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP CLINICAL TRIAL (PUVENAFLD)

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SYNOPSIS

Title **THE EFFECT OF VITAMIN E AND DOCOSAHEXAENOIC ACID ETHYL ESTER ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) - A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP CLINICAL TRIAL**

STUDY ACRONYM: PUVENAFLD

PROTOCOL NUMBER: 2017-1088 **IND NO.145428**

VERSION 3.0 **DATE MAY 10, 2021**

IND SPONSOR-INVESTIGATOR: Naga Chalasani, MD **Clinicaltrials.gov: NCT04198805**

STUDY PHASE/TYPE
PHASE II /PROOF-OF-CONCEPT INTERVENTIONAL STUDY

PLANNED START OF STUDY Q3 2019 **ESTIMATED STUDY DURATION: 3-4 YEARS**

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STUDY SITES: US, MULTI - CENTER

RESEARCH AREA/APPLICATION FIELD: NON-ALCOHOLIC FATTY LIVER DISEASE/ PREVENTION OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)

RATIONALE
This study will examine the combination of Vitamin E and Docosahexaenoic Acid Ethyl Ester (DHA EE) to improve symptoms of non-alcoholic fatty liver disease (NAFLD). This combination has not previously been tested but may provide benefit to patients with NAFLD by antioxidant and anti-inflammatory mechanisms of action.

PRIMARY OBJECTIVE
To determine the effect of the combination of vitamin E and omega-3 fatty acid, DHA EE versus placebo on reducing liver fat content after 6 months of intervention in adults with NAFLD.

SECONDARY OBJECTIVES

- To determine effect of Vitamin E alone on reducing liver fat content after 6 months of intervention in adults with NAFLD.
- To determine effect of DHA EE alone on reducing liver fat content after 6 months of intervention in adults with NAFLD.
- To determine the change after 6 months of Vitamin E and /or DHA EE intervention in anthropometric, metabolic, hepatologic, nutrient, and inflammatory parameters, and quality of life status of adults with NAFLD.

STUDY DESIGN
Multi-center, randomized, double-blind, placebo-controlled, parallel, 4-group intervention study in adults with NAFLD

STUDY POPULATION
≥18 years old male or female subjects with presence of fatty liver (*min. 12% liver fat content)* by magnetic resonance imaging proton density fat fraction (MRI-PDFF)

SAMPLE SIZE
Total number of subjects: 200 (n=65/65/35/35 for Combination/Placebo/DHA only/Vitamin E only groups)

NUMBER OF SITES
Estimated 16 US sites

INVESTIGATIONAL DRUG PRODUCT
1. Vitamin E [(all-*rac*)-α-tocopheryl acetate] administered orally via softgel capsules
2. Omega-3 fatty acid (DHA EE) administered orally via softgel capsules
3. Vitamin E and DHA EE combination administered orally via separate softgel capsules
4. Matching placebo (soy oil) administered orally via softgel capsules

DOSAGE AND REGIMEN
Vitamin E [(all-*rac*)-α-tocopheryl acetate]1000 IU/day (1000mg/d)
DHA EE 1.89 g/day

DURATION OF SUPPLEMENTATION
6 months

SAFETY PARAMETERS
- Vital signs (e.g. blood pressure, heart rate)
- Serum hematology and biochemistry
- AE/SAE reporting

SUBJECT SELECTION CRITERIA

1.1.1 Inclusion criteria

1. Male or female gender
2. ≥18 years of age
3. A new diagnosis or reconfirmation of previously known fatty liver by imaging (ultrasound or CT or MRI), or by liver biopsy within ≤ 4 years
4. Fibroscan Controlled Attenuation Parameter (CAP) score ≥ 300db
5. Liver fat content (≥ 12%) measured by MRI-PDFF
6. ALT ≥ 40 U/L
7. eGFR/eCreatinine Clearance ≥ 60ml/min
8. Participants with previously diagnosed Type 2 diabetes (up to 50% of sample): they must either be taking anti-diabetic medications, or their fasting (>10 hours) glucose must be ≥ 100 mg/dL at the time of screening
9. Stable weight (±5%) for at least 3 months
10. Subjects willing and able to give written informed consent and to understand, to participate and to comply with the clinical study requirements.

1.1.2 Exclusion criteria

1. Evidence of alternative causes of hepatic steatosis or other forms of chronic liver disease, e.g. Hepatitis B, Hepatitis C (with <3 years treatment)
2. Evidence of acute Hepatitis A
3. Serum ALT or AST ≥ 250 U/L
4. Serum Alkaline Phosphatase > 2 ULN
5. Total bilirubin > 2 ULN in the absence of Gilbert's Syndrome [In patients with Gilbert's Syndrome, direct bilirubin must not exceed 2 ULN]
6. HbA1c > 9.5%
7. Decompensated acute or chronic liver disease
8. Clinical, imaging or histological evidence of cirrhosis
9. Use of anti-NASH drugs (e.g. thiazolidinediones (TZD)) in the 3 months prior to randomization
10. Use of a **non-stable** dose of statins or fibrates in the 3 months prior to randomization
11. Use of fish oil, algal oil or Krill oil supplements, drugs or foods fortified with omega-3s in the 2 months prior to randomization (>200 mg DHA/d and/or > 60mg EPA/d intake by FFQ)
12. Known intolerance to vitamin E or DHA
13. Malabsorption of Vit E (e.g. due to steatorrhea, chronic pancreatitis, severe cholestasis)
14. Vitamin E supplementation of greater than 100 IU/day in the 3 months prior to randomization
15. History of bariatric surgery (jejunoileal bypass or gastric weight loss surgery) or currently undergoing evaluation for bariatric surgery
16. History of biliary diversion

17. Known positivity for antibody to Human Immunodeficiency Virus
18. Patients with coagulopathy (PT ≥ 3 sec. from ULN) or thrombocytopenia (<70K)
19. Contraindication to MRI (implants, metal…)
20. Active, serious medical disease or disease diagnosis of a life-expectancy less than 5 years
21. Ongoing or recent alcohol consumption > 21 drinks (1 drink= 12 oz regular beer, or 5 oz wine, or 1.5 oz distilled spirits) per week in men and > 14 drinks per week in women as per subject self-report as part of medical history.
22. Active substance abuse, such as oral, inhaled or injected illicit drugs (except marijuana), in the year prior to screening
23. Women of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
24. Women who are breastfeeding
25. Any other condition which, in the opinion of the investigator would impede compliance or hinder completion of the study
26. Subjects who are enrolled in an interventional clinical study or have received an investigational new drug or product within the last 30 days prior to screening
27. Participants diagnosed with type 1 diabetes

STUDY PROCEDURES

Study visit overview:

The patient-related activities of the PUVENALFD trial are divided into 5 phases:

1. Screening of eligibility for enrolment (Visit 0),
2. 2nd Screening with MRI-PDFF (Visit 1)
3. Randomization (Visit 2),
4. Phone call at 1 month for compliance
5. Visit at 3 months (Visit 3), and
6. Visit at 6 months (Visit 4)
7. *Screening of eligibility for enrollment (Visit 0):*

All participants undergo a Fibroscan first (≥300db) and must meet criteria for ALT ≥ 40 to assess the eligibility for enrolment into the trial.
Alcohol consumption will be ascertained by subject self-report through medical history. Participants are not allowed to use any prescription or over-the-counter medication, or herbal remedy taken with an intent to improve or treat fatty liver, liver disease, or obesity for the 3 months before randomization. Such agents include but are not limited to thiazolidinediones (TZDs). These agents are not to be used during screening nor for the duration of the trial. If a participant is using a statin or

fibrate medication to improve hyperlipidemia during screening, he/she is required to be on a stable dose in the 3 months before randomization. Participants using anti-diabetic medications must also be on a stable dose in the 3 months before randomization. Poorly controlled diabetic patients will be referred back to their diabetologist to manage hyperglycemia. Use of fish oil, algal oil or Krill oil supplements, drugs, or foods fortified with omega-3s are not allowed for at least 2 months before randomization and are prohibited after randomization and will be assessed by a Food Frequency Questionnaire (FFQ) at screening V0 with <200mg/d DHA and/or <60mg/d EPA intake to qualify for enrollment.

8. 2nd Screening with MRI-PDFF (Visit 1)

For those participants who pass the first screening criteria, an assessment of liver fat percentage by MRI-PDFF will occur to confirm ≥12% liver fat content for trial eligibility. A second ALT measure will be taken to confirm ALT ≥ 40 as well as second alkaline phosphatase, and bilirubin measures. If results exceed more than 50% from first lab results, and are above the normal range, then a third test (unscheduled visit) will be repeated (only for the elevated test(s)) before V2 to determine the direction of the change. If the third test shows a continued increase greater than 25% higher than the second test in ALT, Alk-P or Bilirubin the subject will be disqualified. For those that qualify, all values will be recorded on the CRF.

9. Randomization (Visit 2):

Subjects will be randomly assigned to one of the study interventions or placebo and stratified by Type II diabetes Dx (in no greater than 50% of sample).

10. Phone call at one month for Compliance

11. Visit at 3 months (Visit 3) and visit at 6 months (Visit 4):

For details see Table for Scheduled Assessments

STATISTICAL CONSIDERATIONS

For a within-subject 3.6% absolute reduction in liver fat for the combination group vs. placebo and setting α=0.05 and 80% power, **n=60/arm** for the combination and placebo groups are needed. [1, 2] To estimate secondary outcomes of DHA alone and Vitamin E alone versus placebo with a confidence interval ±3% of liver fat, n=30/arm for the DHA only and Vitamin E only groups are needed. With 10% expected attrition, the total sample size is 200 subjects.

Sample will be stratified by Diabetes Dx (no greater than 50% of sample).

ABBREVIATIONS

AE	Adverse Event
AHRQ	Agency for Healthcare Research and Quality
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AR	Adverse Reaction
AST	aspartate transaminase
AUDIT	alcohol use disorders identification test
BMI	body mass index
CAP	Controlled Attenuation Parameter
CK-18	cytokeratin 18
CRF	Case Report Form
CRO	Clinical Research Organization
CS	Clinically significant
DHA	docosahexaenoic acid
DNP	DSM Nutritional Products
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
e.g.	for example
EPA	eicosapentaenoic acid
EE	ethyl ester
FFQ	Food Frequency Questionnaire
FIB-4	Fibrosis -4 score
GGT	γ -glutamyl-transferase
GCP	Good Clinical Practice
GRAS	Generally Recognized as Safe
HDL-C	high-density lipoprotein cholesterol
IEC/IRB	Independent Ethics Committee/Institutional Review Board
INR	International Normalized Ratio
IOM	Institute of Medicine
IP	Investigational Product
ISF	Investigator Site File
ITT	Intent-to-Treat
IWRS	interactive-web-based response system
LC-PUFA	Long-chain polyunsaturated fatty acid
LDL-C	low density lipoprotein cholesterol
MedDRA	Medical Dictionary for Drug Regulatory Affairs
MRI	magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PBO	placebo
PDFF	proton density fat fraction
PI	Principal Investigator

PP	Per Protocol
PPAR	peroxisome proliferator activated receptors
RBC	red blood cell
RCTs	randomized controlled trials
ROI	regions of interest
ROS	reactive oxygen species
SAE	Serious Adverse Event
SAP	statistical analysis plan
SAR	Suspected Adverse Reaction
SC	Study Coordinator
SF-36	Short form 36
SID	subject identifier
SN	Study Nurse
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TE	transient elastography
TEAE	treatment-emergent adverse events
TGs	triglycerides
TMF	Trial Master File
TNFα	tumor necrosis factor-α
TZDs	thiazolidinediones
UDCA	ursodeoxycholic acid
UL	Tolerable Upper Intake Level
vs	versus

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Introduction

1.2 *Background information*

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive fat accumulation in the liver and is defined by evidence of hepatic steatosis (via imaging or histology) and is not due to secondary liver fat accumulation from excessive alcohol consumption or hereditary disorders (e.g. Wilson's disease) [3]. NAFLD is most commonly associated with metabolic syndrome, consisting of obesity, insulin resistance, elevated blood pressure and dyslipidemia. NAFLD is one of the most common causes of chronic liver disease, globally with a prevalence as high as 30% in Western countries [4]. It includes a spectrum of diseases from steatosis to non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and hepatocellular carcinoma [5]. Non-alcoholic fatty liver does not involve hepatocellular injury in the form of ballooning hepatocytes, whereas NASH is defined by steatosis, inflammation, and hepatocyte injury (ballooning) with or without fibrosis [3]. The causes of NAFLD are likely due to a combination of genetic and physiologic factors, namely those that promote oxidative stress and inflammation such as metabolic syndrome, visceral adiposity, and changes in intestinal microbiota [6]. NAFLD is significantly associated with increased risk of Type II Diabetes and cardiovascular disease, and increased overall mortality compared to age matched controls. There is currently no approved drug treatment for NAFLD or NASH. Dietary restriction for weight loss and increased physical activity are the recommended therapies albeit with limited success.

