PROTOCOL TRACKING SHEET

VERSION NUMBER	VERSION DATE	REASON FOR CHANGE (high-level)
2.2	09/21/2021	DCP Final Approval
2.3	05/05/2021	To remove the Northwestern accrual site; to correct an error in the exclusion criteria; to utilize DCP Protocol Template v4.0 dated 11/19/2020
2.4	12/02/2021	To revise the Fibroscan inclusion criteria; to give participants blood pressure monitors and instructions for self-collection; to utilize DCP Protocol Template v7.0 dated 10/19/2021
2.5	09/12/2022	To utilize current DCP protocol template v11.0 dated 08/18/2022; to add Mayo as accrual site; to update protocol investigators and staff changes; to make MRI/PDFF-MRE optional for participants; to correct minor errors throughout the protocol
2.6	11/03/2022	To utilize current DCP protocol template v12.0 dated 09/21/2022; to correct minor protocol inconsistencies including instructions for stool sample self-collection
2.7	12/20/2022	To clarify in the exclusion criteria that trace ascites documented on a radiology report is permitted; to correct minor errors.
2.8	01/23/2024	To modify inclusion criteria; to include updates from DCP protocol template v 13 & 14; to update Fibroscan® specific language
2.9	03/05/2025	To update protocol language per DCP guidance; to update study personnel; to include updates from DCP protocol template v 16 & v 17
2.10	06/16/2025	To update secondary and exploratory biomarkers; update to DCP protocol template v18.0; to update study staff

DCP Protocol #: NWU20-01-03

Local Protocol #: NCI 20-01-03

***RO*L*E* OF *LI*SINOPRIL IN PR*E*VENTING THE PROGRESSION OF NON-ALCOHOLIC *F*ATTY LIVER DISEASE (NAFLD): RELIEF-NAFLD**

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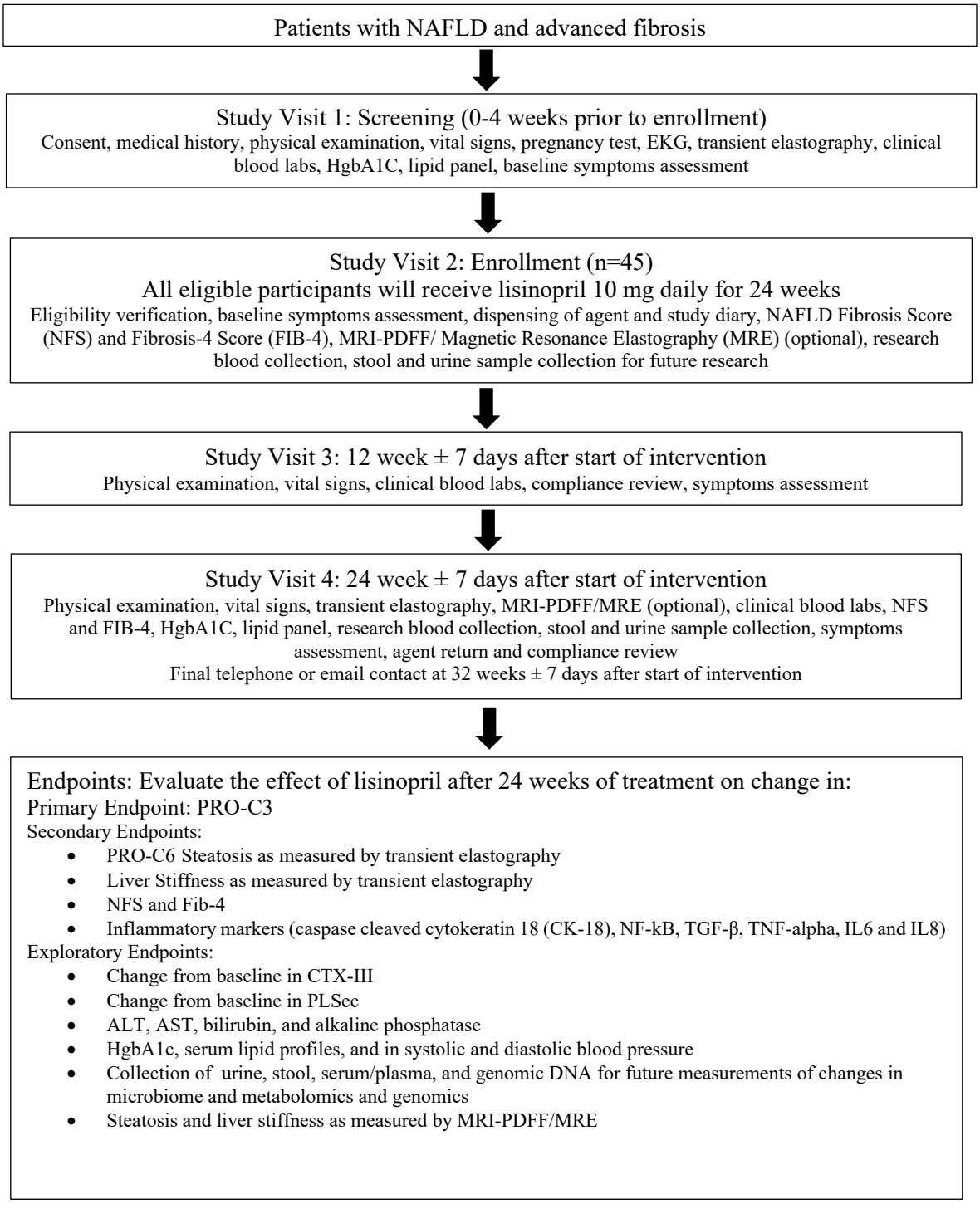
IND# 151831 (exempt)
Agent(s)/Supplier: Lisinopril / NCI DCP

Protocol Version Date: June 16, 2025

Protocol Revision or Amendment # Version 2.10

SCHEMA

NWU20-01-03: Role of Lisinopril in Preventing the Progression of Non-Alcoholic Fatty Liver Disease (NAFLD): RELIEF-NAFLD



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1. OBJECTIVES

1.1 Primary Objective

The primary objective of this study is to determine if NAFLD patients with advanced fibrosis will demonstrate a change in PRO-C3, a marker of liver fibrosis, following 24 weeks of treatment with lisinopril.

1.2 Secondary Objectives

Noninvasive measures of fibrosis and steatosis:

a) Change from baseline in PRO-C6
b) Change from baseline in steatosis, as measured by controlled attenuation parameter (CAP) or liver ultra-sound attenuation (LiSA), determined with transient elastography
c) Change from baseline in liver stiffness as measured with magnetic resonance elastography (MRE)
d) Change from baseline in liver stiffness as measured with transient elastography
e) Changes from baseline in Fibrosis-4 score (FIB-4) and NAFLD fibrosis score (NFS)
f) Change in inflammatory markers (caspase cleaved cytokeratin 18 (CK-18), NF-κB, TGF-β, TNF-alpha, IL6 and IL8)

1.3 Exploratory Objectives

a) Change from baseline in CTX-III
b) Change from baseline in PLSec
c) Change from baseline in steatosis, as measured by magnetic resonance imaging-proton density fat fraction (MRI-PDFF) in participants who undergo optional MRI-PDFF/MRE
d) Change from baseline in markers of liver injury and function: ALT, AST, bilirubin, and alkaline phosphatase.
e) Change from baseline in homeostatic assessment of hemoglobin A1C, serum lipid profiles, and in systolic and diastolic blood pressure.
f) Collection of urine, stool, serum/plasma and genomic DNA for future measurements of changes in microbiome and metabolomics and genomics.

2. BACKGROUND

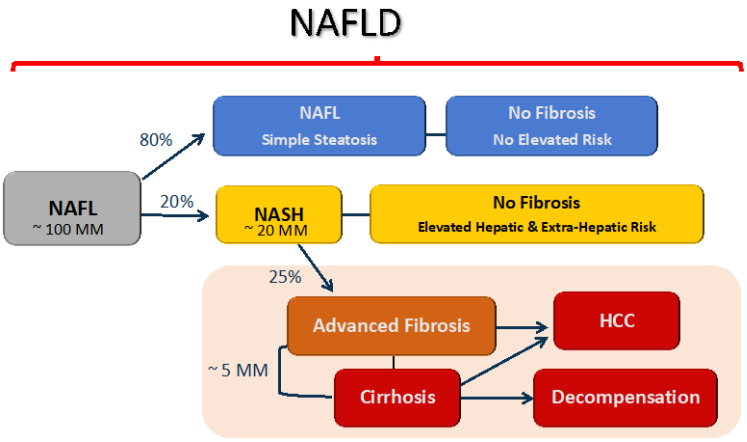
2.1 Study Disease

Hepatocellular carcinoma (HCC) is increasing in prevalence more rapidly than any cancer in the U.S., and liver transplantation for HCC increased up to10-fold between 2004 and 2015 [1, 2]. These increases are due mainly to a dramatic increase in the prevalence of nonalcoholic fatty liver disease (NAFLD) and its advanced form, nonalcoholic steatohepatitis (NASH) [2, 3]. NAFLD affects about 25% of the general population worldwide, and it is the most common cause of chronic liver disease and cirrhosis [4]. NAFLD has a higher incidence in certain U.S. ethnic groups, especially those of Latin American descent, a rise in incidence that is correlated with the rising incidence of obesity and type 2 diabetes [5, 6]. Fatty liver disease has a spectrum, ranging from hepatic steatosis without prominent inflammation to NASH, which also displays inflammation and ballooning of hepatocytes, which can lead to fibrosis, cirrhosis and HCC [3, 7].

Hepatitis C was the leading cause of HCC and HCC-related liver transplantation in the U.S., until the introduction of effective therapies for hepatitis C resulted in a decreased incidence of hepatitis C-related HCC [8]. On the other hand, HCC due to NASH continues to increase steadily in the U.S. Among NAFLD

patients, the incidence of HCC has been estimated to be 0.44 per 1000 person years (range 0.29-0.66), and the incidence in NASH with cirrhosis to be 9-26 per 1000 person years [3, 9]. It is plausible that effective treatment of NAFLD that would prevent progression to NASH would lead to an overall lower incidence of HCC. Thus results of NAFLD therapy would have a similar impact to that of curative treatment of hepatitis C. NAFLD has also been associated with cancers other than HCC: gastric, colonic, pancreatic and uterine, and this association has been found to be independent of diabetes and obesity [10].

NAFLD with advanced fibrosis and NASH with advanced fibrosis are overlapping entities. NAFLD is a term used to cover the entire disease spectrum. The disease starts as simple steatosis (NAFL), which in some patients progresses to the more severe form, NASH; NASH consists of inflammation and hepatocyte injury in addition to steatosis. NASH is believed to be the only form of NAFLD that progresses to fibrosis and cirrhosis. The diagnosis of NASH can only be made conclusively via histologic diagnosis (liver biopsy). However, since only NASH leads to fibrosis, fibrosis detected by noninvasive liver tests is felt to be the equivalent of NASH determined by liver biopsy – and that the noninvasive tests can replace liver biopsy in making the diagnosis of NASH. Therefore, our cohort will consist of likely NASH with advanced fibrosis -- which will be more accurately called NAFLD with advanced fibrosis -- given the lack of liver histology. Please see figure for explanation:



Torres DM, et al. *Clin Gastroenterol Hepatol.* 2012;10(8):837-858.

2.2 Study Agent

Angiotensin-converting enzyme (ACE) inhibitors and blockers, NAFLD, and HCC:
Angiotensinogen produced by the liver is secreted into the circulation in response to low blood pressure or change in sodium concentrations. The enzyme renin is secreted by the kidney to cleave angiotensinogen to the inactive angiotensin I, which can be converted to the active form, angiotensin II, by angiotensin converting enzyme (ACE). Angiotensin II then binds the angiotensin II type 1 receptor (AT_1) on the vascular endothelium, which leads to vasoconstriction, sodium retention, and, eventually, increased blood pressure. ACE inhibitors (ACEis) inhibit the formation of angiotensin II, and angiotensin II receptor blockers (ARBs) block the binding of angiotensin II to its AT_1 receptor [11]. ACEis and ARBs are collectively called renin angiotensin system inhibitors. Interestingly and importantly, these drugs have also reduced morbidity and mortality in cancer patients [11]. Although ACEis and ARBs are important candidates for cancer chemoprevention, their mechanism of action is unknown [11]. Possible mechanisms are anti-inflammatory and immunomodulatory (NF-κB inhibition), anti-angiogenic, and anti-fibrotic activities [12, 13], most of which (except anti-angiogenic activity) are also key pathways in the pathogenesis of NASH, fibrosis and cirrhosis. In animal models, renin angiotensin system inhibitors reduced HCC

tumor burden and progression to higher grades, led to regression of HCC, and restored liver histology [14, 15]. A study of 153 patients with HCC who received radiofrequency ablation found that patients' overall survival was significantly better if they received ARBs and was numerically better if they received ACEis; median time to recurrence of HCC was also significantly better in those who had received ARBs [16]. In a small, prospective, randomized, open-label trial, HCC patients were randomized to an ACEi plus vitamin K vs. no treatment and were followed for up to 54 months; the cumulative HCC recurrence rate was lower in the ACEi-treated patients than in untreated patients (~40% vs. 75% recurrence ($p<0.01$))[17].

From the standpoint of NAFLD and fibrosis, the renin-angiotensin system regulates the activation of hepatic stellate cells and fibrogenesis in animal models [18, 19]. Emerging data have suggested that blocking this system improves histology, especially fibrosis, in NAFLD. For example, a recent small retrospective analysis showed that the use of ACEis or ARBs for treating hypertension in patients with NAFLD was associated with decreased hepatic fibrosis [20]. Losartan, an angiotensin II receptor antagonist, was tested in 7 patients with NASH and hypertension; significant decreases in serum markers of hepatic fibrosis, plasma TGF-β1, and serum ferritin, along with an improvement in aminotransferase levels, were found [21]. Treatment with losartan also led to improvement in hepatic necroinflammation, reduction of hepatic fibrosis, and disappearance of iron deposits [21]. Other small, limited studies have found similar effects in patients with NASH [22, 23].

ACEis and ARBs are generally well tolerated. Common adverse events (AEs) associated with the ACEis and ARBs are generally low grade. However, the ACEis fosinopril [24] and perindopril [25] can cause dry cough, dizziness, hypotension, headache, and hyperkalemia. Rare and serious AEs have included anaphylactic reactions such as angioedema, and hepatic failure. Common AEs associated with the ARBs candesartan [26] and losartan [11] include abnormal kidney function, dizziness, hypotension, and hyperkalemia.

It should also be noted that in 2010 a negative meta-analysis by Sipahi et al. [27] reported that ARB-treated patients were at a modestly increased risk for new cancer vs. control patients (7.2% vs. 6.0%; risk ratio [RR] 1.08, 95% CI 1.01–1.15; p=0.016). However, their conclusion was refuted by multiple meta-analyses, including one by the Food and Drug Administration (FDA), which concluded that there was no increased risk of new cancers in ARB vs. control patients (1.82 per patient year vs 1.84 per patient year; RR 0.99, 95% CI 0.92–1.06) [28, 29]. In addition, Pasternak et al., using patient-level data from Danish registries, came to a similar conclusion that ARB use was not significantly associated with increased risk of cancer; on the contrary, ARB users experiences a reduced cancer mortality rate ratio of 0.77 (95% CI 0.72–0.82) [30].

However, due to the recent large-scale recalls of a number of ARBs and ARB- containing formulations, in response to the finding of genotoxic nitrosamine impurities in these drugs, formed during drug manufacturing processes, this class of ARBs, are expressly excluded from this solicitation. Therefore, we chose the ACEi lisinopril 10 mg oral daily, which has been shown to be a safe and effective antihypertensive dose. Lisinopril was also used in animal data [31]. The effective dose is unknown and therefore we aim to uncover the potential benefit by choosing a higher dose.

2.3 Rationale

Many studies have found that fibrosis is the most important prognostic factor in NAFLD, as it correlates with disease mortality [32-34]. Thus, detection of fibrosis is used for staging of NAFLD and determining treatment strategies. Fibrosis is also related directly to the progression to HCC, and collagen deposition is increased in HCC stroma [35]. Liver fibrosis can be assessed with noninvasive tools. These include the

NAFLD fibrosis score (NFS), Fibrosis-4 (FIB-4) score, and imaging techniques -- transient elastography and magnetic resonance elastography (MRE) -- all of which have been endorsed by the American Association for the Study of Liver Diseases (AASLD) for staging of the disease [36]. The Enhanced Liver Fibrosis score consists of measurement of tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type III procollagen, and hyaluronic acid [37]. PRO-C3 is a serum biomarker that is considered a type III collagen marker. The Enhanced Liver Fibrosis score and PRO-C3 have been used as proof-of-concept fibrosis biomarkers to advance therapeutic agents from phase 2a to phase 2b NASH studies [38]. New data suggest that PRO-C3 can be a biomarker for HCC (see PRO-C3 discussion below).