1.3 *Investigational products*

1.3.1 Vitamin E [(all-*rac*)-α-tocopheryl acetate]

Vitamin E is a fat-soluble vitamin that is synthesized naturally in plants in four tocopheryl forms: α, ß, γ and δ. All-rac-α-tocopheryl acetate has the highest biological activity in animal models [7] and it is the α-tocopheryl form that is used to prevent and treat Vitamin E deficiency in humans. Functionally, Vitamin E is an antioxidant and peroxyl radical scavenger. It is an inhibitor of lipid peroxidation and can also inhibit and modulate intracellular signaling molecules, e.g. protein kinase C, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [7]. α-tocopheryl regulates gene expression of several intracellular enzymes such as 5-lipoxygenase and cyclooxygenase and has anti-inflammatory activity (i.e. decreasing cytokine release and plasma C reactive protein). It is also known to inhibit platelet adhesion and aggregation.

In hepatocytes, Vitamin E suppresses expression of xanthine oxidase, a source of reactive oxygen species (ROS). It inhibits the propagation of peroxyl radicals by donating its phenolic hydrogen and converts the radical to a hydroperoxide, nonradical product [8]. Proliferation and enlargement of hepatic peroxisomes are seen in hepatic steatosis. In patients with NASH, increased fatty acid oxidation and decreased mitochondrial respiratory chain activity, including adenosine triphosphate (ATP) depletion is present. These patients also have higher cytochrome P450 activity which metabolizes long chain fatty acids producing increased ROS. Such increased ROS enhance lipid peroxidation and form aldehyde by-products such as malondialdehyde and increased cytokines e.g. tumor necrosis factor-α (TNF-α) and transforming growth factor-ß (TGF-ß), markers of inflammation. These cytokines can damage mitochondrial DNA and function, leading to a continuous cycle of peroxidation, fatty acid oxidation, and inflammation that result in hepatocyte apoptosis and eventually fibrosis. Vitamin E's antioxidant, anti-inflammatory, and inhibitory intracellular signaling functions prevent the propagation of free radicals and inflammatory processes.

Vitamin E has been shown to have a beneficial effect on NAFLD in previous studies. In a meta-analysis of 5 randomized controlled trials (RCTs), Sato [9] demonstrated that vitamin E significantly reduced liver enzymes: aspartate transaminase (AST), alanine aminotransferase

(ALT) and alkaline phosphatase (ALP), and significantly decreased steatosis, lobular inflammation, and hepatocellular ballooning compared to control or placebo. In this meta-analysis, hepatic fibrosis was improved with Vitamin E but was not statistically significant (p=0.06). In the largest of these trials, Sanyal [10] found that 800 IU Vitamin E (as *RRR*-α-tocopherol) /day for 96 weeks, administered to 84 non-diabetic adults with NASH significantly improved NASH, based on a composite score of histologic features, by 43% compared to 19% in the placebo group (n=83) (p=0.001). The rate of improvement of the comparator, 30 mg/d pioglitazone was only 34% and not significantly different from placebo. Significant reductions in hepatic steatosis and lobular inflammation were seen with both agents, but improvement in fibrosis was not significant. Other smaller studies in adult patients with NAFLD or NASH [11] [12] [13] and [14], given 500-800 IU/day Vitamin E have also shown significant decreases in liver enzymes, steatosis [12] [13] [14], inflammation [12] [13], and ballooning [13]. Two of the trials administered Vitamin E with background ursodeoxycholic acid (UDCA) but guidelines from the American Association of Study for Liver Disease considers UDCA ineffective for NASH treatment[15].
The Institute of Medicine (IOM),[16] has established the Tolerable Upper Intake Level (UL) for Vitamin E as 1000 mg/d of α-tocopheryl (all stereoisomers), equivalent to 1000 IUs by US Pharmacopeia. Few adverse effects are seen below doses of 2100 mg according to IOM. The UK Expert Group on Vitamins and Minerals, [17] also established a safe upper level of consumption of 800 IU. Sanyal's 2010 study [10] which used 800 IU Vitamin E found no differences between groups in adverse events including no difference in number of cardiovascular events. Although a study by Klein [18] of 400IU Vitamin E and Prostate cancer risk found a 17% increased risk in healthy men, other studies have found either no increased risk or decreased incidence in prostate cancer [19] [20] and improved prostate cancer survival [21]. In an 18 year follow up of the Alpha-Tocopheryl, Beta-Carotene (ATBC) Cancer Prevention study [22], α-tocopheryl had no effect on prostate cancer incidence (RR 0.97), no effect on overall mortality and decreased prostate cancer mortality (RR 0.84). Additionally, an Agency for Healthcare Research and Quality (AHRQ) report [23] concluded that trials of Vitamin E altogether had no overall effect on cancer.

1.3.2 DHA Ethyl Ester

Long-chain polyunsaturated fatty acid (LC-PUFA), docosahexaenoic acid (DHA) is an important omega-3 fatty acid for brain, eye and cardiovascular development and health. It significantly reduces triglycerides (TGs), lowers heart rate [24], lowers blood pressure [25], and reduces the risk of cardiac death by an overall 8% [26]. Both DHA and eicosapentaenoic acid (EPA) have anti-thrombotic, anti-inflammatory, and anti-oxidative properties [27]. As NAFLD patients are at significantly greater risk of cardiovascular disease and higher overall mortality, the cardioprotective effects of DHA are significant and may be beneficial in the NAFLD population.

Potential mechanisms for DHA's effects in NAFLD include reduction of TG synthesis via activation of peroxisome proliferator activated receptors (PPAR-α and γ) which accelerates fatty acid oxidation in liver mitochondria [5]. DHA is also known to have an integral role in maintaining and improving cell membrane fluidity, as a fatty acid that is incorporated into the phospholipids of the membrane, thereby optimizing surface receptors and signal transduction pathways in liver cells. The anti-inflammatory role of DHA in NAFLD may be mediated through activation of adiponectin secretion through the PPAR-γ path. Adiponectin improves lipid oxidation and reduces insulin resistance and inflammation [5] [28]. DHA also decreases expression of pro-inflammatory cytokines and increases DHA-derived prostaglandins and leukotrienes which inhibit pro-inflammatory cytokines. DHA-derived resolvins, protectins, and maresins are also anti-inflammatory by stimulating the resolution of inflammation [27]. Many of these mechanisms may contribute to the effects of DHA on metabolic syndrome and improve liver function in NAFLD.

A meta-analysis by Guo [5] demonstrated decreased levels of DHA in NAFLD patients compared to case controls, and their analysis of 10 RCTs showed significant reductions in ALT, AST, TGs,

and liver fat with doses of 1-5 g/d omega-3 supplementation. They showed that with each one gram/day increment in DHA, decreases in liver enzymes, i.e. -7.42 U/L ALT, -5.3 U/L AST, and -7.26% reduction in liver fat were achieved. Other meta-analyses of RCTs [[28] [29, 30] have also shown significant decreases in liver fat and positive results (or positive trends) in liver enzymes: decreases in ALT, AST, GGT, significant decreases in TGs, and increases in high-density lipoprotein cholesterol (HDL) with omega-3 supplementation in NAFLD patients. As reported by Parker et al [30] a 27% reduction of steatosis, measured by ultrasound, was seen across 5 trials with doses of 2-5g/d omega-3 treatment and may be considered clinically significant. In a RCT of omega-3 (DHA+EPA) ethyl esters (Lovaza®, 4g/d), Scorletti [2] demonstrated an overall trend for a decrease in liver fat (ß= -3.64, p=0.1) but compliance and contamination in the placebo group limited the significance of the results. However, a regression analysis of DHA independently showed a significant 1.7% decrease in liver fat for each 1% increase in red blood cell (RBC) DHA (p=0.007); when adjusted for cofactors such as age, gender, body weight, each 1% increase in RBC DHA, was associated with a 3.3% decrease in liver fat (p=0.0001). This relationship was not seen with EPA. A larger RCT of EPA ethyl ester alone [31] in patients with biopsy-confirmed NASH found no significant differences in the NAFLD activity score, steatosis, inflammation, ballooning, or liver enzymes between 2 doses of active (1.8 g/d or 2.7 g/d) and placebo. Although this trial was conducted in patients at a later stage of disease and had a higher than expected placebo response rate, the results collectively may suggest that DHA is the key omega-3 fatty acid to provide benefit for patients with fatty liver, i.e. NAFLD.

1.4 *Rationale for conducting the clinical study*

The combination of Vitamin E and DHA has not been tested in previous clinical trials of adults with NAFLD. This combination may provide optimal benefit for patients with NAFLD due to their associated mechanisms of action, namely Vitamin E's antioxidant action, preventing lipid oxidation of long chain fatty acids such as DHA and thus preventing the propagation of free radicals and ROS. Vitamin E's protection of LC-PUFA DHA therefore assists it in maintaining cell membrane stability and optimal signalling. Their combined anti-inflammatory effects (e.g. inhibiting pro-inflammatory cytokines, increasing adiponectin, and producing docosanoids to resolve inflammation) may also be efficacious for those with metabolic syndrome and NAFLD. The combination of Vitamin E and DHA will specifically be used in this study to determine if a reduction in liver fat occurs after 6 months of co-administration, using a magnetic resonance imaging (MRI) technique, proton density fat fraction (PDFF). PDFF imaging is non-invasive and highly sensitive to detect liver steatosis in patients with NAFLD. PDFF imaging is significantly more accurate than liver ultrasound-based transient elastography (TE) in detecting steatosis (AUROC 0.99 vs. 0.85 by TE-CAP) [32] [33]. It also significantly correlates with histology-determined steatosis (I^2=0.56, p<0.0001) in adults with NAFLD [34]. MRI-PDFF is also an appropriate technique to diagnose and stage disease in those with metabolic syndrome and NAFLD.

The clinical trial is designed to test the combination of Vitamin E and DHA against placebo, to demonstrate efficacy and safety. It is not powered however, to compare the combination against each of the active products for superiority. Each active product will be included as additional treatment arms to gain further insight into their contribution to changes in NAFLD parameters and to collect additional safety data.

2 OBJECTIVES AND ENDPOINTS

2.1 *Primary objective and endpoint variable*

2.1.1 Primary objective

To determine the efficacy of the combination of vitamin E and omega-3 fatty acid (DHA EE) versus (vs) placebo on reducing liver fat content after 6 months of intervention in adults with NAFLD.

2.1.2 Primary endpoint

Decrease in hepatic fat fraction [%] relative to baseline between Vitamin E + DHA combination vs placebo, measured by MRI-PDFF after 6 months of intervention

2.2 *Secondary objectives and endpoint variables*

2.2.1 Secondary objectives

- To determine effect of Vitamin E alone vs Placebo on reducing liver fat after 6 months of intervention in adults with NAFLD.
- To determine effect of DHA alone vs Placebo on reducing liver fat after 6 months of intervention in adults with NAFLD.
- To determine the change after 6 months of DHA EE and /or Vitamin E intervention in anthropometric, metabolic, hepatologic, nutrient, and inflammatory parameters, and quality of life status of adults with NAFLD.