2.3.1. Available Treatment of NAFLD. Weight loss and exercise are recommended for treatment of NAFLD, but these measures are difficult for patients to sustain [7]. Benefits from vitamin E were found in the PIVEN trial [39], but because the long-term effects of vitamin E are unknown, it is not routinely used. Pioglitazone has been beneficial for the treatment of NAFLD, but the adiposity weight gain it causes limits its adoption [40]. Multiple drugs with different mechanisms of action are under investigation. Obeticholic acid, elafibranor, cenicriviroc, belapectin, resmetirom, and aramchol are in phase 3 trials, while selonsertib has failed and showed no efficacy despite advancing to phase 3 testing [41-44]. Interim results with obeticholic acid in a phase 3 trial have been announced, but the magnitude of improvement was modest, and potential side effects were a concern [7, 45]. FDA approval of obeticholic acid for NASH without cirrhosis is expected in 2020.

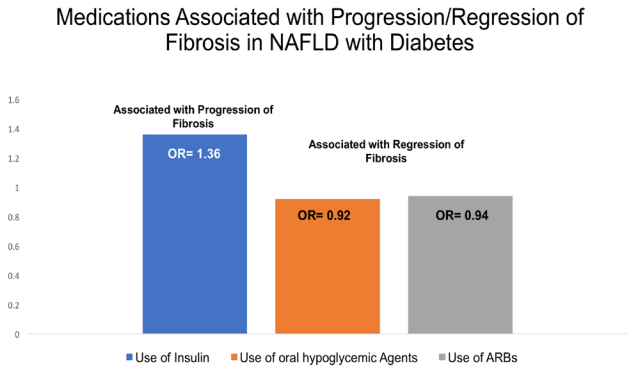


Fig 1: Prelim data: Medications associated with fibrosis Progression or regression in Diabetics with NAFLD

2.3.2. Preliminary Data. We have conducted a preliminary epidemiological study of a large cohort of type 2 diabetes patients with NAFLD, both conditions identified by ICD-9 code. We used scores of noninvasive tests to assess the transition from no advanced fibrosis to advanced fibrosis during the period from baseline to last follow-up. The cutoffs used to assess transition were: aspartate aminotransferase (AST)/alanine aminotransferase (ALT) > 1.4; AST to platelet ratio score > 1.5; FIB-4 > 2.67; and NFS > 0.676. A total of 50,695 patients were included, with median follow up of 84.4 months. Transition from no advanced fibrosis to advanced fibrosis (progression) occurred in 25.8% of patients; transition from advanced fibrosis to no fibrosis (regression) occurred in 6.4%; and no change occurred in the remaining patients. Clinical factors significantly associated with progression were female, older age at baseline, presence of baseline obesity, chronic kidney disease, and coronary artery disease. Use of insulin was associated with progression to advanced fibrosis (OR =1.36, $p<0.001$), whereas use of oral hypoglycemic agents and ARBs was associated with regression of fibrosis (OR= 0.92 and 0.94, respectively; all p values were <0.05) (Fig 1).

Therefore, ACEis and ARBs may be attractive agents for the treatment of NAFLD. Their use could lead to less development and progression of the key pathogenic features of the disease, i.e., inflammation and fibrosis, and eventually to less cirrhosis, carcinogenesis, and HCC. Since NAFLD is the most common cause of HCC in the Western world, preventing progression of NAFLD to its advanced stages by ACEis and/or ARBs has potential for reducing the global burden of HCC.

2.3.3 PRO-C3. Fibrosis is the common consequence of chronic liver diseases leading to impaired liver function [46]. It is the best predictor of progression to end-stage liver disease and adverse liver-related outcomes. Changes in the extracellular matrix (ECM) reflect the development of fibrosis and can be monitored [46]. Circulating collagen fragments that have distinctive epitopes and are released during

collagen formation and degradation correlate with the extent of the ECM change [46]. Some of the collagen fragments that reflect collagen formation are PRO-C3 (N-terminal pro-collagen III peptide), PRO-C5 (C-terminal pro-peptide of type V collagen), PRO-C6 (C-terminal pro-peptide of type VI collagen), and basement membrane biomarker P4NP7S (7 S domain of type IV collagen) [47]. Fragments that reflect collagen degradation are C3M (matrix metalloprotease degradation fragment of collagen III) and C4M (matrix metalloprotease degradation fragment of collagen IV) [47]. PRO-C3 assay, developed by Nordic Bioscience, uses an antibody that specifically recognizes the neo-epitope at the cleavage site of PRO-C3 [48]. Thus, PRO-C3 reflects the biosynthesis of type III collagen and fibrogenesis.

In studies of chronic hepatitis C patients, those who had higher baseline PRO-C3 values (≥20.2 ng/ml) had progression of fibrosis, whereas those who had lower values did not [49, 50]. PRO-C3 is also correlated with hepatic venous pressure gradient, which is an invasive diagnostic and prognostic marker for portal hypertension in cirrhosis [51]. In NASH, PRO-C3 is correlated with all histological features -- steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis (Fig 2. Unpublished data). Recent studies have shown that PRO-C3 can be an accurate biomarker in NASH patients, to distinguish those with advanced fibrosis from those with non-advanced fibrosis [52].Two large NASH clinical trials have used PRO-C3 as a biomarker of treatment response. Reduction in PRO-C3 at week 16 in patients treated with pegbelfermin (pegFGF21) was associated with reduction in hepatic fat, as determined with MRI-proton density fat fraction (MRI-PDFF) [53]. The result of the trial has led to pegFGF21 being advanced into later stage trials. In another randomized phase 2 trial [54], NASH patients received NGM282 (FGF19) or placebo; at week 12, 50% and 68% of the patients given 1 mg or 3 mg, respectively, had improved histological NAFLD activity score by 2 or more points without worsening fibrosis. PRO-C3 values declined at weeks 6 and 12 in these patients.

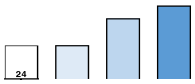


Fig 2: Correlation between PRO-C3 and histology in NASH patients

2.3.4. PRO-C3 and HCC. Fibrosis, especially at later stages (cirrhosis), is a major risk factor for HCC. As mentioned, fibrosis and cirrhosis are the result of the activation of fibroblasts, leading to the deposition of ECM, which is the foundation of HCC development in >80% of cases [55, 56]. The progression to HCC occurs because the cross-linking of ECM makes it stiffer, which incites the HCC process, its progression and metastasis [57, 58]. The cross-linking of ECM is facilitated by many collagens, such as type III collagen, and is catalyzed by enzymes such as lysyl oxidase (LOX), its family members LOX-like (LOXL) 1-4, and transglutaminase 2 [59, 60]. LOXL2-induced tissue stiffness has been associated with metastatic HCC, and HCC patients with elevated transglutaminase 2 have more HCC invasion [61]. Therefore, it is plausible that, as in fibrosis, where type III collagen production is markedly upregulated, those products can be increased in HCC, with corresponding increased amounts of PRO-C3.

Guidelines for screening for HCC, which can lower the mortality of HCC if followed properly, differ among liver societies. The American Association for the Study of Liver Diseases (AASLD) recommends the use of ultrasonography with or without determination of serum alpha-fetoprotein (AFP) values [18], whereas the European Association for the Study of the Liver (EASL) recommends the use of ultrasonography alone. Although AFP is considered the “go to biomarker” in HCC screening, a large proportion of HCC patients (≈40%) do not have elevated AFP values [62]. Therefore, new biomarkers that are related to HCC pathophysiology need to be critically evaluated. Based on the evidence cited above [53, 54], Nordic Bioscience (our industry partner) has developed a sandwich ELISA to measure circulating

cross-linked multimeric pro-peptides of type III collagen (PRO-C6), and it has evaluated PRO-C6 as a biomarker in HCC in comparison with PRO-C3 and AFP.

2.3.5 Preliminary data (provided by Nordic Bioscience) show that PRO-C6, PC3X, PRO-C3 and AFP values were significantly increased in HCC patients compared with those in patients with other liver diseases and in healthy controls (p=0.0002, p<0.0001). Interestingly, in patients with normal AFP (<20 IU/ml), XM-PRO- C3 and PRO-C3 distinguished HCC from cirrhosis with an AUROC of 0.69 and 0.67, respectively. High PC3X and AFP predicted poor progression-free survival (HR for PC3X =1.80, p=0.032; HR for AFP=1.70, p=0.031) and overall survival (HR_{PC3X}=2.12, p=0.024; HR_{AFP}=2.55; p=0.003) (Fig 3). PC3X was independent of AFP (progression-free survival: HR=1.74, p=0.045 and overall survival: HR=2.21, p=0.018), and the markers combined had better prognostic value (progression-free survival: HR=2.66, p=0.004 and overall survival: HR=5.86, p<0.0001).

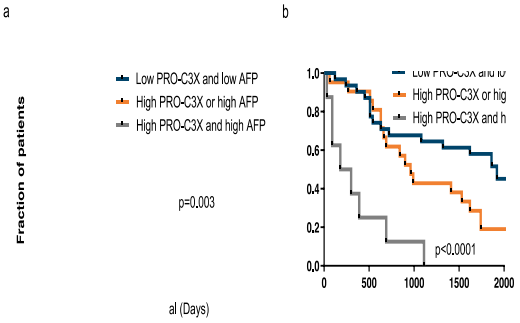


Fig 3: PC3X was independent of AFP in predicting progression free survival and overall survival

2.3.6 Hypothesis. We hypothesize that treatment of NASH with lisinopril (an ACEi) will lead to improvement in PRO-C3 values, which would correlate with decrease in histologic fibrosis. Fibrosis leads to adverse outcomes of liver disease (HCC, liver transplantation, and death) in NAFLD patients.

2.3.7 Rationale for exploratory endpoints:
CTX-III will be analyzed for a change from baseline and end of study. Preliminary data provided by Nordic Bioscience indicates that CTX-III is a non-invasive biomarker applicable for the evaluation of fibrosis regression and progression[63].

A serum protein-based biomarker, Prognostic Liver Secretome signature (PLSec), was developed as a surrogate biomarker for HCC development by utilizing integrative database/pipeline of protein subcellular localization, extracellular secretion to circulation, and tissue type specificity for HCC. The 8-protein PLSec assay implemented in an FDA-approved diagnostic platform, xMAP assay (Luminex), was successfully validated in independent cohorts of 641 patients longitudinally followed for up to 15 years and 1,342 compensated cirrhosis patients from the NCI Early Detection Research Network (EDRN) HCC Early Detection Strategy (HEDS) cohort[64]. PLSec is used as a surrogate endpoint in NCI-funded HCC prevention trials in cirrhotic patients, including MASLD cirrhosis (R01CA255621/NCT05028829, R01CA282178/NCT06015022)

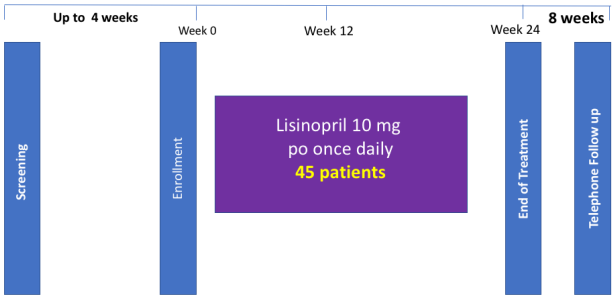
2.3.8 Rationale for alcohol and tobacco use questionnaires:
Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and at visit 4, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

2.3.9 Rationale for COVID-19 assessment:
The NCI, DCP Assessment of COVID-19 exposure and vaccine status at baseline and end of study will be used to determine the potential impact on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

3. SUMMARY OF STUDY PLAN

As per DCP solicitation, this is a proof-of-concept open label trial in subjects with NAFLD and advanced fibrosis. Subjects meeting the entry criteria for the study will receive lisinopril 10 mg orally once daily for 24 weeks as illustrated in Fig 4. Participation in the study includes a 4-week screening period, a treatment period of up to 24 weeks, and a follow-up evaluation 8 weeks post-intervention. Study drug will be dispensed to the subjects by the study pharmacist or designee. Participant compliance with the study drug will be assessed at each visit via diaries and pill counts.

4. PARTICIPANT SELECTION



The Protocol Risk for Auditing for this study is *intermediate.*

Low and Intermediate risk studies require confirmation of eligibility of the first two participants enrolled at each accruing LAO or AO. This review should take place as soon as possible but not more than two weeks following enrollment/randomization. Continuing eligibility review can be determined based on the LAO assessment of AO performance and protocol complexity.

4.1 Inclusion Criteria

4.1.1 Male and female subjects ≥18 years of age

4.1.2 Clinical diagnosis of NASH assessed by the presence of body imaging criteria [ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI)], or liver biopsy up to six months prior to enrollment without suspicious nodules or cancer

4.1.3 Screening transient elastography liver stiffness ≥ 12 kPa (which correlates with F3 fibrosis and more) and < 25 kPa [65]. Historic transient elastography within 0 - 4 weeks prior to the date of the screening visit is acceptable. Patients with liver stiffness ≥ 10 and < 12 kPA with clinical evidence of cirrhosis based on any of the following criteria would also be eligible.

A. Imaging diagnosis of nodular liver with splenomegaly or recanalized umbilical vein
B. MRE ≥ 5 kPa
C. FIB-4 > 2.67 or platelet count < 150,000 mL
D. Liver biopsy < 5 years with METAVIR stage 4 or Ishak stage 5-6

4.1.4 Controlled attenuation parameter score or LiSA of ≥ 260 dB/m and any single component of metabolic syndrome (ATP3 criteria)

or

historic liver biopsy within 0 - 6 months prior to the date of the screening visit consistent with NASH (defined as the presence of steatosis, inflammation, and ballooning), with stage 3-4 fibrosis according to the NASH Clinical Research Network classification (or equivalent).

4.1.5 Participants must have adequate marrow, organ, hepatic and renal function as defined below:

Leukocytes	*≥3,000/microliter*
Absolute neutrophil count	*≥1,500/microliter*
Platelets	*≥75,000/microliter*
Total bilirubin	*within normal institutional limits unless the patient has Gilbert's syndrome*
AST (SGOT)/ALT (SGPT)	*≤8× institutional upper limit of institutional limits*
Glomerular filtration rate	*>30 ml/min*
INR	*≤1.3 unless the patient is on a therapeutic medication*

4.1.6 ECOG performance status ≤ 2 (Karnofsky ≥60%; see Appendix A)

4.1.7 The effects of lisinopril has been shown to be teratogenic in animal models. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.8 Ability to understand and the willingness to sign a written informed consent document. If a participant has impaired decision-making capacity (IDMC), their legal representative may replace them in this process.

4.1.9 Systolic blood pressure ≥90 and ≤160 mm/Hg. Diastolic blood pressure ≥60 and ≤110 mm/Hg.

4.2 Exclusion Criteria

4.2.1 Prior or current use of an ACEi or ARB within 0 - 24 weeks prior to enrollment.

4.2.2 Glomerular filtration rate ≤ 30 ml/min (for both male and female participants).

4.2.3 History of decompensated liver disease, including ascites, hepatic encephalopathy, or high-risk variceal bleeding.

NOTE: Trace ascites documented by radiology is permitted.

4.2.4 History of other causes of liver disease, including but not limited to alcoholic liver disease, hepatitis B, hepatitis C, autoimmune disorders (primary biliary cholangitis, primary sclerosing cholangitis, or autoimmune hepatitis), drug-induced hepatotoxicity, Wilson's disease, iron overload, or alpha-1-antitryspin deficiency.

4.2.5 History of liver transplantation.

4.2.6 History of HCC diagnosis.

4.2.7 History of weight reduction surgery in the past 2 years or planned during the study.

4.2.8 Within 6 months prior to the date of the screening visit, there must be no history of the following cardiac events: unstable angina; myocardial infarction, coronary artery bypass surgery or coronary

angioplasty; transient ischemic attack or cerebrovascular accident; emergency room visit or hospitalization for confirmed cardiovascular disease

4.2.9 Participants taking vitamin E ≥800 IU/day must be on a stable dose, defined as no changes in prescribed dose, new vitamin E-containing medications, or discontinuation for at least 180 days prior to the date of the screening visit and throughout study participation.

4.2.10 Participants taking anti-diabetic medications must be on a stable dose for at least 90 days prior to the date of the screening visit and in the period between the date of the screening visit and enrollment

4.2.11 Current alcohol consumption >21 oz/week for males or >14 oz/week for females [1 oz/30 mL of alcohol is present in one 12 oz/360 mL beer, 4 oz/120 mL glass of wine, and a 1oz/30 mL measure of 40proof (20%) alcohol].

4.2.12 Participants may not be receiving any other investigational agents, at the time of the screening visit, or in the prior 30 days, or within 5 half-lives of the prior investigational agent (whichever is longer).

4.2.13 History of allergic reactions attributed to compounds of similar chemical or biologic composition to lisinopril.

4.2.14 Uncontrolled intercurrent illness or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.15 History of HIV infection. HIV patients may develop fatty liver as well as advanced fibrosis due to many causes including metabolic syndrome, hyperuricemia, HIV-related lipodystrophy, genetic polymorphisms, medications, and HIV itself [66]. As the natural history of fatty liver in this population is largely unknown, these patients will be excluded from this study.