2.2.2 Secondary endpoints

- Decrease in hepatic fat fraction [%] relative to baseline between Vitamin E vs placebo, measured by MRI-PDFF after 6 months of intervention
- Decrease in hepatic fat fraction [%] relative to baseline between DHA vs placebo, measured by MRI-PDFF after 6 months of intervention
- Change after 6 months of DHA EE and/ or Vitamin E intervention in:

- Anthropometric measures (body weight, waist-to hip ratio, waist circumference, body mass index (BMI)
- Insulin resistance
- Liver enzymes
- Fibrosis-4 (FIB-4) score
- Plasma Vitamin E concentration
- Plasma DHA concentration
- Lipid profile (e.g. HDL-C, low density lipoprotein (LDL-C), TGs, oxidized LDL)
- Health related quality of life score (Short form (SF-36))
- Dietary Intake Levels of LC-PUFA (i.e. DHA and EPA) as measured by the Food Frequency Questionnaire (FFQ)
- Inflammatory markers (e.g. cytokeratin 18 (CK-18), TNFα, IL-1β)

2.2.3 Exploratory Endpoints

- Transcriptomic analysis of the PBMC gene expression
- PNPLA3 genotype
- HSD17b
- Haptogloblin
- LC-PUFA-derived oxylipins

2.3 *Safety objectives and endpoint variables*

2.3.1 Safety objective(s)

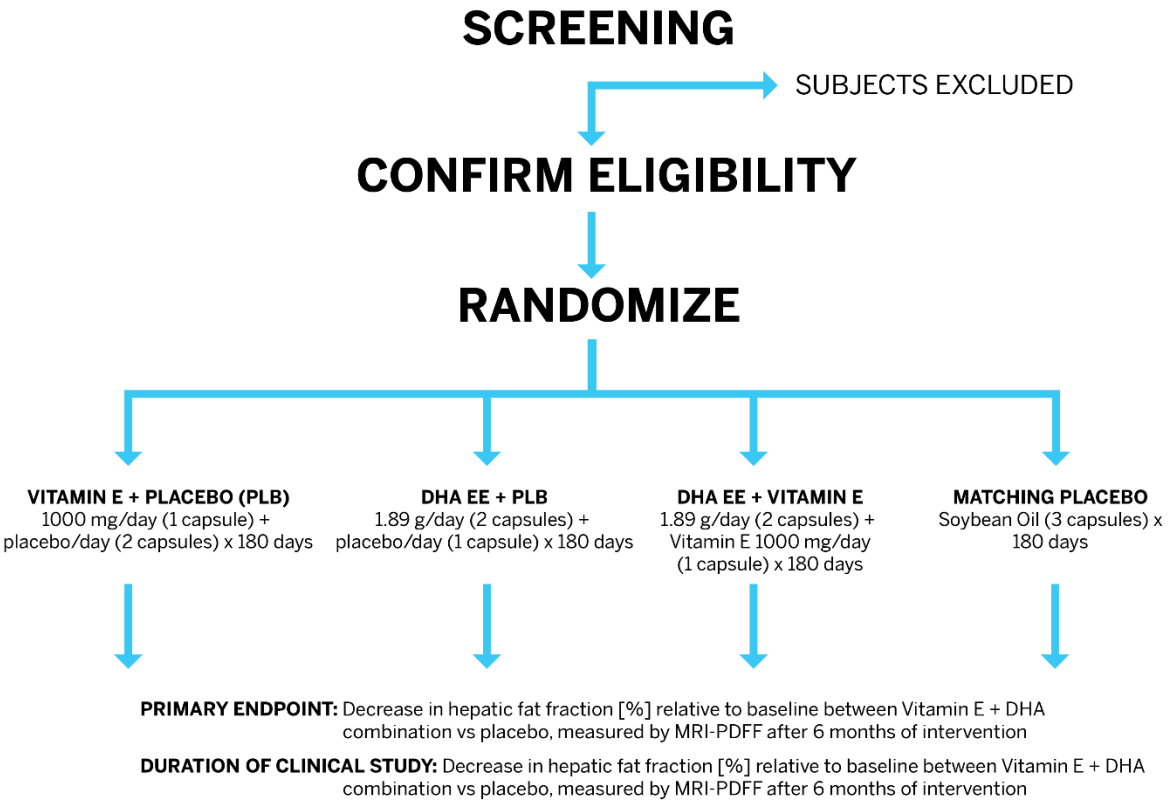
Safety objectives are to examine any treatment-emergent adverse events (TEAEs), and changes in clinical safety laboratory parameters

2.3.2 Safety endpoint variable(s)

- Vital signs
- Serum hematology and biochemistry
- AE/SAE reporting

3 STUDY DESIGN

The study is a multi-center, randomized, double-blind, placebo-controlled, parallel, 4-group intervention study in adults with NAFLD.



3.1 *Duration of clinical study*

The project may take up to 3-4 years to complete, depending upon recruitment time.

3.2 *Duration of clinical study per subject*

Total duration of the study per subject is 6 months consisting of 2 screening visits, randomization/baseline visit, a one-month phone call, a visit at 3 months and a visit at 6 months.

4 SUBJECTS AND SITE

4.1 *Number of subjects*

Total number of subjects to be enrolled: 200 (unequal allocation: 65/65/35/35 for Combination/Placebo/DHA only/Vitamin E only groups, respectively). With an estimated 10% attrition, approximately 180 subjects are expected to complete the study.

4.2 *Clinical study population*

4.2.1 Inclusion criteria

1. Male or female gender
2. ≥18 years of age
3. A new diagnosis or reconfirmation of previously known fatty liver by imaging (ultrasound or CT or MRI), or by liver biopsy within ≤ 4 years
4. Fibroscan Controlled Attenuation Parameter (CAP) score ≥ 300db
5. Liver fat content (≥ 12%) measured by MRI-PDFF
6. ALT ≥ 40 U/L
7. eGFR/Creatinine Clearance ≥ 60ml/min
8. Participants with previously diagnosed Type 2 diabetes (up to 50% of sample): they must either be taking anti-diabetic medications, or their fasting (>10 hours) glucose must be ≥ 100 mg/dL at the time of screening
9. Stable weight (±5%) for at least 3 months
10. Subjects willing and able to give written informed consent and to understand, to participate and to comply with the clinical study requirements.

4.2.2 Exclusion criteria

1. Evidence of alternative causes of hepatic steatosis or other forms of chronic liver disease, e.g. Hepatitis B, Hepatitis C (with <3 years treatment)
2. Evidence of acute Hepatitis A
3. Serum ALT or AST ≥ 250 U/L
4. Serum Alkaline Phosphatase > 2 ULN
5. Total bilirubin > 2 ULN in the absence of Gilbert's Syndrome [In patients with Gilbert's Syndrome, direct bilirubin must not exceed 2 ULN]
6. HbA1c > 9.5%
7. Decompensated acute or chronic liver disease
8. Clinical, imaging or histological evidence of cirrhosis
9. Use of anti-NASH drugs (e.g. thiazolidinediones (TZD)) in the 3 months prior to randomization
10. Use of a **non-stable** dose of statins or fibrates in the 3 months prior to randomization
11. Use of fish oil, algal oil or Krill oil supplements, drugs or foods fortified with omega-3s in the 2 months prior to randomization (>200 mg DHA/d and/or > 60mg EPA/d intake by FFQ)
12. Known intolerance to vitamin E or DHA
13. Malabsorption of Vit E (e.g. due to steatorrhea, chronic pancreatitis, severe cholestasis)
14. Vitamin E supplementation of greater than 100 IU/day in the 3 months prior to randomization
15. History of bariatric surgery (jejunoileal bypass or gastric weight loss surgery) or currently undergoing evaluation for bariatric surgery
16. History of biliary diversion
17. Known positivity for antibody to Human Immunodeficiency Virus

18. Patients with coagulopathy (PT ≥ 3 sec. from ULN) or thrombocytopenia (<70K)
19. Contraindication to MRI (implants, metal…)
20. Active, serious medical disease or disease diagnosis of a life-expectancy less than 5 years
21. Ongoing or recent alcohol consumption > 21 drinks (1 drink= 12 oz regular beer, or 5 oz wine, or 1.5 oz distilled spirits) per week in men and > 14 drinks per week in women as per subject self-report as part of medical history.
22. Active substance abuse, such as oral, inhaled or injected illicit drugs (except marijuana), in the year prior to screening
23. Women of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
24. Women who are breastfeeding
25. Any other condition which, in the opinion of the investigator would impede compliance or hinder completion of the study
26. Subjects who are enrolled in an interventional clinical study or have received an investigational new drug or product within the last 30 days prior to screening
27. Participants diagnosed with type 1 diabetes

4.3 *Randomization, blinding and treatment allocation*

Subjects will be randomly assigned to one of four treatment arms, either 1000 mg Vitamin E, 1.89 g DHA EE, 1000 mg Vitamin E + 1.89 g DHA EE, or placebo (PBO) based on a computer-generated randomization scheme, i.e. interactive-web-based response system (IWRS). Subjects will be stratified by Diabetes, Type II diagnosis (in no greater than 50% of sample). Enrolment will be competitive across study sites.

4.4 *Unblinding procedure*

One unblinded representative from the participating Clinical Research Organization (CRO) who is not participating in the study will maintain access to the computer- generated randomization code, in case unblinding is necessary. In an emergency situation where unblinding is needed, the site's Principal Investigator
(PI) is instructed to contact the CRO. Only the PI will become unblinded to that subject's treatment; other site staff and study team are to remain blinded.

4.5 *Number of sites*

An estimated 16 sites in the US will be recruited for this study based on MRI capabilities and expertise in NAFLD. For a sample size of 200 subjects, and an approximate recruitment time of 24 months, it is anticipated that each site will recruit an estimated **1-2 subjects/month**.

5 INVESTIGATIONAL DRUG PRODUCT AND REGIMEN

5.1 *Investigational product (IP)*

5.1.1 Investigational drug product names and formulations

1.Vitamin E [(all-*rac*)-α-tocopheryl acetate], 1000 mg [1000 IU/day], API grade, DMF # 030673 in a 1 g soft gel capsule containing also natural citrus flavoring agent, and shell colorant, manufactured by Catalent Pharma and supplied by DSM Nutritional Products Ltd.

2.DHA EE, 947 mg API grade (derived from DHASCO® marine microalgae, *Crypthecodinium cohnii*) in a 1 g soft gel capsule containing, natural citrus flavoring agent, and shell colorant,

manufactured by Catalent Pharma and supplied by DSM Nutritional Products. Dose of DHA EE is 1.89 g/d (2 capsules).

5.1.2 Other product name(s) and formulation(s)

The placebo comparator is Soybean oil in a 1 g soft gel capsule containing natural citrus flavoring agent, and shell colorant, manufactured by Catalent Pharma and supplied by DSM Nutritional Products.

5.1.3 Packaging and labelling

The IP is packaged in blister cards to enable blinding of the 4 arms of the trial. The combination of Vitamin E and DHA EE requires 3 softgel capsules to be taken /day. Thus, all other arms must have 3 capsules/day. Each blister card will contain a 2-week supply of capsules (42 softgels). Each subject kit will contain 13 blister cards plus an extra 1-week supply of capsules (one 1/2 blister card) to account for any potential missed dose or loss. The subject kit will contain the following information on the label:

- *Limited by Federal law, for investigational use only*
- *DSM Nutritional Products & address*
- *Product name and instructions for use*
- *Protocol number*
- *kit number*
- *Subject ID (to be filled in)*
- *Randomization # (to be filled in)*
- *Storage conditions / keep away from children*

5.1.4 Handling and storage conditions

Store in a cool, dry place (60-77°F). Capsules should not be kept for prolonged periods at high temperatures.

5.2 *Drug regimen*

5.2.1 Rationale for dose selection

Oral vitamin E intake at 1000 IU daily dose is safe, as confirmed by the US Food and Nutrition Board of the Institute of Medicine (IOM), that set the Tolerable Upper Limit (UL) at 1000 mg/d of α-tocopheryl in any supplemental form. This dose is within the range of vitamin E dosage that has been tested for the treatment of NAFLD and NASH in previous studies, and Practice Guidance from the American Association for the Study of Liver Diseases states that Vitamin E administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH. The dose of DHA EE (1.89 g/d) is also within the range of doses tested for treatment of NAFLD in previous studies and is below the Generally Recognized as Safe (GRAS) affirmation level (FDA 21CFR 184, docket #86G-0289, 1997). As there is no proven pharmacologic therapy for NAFLD, using a placebo for comparative purposes is justified.