4.2.16 Women who are pregnant or breastfeeding. Pregnant women are excluded from this study because lisinopril is an ACE Inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with lisinopril. Breastfeeding should be discontinued if the mother is treated with lisinopril.

4.2.17 Systolic blood pressure ≥161 mm/Hg. Diastolic blood pressure ≥111 mm/Hg.

4.2.18 Participants taking Lithium.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.4 Recruitment

Participants will be recruited from all eligible patients who have NAFLD as defined per the AASLD guidelines [36]. We will recruit patients from the Fatty Liver Program and hepatology clinics in the 4 centers. A large proportion of our population is of Latin American descent (~30% average) providing a unique opportunity to test an intervention for a disease and subsequent cancer that is the most burdensome to this large, growing, and understudied demographic group [67]. We will provide CIRB approved translations of the informed consent and all protocol materials for Spanish-speaking participants. We will utilize short form consenting process to enroll other non-English speaking participants as needed. Given

the large volume of patients with NAFLD and NAFLD with advanced fibrosis at our clinical centers, there is large number of patients potentially eligible for this trial. The number of NAFLD patients followed in the Fatty Liver Program and hepatology clinics in the 4 centers is approximately 2500-3500 per year. Overall, The Cedars-Sinai health system has 11,200 patients with ICD-9 and ICD-10 codes consistent with NAFLD/NASH. Of these, only 15% are receiving ACEis or ARBs. The Duke University health system has evaluated over 11,000 patients with ICD-9 and ICD-10 codes consistent with NAFLD/NASH in addition to the NASH CRN Database and Duke NAFLD Clinical Database and Biorepository cohorts. The Mount Sinai health system has more than 10 million people in its medical records, with many outreach clinics. Six Fibroscans® travel in the metropolitan area in multiple clinics to recruit patients with NAFLD. The Mayo Clinic Division of Gastroenterology is staffed with 24 hepatologists who see patients with NAFLD / NASH and cirrhosis. There is a dedicated NAFLD Clinic staffed by 4 hepatologists. The Mayo Clinic NAFLD registry will utilize ICD-9 and ICD-10 codes consistent with NAFLD / NASH for case-finding subjects who may be eligible to participate in this study.

We assume that the same sampling frame will be available for the proposed study. Thus, the large population of NAFLD patients provide assurance that our source population will meet our recruitment needs.

4.4.1 Recruitment and Feasibility

Recruitment will occur over 18-24 months. Medical records of patients with NAFLD will be reviewed for age, transient elastography results, ACEis and ARB use, concomitant medications, and medical contraindications. The investigator will obtain information through oral or written communication with the prospective subject or legally authorized representative. Patients deemed potentially eligible will be approached in clinic during their regularly scheduled appointment or contacted by phone to discuss study participation. A study information packet including an introductory letter and lay summary of the study may be mailed to potentially eligible participants. Interested patients will be invited to schedule a Screening Visit. We will provide CIRB approved translations of the informed consent and all protocol materials for Spanish-speaking participants. For patients who agree and consent into the study, copies of each form will be given to the patient and placed in the medical record. Screening procedures may be performed 0-4 weeks prior to enrollment.

4.4.2 Pre-Screen Medical Chart Review- Initial determination of eligibility and recruitment

Patients with NAFLD over age 18 who have an upcoming usual care appointment with a study physician will be identified and then further pre-screened for eligibility as specified in the inclusion and exclusion criteria, based on the most updated information in their medical record. The medical records will be reviewed by the clinical research staff to determine initial eligibility based on age, transient elastography results, ACEis and ARB use, concomitant medications, medical contraindications, and laboratory results.

The investigator will obtain information through oral or written communication with the prospective subject or legally authorized representative. Patients deemed potentially eligible will be approached in clinic during their regularly scheduled appointment or contacted by phone to discuss study participation. Study staff will introduce the study and answer questions. A study information packet including an introductory letter and lay summary may be mailed to potentially eligible participants. Patients will be encouraged to review the consent documents with family, friends, and/or other physicians. The patient will be told that the decision to join or not join the study will not affect the medical treatment that s/he receives, and that s/he can withdraw from the study at any time.

4.4.3 Consent and Final Determination of Eligibility

The study physician will review the study details and answer any questions regarding the trial after all clinical options have been presented to the patient. After the patient's questions have been answered, study staff will obtain signatures on the consent documents from patients who wish to participate in the study or their legally authorized representative. Copies of each document will be given to the patient and placed in the medical record. We anticipate that we will obtain consent from 65 potentially eligible patients to successfully enroll 45 participants across all 4 sites. Written consent will be obtained before any research related procedures are initiated. After consent has been obtained, a final assessment of eligibility will be conducted.

The study physician will thoroughly review the patient's medical history, concomitant medications, laboratory test results, and evaluate all inclusion and exclusion criteria. All screening procedures including: clinical and research blood specimen collection, urine/stool sample collection, completion of alcohol and tobacco use questionnaires, medical history, physical exam, vital signs, EKG, transient elastography, baseline symptoms assessment, NFS, and FIB-4 will be performed 0-4 weeks prior to enrollment. In general, the definition of NAFLD require that patients do not drink alcohol excessively. Nevertheless, inconclusive data suggest that even minimal amounts of alcohol affect the disease outcome. Therefore, alcohol questionnaire should be collected. The inclusion of the tobacco questionnaire is important because patients with NAFLD, including those with advanced fibrosis, have increased likelihood of cardiac events. Since tobacco use plays a significant role in cardiac events, such data is needed. In addition, because ACEis are known to be teratogenic, women who are able to become pregnant will be required to have a serum or urine pregnancy test 0-4 weeks prior to enrollment. Women who are ≥ 50 years old and have not had a menstrual cycle in the past year or who have had a hysterectomy, both ovaries removed, or a tubal ligation will not be required to have a pregnancy test. Women who are pregnant will be excluded from the study. Following eligibility screening, consented patients who qualify for the study will be registered and enrolled in the study.

There will be four study visits. Study Visit 1 (screening, consent, and baseline measurements) will occur 0-4 weeks prior to enrollment. Study Visit 2 (enrollment, agent dispensing, and baseline measurements) will occur 0-4 weeks after screening. Follow-up visits will be scheduled at 12 weeks (± 7 days) and 24 weeks (± 7 days) from agent start. Study visits, scheduled at the participating clinic or clinical research center, will coincide whenever possible with usual care appointments to reduce travel time and increase compliance with the study protocol.

Participants will receive $100 when they complete each study visit for their time, transportation, parking, and other expenses related to the study. Participants who complete the entire study will receive $400.

4.5 Planned Accrual

We are expecting the composition of our study population to be 80% white, 9% Black or African American, and 8% Asian (Table 2). These proportions are based on NAFLD databases at CSMC, Duke, Mount Sinai, and Mayo Clinic and are aligned with the racial proportions of NAFLD patients reported in the literature [4]. We are expecting that 30% of our study population will be Hispanic or Latino. These proportions are representative of the NAFLD patients seen at CSMC, Duke, Mount Sinai, and Mayo Clinic and correspond to those estimated in the US population.

- Cedars-Sinai: 90% White, 3% Black, 4% Asian, 3% Other; 10% Hispanic or Latino
- Duke University Medical Center: 97% White, 1% Black, 1% Asian, 1% Other
- Mount Sinai Medical Center: 93% White, 3% Black, 2% Asian, 2% Other, 10% Hispanic or Latino
- Mayo Clinic: 97% White, 1% Black, 1% Asian, 1% Other

In the unlikely event that we are unable to reach our recruitment goal, we have active collaborations with other community non-profit liver centers that we could utilize for recruitment, if needed. Assuming that ~10% of the study participants drop-out, we expect to have data on a final sample of 40 men and women completing the 24 weeks visit.

DOMESTIC PLANNED ENROLLMENT REPORT

Racial Categories	Not Hispanic or Latino: Female	Not Hispanic or Latino: Male	Hispanic or Latino: Female	Hispanic or Latino: Male	Total
American Indian/Alaska Native	0	0	0	0	0
Asian	2	2	0	0	4
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	2	2	0	0	4
White	13	8	9	6	36
More Than One Race	0	1	0	0	1
Total	17	13	9	6	45

5. REGISTRATION PROCEDURES

5.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually which is done via the Registration and Credential Repository (RCR).

To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Rave or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	**IVR (Investigator)**	**NPIVR (Non-physician Investigator)**	**AP (Associate Plus)**	**A (Associate)**	**AB (Associate Basic)**
FDA Form 1572	✔	✔			

Documentation Required	IVR (Investigator)	NPIVR (Non-physician Investigator)	AP (Associate Plus)	A (Associate)	AB (Associate Basic)
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to participate in all CP-CTNet clinical trials.

All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be registered in the RCR.

Personnel associated with the five registration types include, but is not limited to, the following:

- **Investigator (IVR)** — MD, DO, or international equivalent
- **Non-Physician Investigator (NPIVR)** — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD)
- **Associate Plus (AP)** — clinical site staff (e.g., RN or CRA) with data entry access to RAVE. Also includes site administrator, data administrator, and consenting person. Individuals with an auditing role should register as an AP.
- **Associate (A)** — other clinical site staff involved in the conduct of NCI-sponsored trials.
- **Associate Basic (AB)** — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems

In addition, the site -protocol Principal Investigator (PI) must meet the following criterion:
- Active registration status
- The IRB number of the CIRB (IRB of record) listed on their Form FDA 1572

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

6. NCI CENTRAL INSTITUTIONAL REVIEW BOARD

The NIH policy on the Use of a Single Institutional Review Board for Multi-Site Research https://grants.nih.gov/grants/guide/notice-files/not-od-16-094.html became effective on January 25, 2018. In compliance with this policy, NCI Central IRB (NCI CIRB) is the sole IRB of record for all accruing sites conducting clinical trials through the CP-CTNet, all CP-CTNet U.S.-based sites must be members of the NCI NCI CIRB, and utilize the Cancer Prevention and Control CIRB as their IRB of record. International sites should submit Research Ethics Board (REB) approval to the DCP Regulatory contractor following country-specific regulations.

Signatory Institutions must submit a Study Specific Worksheet (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the PIs at the Signatory Institution and the Regulatory Contractor. In order for the SSW approval to be processed, the Signatory Institution must inform which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the CIRB prior to implementation.

7. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported AEs and potential risks are described below.

7.1 Dose Regimen and Dose Groups

Patients will receive lisinopril 10 mg orally daily for 24 weeks after enrollment.

7.2 Lisinopril Administration

Patients will self-administer the 10 mg pill of lisinopril daily. Patients will be instructed to take the study agent in the morning prior to breakfast. Lisinopril should be taken at approximately the same time each day. The absorption of lisinopril tablets is not affected by food. Each participant will be given one bottle of 100 pills at Study Visit 2 (84-day supply + buffer) and one bottle of 100 pills (84-day supply + buffer) at Study Visit 3 (week 12).

7.3 Run-in Procedures

None.

7.4 Contraindications

Angiotensin-converting enzyme inhibitors may cause hypersensitivity, usually manifested as a result of alterations in kinin generation in sensitive individuals; there is no evidence of a specific immune-mediated reaction. However, such reactions can be potentially life-threatening, even if they are not true 'allergic' reactions. Lisinopril is contraindicated in patients with a history of ACE-inhibitor induced angioedema, hereditary angioedema, or idiopathic angioedema. Risk of angioedema may also be higher in patients with a history of angioedema unrelated to ACEis. If angioedema occurs, ACEi therapy should be

halted and appropriate treatment instituted. The incidence of ACE-inhibitor induced angioedema is higher in people of African descent than in other racial/ethnic groups.

7.4.1 Diabetes mellitus, hyperkalemia, renal artery stenosis, renal disease, renal failure, renal impairment

Lisinopril should be used with caution in patients with risk factors for hyperkalemia. ACEis can elevate serum potassium concentrations and could worsen pre-existing conditions. Risk factors for the development of hyperkalemia include renal insufficiency, diabetes mellitus, and the concomitant use of potassium-sparing diuretics, potassium supplements, and/or potassium-containing salt substitutes, or other drugs that may increase serum potassium. Renal function and serum potassium are monitored throughout the trial. We exclude patients with moderate to severe renal impairment or renal failure (CrCl 30 mL/minute or less). Treatment with ACEis has demonstrated favorable effects on the progression of renal disease in diabetic and nondiabetic patients; however, minor increases in BUN and serum creatinine may occur. These effects, more commonly reported in patients with renal artery stenosis or those receiving concomitant diuretic therapy, are usually reversible and are not considered a reason to withhold therapy unless accompanied by hyperkalemia.

7.4.2 Autoimmune disease, bone marrow suppression, collagen-vascular disease, immunosuppression, scleroderma, systemic lupus erythematosus (SLE)

Neutropenia and/or agranulocytosis have been reported during therapy with ACEis. This effect rarely occurs in uncomplicated patients but more frequently in patients with renal impairment especially if they also have a collagen-vascular disease (e.g., systemic lupus erythematosus (SLE) or scleroderma) or are receiving concomitant immunosuppression. Data from clinical trials of lisinopril are insufficient to show that the drug does not cause agranulocytosis. Therefore, complete blood counts will be established prior to and during lisinopril therapy.

7.4.3 Acute myocardial infarction, aortic stenosis, cardiomyopathy, cerebrovascular disease, coronary artery disease, heart failure, hyponatremia, hypotension, hypovolemia

It is recommended that lisinopril should be used cautiously in patients who exhibit hyponatremia or hypovolemia, in part because inhibition of aldosterone production would be expected to exacerbate both conditions. We excluded these patients from our study.

7.4.4 Surgery

In patients undergoing major surgery or during anesthesia with agents that produce hypotension, lisinopril may block angiotensin II formation secondary to compensatory renin release. Hypotension considered to be due to this mechanism can be corrected by volume expansion.

7.4.5 Jaundice

ACE inhibitors such as lisinopril have rarely been associated with a syndrome that starts with cholestatic jaundice or hepatitis and progresses to fulminant hepatic necrosis and sometimes death. The mechanism of this syndrome is not understood. Patients receiving ACEis who develop jaundice or marked elevations of hepatic enzymes will discontinue the ACEi and receive appropriate medical treatment.

7.4.6 Pregnancy, Breastfeeding

Pregnant women are excluded from this study because lisinopril is an ACE Inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with lisinopril. Breastfeeding should be discontinued if the mother is treated with lisinopril.

7.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

7.6 Dose Modification

The study agent will be stopped in the event of an AE ≥ grade 3 considered possibly, probably, or definitely related to the study agent. The study agent will be restarted after resolution. In the event of a ≥ grade 2 cough, the study agent will be stopped for 7 days and restarted if patient does not tolerate, they will be a screen fail. If the participant develops ≥ grade 2 hypotension, the dose will be modified to 10 mg of Lisinopril every other day for two weeks. If hypotension resolves after 2 weeks, the participant may re-start 10 mg Lisinopril daily. Blood pressure is measured on a weekly basis for the first 12 weeks of intervention. If a participant develops hypotension at any point during the intervention, blood pressure will be measured weekly for the duration of the intervention. Participants may self-collect blood pressure readings at home or at a nearby pharmacy. Participants will be given a blood pressure monitor, instructed on its use, and given instructions on recording and reporting their blood pressure within the participant diary.

7.7 Adherence/Compliance

Compliance will be measured by pill counts and patient diaries. Compliance is defined as 85% of the total dose with all doses of the study agent. A participant diary will be used to monitor daily compliance. Participants will be instructed to record the time the study drug was taken each day in the diary, as well as any potential side effects. Diaries will be reviewed and compliance will be assessed at Study Visits 2-5.

Phone calls will be made to study participants at weeks 1, 2, 8, 16, and 20 after start of intervention to monitor compliance. A window of ±3 days is allowed. Participants will be asked if they missed any doses of medication, and the dates of all missed doses will be recorded. Participants will also be reminded to bring any leftover study drugs and the study diary with them to each visit. The study agent diary will be reviewed with participants at every visit. Please see Appendix E for suggested language for participant communication.

All participants that receive a study agent for at least one dose of study agent will be evaluable for toxicity.

8. PHARMACEUTICAL INFORMATION

8.1 Lisinopril (IND #151831, NCI, Division of Cancer Prevention)

Lisinopril, originally approved in 1987, is an angiotensin converting enzyme (ACE) inhibitor. Angiotensin I is an inactive prohormone that is part of the renin-angiotensin system responsible for cardiovascular blood pressure. It undergoes site selective proteolysis by angiotensin converting enzyme to

form active angiotensin II. Angiotensin II then proceeds to bind to its corresponding receptor on the vascular endothelium resulting in vasoconstriction, sodium retention, and ultimately increased blood pressure. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Consequently, preventing the formation of angiotensin II counteracts multiple cardiovascular processes consistent with lisinopril's approvals for the treatment of hypertension, heart failure, and heart attack.

Following oral administration of lisinopril, peak serum concentrations occur within about 7 hours. Upon multiple dosing lisinopril exhibits an effective half-life of 12 hours, thereby supporting once daily dosing. The mean extent of absorption of lisinopril is approximately 25 percent, with large inter-subject variability (6–60 percent). Lisinopril absorption is not influenced by the presence of food. Lisinopril does not undergo metabolism and is excreted unchanged entirely in the urine.

Lisinopril is a white to off-white, crystalline powder that is incorporated into a 10 mg tablet for once daily oral administration. The tablet is provided by Solco Healthcare and also includes inactive excipients that are generally recognized as safe, including calcium phosphate, magnesium stearate, mannitol, and starch. The 10 mg dose is recommended as the starting dose for monotherapy treatment for hypertension [https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=e299d477-a612-4af4-b6aa-f5addc8a736d].

8.2 Reported Adverse Events and Potential Risks

According to the lisinopril drug label, patient reports of adverse events that were 2% greater with lisinopril than placebo in hypertension studies were: headache (5.7% vs 1.9%), dizziness (5.4% vs 1.9%), and cough (3.5% vs 1.0%). Clinical laboratory test findings of hypertension patients revealed 2.2% had hyperkalemia and 2.0% had minor increases in blood urea nitrogen that were reversible upon discontinuation. Additional adverse events occurring in ≥1% of hypertension or heart failure patients included fatigue, orthostatic hypotension, constipation, dry mouth, and diarrhea. ACEis have caused rare but serious events of angioedema and other anaphylactoid reactions as well as hepatic failure developing subsequent to cholestatic jaundice, hepatitis, or marked elevations of hepatic enzymes.

The risks and discomforts of frequent phlebotomy: Blood collection by venipuncture is associated with mild discomfort, and the possibility of localized bruising, phlebitis, or extravasation. The risk of infection or fainting is extremely low.

Transient elastography has no known side effects: No adverse events have been associated with transient elastography

MRI-PDFF and MRE are optional and may cause discomfort especially with the noise during the test. Earplugs and/or headphone with music will be offered to the patients. There might be incidental findings on those MRIs. If so, the patient will be given a report of the MRI to provide to the patient's primary care physician.

8.3 Availability

Lisinopril (10 mg tablets, 100 tablets/bottle) is an investigational agent supplied to investigators by NCI, DCP.

8.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of CIRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or his/her authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the sites will make a request. Agents are requested by completing the DCP Clinical Drug Request form: https://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/Investigational-Agent-Request.docx (to include complete shipping contact information) and e-mailing the form to the DCP agent repository contractor:

MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 753-7600
E-mail: NCI.DCP@mriglobal.org
Emergency Telephone: (816) 360-3800

8.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the Research Pharmacist at each site. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

8.6 Packaging and Labeling

Lisinopril 10 mg tablets will be packaged by NCI, DCP.

8.7 Storage

Lisinopril will be store at controlled room temperature, 15–30°C (59–86°F) and protected from moisture. The CP-CTNet Lead Academic Organization, the DCP agent repository contractor, DCP Medical Monitor and Nurse Consultant should be notified in the event of a Temperature Excursion.

8.8 Registration/Randomization

This trial will use a web-based Registration/Randomization System, developed and maintained by the CP-CTNet Data Management, Auditing, and Coordinating Center (DMACC). The Help Desk includes technical personnel and administrators of the registration programs at the Data Management Center in Amherst, NY, USA. The Help Desk is available round the clock 7 days per week, except for New Year's Eve, Memorial Day, Independence Day, Thanksgiving Day, and Christmas Day.

Frontier Science Randomization Help Desk
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716 834 0900 Extension 7301
Email: UserSupport_CP-CTNet@frontierscience.org

Note: The Registration and Randomization process is documented in the "Stars User Guide".

8.9 Blinding and Unblinding Methods

Not applicable, this is an open-label study.

8.10 Agent Destruction/Disposal

At the completion of investigation, all unused study agent will be destroyed according to local institutional procedures. Destruction records must be provided to MRIGlobal at the end of the study.

9. CLINICAL EVALUATIONS AND PROCEDURES

9.1 Schedule of Events

Evaluation/ Procedure	**Visit 1 Screening** (0-4 weeks prior to enrollment)	**Visit 2 Enrollment** (start of intervention)	**Visit 3** (12 week ± 7 days after start of intervention)	**Visit 4** (24 week ± 7 days after start of intervention)	**Post Treatment Follow up** (Week 32 ± 7 days after start of intervention)
Informed Consent	X				
Assess Eligibility	X	X			
Medical History	X				
Physical Exam	X		X	X	
Vital Signs[1]	X		X	X	
Blood Pressure self-collection			X[2]		
Serum or Urine Pregnancy Test[3]	X				
Medication Review	X	X	X	X	
Routine Blood Tests[4]	X		X	X	
HgbA1C and Lipid Panel	X			X	
Research Blood Collection[5]		X		X	
EKG	X				
NFS and FIB-4		X		X	
Transient Elastography[6]	X			X	
MRI-PDFF/MRE (optional)		X[7]		X	
Stool and urine sample collection		X		X	
Dispense study agent/diary		X	X		
Collect leftover study agent/diary			X	X	
Review of study agent diary/Dosing Compliance		X	X	X	
Adverse events/baseline symptoms assessment	X	X	X	X	
Baseline Alcohol and Tobacco Questionnaires	X				
Follow-Up Alcohol and Tobacco Questionnaires				X	
COVID-19 Assessment	X			X	
Telephone or Email Contact[8]		X			X

[1]Vital signs including: Performance status (ECOG), height, weight, blood pressure, respiration rate, heart rate, and temperature.
[2]Participants will self-collect blood pressure readings at home or at a nearby pharmacy once per week for the first 12 weeks of intervention. Participants will be given a blood pressure monitor, instructed on its use, and given instructions on recording and reporting their blood pressure within the participant diary. See section 7.6 on how to address hypotension.
[3]For women who are able to become pregnant. Women ≥ 50 years of age who have not had a menstrual period in the past year; and women who have had a hysterectomy, both ovaries removed, or a tubal ligation; will not be required to have a pregnancy test. Women who are pregnant will be excluded from the study
[4]Routine blood tests: Complete Metabolic Panel (AST, ALT, Alkaline Phosphatase, Total bilirubin, creatinine, potassium.) Complete blood counts (white blood count, hemoglobin, hematocrit and Platelets) and INR.
[5]Up to twelve mL of fasting blood collected for research: 5 mL for PRO-C3, PRO-C6, CTX-III, PLSec, 2mL for Genetic Sampling (DNA), and 5mL for Inflammatory Markers TNF-alpha, TGF-beta, NF-kB, IL-6, and IL-8. Genetic Sampling (DNA) only collected at baseline.
[6]Transient elastography may be performed within 4 weeks of screening visit.
[7]Baseline MRI-PDFF/MRE are optional and may be performed 0-12 weeks prior to enrollment if the participant has not experienced more than 10% weight loss.
[8]Telephone or email contact: The participant will be asked at registration if they would like to receive reminder emails or text messages regarding the study medication. According to subject preference, messages will be sent daily for the first week; and weekly thereafter. In addition, emails or telephone calls will be made to study participants at weeks 1, 2, 8, 16, and 20 after start of intervention to monitor compliance. A window of ±3 days is allowed. Participants will also be reminded to bring any leftover study drugs and the study diary with them to each visit. The final follow-up contact will be made at week 32 ± 7 days from start of intervention.

9.2 Baseline Testing/Pre-Study Evaluation

9.2.1 Pre-Screening Evaluation

Patients who have an upcoming care appointment with a study physician will be identified and pre-screened for eligibility based on the most updated information in their medical charts. The medical records will be reviewed by the clinical research staff to determine initial eligibility. Medical charts will be reviewed for 1) age, 2) transient elastography results, 3) ACEis and ARB use, 4) concomitant medications, 5) medical contraindications, and 6) laboratory results.

Patients deemed potentially eligible will be approached in clinic during their regularly scheduled appointment or contacted by phone to discuss study participation. Study staff will introduce the study and answer questions. A study information packet including an introductory letter and consent form may be mailed to potentially eligible participants when deemed appropriate by study staff. Patients will be encouraged to review the consent documents with family, friends, and/or other physicians. The patient will be told that the decision to join or not join the study will not affect the medical treatment that s/he receives, and that s/he can withdraw from the study at any time.

9.2.2 Study Visit 1: Screening (0-4 weeks prior to enrollment)

Pre-Study procedures include:

- Informed consent
- Medical History
- Physical Examination including Vital signs: ECOG performance status, height, weight, heart rate, respiratory rate, blood pressure, and temperature.
- Serum or urine pregnancy test for women of who are able to become pregnant. Women ≥ 50 years of age who have not had a menstrual period in the past year; and women who have had a hysterectomy, both ovaries removed, or a tubal ligation; will not be required to have a pregnancy test. Women who are pregnant will be excluded from the study.
- Review of baseline medications and symptoms assessment
- Routine Blood Tests: Complete Metabolic Panel (AST, ALT, Alkaline Phosphatase, Total bilirubin, creatinine, potassium.) Complete blood counts (white blood count, hemoglobin, hematocrit and Platelets) and INR to establish baseline levels prior to study intervention

- HgbA1C and lipid panel (total cholesterol, HDL, LDL, triglycerides) to establish baseline levels prior to study intervention
- EKG
- Transient Elastography may be completed 4 weeks prior to screening visit.
- Baseline Alcohol and Tobacco Questionnaires (See Appendix B)
- Baseline COVID-19 Assessment (See Appendix D)
- Adverse events and baseline symptoms assessment

9.2.3 Study Visit 2: Enrollment (0-4 weeks after Screening)

- Eligibility Verification: eligible participants who meet all inclusion/exclusion criteria will be enrolled during the visit. All enrolled participants will receive 10mg lisinopril daily for 24 weeks.
- Review of baseline medications and symptoms assessment
- NFS and FIB-4
- *Twelve mL of fasting blood collected for research: 5 mL for PRO-C3, PRO-C6, CTX-III, PLSec, 2mL for Genetic Sampling (DNA), and 5mL for Inflammatory Markers TNF-alpha, TGF-beta, NF-kB, IL-6, and IL-8
- Stool and urine sample collection for future research
- Optional baseline MRI-PDFF/MRE (results of previous MRI-PDFF/MRE can be used if done 0-12 weeks prior to enrollment if the patient has not experienced more than 10% weight loss)
- Dispensing of study agent and diary (See Appendix E).

*Participants will be asked to fast at least 8 hours prior to blood collection.

9.3 Evaluation During Study Intervention

9.3.1 Study Visit 3 (12 weeks ± 7 days after start of study intervention)

- Physical Examination including Vital signs: ECOG performance status, height, weight, heart rate, respiratory rate, blood pressure, and temperature.
- Review of medications and symptoms assessment
- Routine Blood Tests: Complete Metabolic Panel (AST, ALT, Alkaline Phosphatase, Total bilirubin, creatinine, potassium.) Complete blood counts (white blood count, hemoglobin, hematocrit and Platelets) and INR to monitor for potential adverse events and to assess potential effects of the study intervention
- Collection of study agent and diary and review of study diary and dosing compliance.
- Dispensing of study agent and diary.

9.3.2. Telephone or Email Contact

- Participants will receive daily reminder emails or text messages during the first week of intervention and weekly thereafter.
- Emails or telephone calls will be made to study participants at weeks 1, 2, 8, 16, and 20 after start of intervention to monitor compliance, review changes in concomitant medications, and adverse events. A window of ±3 days is allowed. Participants will also be reminded to bring any leftover study drugs and the study diary with them to each visit.
- Please refer to Appendix F for suggested language for participant communications.

9.3.3 Blood Pressure Self-Collection

Participants will self-collect blood pressure readings at home or at a nearby pharmacy once per week for the first 12 weeks of intervention. Participants will be given a blood pressure monitor, instructed on its

use, and given instructions on recording and reporting their blood pressure within the participant diary. See section 7.6 on how to address hypotension.

9.4 Evaluation at Completion of Study Intervention

9.4.1 Study Visit 4 (24 weeks ± 7 days after start of study intervention)

- Physical examination and vital signs: ECOG performance status, height, weight, blood pressure, and temperature.
- Review of medications
- Routine Blood Tests: Complete Metabolic Panel (AST, ALT, Alkaline Phosphatase, Total bilirubin, creatinine, potassium.) Complete blood counts (white blood count, hemoglobin, hematocrit and Platelets) and INR to monitor for potential adverse events and to assess potential effects of the study intervention
- HgbA1C and Lipid Panel (total cholesterol, HDL, LDL, triglycerides) to assess potential effects of the study intervention
- *Ten mL of fasting blood collected for research: 5 mL for PRO-C3, PRO-C6, CTX-III, PLSec, and 5mL for Inflammatory Markers TNF-alpha, TGF-beta, NF-kB, IL-6, and IL-8
- Stool and urine sample collection for future research
- NFS and FIB-4
- Optional MRI-PDFF/MRE
- Transient Elastography
- Follow-Up Alcohol and Tobacco Questionnaires (see Appendix B)
- Follow-Up Covid-19 Assessment (see Appendix D)
- Collection of study agent and diary and review of study diary and dosing compliance (see Appendix E).
- Adverse events and symptoms assessment.

*Participants will be asked to fast at least 8 hours prior to blood collection.

9.5 Post-intervention Follow-up Period

- Participants will be contacted at week 32 ± 7 days which will be 8 weeks from stopping the intervention to get update on their health and safety.

9.6 Methods for Clinical Procedures

9.6.1. Transient elastography Subjects will undergo transient elastography with Controlled Attenuated Parameter (CAP) or LiSA at screening Visit 1 to document steatosis and stiffness prior to enrollment and at Study Visits 4. In a standard transient elastography test, an elastic shear wave propagates through the liver, and its velocity, which is directly related to tissue stiffness, is measured on a scale from 1.5 to 75 kPa, with higher numbers indicating increased stiffness. A median of 10 measurements is used for the test result. As propagation of the wave is disrupted by fat, liver fat content can also be estimated using transient elastography on a scale from 100 to 400 dB/m (CAP or LiSA score), with higher numbers indicating increased steatosis. Subjects will be asked to fast for 3 hours prior to the procedure. The patient will be tested in a supine position and 10 measurements will be taken.

9.6.2 MRI-PDFF and MRE. At Baseline and Study Visit 4, subjects may choose to undergo optional MRI-PDFF and MRE. Both tests are performed on a standard MR platform, and use specialized software to measure total liver fat content and liver fibrosis, respectively. The MRI-PDFF software corrects for differences water and fat proton densities and then calculates the fat fraction. MRI-PDFF is now

considered a gold standard for primary outcome in phase 2 clinical trials of NAFLD. MRE uses shear waves from low vibrations propagated through the liver to measure tissue motion. Wave images are generated and automatically processed into cross-sectional, color-coded elastograms depicting liver stiffness on a scale measured in kPa. This technique proved highly accurate in discriminating between histologic grades of fibrosis, including in patients with advanced fibrosis and cirrhosis. Subjects will be asked to fast for 8 hours prior to the procedure.

10. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

PRO-C3, PRO-C6 and CTX-III: These biomarkers have been discussed above in Sections 2.3.3 and 2.3.4. PRO-C3 and PRO-C6 will be quantified in human serum with novel, fully automated electrochemiluminescence (Elecsys) immunoassays on the cobase 601 analyzer at Nordic Bioscience. Each assay uses monoclonal antibodies either in competitive format (PRO-C3) or in a sandwich format, and has a total run duration of 18 min or 27 min.

PDFFMRI-PDFF/MRE: MRI-determined proton density fat fraction (MRI-PDFF) has been widely used and useful in NAFLD/NASH clinical trials [45, 68, 69]. MR elastography (MRE) is an emerging technique that has accurately detected fibrosis in NAFLD [70]. NAFLD/NASH Phase IIA trials have adopted changes in non-invasive tests (particularly MRI-PDFF) as primary outcomes, while liver enzymes, inflammatory and fibrosis biomarkers, and MRE changes have been used as secondary outcomes [71]. MRI techniques have rapidly become the preferred method in NASH trials because they avert the invasive nature and poor patient acceptance of liver biopsy. MRI can accurately assess the degree of steatosis and fibrosis; show dynamic changes within a relatively short time; and potentially detect changes in steatosis, at a minimum, as well as steatohepatitis. MRI techniques have been found accurate in overtly obese patients [72]. A proof-of-concept, single-center study by Patel et. al. (5) found that a 29% reduction in liver fat, as measured with MRI-PDFF, was associated with a 2-point decrease in the histological NAFLD activity score, which is the gold standard histological assessment used in previous clinical trials. In a recent secondary analysis of 54 patients collected from multiple centers in which selonsertib was tested for 24 weeks, MRI-PDFF changes had an AUROC 0.70 of predicting histologic improvement [73]. A recent randomized controlled trial of MGL-3196 (a thyroid hormone receptor B) vs. placebo for 36 weeks found that, in patients who received MGL-3196, MRI-PDFF (≥ 30% fat reduction) at week 12 predicted resolution of NASH at week 36 (p=0.001). MGL-3196 PDFF responders were also more likely to have a reduction in other components of NASH (ballooning, inflammation) [44].