5.2.2 Dosage regimen and dose adjustment

Subjects will be provided blister cards of capsules with a 2-week supply per blister card and will be instructed to take 3 capsules/day. All capsules will look identical to maintain blinding. Subjects assigned to Vitamin E will take 1 active and 2 PBO capsules. Subjects assigned to DHA EE will take 2 active and 1 PBO capsules. Subjects assigned to the combination will take 1 Vitamin E and 2 DHA EE capsules. Subjects assigned to PBO will take 3 PBO (soybean oil) capsules.

Subjects will be supplied with 13 cards, plus one extra ½ card (in case of a lost/misplaced card or late visit) in a blinded kit. Each study site will receive approximately 12 kits initially, and will be resupplied, depending upon enrolment. Kits will be labelled with a unique identifier (kit #) linked to the randomization (IWRS).

5.2.3 Route of administration

Three softgels per day are to be taken by mouth with food at the same time each day.

5.2.4 Dosing duration

The duration of IP administration in the trial is 180 +3 days. IP will not be provided after the subject completes the study.

5.3 *Dispensing and accountability*

The study sites will receive a *Product Dispensing and Return Log* to account for investigational product dispensed and returned to the site. Individual Product Disposition Sheets must be completed for each subject and must be kept up to date. It lists the subject ID, the amount of IP and date dispensed to the subject and the amount of product and date returned. Product inventory will also be tracked via the IWRS. Subjects will be instructed to return all empty and unused blister cards to their clinic at the 3 month visit and at the end of the study.

The monitor will inspect the amount of IP dispensed, returned and on stock at the site (including empty kits, partly used, and used kits) during monitoring visits. The investigator must discard all unused product at the end of the study, according to site policy and as agreed with the Sponsor. Unused means undispensed kits. Therefore, study sites may discard any previously dispensed kits (containing both empty blister packs and unused returned product) once final product accountability has been conducted by the study monitor. Document the destruction of the returned kits (documentation must include the kit number, date of destruction and clinic staff member responsible for product disposal).

5.4 *Compliance*

Compliance will be determined by capsule counts and by DHA and α-tocopheryl blood levels. Subjects must take a minimum of 80% of capsules in order to be compliant. DHA and α-tocopheryl blood levels will be used to confirm accuracy of the capsule counts. A subject may be withdrawn if non-compliant. Non-compliant subjects will not be replaced and may be removed from the per-protocol analysis.

5.5 *Warnings and precautions*

Subjects must be eligible for MRI scanning in this study.

5.5.1 Summary of Known Potential Risks with Study Medication

Vitamin E (study drug) is an essential nutrient found in highest quantities in sunflowers, almonds, hazelnuts, and peanuts. It is essential for the body's cells to function.

To our knowledge, there are two published studies with 800 IU/day of a-tocopherol, both in NAFLD and NASH (PIVENS and TONIC)

In the **PIVENS** trial, 84 adults with biopsy proven NASH were administered 800 IU/d of a-tocopherol for 96 weeks (Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010; 362:1675-1685

AEs by severity	Placebo (N=83)	Attribution to Study Drug	Vitamin E (N=84)
Mild	20	Not Related	24
Moderate	20	Not Related	19
Severe	10	Not Related	7 Gastroenteritis 1; Hyperglycemia 1; Pain 3; Syncope 1; Urticaria 1
Life threatening	0	Not Related	2 Cardiac ischemia/infarction 1 Liver dysfunction 1
Fatality	0	Not Related	1 (Cause of death: Pneumonia and liver failure secondary to sepsis from gram negative bacteremia. Deemed unrelated to the study drug).

In the TONIC trial (Lavine, 2011), 58 children with biopsy proven NAFLD received 800 IU/day of a-tocopherol for 96 weeks.

AEs by severity	Placebo (N=58)	Attribution to Study Drug	Vitamin E (N=58)
Mild	6	Not Related	10
Moderate	12	Not Related	7
Severe	9	Not Related	3 (Bronchospasm 1, elevated blood glucose 1, hepatotoxicity 1); Note: No specific details of this Hepatotoxicity SAE are available from the publication. In this paper (supplemental table eTable2), it was reported that there was only one patient with ALT > 2 ULN in vitamin E group as opposed to 5 in placebo group (p=0.09). No cases of bilirubin > 3 mg/day in either group.

Vitamin E administered prophylactically or therapeutically within the range of the tolerable upper limit (i.e., ≤1000 mg) established for adults by the Institute of Medicine, has evidenced very low toxicity in humans. Summaries of the adverse events reported by double-blind randomized-controlled trials of vitamin E, including dl-α-tocopheryl acetate, are included in **tables 5.5 and 5.6 of the dl- Alpha- Tocopherol Acetate Investigator's Brochure**. Overall, oral

administration of therapeutic levels of vitamin E, including use for up to one year in NAFLD patients at dose levels of 800mg/d, has shown very few AEs. Additionally, **Section 5.2.1 of the Investigator's Brochure** discusses potential adverse effects involving blood coagulation from oral intake of high-dose vitamin E and aspirin, anti-coagulant drugs and/or vitamin K deficiency.

When given in tablets, vitamin E may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

Side effects	Frequent >20% of participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious			
Less Serious			blood coagulation with medications
Minor			Fatigue, headache, flatulence, gastrointestinal disorders

DHA EE (study drug) is an essential nutrient found in highest quantities in salmon, mackerel, oysters and anchovies. It is essential for the body's cells to function.

The Investigator Brochure for DHA EE in Tables 5-1 and 5-2 lists published studies in healthy adults and in those with various medical conditions with DHA and include dose, duration and safety outcomes. The maximal dose tested was 12g/d DHA in a prostate cancer study of 6 months duration; one SAE of urinary obstruction in one subject was reported and considered unrelated to DHA administration. There have been no deaths associated with the use of DHA in these studies. Table 5-5 in the IB provides a frequency table of reported AEs by SOC and PT in 556 subjects (DHA n=379; PBO n=220); the most commonly reported AEs attributed to DHA are gastrointestinal disorders. Additionally, Table 5-6 provides a frequency table of SAEs by SOC and PT across the same studies. A total of 9 subjects who received DHA reported 15 SAEs with the most frequent SOC, infections and infestations. Scorletti, et al, 2014 reported no SAEs attributed to omega-3 administration (Omacor® 4g/d: 1.52g DHA EE and 1.84g EPA EE) over 15-18 months in NAFLD patients.

Literature citations for toxicology studies with DHA are found in Module 2, 24-Nonclinical overview, section 2.4.6 and cited publications can be found in Module 4, 43-lit-ref.

When given in tablets, DHA EE may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

Side Effects	Frequent >20% of participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious			
Less Serious			

Minor			Eructation, Gas, Bloating

5.6 *Concomitant treatments/supplements and restrictions*

Participants are not allowed to use any prescription or over-the-counter medication, or herbal remedy taken with an intent to improve or treat fatty liver, liver disease or obesity for the 3 months before randomization. Such agents include but are not limited to, TZDs. These agents are not to be used during screening nor for the duration of the trial. **If a participant is using a statin or fibrate medication to improve hyperlipidemia during screening, he/she is required to be on a stable dose in the 3 months before randomization. Additionally, participants are allowed to continue on prescription anti-hyperlipidemic agents, if they are on a stable dose**. **Participants on antidiabetic medication are allowed in the trial if they are on a stable dose in the 3 months prior to randomization.** Use of fish oil, algal oil or Krill oil supplements, drugs, e.g. Lovaza® or foods fortified with omega-3s are not allowed for at least 2 months before randomization and are prohibited after randomization. Fatty fish intake (e.g. salmon, tuna, herring, etc) is restricted to <2 fish meals/week. Participants are interviewed with a FFQ for LC-omega-3s at screening, randomization, and at every visit to document the quantity of LC-omega-3s consumed. Vitamin E supplementation of greater than 100 IU/day are not allowed in the 3 months prior to randomization and are prohibited after randomization.

6 STUDY PROCEDURES

6.1 *Recruitment procedures*

Subjects will be recruited from hepatology clinics that are experienced in diagnosing and treating NAFLD and NASH and that have qualified MRI facilities. It is anticipated that sites will pre-screen subjects from their existing databases to identify potential subjects for this study. It is expected that an advertising and/or social media campaign will be needed also to assist with recruitment. Each site will create a recruitment plan in order to meet study recruitment goals.

6.2 *Schedule of assessments*

Visits	V0 Screening[+]	2nd Screening V1[+]	V2 Randomization	Phone call at 1 mos[3]	V3 3mos	V4 6mos
Day**	-28 to -14	-21 to -5		30±3	90 -7 to +3	180 -7 to +3
Informed Consent	X					
Medical History	X					
Physical Examination	X				X	
LIVER FIBROSCAN	X					
MRI-PDFF for Liver fat		X				X
Clinical chemistry*	X	ALK-P, AST, ALT, Bili only			X	X
HbA1c and Insulin	X					X
Hep A (HAVIgM)[#]	X					

Hematology*	X				CBC	X
Coagulation: INR	X					
Urine pregnancy test (DipStick)	X		X			
Skinner questionnaire[2]	X					
AUDIT questionnaire^	X			X	X	X
Vital signs	X		X		X	X
Lipid panel* including oxidized LDL		X				X
Plasma/serum for inflammatory markers		X				X
Anthropometrics	X				X	X
RANDOMIZATION			X			
DHA & α-tocopheryl determination and oxylipins[1]		X			X	X
Product dispense			X			
Compliance-Pill count				X	X	X
Adverse event reporting		X	X	X	X	X
ConMeds			X	X	X	X
FFQ	X		X	X	X	X
SF-36 questionnaire			X			X
Biosample collection –for exploratory analyses and future research use¶		X				X

* fasted blood draw required and ALT, AST, Alk-P, bilirubin is measured 2x;
[+]values obtained at screening (except vital signs and FFQ) will be considered 'baseline' values;
[#]HAVIgM to be performed unless negative results in past 3 years;
[1]oxylipins measured only at V1 and V4.
[2]Skinner Lifetime Drinking Questionnaire and AUDIT given at V0 Screening
[3] Reminder phone calls at months 2,4 and 5are advised to assist with retention and check on subject compliance, no diet fluctuations, ConMeds, and AEs. Only deviations, ConMeds, and AEs are to be recorded in the eCRF from such phone calls.
** Additional time may be allowed between study visits with approval and subsequent protocol deviation.
^ Audit questionnaire - Self-report version is collected at V0, V3 and V4 and the interview is only conducted during the 1-month phone call
¶ Refer to lab manual for sample collection table - Exploratory analyses include gene expression at V1 and V4 in a sub-set of 40 subjects, and PNPLA3, HSD17b, haptoglobin genotyping at V1.

6.3 *Screening and eligibility*

6.3.1 Obtaining informed consent

Subjects who are foreseen to fulfil the inclusion / exclusion criteria for enrolment into the clinical study will be asked to give informed consent in writing prior to any clinical study specific procedures at the screening visit. Both the subject (or legal representative) and investigator will sign and date the informed consent form. All subjects who have signed the informed consent form will be listed on the *Subject Screening and Enrolment Log*.

6.3.2 Visit 0 – Screening examinations

The following assessments are done at the screening visit (up to 28 days prior to randomization):

1. Inclusion/Exclusion criteria
2. Informed Consent
3. Medical History
4. Physical Exam
5. Liver Fibroscan
6. Fasted Blood draws (>10 hours) for Clinical Chemistry (including HbA1c, insulin, and eGFR/eCreatinine Clearance), Hematology (including Protime and International Normalized Ratio (INR)) and Hep A (HAVIgM) test unless negative results obtained in past 3 years
7. Skinner Lifetime Drinking Questionnaire
8. AUDIT Questionnaire – Self-Reported Version
9. Urine Pregnancy test (for females of child-bearing potential who are not post-menopausal or surgically sterilized)
10. Vital signs (heart rate, blood pressure)
11. Anthropometrics: body weight [kg], waist-to hip ratio, waist circumference [cm or inches],], body mass index (BMI)[kg/m^2]
12. FFQ (<200mg/d DHA and/or<60mg/d EPA to qualify) see Appendix 1
13. FIB-4 score calculated

6.3.3 Visit 1- 2nd Screening

A second fasted blood draw (>10 hours) for ALT, AST, Alkaline phosphatase, and Bilirubin will be taken to confirm the first lab results at V0. If results exceed more than 50% from first lab results, and are above the normal range, then a third test (unscheduled visit) will be repeated (only for the elevated test(s)) before V2 to determine the direction of the change. If the third test shows a continued increase greater than 25% higher than the second test in ALT Alk-P or Bilirubin the subject will be disqualified. For those that qualify, all values will be recorded on the CRF.