In this study, MRI-PDFF and MRE are optional and may be read locally at each site (Cedars-Sinai, Duke University, Mount Sinai, and Mayo Clinic). These centers have expert radiologists who have extensive experience in these techniques. Variability is not an issue, as the precision and reproducibility of these MRI techniques have been established. Given the extensive expertise in reading clinical MRIs at the Network institutions, central reading will not be needed.

Transient Elastography: Vibration-controlled transient elastography (VCTE), usually referred to as transient elastography or (Fibroscan®), measures the speed of a mechanically generated shear wave across the liver to derive a liver stiffness measurement, which generally correlates with hepatic fibrosis burden [74]. The measured attenuation of ultrasound signal through the liver is used to derive the controlled attenuation parameter (CAP) or LiSA, which can be assessed simultaneously with liver stiffness measurements as a marker of hepatic steatosis [75]. The noninvasive VCTE uses ultrasound technology to determine steatosis and fibrosis in a few minutes, with an immediate result. Multiple studies have shown that VCTE is accurately detects the degree of steatosis and fibrosis, and it has been used in clinical trials to monitor longitudinal changes of these characteristics [76]. In one study, baseline fibrosis measurements using VCTE in 258 cirrhotic patients predicted the risk of HCC [77]. Similarly, in a prospective study of

866 hepatitis C virus patients, increased stiffness on VCTE correlated with the incidence of HCC [58]. Our inclusion criteria include NASH patients with VCTE stiffness of ≥12 kPa, which correlates with F3 fibrosis and more [65]. While MRI-PDFF and MRE are more accurate and widely acceptable outcomes for Phase III NAFLD/NASH trials, using VCTE (transient elastography) will help to assess the presence of advanced fibrosis. In addition, as it is a point of care test and an evolving secondary outcome, having MRI and transient elastography in this proof of concept study will facilitate the biomarker plan for future studies.
Transient elastography examinations will be performed at the screening visit and at the end of treatment. Median liver stiffness in kilopascals (kPa), interquartile range/median value, and success rate (number of valid shots/total number of shots) will be recorded. The median CAP or LiSA and the interquartile range of CAP or LiSA values will be recorded from transient elastography examinations.

Inflammatory markers: We will measure NASH- and HCC-related inflammatory markers and cytokines (TNF-alpha, TGF-beta, NF-kB, IL-6, and IL-8) with enzyme-linked immunosorbent assays (ELISA) [78-80]. Other common markers of liver cell injury that have been used in NASH patients will also be measured: caspase-cleaved and total K18 will be determined with the M65 and M30 (apoptosense) ELISA [81]. These inflammatory markers and cytokines will be measured at baseline and at end of treatment. To assess the effect of lisinopril treatment, we will compare these inflammatory markers and cytokines in pre- and post-lisinopril-treatment samples.

Liver enzymes measurements*:* Liver enzymes (ALT, AST, and alkaline phosphate) and total bilirubin will be monitored. Improvement in ALT correlates with histological improvement in NAFLD [82].

NFS and FIB-4: The FIB4 index was developed as a noninvasive panel to stage liver disease in subjects with HIV-hepatitis C virus co-infection [83]. The index has been validated as an accurate biomarker in NAFLD [84]. It relies on the patient's age, AST, ALT levels and the platelet count, which are routinely measured and available for virtually all subjects with liver disease. FIB-4 correlates with histologic progression and regression in NAFLD/NASH patients [85]. Similarly, the NAFLD fibrosis score (NFS) is a clinically applicable method to differentiate patients with and without prognostically significant NAFLD [86]. The fibrosis score is a formula that contains age, BMI, diabetes, AST/ALT ratio, albumin and platelet count. The higher the score the more advanced the fibrosis; improvement in the score is expected to correlate with regression of fibrosis.

Prognostic Liver Secretome signature (PLSec): A serum protein-based biomarker, was developed as a surrogate biomarker for HCC development by utilizing integrative database/pipeline of protein subcellular localization, extracellular secretion to circulation, and tissue type specificity for HCC

10.1 Primary Endpoint

Measure the change in PRO-C3 values over 24 weeks in NAFLD patients with advanced fibrosis treated with lisinopril.

10.2 Secondary and Exploratory Endpoints

Measure noninvasive measures of fibrosis and steatosis:

- Change from baseline in PRO-C6
- Change from baseline in steatosis, as measured by controlled attenuation parameter (CAP) or LiSA, determined with transient elastography
- Change from baseline in liver stiffness as measured with magnetic resonance elastography (MRE)
- Change from baseline in liver stiffness as measured with transient elastography

- Changes from baseline in NFS and Fib-4
- Change in inflammatory markers (caspase cleaved cytokeratin 18 (CK-18), NF-kB, TGF-β, TNF-alpha, IL6 and IL8)

Exploratory Endpoints

- Change from baseline in CTX-III.
- Change from baseline in PLSec
- Change from baseline in steatosis, as measured by magnetic resonance imaging-proton density fat fraction (MRI-PDFF) in participants who undergo optional MRI-PDFF/MRE
- Change from baseline in markers of liver injury and function: ALT, AST, bilirubin, and alkaline phosphatase
- Change from baseline in homeostatic assessment of hemoglobin A1C and serum lipid profiles. and in systolic and diastolic blood pressure
- Collect urine, stool, serum/plasma, and genomic DNA for future measurements of changes in microbiome and metabolomics and genomics.

10.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

10.4 Off-Study Criteria

Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure) or pregnancy.

10.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

11. CORRELATIVE/SPECIAL STUDIES

11.1 Rationale for Methodology Selection

PRO-C3 and PRO-C6 are novel biomarkers discovered by Nordic bioscience and will be performed based on their methodology used to developed these tests

We follow the state of art methods in assessing inflammatory biomarkers or in performed MRI-PDFF, MRE and transient elastography, as detailed in Section 10.

11.2 Comparable Methods

Not applicable.

12. SPECIMEN MANAGEMENT

12.1 Laboratories

1) Clinical study blood tests will be done for a Comprehensive Metabolic Panel (CMP), Complete blood count (CBC), INR, Lipid Panel, Hemoglobin A1C at the CSMC, Duke University, Mount Sinai and Mayo Clinic clinical laboratories.

2) The Fatty Liver Program at Cedars-Sinai Medical Center located in the Davis Building will receive all samples collected for research from Duke, Mount Sinai, and Mayo Clinic. The Cedars-Sinai Medical Center Biobank will store and distribute samples to central labs (Dr. Seki Laboratory and Nordic Bioscence) for assays listed below. Details of shipping are provided in Section 12.3.

3) Measurement of inflammatory biomarkers will be conducted in the laboratory of Dr. Ekihiro Seki, MD, PhD. Dr. Seki's laboratory occupies 1300 square feet of space (Room 2099, 2099A, 2029). Dr. Seki also has access to the laboratory space in the Division of Digestive and Liver Diseases and Department of Medicine, which is located on the second floor of the Davis Research Building at the Cedars-Sinai Medical Center.

4) Measurements of PRO-C3, PRO-C6, and CTX-III: Will be quantified in human serum with novel, fully automated electrochemiluminescence (Elecsys) immunoassays on the cobase 601 analyzer at Nordic Bioscience. Each assay uses monoclonal antibodies either in competitive format (PRO-C3) or in a sandwich format, and has a total run duration of 18 min or 27 min.

5) Measurements of PLSec will be performed using 35 uL of serum by the xMAP/MAGPIX system (Luminex) at the UTSW BioCenter CLIA lab.

12.2 Collection and Handling Procedures

12.2.1 **Blood samples for clinical blood tests** will be obtained for a Comprehensive Metabolic Panel (CMP), Complete blood count (CBC), and INR, at Study Visits 1, 3 and 4. Lipid Panel and hemoglobin A1C will be obtained at Study Visits 1 and 4.

12.2.2 **Blood samples for research** including inflammatory markers, and indicators of fibrosis markers (PRO-C3, PRO-C6, CTX-III, and PLSec)will be collected at Study Visits 2 and 4. Blood samples for research for genetic sampling will be collected at Study Visit 2. Participants will be asked to fast at least 8 hours prior to sample collection.

12.2.3 **Fib-4 calculation** will be performed at Study Visits 2 and 4. https://www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis

12.2.4 **NFS calculation** will be performed at Study Visits 2 and 4. https://www.mdcalc.com/nafld-non-alcoholic-fatty-liver-disease-fibrosis-score

12.2.5 **Urine samples** will be self-collected at Visits 2 and 4. Approximately 20-30mL of urine (first void catch) will be self-collected at each visit in a sterile urine specimen container. All specimens will be shipped to CSMC as specified in section 12.3 and will be stored in -80°C freezer for future studies measuring changes in microbiome and metabolomics.

12.2.6 **Stool samples** will be self-collected at Visits 2 and 4. All specimens will be shipped to CSMC as

specified in section 12.3 and will be stored in -80°C freezer for future studies measuring changes in microbiome and metabolomics. Please see Appendix G for instructions for at-home stool sample collection.

12.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

Manaf Alsudaney
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Manaf.Alsudaney@cshs.org
8900 Beverly Blvd., Suite 250.251I
Los Angeles CA 90048
Office: 310-967-7454
Fax: 310-423-0766

All samples and specimens collected at accrual sites will be shipped quarterly to Cedars-Sinai by FedEx Priority Overnight® service. Samples will be stored in the Fatty Liver Program -80 freezer in the Davis Building until the time of analysis. The PRO-C3, PRO-C6, and CTX-III samples will be shipped quarterly by FedEx Priority Overnight® service (along with others) to Cedars-Sinai. Cedars-Sinai will ship those samples to Nordic Bioscience as one batch at the end of study. Samples for measurement of PLSec will be shipped to the UT Southwestern BioCenter CLIA lab.

12.4 Tissue Banking

The NCI reserves the right to require the transfer of biologic specimens and data, or true copies of such data, acquired from research supported under this award to an eligible third party. This transfer can occur in order to preserve the specimens and data and/or to continue the research. Third parties supported under this award must be informed of this right.

13. REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not the untoward occurrence is considered drug related. Thus, an AE can include any unfavorable sign (e.g., an abnormal laboratory finding), symptom, or clinical outcome temporally associated with the use of a test drug, active control, or placebo, regardless of whether the event is thought to be related to the drug. An AE can arise with the use of a drug or biologic (e.g., use for a purpose other than FDA-approved indication or in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) will not be reported as AEs. Any lab value of unknown clinical significance will continue to be investigated/followed-up further for a final determination, if possible. (See the DCP Baseline and Adverse Event Reporting Guidelines [https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms] for more detail on reporting abnormal clinical laboratory values.)

A list of AEs that have occurred or might occur can be found in Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

13.1 Adverse Events

13.1.1 Reportable AEs

All AEs that occur after the informed consent is signed will be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

13.1.2 AE Data Elements:

The following data elements will be required for AE reporting.

1. AE verbatim term
2. NCI Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) AE term (MedDRA lowest level term)
3. CTCAE (MedDRA) System Organ Class (SOC)
4. Event onset date and event ended date
5. Treatment assignment code (TAC) at time of AE onset
6. Severity grade
7. Attribution to study agent (relatedness)
8. Whether or not the event was reported as a SAE
9. Whether or not the subject dropped due to the event
10. Outcome of the event

13.1.3 Severity of AEs

13.1.3.1 Identify the AE using the CTCAE version 5.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v5.0. as stated below.

CTCAE v5.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

13.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: unrelated, unlikely, possible, probable, definite. Criteria for these classifications are provided in DCP's *Serious Adverse Event Report Form: Instructions for Completion* **(https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms).**

13.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

13.1.6 Collection of AEs

Adverse events will be collected for a period of 8 weeks after stopping study agent.

13.2 Serious Adverse Events

13.2.1 DEFINITION: Regulations at 21 CFR §312.32 defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
  (According to FDA safety guidance, an AE is considered life-threatening if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death. Example: An allergic reaction resulting in angioedema of the larynx, allergic bronchospasm or anaphylaxis is considered life-threatening; however, an allergic reaction resulting only in a localized rash is not life-threatening.)
- In patient hospitalization or prolongation of existing hospitalization
  (NCI, DCP uses admission or stay (including emergency room) equal to or greater than 24 hours as the definition of hospitalization. Exceptions are hospitalization for treatment of a pre-existing condition [unless the condition increased in severity on study], outpatient surgery, planned/elective procedures, and procedures described in the protocol [e.g., pharmacokinetic sampling, surgery] even if the hospital stay is of the described length; however, it does include events resulting from any of these that fulfill other serious outcome criteria, e.g., prolongation of hospitalization or life-threatening.)
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the Participant and may require intervention to prevent one of the other outcomes listed above.

13.2.2 Reporting SAEs to DCP

13.2.2.1 The accruing LAO and all Affiliated Organizations (AOs) will report SAEs on the DCP SAE Report Form as described in DMACC's CP-CTNet SOP 02-01, found at CP-CTNet SOP 02-01

Reporting Serious Adverse Events (cp-ctnet-dmacc.org) and DCP's *Serious Adverse Event Report Form: Instructions for Completion and Submission* found at https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms.

13.2.2.2 Contact the DCP Medical Monitor, Protocol PI, and DCP Regulatory Contractor's Safety Department within 24 hours of knowledge of the event. Contact via email is preferred, but phone contact is acceptable.

Luz Maria Rodriguez, MD, FACS
Scientific Lead and Medical Monitor
NCI/Division of Cancer Prevention
9609 Medical Center Dr.
Rm 5E-228
Bethesda, MD 20892
Telephone: (240) 276-7039
Fax: (240) 276-7848
E-mail: rodrigul@mail.nih.gov

The contact information for the DCP Regulatory Contractor's Safety Department is: phone: 650-691-4400 x133; email: safety@ccsainc.com).

Include the following information when contacting the DCP Medical Monitor, Protocol PI, and DCP Regulatory Contractor's Safety Department:

- Participant ID
- Date and time of SAE onset
- Date and time the accruing LAO or AO was notified about the SAE by the study participant or other person(s)
- Name of person reporting the SAE
- Call back phone number and email address
- Accruing LAO or AO at which the subject is enrolled
- DCP protocol number
- Title of protocol
- Suspected drugs (if any)
- Description of the SAE, including attribution to the Investigational Agent

13.2.2.3The accruing LAO and AOs will email written SAE reports to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and DCP Regulatory Contractor's Safety Department within 48 hours of learning of the event using the Word SAE Report Form.

13.2.2.4 The DCP Medical Monitor and the DCP Regulatory Contractor will determine which SAEs require submission to FDA or the manufacturer as expedited safety reports.

13.2.2.5 The accruing LAO and AOs will comply with applicable regulatory requirements related to reporting SAEs to the CIRB and local IRB/IEC as applicable. Specifically, if an SAE meets the definition of an unanticipated problem (UP; i.e., requires expedited reporting to FDA or the manufacturer as a safety report [serious, unexpected, and related to a study agent]), then it needs to be reported to the CIRB by the Signatory Institution PI at the accruing LAO or AO where the SAE occurred (see SOP 02-02 *Reporting*

Protocol Deviations for more information). In addition to CIRB requirements, UPs must be reported to the accruing LAO's or AO's local IRB per local requirements.

13.2.3 Follow-up of SAE

Accruing LAO or AO staff should send follow-up reports as requested when additional information is available. Additional information will be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and DCP Regulatory Contractor's Safety Department as soon as available.

14. STUDY MONITORING

14.1 Data Management

This study will report clinical data using Medidata RAVE, a cloud–based clinical trials data management system managed by the DMACC. RAVE will be the database of record for the protocol and subject to NCI and FDA audit. All RAVE users will be trained to use the system and will comply with the instructions in the guidelines provided to the LAO by the DMACC as well as applicable regulatory requirements such as 21 CFR; Part 11.

14.2 Case Report Forms

The System Variable and Attribute Report (SVAR) template will be used to create the study-specific eCRFs or SVAR workbook. DMACC will contact the LAO to determine if a meeting is needed to discuss the trial and eCRFs before the DMACC creates the draft SVAR.

More detailed information about the SVAR development process is available at: Program Resources | CP-CTNet DMACC Website (cp-ctnet-dmacc.org)

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14.3 Source Documents

All source documents will be collected and stored in the Clinical Research Office of the site where the participant was accrued. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data. Questionnaires completed in person may be completed directly on the paper CRF and need not be transcribed from separate source documentation.

14.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, approved by the DCP, and is on file there. Any future changes will be forwarded for review.

14.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

14.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence of study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as CIRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

14.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

Not applicable.

14.8 Genomic Data Sharing Plan

Not applicable. No specific genomic studies are planned at this time.