All participants will undergo a Fibroscan (≥300db) at the first screening visit, V0 and then, after liver enzymes and all other labs, are confirmed to be within eligibility cut-offs, (V1) an assessment of liver fat percentage (min. 12%) by MRI-PDFF examination will occur. MRI scans will be sent to a Central reader to assess the eligibility for enrolment into the trial.

Fasted blood draws (>10 hours) for Fatty Acid, oxylipins and Vitamin E analyses, Lipid panel, PBMC gene expression (in a sub-set of 40 subjects), and Inflammatory markers will occur at V1. Blood analysis of PNPLA3, HSD17b and haptoglobin will also occur at Visit 1.

6.3.4 Assessment of eligibility and randomization procedure

Based on screening examinations, imaging and lab results, the investigator will assess the subject's eligibility to be entered into the clinical study. On the *Subject Screening and Enrolment Log*, the decision whether to randomize the subject into the clinical study or not is documented. For all eligible subjects, values obtained at screening (except vital signs and FFQ) will be considered 'baseline' values. The vital signs measured at randomization will be considered baseline values. The SF-36 and FFQ at randomization will be considered baseline values.

The study staff will utilize a computer-generated IWRS to randomize all eligible subjects. Subjects will be stratified by Type 2 Diabetes diagnosis (up to 50% of sample). Subject information (e.g. visit date, gender, diabetes) will be entered into the IWRS and the subject

identifier (SID) and a randomization code will be generated and recorded in the subject source documents and the electronic Case Report Form (eCRF).

6.4 *Intervention phase*

6.4.1 Visit 2 Randomization

The following assessments are done during Randomization:

1. Urine Pregnancy test (females of child-bearing potential who are not post-menopausal or surgically sterilized)
2. Vital signs
3. FFQ (<200mg/d DHA and/or <60mg/d EPA to qualify) see Appendix 1
4. SF-36 Questionnaire (Quality of Life) see Appendix 2
5. Product dispensation
6. AEs
7. Concomitant Medications

6.4.2 Phone call at one month

The following assessments are done at the one-month phone call:

1. AUDIT Questionnaire – Interview Version
2. Compliance (pill count check, can do by Skype or request photo of blister cards consumed)
3. Adverse Events (including questions related to bleeding and infections)
4. Con Meds
5. FFQ

Reminder phone calls at months 2,4 and 5 are advised to assist with retention and check on subject compliance, no diet fluctuations, ConMeds, and AEs. Only deviations, ConMeds, and AEs are to be recorded in the eCRF from such phone calls.

6.4.3 Visit 3 (3mos Visit)

The following assessments are done during the 3 mos Visit:

1. Physical exam
2. Vital signs (heart rate, blood pressure)
3. Anthropometrics
4. FFQ (see Appendix 1)
5. Fasted Blood draws (>10 hours) for Clinical chemistry, CBC, Fatty Acid (DHA) and Vitamin E analyses
6. AUDIT Questionnaire – Self- Report Version
7. AEs
8. Concomitant Medications
9. Compliance (pill count- subjects to return all used and unused cards in the kit)

6.4.4 Visit 4 (6mos Visit)

The following assessments are done during the 6 mos Visit:

1. MRI-PDFF
2. Fasted Blood draws (>10 hours) for Clinical Chemistry (including HbA1c and insulin), Hematology, Fatty Acid, oxylipins and Vitamin E analyses, Lipid panel, gene expression (sub-set of 40 subjects), and Inflammatory markers
3. Vital signs (heart rate, blood pressure)
4. Anthropometrics
5. FFQ (Appendix 1)
6. FIB-4 score (calculated)

7. AUDIT Questionnaire – Self- Report Version
8. AEs
9. Concomitant Medications
10. Compliance (pill counts- subjects to return all used and unused cards in the kit)
11. SF-36 Questionnaire (Appendix 2)

7 KEY MEASUREMENTS AND ASSESSMENTS

7.1 *Blood samples*

7.1.1 Blood sample collection

Fasted (>10 hours) plasma and serum samples will be collected from each subject at Screening V0, second Screening V1, Visit 3, and Visit 4.

7.1.2 Blood sample storage and shipment

Whole blood (between 10-25 ml, depending on the visit) will be drawn and processed at the clinical site, according to a central lab manual. Plasma and serum or whole blood will be shipped either ambient or frozen on dry ice to the central lab the day it is drawn and should be received within 24 hours at the central lab.

7.1.3 Blood sample assessment

Clinical chemistry: Glucose, HbA1c, ALT, AST, GGT, ALK-P, eGFR/creatinine clearance, creatinine, insulin, bilirubin, (**note**: for **ALT and AST** reference ranges for HH flags are to be used to determine clinical significance (CS))
Lipid panel: total cholesterol, HDL-cholesterol, LDL-cholesterol, oxidized LDL, TGs;
Hematology: standard panel including complete blood count with automated differential including platelets, and coagulation panel INR, and Hep A (HAVIgM) test at screening, and **Inflammatory markers** (e.g. CK-18, TNFα, IL-6) will be analysed by a central lab.

Fatty acid analyses and analyses of LC-PUFA-derived oxylipins will be performed using plasma samples at the DSM Clinical Laboratory, 6480 Dobbin Rd. Columbia MD 21045. Approximately 1.5 mL of plasma/subject/time point (V1, V3, V4) will be aliquoted and shipped frozen on dry ice in batches from the central lab to DSM.

500uL plasma for **α-tocopheryl** measurement (from V1, V3, V4) will be shipped frozen on dry ice in batches from the central lab to DSM Nutritional Products:

DNP R&D Analytics, NIC-RD/A
Sample Registration Office,
c/o Warenannahme, Bldg. 214
Wurmisweg 576
CH-4303 Kaiseraugst / Switzerland
Phone: + 41 (0) 61 815 8629
Fax: + 41 (0) 61 815 7441

Transcriptome analysis of Gene Expression: in a subset of 40 subjects (~10 subjects/treatment arm), whole blood (~2ml) will be collected in PAXgene blood RNA tubes. Samples will be collected at Screening V1 and at Visit 4 and will be shipped frozen using dry ice along with other blood samples to the central lab the day it is drawn. Samples will be batched shipped frozen from the central lab to DSM Nutritional Products, Kaiseraugst, Switzerland for analysis.

Genotype analyses
An aliquot of whole blood (0.5ml) from each subject will be used for DNA extraction to identify those with single-nucleotide polymorphisms (SNP) i.e. PNPLA3, HSD17b and haptoglobin. Samples will be collected at Screening V1 and will be shipped frozen using dry ice along with other blood samples to the central lab the day it is drawn. Samples will be batched shipped frozen from the central lab to Indiana University for analysis.

An additional 1 ml plasma (aliquoted into [2] 0.5ml tubes) will also be collected at V1 and V4, shipped frozen on dry ice to central lab and stored for future NAFLD biomarker use.

7.2 *MRI-PDFF*

7.2.1 Assessment of liver fat percentage by MRI-PDFF

All subjects will undergo MRI-PDFF to determine eligibility for the trial, after all the V0 and V1 screening procedures have been completed to confirm eligibility. A minimum 12% liver fat is required for trial eligibility. One standard MRI protocol will be used at all sites and one blinded central MRI reader will analyse all MRI assessments. A study-specific MRI-PDFF protocol will be developed for all participating sites. Changes in liver fat % assessed by MRI-PDFF will be measured in localized regions of interest (ROI) within each of the liver segments. MRI-PDFF will also be performed at Visit 4 (6 mos) using the same procedures.

7.3 *Other key assessments*

7.3.1 Liver Fibroscan

Transient elastography (Fibroscan), a rapid measure of liver tissue stiffness, will be used at Visit 0 to assess for eligibility in the trial and will be performed **prior to MRI-PDFF**. Qualifying cut off score on Fibroscan is CAP ≥300db. If the subject meets this cut off, then the MRI-PDFF is performed at the second screening visit.

7.3.2 Liver Fibrosis score

Fibrosis-4 score (FIB-4) predicts changes in fibrosis with high specificity. The FIB-4 score is calculated as Age x AST/platelet count x √ALT. The FIB-4 score will be calculated in the eCRF at Visit 0 and Visit 4.

7.3.3 Anthropometrics

Height, body weight, waist and hip circumference will be measured at Visit 0, 3 and 4 at the site. Waist to hip ratio and BMI will be calculated. Sites are advised to counsel the subjects to avoid major fluctuations in diet and exercise during the study.

7.3.4 Alcohol Consumption

At Visit 0, subjects will provide their typical weekly alcohol consumption as part of medical history to confirm eligibility requirements (>21 drinks per week in men and >14 drinks per week in women is exclusionary). Additionally, the Skinner lifetime drinking history questionnaire will be administered via a structured interview and subjects will be provided the AUDIT test – Self Report Version questionnaire to complete (with cards to remind study participants of drink equivalents) in order to determine baseline alcohol consumption history/patterns.

Following randomization, the AUDIT test will be administered at 3 additional time points during the study to monitor alcohol consumption patterns. During the one-month phone call, the AUDIT test – Interview Version will be administered by study staff. At Visits 3 and 4, the AUDIT test – Self Report Version will be provided to the subject to complete in-clinic.

Data from the AUDIT questionnaire will be used to assess whether alcohol consumption modified the treatment response.

7.3.5 Urine sample collection and storage

For all females of child-bearing potential, a urine pregnancy test (dipstick) will be conducted at the site at both Screening (Visit 0) and Randomization (Visit 2) to determine eligibility in the trial. A positive pregnancy test during screening or at randomization is exclusionary for trial eligibility. Negative results will be recorded in the eCRF.

7.3.6 Questionnaires

The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36), a quality of life assessment will be administered to subjects at Visit 2 and 4. See Appendix 2.

A validated FFQ [35] to measure LC-PUFA dietary intake (namely DHA and EPA) will be administered at visits V0, V2, phone call at 1mos, V3 and V4. See Appendix 1.

8 EARLY SUBJECT WITHDRAWAL

Subjects have the right to withdraw from the study at any time for any reason, without being obliged to give reasons and without penalty or loss of benefits to which they are entitled. The investigator also has the right to withdraw subjects from the study if this is in the best interest of the subject.

Any of the following conditions leads to premature withdrawal:

- request by subject to discontinue for any reason during the study (withdrawal of consent)
- erroneously included / randomized subject
- adverse event or concurrent illness that, in the opinion of the investigator, warrants the subject's withdrawal from intervention
- intake of concomitant medications/dietary supplements prohibited by the protocol
- volunteers who do not follow the requirements of the investigator, especially those concerning safety and/or if the subject, after his enrolment is uncooperative or not willing to comply with the protocol (non-compliant)
- failure to return (lost to follow-up)
- request by the Sponsor or regulatory agencies for termination of supplementation of an individual patient or all patients under this protocol
- pregnancy, i.e. a protocol exclusion criterion

The circumstances of any discontinuation must be documented in detail in the corresponding study disposition form in the eCRF. Whenever a subject is withdrawn from the study for whatever reason, every effort will be made to perform a final assessment to include standard medical and laboratory tests (**including a final MRI, if possible**) which would have been done upon normal completion of the study, i.e. V4. The data of the withdrawn subjects will be documented in the clinical study report and may be used in statistical analyses.

Note: Subjects who withdraw prematurely from the study will not be replaced.

9 DATA PROCESSING AND STATISTICAL CONSIDERATIONS

9.1 *Case Report Form*

9.1.1 Electronic Data Capture

It is the responsibility of the investigator(s) to ensure the completeness and accuracy of the eCRF. One eCRF must be compiled for each subject participating in the study.

Data required for the analysis will be acquired and transferred electronically to a central database by means of an Electronic Data Capture system (the "EDC-tool"). The system operates over the Internet according to the principle of online data capture. Data entered by investigators or data managers into local computers are directly transferred over the Internet to a central database, without any permanent local storage.

High security standards for the transfer and storage of study data are guaranteed by the use of technologies, such as encrypted data transfer, firewalls and periodic backup to protect centrally stored data. Writing access to the system will be limited to authorized personnel and the system will automatically keep an audit trail of all entries and corrections to the eCRF. Training in the use of the EDC-tool will be provided by the CRO and local monitor.

The EDC-tool will comprise an eCRF, designed specifically for the present study.

The EDC-tool is operated under an electronic signature, consisting of the combination of an identification code and a password. In relation to the present study, passwords and electronic signatures will be distributed to users of the EDC tool, and used for entering, modifying, or viewing study data.

It is the responsibility of individuals who receive an electronic signature to keep their password secret, i.e., not to reveal it to third parties, and to access the EDC-tool, to enter or modify study data using the EDC-tool only under their personal identification code and password.

9.2 *Data Management*

The CRO is responsible for the data management of the study. The CRO will design the eCRF with the PI, conduct quality control and review of the eCRF, and create a Data Management Plan, including programming and validation of the database, user training, data handling and edit checks, data cleaning and query generation and processing, medical coding, SAE reconciliation and database closure. Key site and CRO personnel will be trained on the eCRF and the EDC system.

9.3 *Statistical Analysis*

9.3.1 Statistical hypotheses and methods

The primary hypothesis of the study is that Vitamin E and Omega-3 fatty acid (DHA EE) in combination will reduce hepatic fat fraction greater than placebo after 6 months of intervention in adults with NAFLD.

In general, continuous variables will be summarized with the mean, standard deviation, median and range. Categorical variables will be summarized with counts and percentages. SAS® version *9.4* or higher will be used to analyse the data.

9.3.2 Primary endpoint analysis

Decrease in hepatic fat fraction [%] relative to baseline between Vitamin E + DHA EE combination vs. placebo, measured by MRI-PDFF after 6 months of intervention. The decrease will be analysed with a linear mixed model with the hepatic fat fraction in % at 6 months as response variable. The hepatic fat fraction in % at baseline, treatment group and diabetes status will be included as fixed effects. Site will be included as random effect. This representation is formally identical to an analysis that uses the change in hepatic fat fraction in % (6 mos vs. baseline) as response variable and the hepatic fat fraction in % at baseline, treatment group and diabetes status as fixed effects and site as random effect [Laird, N. Further comparative analyses of pre-test post-test research designs. The American Statistician 1983, 37: 329-330; O'Connell, N. Methods for Analysis of Pre-Post Data in Clinical Research: A Comparison of Five Common Methods. Journal of Biometrics and Biostatistics, 2017. 8(1):1-8.]. However, this representation is chosen since it allows for easier variance-stabilizing transformations if necessary.

Statistical tests of significance will be 2-tailed at a 5% significance level.
With blinded data, the residuals of the model will be analysed visually (QQ-plots, residual plots) to determine if the model assumptions (normality, variance, homogeneity) are met. If necessary, variance-stabilizing transformations will be considered.

9.3.3 Secondary endpoint variable(s) and analyses

- Differences in hepatic fat fraction [%], measured by MRI-PDFF after 6 months of intervention, between DHA and placebo will be estimated with the same model as the primary endpoint. The estimated difference will be displayed with its 95% confidence interval.
- Differences in hepatic fat fraction [%], measured by MRI-PDFF after 6 months of intervention, between Vitamin E and placebo will be estimated with the same model as the primary endpoint. The estimated difference will be displayed with its 95% confidence interval.
- Between group differences in anthropometric measures, after 3 and 6 months of DHA EE and/or Vitamin E intervention will be analysed with the same approach as the primary endpoint, using the relevant anthropometric measure at baseline as the respective baseline covariate and gender as a categorical covariate.
- Between group differences in inflammatory markers after 6 months of intervention and liver enzymes after 3 and 6 months of DHA EE and/or Vitamin E intervention will be analysed with the same approach as above and diabetes status as a categorical covariate included in the model.
- Between group differences in the FIB-4 score after 6 months of DHA EE and/or Vitamin E intervention will be analysed with the same approach as above.
- Between group differences in plasma DHA and plasma α-tocopheryl after 3 and 6 months of intervention will be analysed with the same approach as above.
- Between group differences in SF-36 quality of life score after 6 months of DHA EE and/or Vitamin E intervention will be analyzed by ANCOVA with baseline score included in the model.
- Between group differences in dietary intake of LC-PUFA (i.e. DHA and EPA) will be assessed by the FFQ and analysed over time by repeated measures ANOVA with baseline level and site included in the model.

With blinded data, the residuals of the models will be analysed visually (QQ-plots, residual plots) to determine if the model assumptions (normality, variance homogeneity) are met. If necessary, variance-stabilizing transformations or non-parametric methods will be considered.

9.3.4 Exploratory variables

Transcriptome analysis of gene expression will be performed in peripheral blood mononuclear cells in a subset of (n=40) subjects (~10 subjects/ treatment arm). Changes in gene expression from baseline to end of treatment (6 months) will be examined. The analyses will be conducted by DSM. Briefly, differentially expressed genes will be analysed by ANOVA. The false discovery rate (q values) will be used to correct for multiple testing. Using the global gene expression profiling results, an extensive pathway analysis (e.g. MetaCore program and/or Ingenuity Pathway Analysis) will be conducted to better understand gene regulation, mechanisms of action and activated pathways by the various treatments in this NAFLD population.

Between group differences in plasma LC-PUFA-derived oxylipins will be analysed by ANCOVA using the relevant baseline oxylipin level as a covariate.

Sub-set analyses of PNPLA3, HSD17b and haptoglobin SNPs will be conducted on the Primary endpoint to assess potential genotypic responses to treatment.

9.3.5 Safety variables

All SAEs and AEs will be summarized by group. The number of subjects experiencing any events will be examined and the frequency of occurrence by system organ class will be tabulated using Medical Dictionary for Drug Regulatory Affairs (MedDRA) codes.

Vital signs, Hematology, Lipid panel, and Clinical Chemistry data will be summarized using descriptive statistics by group per timepoint including change from baseline at 6 months with group differences analysed using Least Squares Means and 95% Confidence Intervals.

A sub-set analysis will be performed in those with elevated baseline TGs to assess changes with the intervention over 6 months.

9.3.6 Compliance Analysis

A compliance analysis will be performed on all randomized participants who completed the study (6 mos) and ingested at least 80% of the study product using plasma DHA levels (µg/ml) and plasma α-tocopheryl (µmol/L) to confirm accuracy of the pill counts. ANOVA models will be employed with treatment as a factor. Frequency counts and percentages will also be presented for subjects who were at least 80% compliant by treatment group. Differences in this categorical outcome will be assessed using Fisher's Exact Test.

9.3.7 Sample size:

Le et al.[1] report that the variability (standard deviation) of the within-subject change in liver fat [%] for a period of 24 months in a similar population and with the same measurement method (MRI-PDFF) is 7%. Expecting a within-subject 3.64% absolute reduction in liver fat for the combination group vs. placebo [1, 2] and setting α=0.05 and 80% power, n=60/arm for the combination and placebo groups are needed (G*power 3.1.9.2, two-sample t-test).

To estimate the secondary endpoints (effect vs. placebo of the components (DHA EE alone and Vitamin E alone)) with a given precision (confidence interval width) of ±3% of liver fat, we need n=30/arm for the DHA EE only and Vitamin E only groups, leading to a necessary sample size of 180. With 10% expected attrition, the total sample size is 200 subjects.

Subjects will be stratified by Type II Diabetes diagnosis (no greater than 50% of sample). Subjects who withdraw prematurely from the study will not be replaced.

9.3.8 Study Populations

- The Intent-to-Treat (ITT) population will include all randomized subjects who receive at least one dose of study product, according to the product to which they were randomized. The primary efficacy analysis will be on the ITT population.

- The Per-Protocol (PP) population will include subjects who completed the study according to the protocol. The subject's data will be excluded from the per protocol analyses if the subject fails to meet one or more of the inclusion criteria at enrollment or meets one or more of the exclusion criteria. Data will be excluded if the subject has other significant protocol deviations or significant non-compliance with taking the study product (to be determined at a blinded data review meeting).

- The Safety population will include all subjects randomized into the study who received at least one dose of study product, according to the product they actually received.

9.3.9 Poolability of Data

It is considered that data will be pooled across sites for all analyses, unless significant deviations or imbalance in one or more sites is identified. Such discrepancies will be reviewed at the blinded data review meeting. Heterogeneity of the variability across sites will be investigated, and assumptions regarding the homogeneity of the variability across sites will be relaxed in the applied statistical model if necessary.

A statistical analysis plan (SAP) will be written by the statistician of the trial and will be finalized prior to database closure. The SAP will detail the handling of missing data and will outline all analyses to be conducted.

10 SAFETY

10.1 *Definitions and Standards*

Adverse Event (AE):
Any untoward medical occurrence in a subject involved in a clinical study administered an investigational product, whether or not related to this product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding for example), subjective and objective symptom, or disease temporally associated with the use of a product, accidents, and whether or not considered related to the product or study-related procedure and reported by the subject or observed by the investigator.

Adverse Reaction (AR):

All noxious and unintended responses to a product related to any dose should be considered as AR. ARs are a subset of all suspected AEs for which there is reason to conclude that the investigational product caused the event.

Suspected Adverse Reaction (SAR)
SAR means any AE for which there is a reasonable possibility that the product or clinical trial material caused the AE. A SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by an investigational product.

Serious Adverse Event (SAE):
Any AE that at any dose fulfils at least one of the following criteria:

- Is fatal (results in death)
  (*note*: death is an outcome, not an event)
- is life-threatening
  (*note*: the term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which could hypothetically have caused death had it been more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
  (*note*: "inpatient hospitalization" refers to an unplanned, overnight hospitalization)
- results in persistent or significant disability/incapacity
  (*note:* the term means substantial disruption of one's ability to conduct normal life function)
- is a congenital anomaly/birth defect
  (*note:* congenital anomaly/birth defect in offspring of subject taking the product regardless of time to diagnosis)
- is medically significant
  (*note*: Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definitions above).

Suspected Unexpected Serious Adverse Reaction (SUSAR):
All suspected adverse reactions that are both unexpected and serious.

Unexpected Adverse Reaction:
An adverse reaction, the nature, or severity or incidence of which is not consistent with the applicable product information (e.g. Investigator's Brochure).

10.2 *Adverse event assessment*

Expectedness:
An unexpected AE is an event of which the nature or severity is not consistent with the applicable product information.

Causality Assessment:
The causality assessment of an AE to the investigational product will be rated as follows:

- **No (Not related):** The temporal relationship of the clinical event to product administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.
- **Yes (Related):** The temporal relationship of the clinical event to product administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.
- **Suspected** There is a reasonable possibility that the investigational product caused the observed event. Suspected implies a lesser degree of certainty about the causality of the observed event

Severity / Intensity:

The severity / intensity of AEs will be graded on a five-point-scale:

- Mild or Grade 1: discomfort noted, but no disruption to normal daily activities.
- Moderate or Grade 2: discomfort sufficient to reduce or affect normal daily activities.
- Severe or Grade 3: inability to work or perform normal daily activities.
- Life threatening or Grade 4
- Death or Grade 5

Outcome of event:

The outcome of an event will be classified as follows:

- Recovered
- Recovered with sequelae
- Ongoing
- Fatal
- Unknown / Lost to follow-up

10.3 *Safety Management*

10.3.1 Procedures

All AEs occurring during clinical studies are recorded in the eCRF and are classified according to the Common Terminology for Adverse Events (CTCAE) system. AEs should be captured from the screening visit (V1) to the end of the study. Refer to the https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf and https://livertox.nih.gov/Severity.html, for additional information.

CTCAE Terms

An Adverse Event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each CTCAE v4.0 term is a MedDRA LLT (Lowest Level Term).

CTCAE Grades

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE. Grade 5 (Death) is not appropriate for some AEs and therefore is not an option.

A Semi-colon indicates 'or' within the description of the grade.
A single dash (-) indicates a Grade is not available. Not all Grades are appropriate for all AEs. Therefore, some AEs are listed with fewer than five options for Grade selection.

Definitions
A brief Definition is provided to clarify the meaning of each AE term. A single dash (-) indicates a Definition is not available.

Navigational Notes
A Navigational Note is used to assist the reporter in choosing a correct AE. It may list other AEs that should be considered in addition to or in place of the AE in question. A single dash (-) indicates a Navigational Note has not been defined for the AE term.