15. STATISTICAL CONSIDERATIONS

15.1 Study Design/Description

This is a single arm trial with the primary objective to determine the change in PRO-C3 values over 24 weeks in NAFLD patients with advanced fibrosis treated with lisinopril. Eligible patients who consent to study participation will be treated with 10 mg daily lisinopril for 24 weeks. Levels of PRO-C3 will be evaluated at baseline (pre-treatment) and following completion of treatment (post-treatment). The primary endpoint is the change in PRO-C3 biomarker, which is expected to decrease with lisinopril treatment. A single arm design was chosen because PRO-C3 is expected to remain constant or increase over 24 weeks without treatment, and a reduction in PRO-C3 will be clearly attributed to treatment.[48, 52]

15.2 Randomization/Stratification

Not applicable.

15.3 Sample Size

Power calculations are based on a paired *t*-test comparing pre- to post-treatment PRO-C3 levels. The variability at both time points is assumed to be similar to Luo et al [48], who reported mean $\pm$ SEM PRO-C3 levels for F3 and F4 groups of approximately 32 ± 7.5 (n=31) and 32± 8 (n=29), respectively, which correspond to SD of 41.8 and 43.1 ng/ml. The following assumptions are made: 1) SD=42.5 in this study population of F3 and F4 patients; 2) PRO-C3 is log-normally distributed because its distribution is skewed (see Fig 1a; Luo et al;[48] and 3) within-subject correlation between baseline and 24 weeks is moderate ($\rho = 0.5$). Power calculations were performed on the log scale, and then back-transformed to the original scale as described in [87] . Under these assumptions, the baseline mean of 32ng/ml corresponds to μ_{log} = 2.96 on the log scale, and the pooled SD of the changes (i.e. of the paired differences) is SD_p=42.5 on the raw scale, and $SD_{p,log}$=1.01 on the log scale. Sample size of n=40 evaluable subjects

would provide 90% power with two-sided α=0.05 to detect a standardized effect size of Δ= 0.53, which corresponds to a 13.2 ng/ml change in PRO-C3. For example, if the baseline value of PRO-C3 is 32ng/ml, a reduction from 32 to 18.8 ng/ml can be detected. The 18.8 ng/ml value is just above the values that Luo et al;[48] have reported for F0-F2 patients, which ranged from 13.5 to 14.8ng/ml, respectively. Assuming ~10% attrition based on similar studies, we plan to enroll 45 subjects.

15.4 Primary Objective, Endpoint(s), Analysis Plan

PRO-C3 levels will be assessed at baseline and following 24 weeks of treatment. Descriptive statistics will be used to summarize changes and values at each time point. The primary analysis will be performed using a paired *t*-test to compare the pre- to post-treatment changes in PRO-C3 levels. PRO-C3 levels will be log-transformed to satisfy the normality assumption. If log-transformation does not satisfy the normality assumption, a signed rank test will be used.

15.5 Secondary and Exploratory Objectives, Endpoints, Analysis Plans

Secondary and exploratory endpoints include inflammatory biomarkers and measures of liver stiffness and steatosis, which are continuous measures and will be obtained at baseline and after treatment. Descriptive statistics, including means, 95% confidence intervals, or median and inter-quartile range (IQR), will be used to summarize changes as well as biomarker values at each time point. To determine whether changes in these biomarkers occurred following treatment, paired t-test will be used as described for the analysis of the primary endpoint. Biomarker values may be transformed to satisfy the normality assumption. If no suitable transformation can be found, a signed rank test will be used. Changes in categorical variables, e.g. steatosis grade, will be analyzed using the Stuart-Maxwell test of marginal homogeneity. For the analysis of inflammatory biomarkers, tests will be adjusted for multiple comparisons to control the false discovery rate (FDR), e.g. using the method of Romano (85) or a similar method.

Linear regression models will be used to identify baseline characteristics (e.g. steatosis degrees on CAP or LiSA and MRI-PDFF, stiffness assessed via transient elastography and MRE, NFS and Fib-4) that are associated with biomarker changes. Model selection will be conducted according to the purposeful model selection approach to a parsimonious set of predictors[88]. Model fit will be assessed using standard regression diagnostics. Changes in categorical outcomes from pre- to post-treatment will also be categorized as improvement vs. no improvement (i.e. no change or worsening in steatosis grade), and logistic regression models will be used to examine association with baseline characteristics.

With a sample size of n=40, there will be 90% power with two-sided α =0.05 to detect a standardized effect size of Δ =0.53, which is considered a medium effect size. For inflammatory biomarkers, testing with a Bonferroni-corrected α=0.05/6=0.008 level would allow 90% power to detect a standardized effect size of Δ=0.65, controlling the probability of falsely rejecting at least one true null hypothesis (i.e. Type I error). Using the method of Romano to adjust for multiple comparisons (86, 87), there will be 83% power with a two-sided α=0.05 to detect ≥2 markers under the assumption that 3 of the 6 markers are truly changed from pre- to post-treatment and their common standardized effect size is 0.72.

15.6 Reporting and Exclusions

Definition of compliance is clearly stated above. Non-compliance is sufficiently addressed. Particular consideration has been also given above to dropouts, drop-ins, and lost-to-follow up. Handling of missing data or data from non-compliers is described.

15.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of lisinopril.

15.8 Evaluation of Response

All participants included in the study must be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible.

All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

Subanalyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, subanalyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported. For all measurements of response, the 95% confidence intervals should also be provided.

15.9 Interim Analysis

No interim analyses are planned.

15.10 Ancillary Studies

No ancillary studies are planned.

16. REGULATORY AND ETHICAL CONSIDERATIONS

16.1 Required Documents

Besides the regulatory information that will be entered into the Registration and Credential Repository (see Section 5.1), the following documents are also required:

16.1.1 Documentation of Federal Wide Assurance (FWA) number for the LAO andall AOs.

16.1.2 Signed Investigator's Brochure/Package Insert acknowledgement form.

16.1.3 Delegation of Tasks Log form for the Lead Accruing Organization and all Accruing Sites signed by the PI for each site and initialed by all study personnel listed on the form.

16.2 Informed Consent

All potential study participants will be given a copy of the CIRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Individuals who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option should be included within the informed consent document.

Prior to study initiation, the informed consent document will be reviewed and approved by NCI, DCP, and the NCI CIRB. The NCI CIRB approves a model consent for each protocol. Each Signatory Institution inserts their CIRB-approved institutional boilerplate language into the model consent to create the CIRB-approved consent. If the model informed consent document is amended, Signatory Institutions will use the revised model informed consent document and insert their CIRB-approved institutional boilerplate language at the time the change becomes active.

The NCI CIRB is the IRB of record and is the only IRB authorized to approve changes the protocol or informed consent document. Institutions may require additional oversight that involves the local IRB, but the local IRB is not responsible for any regulatory-required IRB actions.

16.3 Collection of Regulatory Documents

Regulatory documents will be collected by the DCP regulatory contractor and reviewed for completeness and accuracy.

16.4 Other

This trial will be conducted in compliance with the protocol, the International Conference on Harmonisation's (ICH) Good Clinical Practice (GCP) guidelines, and the applicable regulatory requirements.

17. ROSTER MANAGEMENT

The LAO is responsible for establishing, maintaining, and monitoring all its members that participate in CP-CTNet studies. The LAO will have a "real-time," comprehensive, consolidated roster of all its members with their relevant Cancer Therapy Evaluation Program (CTEP) institution codes, associated investigators, and research staff. This roster information is used for determining compliance with monitoring requirements

The LAO's organizational rosters will be managed by the CP-CTNet Roster Management System website (https://applications.prevention.cancer.gov/cp-ctnet) Requests to add memberships to a roster will be done via this website. All requests require that the following documents be uploaded:

- Consortium Letter of Commitment
- Site Letter of Commitment
- CV/NIH Biosketch

18. FINANCING, EXPENSES, AND/OR INSURANCE

All research related costs associated with participating in this study will be paid for, and will not be the responsibility of the participant (except standard of care visits). However, it is possible that injury may

result from participating in this study. Any expenses incurred as a result of related injury will be the responsibility of the study participant and/or their insurance carrier.

REFERENCES

1. Ma J, Siegel RL, Islami F, Jemal A. Temporal trends in liver cancer mortality by educational attainment in the United States, 2000-2015. Cancer. 2019;125(12):2089-98. doi: 10.1002/cncr.32023. PubMed PMID: 30957228.
2. Yang JD, Larson JJ, Watt KD, Allen AM, Wiesner RH, Gores GJ, Roberts LR, Heimbach JA, Leise MD. Hepatocellular Carcinoma Is the Most Common Indication for Liver Transplantation and Placement on the Waitlist in the United States. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(5):767-75 e3. doi: 10.1016/j.cgh.2016.11.034. PubMed PMID: 28013117; PMCID: PMC5401787.
3. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. Epub 20160222. doi: 10.1002/hep.28431. PubMed PMID: 26707365.
4. Setiawan VW, Stram DO, Porcel J, Lu SC, Le Marchand L, Noureddin M. Prevalence of chronic liver disease and cirrhosis by underlying cause in understudied ethnic groups: The multiethnic cohort. Hepatology. 2016;64(6):1969-77. doi: 10.1002/hep.28677. PubMed PMID: 27301913; PMCID: PMC5115980.
5. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A, Nader F. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019. doi: 10.1016/j.jhep.2019.06.021. PubMed PMID: 31279902.
6. Setiawan VW, Stram DO, Porcel J, Lu SC, Le Marchand L, Noureddin M. Prevalence of chronic liver disease and cirrhosis by underlying cause in understudied ethnic groups: The Multiethnic Cohort. Hepatology. 2016. doi: 10.1002/hep.28677. PubMed PMID: 27301913.
7. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005-23. doi: http://dx.doi.org/10.1002/hep.25762. PubMed PMID: 364901700.
8. Noureddin M, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, Setiawan VW, Tran T, Ayoub WS, Lu SC, Klein AS, Sundaram V, Nissen NN. NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances. The American journal of gastroenterology. 2018;113(11):1649-59. doi: 10.1038/s41395-018-0088-6. PubMed PMID: 29880964.
9. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. Gastroenterology. 2018;155(6):1828-37 e2. doi: 10.1053/j.gastro.2018.08.024. PubMed PMID: 30144434; PMCID: PMC6279617.
10. Allen AM, Hicks SB, Mara KC, Larson JJ, Therneau TM. The risk of incident extrahepatic cancers is higher in non-alcoholic fatty liver disease than obesity - A longitudinal cohort study. Journal of hepatology. 2019. doi: 10.1016/j.jhep.2019.08.018. PubMed PMID: 31470068.
11. Chiang YY, Chen KB, Tsai TH, Tsai WC. Lowered cancer risk with ACE inhibitors/ARBs: a population-based cohort study. J Clin Hypertens (Greenwich). 2014;16(1):27-33. doi: 10.1111/jch.12228. PubMed PMID: 24304931.
12. Bataller R, Sancho-Bru P, Gines P, Brenner DA. Liver fibrogenesis: a new role for the renin-angiotensin system. Antioxid Redox Signal. 2005;7(9-10):1346-55. doi: 10.1089/ars.2005.7.1346. PubMed PMID: 16115040.
13. Ahmadian E, Khosroushahi AY, Eftekhari A, Farajnia S, Babaei H, Eghbal MA. Novel angiotensin receptor blocker, azilsartan induces oxidative stress and NFkB-mediated apoptosis in hepatocellular carcinoma cell line HepG2. Biomed Pharmacother. 2018;99:939-46. doi: 10.1016/j.biopha.2018.01.117. PubMed PMID: 29710494.

14. Saber S, Mahmoud A, Helal N, El-Ahwany E, Abdelghany R. Liver Protective Effects of Renin-Angiotensin System Inhibition Have No Survival Benefits in Hepatocellular Carcinoma Induced By Repetitive Administration of Diethylnitrosamine in Mice. Open Access Maced J Med Sci. 2018;6(6):955-60. doi: 10.3889/oamjms.2018.167. PubMed PMID: 29983784; PMCID: PMC6026411.
15. Saber S, Mahmoud AAA, Goda R, Helal NS, El-Ahwany E, Abdelghany RH. Perindopril, fosinopril and losartan inhibited the progression of diethylnitrosamine-induced hepatocellular carcinoma in mice via the inactivation of nuclear transcription factor kappa-B. Toxicol Lett. 2018;295:32-40. doi: 10.1016/j.toxlet.2018.05.036. PubMed PMID: 29859236.
16. Facciorusso A, Del Prete V, Crucinio N, Muscatiello N, Carr BI, Di Leo A, Barone M. Angiotensin receptor blockers improve survival outcomes after radiofrequency ablation in hepatocarcinoma patients. Journal of gastroenterology and hepatology. 2015;30(11):1643-50. doi: 10.1111/jgh.12988. PubMed PMID: 25974743.
17. Yoshiji H, Noguchi R, Ikenaka Y, Kaji K, Shirai Y, Aihara Y, Yamao J, Toyohara M, Mitoro A, Sawai M, Yoshida M, Morioka C, Fujimoto M, Uemura M, Kawaratani H, Tsujimoto T, Fukui H. Soluble VEGF receptor-2 may be a predictive marker of anti-angiogenic therapy with clinically available safe agents. Oncol Lett. 2011;2(1):69-73. doi: 10.3892/ol.2010.196. PubMed PMID: 22870131; PMCID: PMC3412527.
18. Moreno M, Gonzalo T, Kok RJ, Sancho-Bru P, van Beuge M, Swart J, Prakash J, Temming K, Fondevila C, Beljaars L, Lacombe M, van der Hoeven P, Arroyo V, Poelstra K, Brenner DA, Gines P, Bataller R. Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats. Hepatology. 2010;51(3):942-52. doi: 10.1002/hep.23419. PubMed PMID: 20044807.
19. Oakley F, Teoh V, Ching ASG, Bataller R, Colmenero J, Jonsson JR, Eliopoulos AG, Watson MR, Manas D, Mann DA. Angiotensin II activates I kappaB kinase phosphorylation of RelA at Ser 536 to promote myofibroblast survival and liver fibrosis. Gastroenterology. 2009;136(7):2334-44 e1. doi: 10.1053/j.gastro.2009.02.081. PubMed PMID: 19303015.
20. Goh GB, Pagadala MR, Dasarathy J, Unalp-Arida A, Sargent R, Hawkins C, Sourianarayanane A, Khiyami A, Yerian L, Pai R, McCullough AJ, Dasarathy S. Renin-angiotensin system and fibrosis in non-alcoholic fatty liver disease. Liver international : official journal of the International Association for the Study of the Liver. 2015;35(3):979-85. doi: 10.1111/liv.12611. PubMed PMID: 24905085.
21. Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, Hasegawa T, Tokusashi Y, Miyokawa N, Nakamura K. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. Hepatology. 2004;40(5):1222-5. doi: 10.1002/hep.20420. PubMed PMID: 15382153.
22. Alam S, Kabir J, Mustafa G, Gupta U, Hasan SK, Alam AK. Effect of telmisartan on histological activity and fibrosis of non-alcoholic steatohepatitis: A 1-year randomized control trial. Saudi J Gastroenterol. 2016;22(1):69-76. doi: 10.4103/1319-3767.173762. PubMed PMID: 26831610; PMCID: PMC4763532.
23. Georgescu EF, Ionescu R, Niculescu M, Mogoanta L, Vancica L. Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. World journal of gastroenterology. 2009;15(8):942-54. doi: 10.3748/wjg.15.942. PubMed PMID: 19248193; PMCID: PMC2653406.
24. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. Science. 2011;332(6037):1519-23. doi: 10.1126/science.1204265. PubMed PMID: 21700865; PMCID: 3229276.
25. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology. 2011;141(4):1249-53. doi: 10.1053/j.gastro.2011.06.061. PubMed PMID: 21726509.
26. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. The Journal of clinical investigation. 2004;114(2):147-52. doi: 10.1172/JCI22422. PubMed PMID: 15254578;