Activities of Daily Living (ADL)
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

SAEs are reported in the eCRF and processed according to the applicable FDA requirements governing the conduct of clinical studies. All SAEs will be followed until resolution or at the latest 30 days after the subject's completion in the study.

SUSARs are reported and processed according to the FDA requirements governing the conduct of clinical studies.

When an unexpected SAE not previously listed in the Investigator's Brochure (IB) occurs in a multi-center clinical study, all investigational sites currently involved in the study should be notified by the study Sponsor or medical monitor within one week of receipt of the unexpected SAE.

SAE and SUSARs are also reported to a third-party vendor (referenced as Pharmacovigilance Vendor (UBC)) per flowchart below.

SAEs and SUSARs must be recorded on the electronic SAE Form. Following case processing, a US FDA MedWatch Form 3500A will be generated from the Pharmacovigilance Vendor for SUSARs. The event is entered into the PV safety database for filing and further expedited reporting by IND Sponsor-Principal Investigator.

The directions for completing the SAE Form are detailed in the "CRF Guidelines". This guideline will be available to all sites in the study.

For SUSARs, study treatment may be unblinded prior to reporting the case to the applicable regulatory authorities and IRB/IEC. Please see section 4.4 for unblinding procedures.

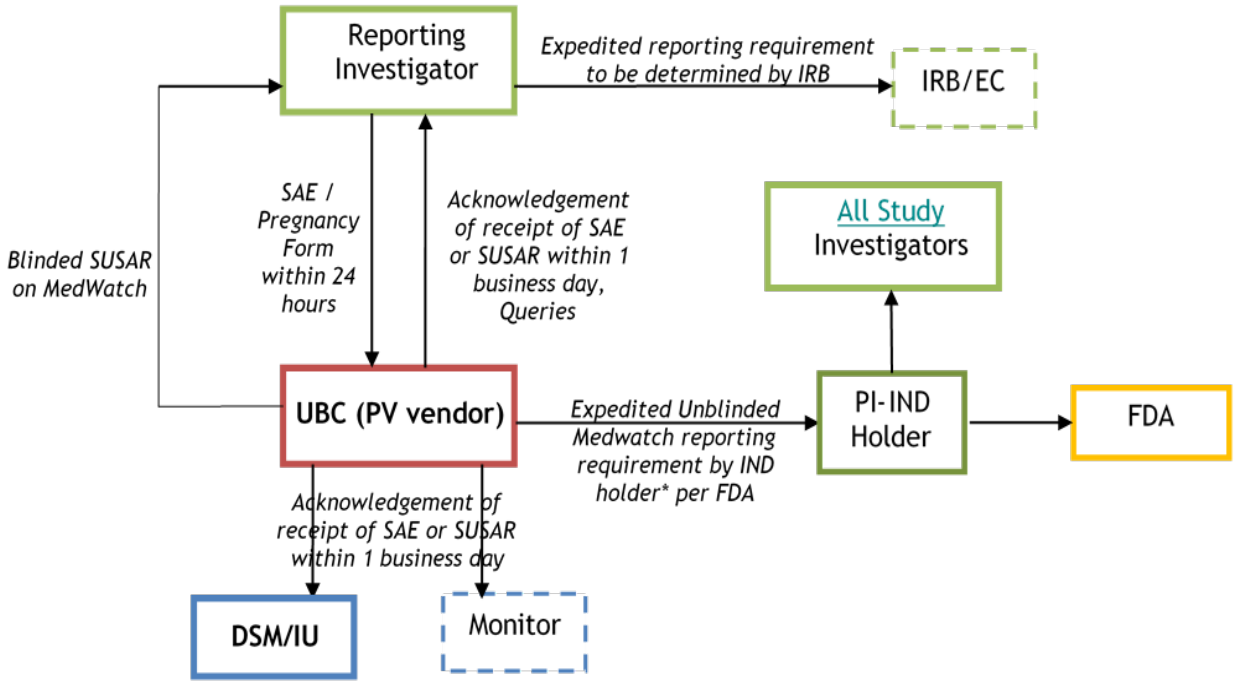
A Data Safety Monitoring Board (DSMB) will be arranged to review the safety information in the trial on a bi-annual basis. Details of the DSMB's responsibilities will be outlined in a DSMB plan.

SAEs will be reviewed by the safety officer of the study and the DSMB.

During or at the end of each study, an SAE reconciliation between data entered in the study database and the data entered in the pharmacovigilance database will be performed if any SAE has occurred in the study. All discrepancies found during the reconciliation are documented in an SAE reconciliation report and corresponding queries are sent to the investigators for clarification.

Since pregnancy is an exclusion criterion in the study, then for any case of pregnancy occurring during the conduct of the study, the course of the pregnancy and the outcome of both the mother and the offspring have to be documented in the eCRF and on the Pregnancy Form. This form will be provided to sites in the study, if needed.

The directions for completing the Pregnancy Form are detailed in the "Guidelines for Completing the Pregnancy Form". This guideline will be provided to sites in the study when needed.



10.3.2 Treatment and Holding/Stopping Rules for Drug Induced Liver Injury (DILI)

See the chart below for DILI monitoring and stopping guidelines:

Treatment Emergent ALT Elevation	Treatment Emergent Total Bilirubin	Liver Related Symptoms	Management Recommendation
Normal/near normal baseline: ALT ≥ 5 ×ULN Elevated baseline: ALT ≥3x baseline or ≥300 U/L whichever occurs first	Normal Patients with Gilbert's syndrome: No change in baseline total bilirubin	None	• Repeat ALT, AST, ALP, TBL, in 2-5 days. • Follow-up for symptoms • Initiate evaluation for other etiologies of abnormal liver tests
Normal/near normal baseline: ALT ≥ 3 ×ULN Elevated baseline: ALT ≥2x baseline or ≥300 U/L whichever occurs first	Normal Patients with Gilbert's syndrome: No change in baseline total bilirubin	Severe fatigue, nausea, vomiting, right upper quadrant pain, tenderness, fever, rash, and/or eosinophilia (> 5%)	• Repeat ALT, AST, ALP, TBL, in 2-5 days. • Follow-up for symptoms • Initiate evaluation for other etiologies of abnormal liver tests
Normal/near normal baseline: ALT ≥ ×8 ULN Elevated baseline: ALT ≥5x baseline or ≥500 U/L whichever occurs first	Normal Patients with Gilbert's syndrome: No change in baseline total bilirubin	None	• Interrupt study drug • Initiate close monitoring and workup for competing etiologies • Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal/near normal baseline: ALT ≥ ×3 ULN Elevated baseline: ALT ≥2x baseline or ≥300 U/L whichever occurs first	TBL ≥2x ULN Patients with Gilberts syndrome: Doubling of direct bilirubin or increased INR to >1.5	Severe fatigue, nausea, vomiting, right upper quadrant pain, tenderness, fever, rash, and/or eosinophilia (> 5%)	• Interrupt study drug • Initiate close monitoring and workup for competing etiologies • Study drug can be restarted only if another etiology is identified and abnormalities return to baseline.
Normal/near normal baseline: ALT ≥ ×5 ULN Elevated baseline: ALT ≥3x baseline or ≥300 U/L	Normal or Elevated	Severe fatigue, nausea, vomiting, right upper quadrant	• Interrupt study drug • Initiate close monitoring and workup for competing etiologies • Study drug can be restarted only

(whichever occurs first)		pain, tenderness, fever, rash, and/or eosinophilia (> 5%)	if another etiology is identified and abnormalities return to baseline.

Source: Modified from Table 1 in REGEV et al, 2019 [36]

* If a patent lives in a remote area, laboratory testing can be performed locally, and the results should be promptly communicated to the investigator site

Study Stopping Rules:

The DSMB will review clinical safety data on an ongoing basis to identify potential safety issues arising during the conduct of the study. Including but not limited to the following:

1. 1 subject receiving the active treatment experiences a grade 5 CTCAE during the clinical trial that it is likely attributable to the study drug(s), OR
2. 2 or more subjects receiving the active treatment experience a grade 4 CTCAEs likely attributable to the study drug(s), OR
3. 3 or more subjects receiving the active treatment experience a grade 3 CTCAEs likely attributable to the study drug(s).

10.3.3 Responsibilities

- The Principal Investigator (PI) ensures that SAE reporting procedures outlined in the study protocol are adhered to and that all required documentation is up-to-date and that regulatory and IRB/IEC SAE reporting procedures are followed.
- All study personnel at the study sites (Study Coordinator, Study Nurse, PI, or designee) who are in contact with clinical trial subjects are responsible for collecting AE information from the subject at each scheduled site visit or during telephone calls with the subject. Therefore, clinical research personnel or delegates (e.g. CRO) who initiate and monitor the study are responsible for explaining the procedures for reporting and evaluating AEs to the PI, and all study personnel who will be in contact with the subjects/patients.
- During the course of the study complete reports of all AEs should be entered in the subject's/patient's site source documents, and on the appropriate study case report forms (CRFs).
- A physician is responsible for: identifying and evaluating the severity (mild, moderate, or severe) and clinical importance of the AE, taking appropriate medical action(s), and for notifying a clinical research monitor, CRO and PV vendor immediately of an SAE, as specified in the protocol, and also for notifying the IRB/IEC. A copy of the source documents and related records should be supplied with the SAE report to the PV Vendor.
- Likewise, a physician indicates the causality (relationship) of the AE to the study product.
- For any laboratory abnormality, the PI will make a judgement as to its clinical significance. If the laboratory value is thought to be clinically significant, the IND Sponsor or medical monitor may be consulted, and an assessment will be made by the PI, as to its relationship to investigational product administration, and it will be documented on the AE page of the eCRF.

- The PI should comply with applicable regulatory requirement(s) related to the reporting of SAEs to the regulatory authority(ies) and the IRB /IEC.
- At completion of the study, the final lists of AEs and SAEs will be MedDRA coded by the CRO or PV vendor.
- The Study Protocol defines AEs (including SAEs) and instructs the PI and study personnel to evaluate and record the occurrence of all AEs during the study. The AEs should be recorded in the patients' site source documents and on the appropriate AE page of the eCRF. The protocol defines the circumstances under which the blind may be broken and by whom when unblinding is deemed necessary for evaluating and managing a particular SAE or SUSAR. The Study Protocol also defines the follow-up period for SAEs.
- The Monitor(s) should review all completed CRF data and should compare CRF entries with information recorded in the source documents. Any discrepancies or omissions in either data source should be discussed with the site personnel who should make the appropriate corrections to the documents. Any recorded SAE or SUSAR which has not been reported to CRO or PV Vendor should be discussed with the PI and should be reported immediately to the CRO or PV Vendor specified in the protocol, and also should be reported by the PI to the IRB/IEC.

11 ETHICAL CONSIDERATIONS

11.1 *Local Regulations and Declaration of Helsinki*

This clinical study will be conducted in compliance with the ethical principles that have their origin in the Declaration of Helsinki, Title 21 of the Code of Federal Regulations §§ 50, 54, 56, and 312, and International Conference on Harmonisation E6, Good Clinical Practice.

11.2 *GCP Management Directive*

The Principal Investigator will ensure that this clinical study is conducted in conformance with the principles of GCP, as outlined below:

1. Clinical studies should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the principles of GCP (where applicable) and the applicable laws and regulatory requirements governing the conduct of human studies;
2. Foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and the society;
3. The rights, safety, and well-being of the subjects involved in clinical studies are the most important considerations and should prevail over interests of science and society;
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical study;
5. Clinical studies should be scientifically sound, and described in a clear, detailed protocol;
6. Clinical studies should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favorable opinion;

7. The medical care given to, and medical decisions made on behalf of, subjects involved in clinical studies should always be the responsibility of a qualified physician;
8. Each individual involved in conducting clinical studies should be qualified by education, training, and experience to perform his or her respective task(s);
9. Freely given written informed consent should be obtained from every subject prior to participation in a clinical study;
10. All information from clinical studies should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification;
11. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable laws and regulatory requirements;
12. Investigational products should be manufactured, handled, and stored in accordance with DNP quality standards, or as applicable, good manufacturing practice (GMP); IP(s) should be used in accordance with the approved protocol;
13. Systems with processes and procedures (such as SOPs) that assure the quality of every aspect of clinical studies should be implemented.

11.3 *Informed Consent*

It is the responsibility of the investigator, or a person designated by the investigator (if acceptable by local regulations), to obtain written informed consent from each subject participating in this clinical study, or from the subject's legally acceptable representative, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. No study procedures may be performed prior to obtaining the informed consent. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason.

11.4 *IRB/IEC approval*

This protocol and any accompanying material provided to the subject (such as subject information sheets or descriptions of the study used to obtain informed consent) as well as any advertising or compensation given to the subjects, will be submitted by the investigators to an IRB/IEC. Approval from the IRB/IEC will be obtained before starting the study and will be documented in a letter to the investigators specifying the date on which the IRB/IEC met and granted the approval.

Any modifications (amendments) made to the protocol after receipt of the IRB/IEC approval must also be submitted by the investigators to the IRB/IEC in accordance with local procedures and regulatory requirements.

11.4.1 Risk-benefit assessment

Oral vitamin E intake at the dose in the study is safe, as confirmed by the US Food and Nutrition Board of the Institute of Medicine (IOM). The DHA dose is also safe and below the GRAS affirmation level. There are no approved treatments for NAFLD. Minimal risks such as bruising at the blood draw site or gastrointestinal effects (e.g. gassiness, reflux) are anticipated in this study.

11.5 *Confidentiality*

11.5.1 Data

All information regarding the nature of the proposed investigation provided by the Sponsor or CRO to the Investigator (with the exception of information required by law or regulations to be disclosed to the IRB/IEC, the subject, or the appropriate regulatory authority) must be kept in confidence by the Investigator in accordance with all applicable laws and regulations as specified in the Clinical Study Agreement.

11.5.2 Subject Anonymity

The anonymity of participating subjects must be maintained. Subjects will be identified by an assigned subject number (and optionally including their initials) on CRFs and other documents submitted to the CRO. Documents that will not be submitted to the CRO and that identify the subject (e.g., the signed informed consent document) must be maintained in strict confidence by the Investigator, except to the extent necessary to allow auditing by the appropriate regulatory authority, the Sponsor or its designee and in accordance with applicable regulatory requirements.

11.6 *Subjects' compensation / remuneration*

Minimal compensation for transportation costs or a meal may be provided to the subjects to facilitate attending the scheduled study visits and a small stipend may be awarded upon completion of scheduled study visits.

11.7 *Registration of study in a public clinical trial database*

This study will be registered at http://clinicaltrials.gov - https://clinicaltrials.gov/ct2/show/NCT04198805

12 STUDY DOCUMENTATION AND RECORD KEEPING

12.1 *Protocol amendments*

Any modification to the agreed protocol must be approved in writing by the IND Sponsor-Principal Investigator. Amendments will be submitted to the IRB/IEC and FDA, as required. These procedures must be fulfilled before any modification is put into effect.
Note: Sites must update the Protocol Amendments Tracking Form Log for each amendment.

12.2 *Investigator Site File*

The Investigators must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories (1) Investigator's Site File (ISF), and (2) source documents.

1. The ISF will contain the protocol/amendments, CRFs and Query Forms, IRB/IEC and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence, etc.

2. Source documents (usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs) would include subjects' records,

physician's, nurse's and research assistant’s notes, appointment book, original laboratory reports, X-rays, pathology, and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrolment logs.

The Investigator should retain the study documents of the ISF for the retention time specified in their contract.

Should the Investigator wish to assign the study records to another party or move them to another location, CRO must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made with the Investigator and CRO to store these in a sealed container(s) outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the subject, appropriate copies should be made for storing outside the site.

12.3 *Source document and source data verification*

According to the standards of the data protection law, all data obtained in the course of a clinical study must be treated with discretion in order to guarantee the rights of the subject's privacy. The Investigator should agree to allow the monitor/auditor/inspector to have access to any or all of the clinical study materials needed for source data verification and proper review of the clinical study progress.

12.4 *Insurance and Funding*

Liabilities, in connection with the clinical study will be covered by an insurance policy with each clinical site in the event of a subject suffering any significant injury, which is proven as being a direct result of study participation (as specified in the Clinical Study Agreement). Funding for this trial is provided by DNP.

12.5 *Monitoring*

It is understood that the responsible monitor will contact and visit the Investigator regularly and will be allowed, on request, to review the various records of the trial (CRFs and other pertinent data) provided that subject confidentiality is maintained in accordance with local requirements and as specified in the contract.

The Study Coordinator (SC) or Study Nurse (SN) at the site of the investigation will be instructed by the monitor to ensure that the following responsibilities and tasks are carried out:

- Monitoring the study supplies and, if requested, returning all undispensed supplies to manufacturer or CRO at completion of the study.
- Maintaining all records of the study.
- Checking source documents for legibility and completion at the time they are received from the investigator. After review of study data with the investigator, the SC/SN will complete the formal eCRFs.
- If required by the study, removing tear-off labels on receipt of double-blind labelled investigational product prior to its being dispensed to the subject/patient.
- Contacting patients to remind them of their scheduled visits and obtaining a final disposition for every subject/patient who is entered in the study.

- Checking for reasonableness and completeness of source documents and of recorded CRF data before CRFs are reviewed by a monitor at each routine monitoring visit.

It will be the monitor's responsibility to review the study documents (e.g. CRFs) at regular intervals throughout the study, to verify the adherence to the protocol and the legibility, completeness, consistency, and accuracy of the data being entered on them. The monitor must have access to laboratory test reports and other subject records needed to verify the entries on the CRF.

The investigator (or his/her deputy) facilitates the monitoring tasks including the source document verification and agrees to cooperate with the monitor to ensure that any issues detected in the course of these monitoring visits are resolved.

12.6 *Quality assurance and quality control*

All IP used in clinical studies are subjected to quality control. Quality assurance audits may be performed by any health authority during the course of the clinical study or after its completion (see Archiving).

The Investigator agrees to comply with regulatory requirements in terms of auditing of the clinical study. This includes access to the source documents for source data verification.

12.7 *Final Study Report*

When all completed CRFs have been collected and data have been analysed, the results of this clinical study are to be documented in a comprehensive study report and manuscript.

12.8 *Archiving*

The study documents will be retained for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

The Trial Master File (TMF) shall be retained for a longer period, where so required by other applicable legal or regulatory requirements and/or as specified in the contract of the Investigator.

The ISF will be retained according to Section 12.2

12.9 *Publication*

INSTITUTION and SPONSOR-INVESTIGATOR agree that the first publication, lecture, manuscript, poster presentation or other disclosure or dissemination of the data or results of this study (collectively, “PUBLICATION”) shall be made in conjunction with the presentation of a joint multi-centre publication of the results with the investigators and institutions from all STUDY sites contributing data (as specified in the Clinical Study Agreement). In this case, a coordinating investigator and authorship will be designated by mutual agreement.

13 CONDITIONS FOR TERMINATING THE STUDY

Both the IND Sponsor-Investigator and the Site Investigator reserve the right to terminate the clinical study at any time. Should this be necessary, both parties will arrange the procedures on an individual project basis after review and consultation. In terminating the project, IND Sponsor-Investigator, DNP and the Site Investigator will assure that adequate consideration is given to the protection of the subject's interests (as specified in the contract).

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15 APPENDICES

15.1 *Appendix 1*

DHA Food Frequency Questionnaire©

Subject ID #: _____ **Initials:** ____ ____ ____ (F M L) **Date:** ____ ____ ____ (MM DD YY)

Estimate your consumption over the past 2 months of the following foods. Use the food model forms to help estimate portion sizes.

		Servings	DHA		EPA	
1.	How many 3oz servings of the following fish do you eat **monthly**? bluefish, blue fin tuna, cisco, smoked, herring, mackerel, pollock, sardines, salmon, whitefish	_____	x 22	_____	x 14	_____
2.	How many 3oz servings of the following fish do you eat **monthly**? bass, calamari, catfish, drumfish, flounder, grouper, halibut, mussels, perch, redfish, rockfish, shark, snapper, sole, squid, swordfish, trout, tuna, canned (6oz can), whiting	_____	x 10	_____	x 5	_____
3.	How many 3oz servings of the following fish/shellfish do you eat **monthly**? carp, clams, cod, crab, crayfish, fish patties/squares, fish sticks, haddock, lobster, mullet, oysters, pike, pompano, scallops, shrimp (14 med.), sturgeon	_____	x 5	_____	x 6	_____
4.	How many 3oz servings of liver (chicken, turkey or beef) do you eat **monthly**?	_____	x 7	_____	x 2	_____
5.	How many egg yolks do you eat **weekly** (including eggs yolks used in cooking)?	_____	x 3	_____	x 0.25	_____
6.	How many 3oz servings of chicken, turkey or other poultry (not including livers) do you eat **weekly**?	_____	x 5	_____	x 3	_____
7.	Any omega-3 dietary supplements or functional foods (i.e., flax, fish oil, Neuromins, DHA Gold, high DHA eggs)? amount or strength _____ frequency _____			**mg/d**		**mg/d**
	Sub-Total:			_____ DHA (mg/d)		_____ EPA (mg/d)
	TOTAL= Rounded to Nearest Whole #:			_____ DHA (mg/d)		_____ EPA (mg/d)

15.2 *Appendix 2*

SF-36(tm) Health Survey

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each
one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents
your response.
Patient Name: _____
SID#: _____ Date: _____
Person heling to complete this form: _____
1. In general, would you say your health is:
▫▫Excellent
▫▫Very good
▫▫Good
▫▫Fair
▫▫Poor
2. Compared to one year ago, how would you rate your health in general now?
▫▫Much better now than a year ago
▫▫Somewhat better now than a year ago
▫▫About the same as one year ago
▫▫Somewhat worse now than one year ago
▫▫Much worse now than one year ago
3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
c. Lifting or carrying groceries.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
d. Climbing several flights of stairs.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
e. Climbing one flight of stairs.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
f. Bending, kneeling or stooping.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
SF-36 2
g. Walking more than one mile.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
h. Walking several blocks.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
i. Walking one block.
▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
j. Bathing or dressing yourself.

▫▫Yes, limited a lot.
▫▫Yes, limited a little.
▫▫No, not limited at all.
4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?
a. Cut down the amount of time you spent on work or other activities?
▫▫Yes ▫▫No
b. Accomplished less than you would like?
▫▫Yes ▫▫No
c. Were limited in the kind of work or other activities
▫▫Yes ▫▫No
d. Had difficulty performing the work or other activities (for example, it took extra time)
▫▫Yes ▫▫No
5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?
a. Cut down the amount of time you spent on work or other activities?
▫▫Yes ▫▫No
b. Accomplished less than you would like
▫▫Yes ▫▫No
c. Didn't do work or other activities as carefully as usual
▫▫Yes ▫▫No
6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?
▫▫Not at all
▫▫Slightly
▫▫Moderately
▫▫Quite a bit
▫▫Extremely
7. How much bodily pain have you had during the past 4 weeks?
▫▫Not at all
▫▫Slightly
▫▫Moderately
▫▫Quite a bit
▫▫Extremely
SF-36 3
8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?
▫▫Not at all
▫▫Slightly
▫▫Moderately
▫▫Quite a bit
▫▫Extremely
9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks
a. did you feel full of pep?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
b. have you been a very nervous person?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
c. have you felt so down in the dumps nothing could cheer you up?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time

d. have you felt calm and peaceful?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
e. did you have a lot of energy?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
f. have you felt downhearted and blue?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
SF-36 4
g. did you feel worn out?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
h. have you been a happy person?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
i. did you feel tired?
▫▫All of the time
▫▫Most of the time
▫▫A good bit of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?
▫▫All of the time
▫▫Most of the time
▫▫Some of the time
▫▫A little of the time
▫▫None of the time
11. How TRUE or FALSE is each of the following statements for you?
a. I seem to get sick a little easier than other people
▫▫Definitely true
▫▫Mostly true
▫▫Don't know
▫▫Mostly false
▫▫Definitely false
b. I am as healthy as anybody I know
▫▫Definitely true
▫▫Mostly true
▫▫Don't know
▫▫Mostly false
▫▫Definitely false
c. I expect my health to get worse
▫▫Definitely true
▫▫Mostly true

▫▫Don't know
▫▫Mostly false
▫▫Definitely false
d. My health is excellent
▫▫Definitely true
▫▫Mostly true
▫▫Don't know
▫▫Mostly false
▫▫Definitely false