PMCID: 449757.
27. Sipahi I, Debanne SM, Rowland DY, Simon DI, Fang JC. Angiotensin-receptor blockade and risk of cancer: Meta-analysis of randomised controlled trials. The Lancet Oncology. 2010;11(7):627–36. Epub 2010/06/15. doi: 10.1016/s1470-2045(10)70106-6. PubMed PMID: 20542468; PMCID: PMC4070221.
28. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, Bass NM, Nonalcoholic Steatohepatitis Clinical Research N. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943-51. doi: 10.1002/hep.25772. PubMed PMID: 22505194; PMCID: 3407289.
29. Bangalore S, Kumar S, Kjeldsen SE, Makani H, Grossman E, Wetterslev J, Gupta AK, Sever PS, Gluud C, Messerli FH. Antihypertensive drugs and risk of cancer: Network meta-analyses and trial sequential analyses of 324,168 participants from randomised trials. The Lancet Oncology. 2011;12(1):65–82. Epub 2010/12/03. doi: 10.1016/s1470-2045(10)70260-6. PubMed PMID: 21123111.
30. Pasternak B, Svanstrom H, Callreus T, Melbye M, Hviid A. Use of angiotensin receptor blockers and the risk of cancer. Circulation. 2011;123(16):1729–36. Epub 2011/04/13. doi: 10.1161/CIRCULATIONAHA.110.007336. PubMed PMID: 21482967.
31. El-Demerdash E, Salam OM, El-Batran SA, Abdallah HM, Shaffie NM. Inhibition of the renin-angiotensin system attenuates the development of liver fibrosis and oxidative stress in rats. Clin Exp Pharmacol Physiol. 2008;35(2):159-67. Epub 2007/09/29. doi: 10.1111/j.1440-1681.2007.04797.x. PubMed PMID: 17900296.
32. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2015;149(2):389-97 e10. doi: 10.1053/j.gastro.2015.04.043. PubMed PMID: 25935633; PMCID: PMC4516664.
33. Hagstrom H, Nasr P, Ekstedt M, Hammar U, Stal P, Hultcrantz R, Kechagias S. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. Journal of hepatology. 2017;67(6):1265-73. doi: 10.1016/j.jhep.2017.07.027. PubMed PMID: 28803953.
34. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology. 2017;65(5):1557-65. doi: 10.1002/hep.29085. PubMed PMID: 28130788; PMCID: PMC5397356.
35. Noureddin M, Rinella ME. Nonalcoholic Fatty liver disease, diabetes, obesity, and hepatocellular carcinoma. Clinics in liver disease. 2015;19(2):361-79. doi: 10.1016/j.cld.2015.01.012. PubMed PMID: 25921668; PMCID: PMC6658171.
36. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology. 2017. doi: 10.1002/hep.29367. PubMed PMID: 28714183.
37. Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology. 2008;47(2):455-60. doi: 10.1002/hep.21984. PubMed PMID: 18038452.
38. Sanyal A, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, Lawitz EJ, Halegoua-DeMarzio D, Kundu S, Noviello S, Luo Y, Christian R. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. Lancet. 2019;392(10165):2705-17. doi: 10.1016/S0140-6736(18)31785-9. PubMed PMID: 30554783.
39. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR, Nash CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. The New

England journal of medicine. 2010;362(18):1675-85. doi: 10.1056/NEJMoa0907929. PubMed PMID: 20427778; PMCID: 2928471.
40. Review Team, LaBrecque DR, Abbas Z, Anania F, Ferenci P, Khan AG, Goh KL, Hamid SS, Isakov V, Lizarzabal M, Penaranda MM, Ramos JF, Sarin S, Stimac D, Thomson AB, Umar M, Krabshuis J, LeMair A. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Journal of clinical gastroenterology. 2014;48(6):467-73. doi: 10.1097/MCG.0000000000000116. PubMed PMID: 24921212.
41. Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, Francque S, Farrell G, Kowdley KV, Craxi A, Simon K, Fischer L, Melchor-Khan L, Vest J, Wiens BL, Vig P, Seyedkazemi S, Goodman Z, Wong VW, Loomba R, Tacke F, Sanyal A, Lefebvre E. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology. 2017. doi: 10.1002/hep.29477. PubMed PMID: 28833331.
42. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E, Network NCR. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2015;385(9972):956-65. doi: 10.1016/S0140-6736(14)61933-4. PubMed PMID: 25468160; PMCID: PMC4447192.
43. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, Romero-Gomez M, Boursier J, Abdelmalek M, Caldwell S, Drenth J, Anstee QM, Hum D, Hanf R, Roudot A, Megnien S, Staels B, Sanyal A, Group G-IS. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor-alpha and -delta, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. Gastroenterology. 2016;150(5):1147-59 e5. doi: 10.1053/j.gastro.2016.01.038. PubMed PMID: 26874076.
44. Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, Alkhouri N, Bansal MB, Baum S, Neuschwander-Tetri BA, Taub R, Moussa SE. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2019. doi: 10.1016/S0140-6736(19)32517-6. PubMed PMID: 31727409.
45. Rinella ME, Loomba R, Caldwell SH, Kowdley K, Charlton M, Tetri B, Harrison SA. Controversies in the Diagnosis and Management of NAFLD and NASH. Gastroenterology & hepatology. 2014;10(4):219-27. PubMed PMID: 24976805; PMCID: 4073533.
46. Friedman SL. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. Nat Clin Pract Gastroenterol Hepatol. 2004;1(2):98-105. doi: 10.1038/ncpgasthep0055. PubMed PMID: 16265071.
47. Karsdal MA, Hjuler ST, Luo Y, Rasmussen DGK, Nielsen MJ, Holm Nielsen S, Leeming DJ, Goodman Z, Arch RH, Patel K, Schuppan D. Assessment of liver fibrosis progression and regression by a serological collagen turnover profile. American journal of physiology Gastrointestinal and liver physiology. 2019;316(1):G25-G31. doi: 10.1152/ajpgi.00158.2018. PubMed PMID: 30160980.
48. Luo Y, Oseini A, Gagnon R, Charles ED, Sidik K, Vincent R, Collen R, Idowu M, Contos MJ, Mirshahi F, Daita K, Asgharpour A, Siddiqui MS, Jarai G, Rosen G, Christian R, Sanyal AJ. An Evaluation of the Collagen Fragments Related to Fibrogenesis and Fibrolysis in Nonalcoholic Steatohepatitis. Sci Rep. 2018;8(1):12414. doi: 10.1038/s41598-018-30457-y. PubMed PMID: 30120271; PMCID: PMC6098042.
49. Nielsen MJ, Veidal SS, Karsdal MA, Orsnes-Leeming DJ, Vainer B, Gardner SD, Hamatake R, Goodman ZD, Schuppan D, Patel K. Plasma Pro-C3 (N-terminal type III collagen propeptide) predicts fibrosis progression in patients with chronic hepatitis C. Liver international : official journal of the International Association for the Study of the Liver. 2015;35(2):429-37. doi: 10.1111/liv.12700. PubMed PMID: 25308921.
50. Nielsen MJ, Kazankov K, Leeming DJ, Karsdal MA, Krag A, Barrera F, McLeod D, George J, Gronbaek H. Markers of Collagen Remodeling Detect Clinically Significant Fibrosis in Chronic Hepatitis

C Patients. PloS one. 2015;10(9):e0137302. doi: 10.1371/journal.pone.0137302. PubMed PMID: 26406331; PMCID: PMC4583995.
51. Leeming DJ, Karsdal MA, Byrjalsen I, Bendtsen F, Trebicka J, Nielsen MJ, Christiansen C, Moller S, Krag A. Novel serological neo-epitope markers of extracellular matrix proteins for the detection of portal hypertension. Alimentary pharmacology & therapeutics. 2013;38(9):1086-96. doi: 10.1111/apt.12484. PubMed PMID: 24099470; PMCID: PMC3935409.
52. Daniels SJ, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, Karsdal MA, Grove JI, Neil Guha I, Kawaguchi T, Torimura T, McLeod D, Akiba J, Kaye P, de Boer B, Aithal GP, Adams LA, George J. ADAPT: An Algorithm Incorporating PRO-C3 Accurately Identifies Patients With NAFLD and Advanced Fibrosis. Hepatology. 2019;69(3):1075-86. doi: 10.1002/hep.30163. PubMed PMID: 30014517.
53. Serai SD, Obuchowski NA, Venkatesh SK, Sirlin CB, Miller FH, Ashton E, Cole PE, Ehman RL. Repeatability of MR Elastography of Liver: A Meta-Analysis. Radiology. 2017;285(1):92-100. doi: 10.1148/radiol.2017161398. PubMed PMID: 28530847; PMCID: PMC5621724.
54. Harrison SA, Rossi SJ, Paredes AH, Trotter JF, Bashir MR, Guy CD, Banerjee R, Jaros MJ, Owers S, Baxter BA, Ling L, DePaoli AM. NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic Steatohepatitis. Hepatology. 2019. doi: 10.1002/hep.30590. PubMed PMID: 30805949.
55. Kang N, Shah VH, Urrutia R. Membrane-to-Nucleus Signals and Epigenetic Mechanisms for Myofibroblastic Activation and Desmoplastic Stroma: Potential Therapeutic Targets for Liver Metastasis? Mol Cancer Res. 2015;13(4):604-12. doi: 10.1158/1541-7786.MCR-14-0542. PubMed PMID: 25548101; PMCID: PMC4398610.
56. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165-78. doi: 10.1242/dmm.004077. PubMed PMID: 21324931; PMCID: PMC3046088.
57. Affo S, Yu LX, Schwabe RF. The Role of Cancer-Associated Fibroblasts and Fibrosis in Liver Cancer. Annu Rev Pathol. 2017;12:153-86. doi: 10.1146/annurev-pathol-052016-100322. PubMed PMID: 27959632; PMCID: PMC5720358.
58. Masuzaki R, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. Hepatology. 2009;49(6):1954-61. doi: 10.1002/hep.22870. PubMed PMID: 19434742.
59. Cox TR, Bird D, Baker AM, Barker HE, Ho MW, Lang G, Erler JT. LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis. Cancer research. 2013;73(6):1721-32. doi: 10.1158/0008-5472.CAN-12-2233. PubMed PMID: 23345161; PMCID: PMC3672851.
60. Grenard P, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S. Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. Journal of hepatology. 2001;35(3):367-75. doi: 10.1016/s0168-8278(01)00135-0. PubMed PMID: 11592598.
61. Wong CC, Tse AP, Huang YP, Zhu YT, Chiu DK, Lai RK, Au SL, Kai AK, Lee JM, Wei LL, Tsang FH, Lo RC, Shi J, Zheng YP, Wong CM, Ng IO. Lysyl oxidase-like 2 is critical to tumor microenvironment and metastatic niche formation in hepatocellular carcinoma. Hepatology. 2014;60(5):1645-58. doi: 10.1002/hep.27320. PubMed PMID: 25048396.
62. Malek NP, Schmidt S, Huber P, Manns MP, Greten TF. The diagnosis and treatment of hepatocellular carcinoma. Dtsch Arztebl Int. 2014;111(7):101-6. doi: 10.3238/arztebl.2014.0101. PubMed PMID: 24622679; PMCID: PMC3957051.
63. Pehrsson M, Manon-Jensen T, Sun S, Villesen IF, Castañé H, Joven J, Patel K, Goodman Z, Nielsen MJ, Bay-Jensen AC, Leeming DJ, Mortensen JH, Karsdal MA. An MMP-degraded and cross-linked fragment of type III collagen as a non-invasive biomarker of hepatic fibrosis resolution. Liver Int. 2022;42(7):1605-17. Epub 20220412. doi: 10.1111/liv.15270. PubMed PMID: 35384259; PMCID:

PMC9324161.
64. Fujiwara N, Kobayashi M, Fobar AJ, Hoshida A, Marquez CA, Koneru B, Panda G, Taguri M, Qian T, Raman I, Li QZ, Hoshida H, Sezaki H, Kumada H, Tateishi R, Yokoo T, Yopp AC, Chung RT, Fuchs BC, Baumert TF, Marrero JA, Parikh ND, Zhu S, Singal AG, Hoshida Y. A blood-based prognostic liver secretome signature and long-term hepatocellular carcinoma risk in advanced liver fibrosis. Med. 2021;2(7):836-50.e10. Epub 20210421. doi: 10.1016/j.medj.2021.03.017. PubMed PMID: 34318286; PMCID: PMC8312635.
65. Siddiqui MS, Yamada G, Vuppalanchi R, Van Natta M, Loomba R, Guy C, Brandman D, Tonanscia J, Chalasani N, Neuschander-Tetri B, Sanyal AJ, Network NCR. Diagnostic Accuracy of Noninvasive Fibrosis Models to Detect Change in Fibrosis Stage. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2019. doi: 10.1016/j.cgh.2018.12.031. PubMed PMID: 30616027.
66. Seth A, Sherman KE. Fatty liver disease in persons with HIV infection. Top Antivir Med. 2019;27(2):75-82. PubMed PMID: 31136997; PMCID: PMC6550355.
67. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. The Journal of clinical investigation. 2005;115(5):1343-51. doi: 10.1172/JCI23621. PubMed PMID: 15864352; PMCID: 1087172.
68. Noureddin M, Yates KP, Vaughn IA, Neuschwander-Tetri BA, Sanyal AJ, McCullough A, Merriman R, Hameed B, Doo E, Kleiner DE, Behling C, Loomba R, Nash CRN. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. Hepatology. 2013;58(5):1644-54. doi: 10.1002/hep.26465. PubMed PMID: 23686698; PMCID: PMC3760979.
69. Tang A, Tan J, Sun M, Hamilton G, Bydder M, Wolfson T, Gamst AC, Middleton M, Brunt EM, Loomba R, Lavine JE, Schwimmer JB, Sirlin CB. Nonalcoholic fatty liver disease: MR imaging of liver proton density fat fraction to assess hepatic steatosis. Radiology. 2013;267(2):422-31. doi: http://dx.doi.org/10.1148/radiol.12120896. PubMed PMID: 368825092.
70. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nature reviews Gastroenterology & hepatology. 2013;10(11):666-75. doi: 10.1038/nrgastro.2013.175. PubMed PMID: 24061203.
71. Younossi ZM, Loomba R, Anstee QM, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE, Friedman SL, Abdelmalek MF, Harrison SA, Sanyal AJ, Lavine JE, Mathurin P, Charlton MR, Goodman ZD, Chalasani NP, Kowdley KV, George J, Lindor K. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. Hepatology. 2018;68(1):349-60. doi: 10.1002/hep.29721. PubMed PMID: 29222917; PMCID: PMC6511364.
72. Allen AM, Shah VH, Therneau TM, Venkatesh SK, Mounajjed T, Larson JJ, Mara KC, Schulte PJ, Kellogg TA, Kendrick ML, McKenzie TJ, Greiner SM, Li J, Glaser KJ, Wells ML, Chen J, Ehman RL, Yin M. The Role of Three-Dimensional Magnetic Resonance Elastography in the Diagnosis of Nonalcoholic Steatohepatitis in Obese Patients Undergoing Bariatric Surgery. Hepatology. 2018. doi: 10.1002/hep.30483. PubMed PMID: 30582669; PMCID: PMC6591099.
73. Jayakumar S, Middleton MS, Lawitz EJ, Mantry PS, Caldwell SH, Arnold H, Mae Diehl A, Ghalib R, Elkhashab M, Abdelmalek MF, Kowdley KV, Stephen Djedjos C, Xu R, Han L, Mani Subramanian G, Myers RP, Goodman ZD, Afdhal NH, Charlton MR, Sirlin CB, Loomba R. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: Analysis of data from a phase II trial of selonsertib. Journal of hepatology. 2019;70(1):133-41. doi: 10.1016/j.jhep.2018.09.024. PubMed PMID: 30291868.
74. Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Ledinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51(2):454-62. doi: 10.1002/hep.23312. PubMed PMID: 20101745.

75. de Ledinghen V, Wong GL, Vergniol J, Chan HL, Hiriart JB, Chan AW, Chermak F, Choi PC, Foucher J, Chan CK, Merrouche W, Chim AM, Le Bail B, Wong VW. Controlled attenuation parameter for the diagnosis of steatosis in non-alcoholic fatty liver disease. Journal of gastroenterology and hepatology. 2016;31(4):848-55. doi: 10.1111/jgh.13219. PubMed PMID: 26514665.
76. Loomba R, Kayali Z, Noureddin M, Ruane P, Lawitz EJ, Bennett M, Wang L, Harting E, Tarrant JM, McColgan BJ, Chung C, Ray AS, Subramanian GM, Myers RP, Middleton MS, Lai M, Charlton M, Harrison SA. GS-0976 Reduces Hepatic Steatosis and Fibrosis Markers in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2018;155(5):1463-73 e6. doi: 10.1053/j.gastro.2018.07.027. PubMed PMID: 30059671; PMCID: PMC6318218.
77. Rinaldi L, Guarino M, Perrella A, Pafundi PC, Valente G, Fontanella L, Nevola R, Guerrera B, Iuliano N, Imparato M, Trabucco A, Sasso FC, Morisco F, Ascione A, Piai G, Adinolfi LE. Role of Liver Stiffness Measurement in Predicting HCC Occurrence in Direct-Acting Antivirals Setting: A Real-Life Experience. Digestive diseases and sciences. 2019;64(10):3013-9. doi: 10.1007/s10620-019-05604-8. PubMed PMID: 30937719.
78. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: A pilot study of the effects of lifestyle modification and vitamin E. Hepatology. 2003;38(2):413-9. doi: http://dx.doi.org/10.1053/jhep.2003.50316. PubMed PMID: 36919862.
79. Stiuso P, Scognamiglio I, Murolo M, Ferranti P, De Simone C, Rizzo MR, Tuccillo C, Caraglia M, Loguercio C, Federico A. Serum oxidative stress markers and lipidomic profile to detect NASH patients responsive to an antioxidant treatment: a pilot study. Oxid Med Cell Longev. 2014;2014:169216. doi: 10.1155/2014/169216. PubMed PMID: 24987492; PMCID: PMC4060161.
80. Kalhan SC, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in nonalcoholic fatty liver disease. Metabolism: clinical and experimental. 2011;60(3):404-13. doi: 10.1016/j.metabol.2010.03.006. PubMed PMID: 20423748; PMCID: PMC2950914.
81. Harrill AH, Roach J, Fier I, Eaddy JS, Kurtz CL, Antoine DJ, Spencer DM, Kishimoto TK, Pisetsky DS, Park BK, Watkins PB. The effects of heparins on the liver: application of mechanistic serum biomarkers in a randomized study in healthy volunteers. Clinical pharmacology and therapeutics. 2012;92(2):214-20. doi: 10.1038/clpt.2012.40. PubMed PMID: 22739141.
82. Loomba R, Sanyal AJ, Kowdley KV, Terrault N, Chalasani NP, Abdelmalek MF, McCullough AJ, Shringarpure R, Ferguson B, Lee L, Chen J, Liberman A, Shapiro D, Neuschwander-Tetri BA. Factors Associated With Histologic Response in Adult Patients With Nonalcoholic Steatohepatitis. Gastroenterology. 2019;156(1):88-95 e5. doi: 10.1053/j.gastro.2018.09.021. PubMed PMID: 30222962; PMCID: PMC6696948.
83. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, M SS, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M, Investigators AC. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43(6):1317-25. doi: 10.1002/hep.21178. PubMed PMID: 16729309.
84. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, Nash Clinical Research N. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(10):1104-12. doi: 10.1016/j.cgh.2009.05.033. PubMed PMID: 19523535; PMCID: PMC3079239.
85. Vilar-Gomez E, Calzadilla-Bertot L, Friedman SL, Gra-Oramas B, Gonzalez-Fabian L, Lazo-Del Vallin S, Diago M, Adams LA. Serum biomarkers can predict a change in liver fibrosis 1 year after lifestyle intervention for biopsy-proven NASH. Liver international : official journal of the International Association for the Study of the Liver. 2017;37(12):1887-96. doi: 10.1111/liv.13480. PubMed PMID: 28544769.
86. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The

NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846-54. doi: 10.1002/hep.21496. PubMed PMID: 17393509.
87. Julious SA, Debarnot CA. Why are pharmacokinetic data summarized by arithmetic means? J Biopharm Stat. 2000;10(1):55-71. doi: 10.1081/BIP-100101013. PubMed PMID: 10709801.
88. Hosmer D. W. LS, & Sturdivant R. X. . Applied Logistic Regression, 3rd Edition: Wiley-Blackwell; 2013.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B
ALCOHOL AND TOBACCO QUESTIONNAIRE INSTRUCTIONS

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- Data collection will be required for all CP-CTNet studies.
    - Data will be collected at baseline and end of every study. Data may also be collected at follow-up visits as determined by each protocol. If you wish to collect additional information beyond these core elements, you may certainly do so. However, all studies need to collect the basic elements in the attached CRFs.
    - The CRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

ALCOHOL ASSESSMENT-- BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
	_____	_____	__ __/__ __/__ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refuse to answer **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refuse to answer
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refuse to answer
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years

   ☐ Refuse to answer
   ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

   _____ years of age

   ☐ Refuse to answer
   ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

- ☐ Wine
- ☐ Liquor
- ☐ Beer
- ☐ Wine cooler
- ☐ Other _____ (enter other type(s) of alcohol you drink)

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

ALCOHOL ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refuse to answer **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refuse to answer
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refuse to answer
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

☐ Refuse to answer
☐ Do not know/Not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini

☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

☐ Yes
☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

☐ Yes In total, for about how many years?_____ If less than 1, write "1."
☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

☐ Yes → In total, for about how many years?_____ If less than 1, write "1."

☐ No

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a whole number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other,

Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

NATIONAL AND LOCAL RESOURCES TO HELP WITH ALCOHOL ABUSE AND ALCOHOLISM

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them.
https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

NATIONAL AND LOCAL RESOURCES TO HELP WITH QUITTING SMOKING

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Staying Smoke-Free for Good for smokers who have recently quit.
- Smoke-free for women, including pregnant women.
- Smoke-free information in Spanish
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov Web site.

APPENDIX C PARTICIPANT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Participant Name:

Diagnosis/Condition: NAFLD

Study Doctor:

Study Doctor Phone #:

NCI Trial #: NWU20-01-03

Study Drug(S): Lisinopril

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX D
COVID-19 ASSESSMENT INSTRUCTIONS

- Data collection will be required for all CP-CTNet studies.
    - Data will be collected at baseline and end of every study. All studies need to collect the elements in the attached eCRFs.
    - The eCRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

CP-CTNET COVID-19 BASELINE ASSESSMENT

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
The following information is being collected for all Cancer Prevention Clinical Trials Network (CP-CTNet) studies. Only information from before study entry should be reported on this form.

Have you had a positive COVID-19 test in the last 3 months?

☐ Yes
☐ No

Have you received a COVID-19 vaccine in the last 3 months?

☐ Yes
☐ No
☐ Prefer not to answer

Comments:
_____
_____

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

CP-CTNET COVID-19 FOLLOW-UP ASSESSMENT

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
The following information is being collected for all Cancer Prevention Clinical Trials Network (CP-CTNet) studies. Only use this form to report information that has become available since the last completion of a CP-CTNet COVID-19 Assessment.

Since your initial study visit, have you had a positive COVID-19 test?

☐ Yes
☐ No

If Yes, was it in the last 3 months?

☐ Yes
☐ No
☐ Prefer not to answer

Since your last study visit, have you received a COVID-19 vaccine?

☐ Yes
☐ No
☐ Prefer not to answer

If Yes, was it in the last 3 months?

☐ Yes
☐ No
☐ Prefer not to answer

Comments:
_____
_____

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX E STUDY DIARY

NWU20-01-03: Role of Lisinopril in Preventing the Progression of Non-Alcoholic Fatty Liver Disease (NAFLD): RELIEF-NAFLD

Please bring your completed diary and your study drug supply, including empty bottles, to every study visit. This will help us keep track of your study drug and how well you are tolerating it.

Protocol Number: NWU20-01-03 **Site: _____**

Participant Study ID: _____ **Participant Name: _____**

Date Study Drug Dispensed: _____ **Participant Signature: _____**

Instructions

Complete one line in the table for each day you take the study drug.
Please contact the study coordinator at << >> if you have any questions.

- Take your study drug once per day in the morning prior to breakfast. Take the tablet at the same time every day. Please swallow the tablet whole and do not chew, crush, or open it.
- Record the date and time of day you took the study drug.
- If you notice any side effects or if you have any other symptoms or comments, please record them in the Side Effects/Comments column and report them to the study staff when they call you.
- If you miss a dose of the study drug, take it as soon as you remember. Do not take the drug if it has been more than 12 hours since you missed your last dose. Wait and take the next dose at your regular time. Do not take 2 doses of the study drug at the same time. Please write the reason for missing a dose in the Side Effects/Comments column.
- For the first 12 weeks of intervention, please record a self-collected blood pressure reading once per week. You may self-collect your blood pressure at a nearby pharmacy or at home using the blood pressure monitor provided and instructions below.
- If your blood pressure reading is below 90 for systolic blood pressure (top number) and below 60 for diastolic blood pressure (bottom number) please call your study physician or another member of the study team. You should also start taking your study medication every other day.

Instructions for taking blood pressure reading using the monitor provided:

- Put cuff on and place that arm comfortably on top of a table.
- Sit with both feet on ground and apart from each other; do not cross your legs while sitting.
- Wait in this position for 5 minutes and relax while taking normal breaths. After 5 minutes press the start button on the monitor.
- The monitor reads and records the blood pressure and pulse. Please note the values for blood pressure in your diary below.
- Please call your physician or another member of the study team if your blood pressure reading is below 90 for systolic blood pressure (top number) and below 60 for diastolic blood pressure (bottom number). You should also start taking your study medication every other day.

Week 1 – Week 12				
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**	**Weekly Blood Pressure Reading**
1		____:____ ☐ a.m. / ☐ p.m.		Week1
2		____:____ ☐ a.m. / ☐ p.m.		
3		____:____ ☐ a.m. / ☐ p.m.		

Week 1 – Week 12				
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**	**Weekly Blood Pressure Reading**
4		____:____ ☐ a.m. / ☐ p.m.		
5		____:____ ☐ a.m. / ☐ p.m.		
6		____:____ ☐ a.m. / ☐ p.m.		
7		____:____ ☐ a.m. / ☐ p.m.		
8		____:____ ☐ a.m. / ☐ p.m.		Week 2
9		____:____ ☐ a.m. / ☐ p.m.		
10		____:____ ☐ a.m. / ☐ p.m.		
11		____:____ ☐ a.m. / ☐ p.m.		
12		____:____ ☐ a.m. / ☐ p.m.		
13		____:____ ☐ a.m. / ☐ p.m.		
14		____:____ ☐ a.m. / ☐ p.m.		
15		____:____ ☐ a.m. / ☐ p.m.		Week 3
16		____:____ ☐ a.m. / ☐ p.m.		
17		____:____ ☐ a.m. / ☐ p.m.		
18		____:____ ☐ a.m. / ☐ p.m.		
19		____:____ ☐ a.m. / ☐ p.m.		
20		____:____ ☐ a.m. / ☐ p.m.		
21		____:____ ☐ a.m. / ☐ p.m.		
22		____:____ ☐ a.m. / ☐ p.m.		Week 4
23		____:____ ☐ a.m. / ☐ p.m.		
24		____:____ ☐ a.m. / ☐ p.m.		
25		____:____ ☐ a.m. / ☐ p.m.		
26		____:____ ☐ a.m. / ☐ p.m.		
27		____:____ ☐ a.m. / ☐ p.m.		
28		____:____ ☐ a.m. / ☐ p.m.		
29		____:____ ☐ a.m. / ☐ p.m.		Week 5
30		____:____ ☐ a.m. / ☐ p.m.		
31		____:____ ☐ a.m. / ☐ p.m.		
32		____:____ ☐ a.m. / ☐ p.m.		
33		____:____ ☐ a.m. / ☐ p.m.		
34		____:____ ☐ a.m. / ☐ p.m.		
35		____:____ ☐ a.m. / ☐ p.m.		
36		____:____ ☐ a.m. / ☐ p.m.		Week 6

Week 1 – Week 12				
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**	**Weekly Blood Pressure Reading**
37		____:____ ☐ a.m. / ☐ p.m.		
38		____:____ ☐ a.m. / ☐ p.m.		
39		____:____ ☐ a.m. / ☐ p.m.		
40		____:____ ☐ a.m. / ☐ p.m.		
41		____:____ ☐ a.m. / ☐ p.m.		
42		____:____ ☐ a.m. / ☐ p.m.		
43		____:____ ☐ a.m. / ☐ p.m.		Week 7
44		____:____ ☐ a.m. / ☐ p.m.		
45		____:____ ☐ a.m. / ☐ p.m.		
46		____:____ ☐ a.m. / ☐ p.m.		
47		____:____ ☐ a.m. / ☐ p.m.		
48		____:____ ☐ a.m. / ☐ p.m.		
49		____:____ ☐ a.m. / ☐ p.m.		
50		____:____ ☐ a.m. / ☐ p.m.		Week 8
51		____:____ ☐ a.m. / ☐ p.m.		
52		____:____ ☐ a.m. / ☐ p.m.		
53		____:____ ☐ a.m. / ☐ p.m.		
54		____:____ ☐ a.m. / ☐ p.m.		
55		____:____ ☐ a.m. / ☐ p.m.		
56		____:____ ☐ a.m. / ☐ p.m.		
57		____:____ ☐ a.m. / ☐ p.m.		Week 9
58		____:____ ☐ a.m. / ☐ p.m.		
59		____:____ ☐ a.m. / ☐ p.m.		
60		____:____ ☐ a.m. / ☐ p.m.		
61		____:____ ☐ a.m. / ☐ p.m.		
62		____:____ ☐ a.m. / ☐ p.m.		
63		____:____ ☐ a.m. / ☐ p.m.		
64		____:____ ☐ a.m. / ☐ p.m.		Week 10
65		____:____ ☐ a.m. / ☐ p.m.		
66		____:____ ☐ a.m. / ☐ p.m.		
67		____:____ ☐ a.m. / ☐ p.m.		
68		____:____ ☐ a.m. / ☐ p.m.		
69		____:____ ☐ a.m. / ☐ p.m.		

Week 1 – Week 12				
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**	**Weekly Blood Pressure Reading**
70		____:____ ☐ a.m. / ☐ p.m.		
71		____:____ ☐ a.m. / ☐ p.m.		Week 11
72		____:____ ☐ a.m. / ☐ p.m.		
73		____:____ ☐ a.m. / ☐ p.m.		
74		____:____ ☐ a.m. / ☐ p.m.		
75		____:____ ☐ a.m. / ☐ p.m.		
76		____:____ ☐ a.m. / ☐ p.m.		
77		____:____ ☐ a.m. / ☐ p.m.		
78		____:____ ☐ a.m. / ☐ p.m.		Week 12
79		____:____ ☐ a.m. / ☐ p.m.		
80		____:____ ☐ a.m. / ☐ p.m.		
81		____:____ ☐ a.m. / ☐ p.m.		
82		____:____ ☐ a.m. / ☐ p.m.		
83		____:____ ☐ a.m. / ☐ p.m.		
84		____:____ ☐ a.m. / ☐ p.m.		
85		____: ____ ☐ a.m. / ☐ p.m.		
86		____:____ ☐ a.m. / ☐ p.m.		
87		____:____ ☐ a.m. / ☐ p.m.		

FOR STUDY TEAM USE ONLY	
Date study drug dispensed:	Date study drug returned:
Number of tablets dispensed:	Number of tablets returned:
Number of tablets that should have been taken:	
Discrepancy/notes: Staff Signature and Date Study Diary Reviewed with Participant:	

Week 13 – Week 24			
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**
1		____:____ ☐ a.m. / ☐ p.m.	
2		____:____ ☐ a.m. / ☐ p.m.	
3		____:____ ☐ a.m. / ☐ p.m.	
4		____:____ ☐ a.m. / ☐ p.m.	
5		____:____ ☐ a.m. / ☐ p.m.	
6		____:____ ☐ a.m. / ☐ p.m.	
7		____:____ ☐ a.m. / ☐ p.m.	
8		____:____ ☐ a.m. / ☐ p.m.	
9		____:____ ☐ a.m. / ☐ p.m.	
10		____:____ ☐ a.m. / ☐ p.m.	
11		____:____ ☐ a.m. / ☐ p.m.	
12		____:____ ☐ a.m. / ☐ p.m.	
13		____:____ ☐ a.m. / ☐ p.m.	
14		____:____ ☐ a.m. / ☐ p.m.	
15		____:____ ☐ a.m. / ☐ p.m.	
16		____:____ ☐ a.m. / ☐ p.m.	
17		____:____ ☐ a.m. / ☐ p.m.	
18		____:____ ☐ a.m. / ☐ p.m.	
19		____:____ ☐ a.m. / ☐ p.m.	
20		____:____ ☐ a.m. / ☐ p.m.	
21		____:____ ☐ a.m. / ☐ p.m.	
22		____:____ ☐ a.m. / ☐ p.m.	
23		____:____ ☐ a.m. / ☐ p.m.	
24		____:____ ☐ a.m. / ☐ p.m.	
25		____:____ ☐ a.m. / ☐ p.m.	
26		____:____ ☐ a.m. / ☐ p.m.	
27		____:____ ☐ a.m. / ☐ p.m.	
28		____:____ ☐ a.m. / ☐ p.m.	
29		____:____ ☐ a.m. / ☐ p.m.	
30		____:____ ☐ a.m. / ☐ p.m.	
31		____:____ ☐ a.m. / ☐ p.m.	
32		____:____ ☐ a.m. / ☐ p.m.	
33		____:____ ☐ a.m. / ☐ p.m.	

Week 13 – Week 24			
Day	**Date**	**Time Study Drug Taken**	**Side Effects/Comments (Please include reason for missed dose)**
34		____:____ ☐ a.m. / ☐ p.m.	
35		____:____ ☐ a.m. / ☐ p.m.	
36		____:____ ☐ a.m. / ☐ p.m.	
37		____:____ ☐ a.m. / ☐ p.m.	
38		____:____ ☐ a.m. / ☐ p.m.	
39		____:____ ☐ a.m. / ☐ p.m.	
40		____:____ ☐ a.m. / ☐ p.m.	
41		____:____ ☐ a.m. / ☐ p.m.	
42		____:____ ☐ a.m. / ☐ p.m.	
43		____:____ ☐ a.m. / ☐ p.m.	
44		____:____ ☐ a.m. / ☐ p.m.	
45		____:____ ☐ a.m. / ☐ p.m.	
46		____:____ ☐ a.m. / ☐ p.m.	
47		____:____ ☐ a.m. / ☐ p.m.	
48		____:____ ☐ a.m. / ☐ p.m.	
49		____:____ ☐ a.m. / ☐ p.m.	
50		____:____ ☐ a.m. / ☐ p.m.	
51		____:____ ☐ a.m. / ☐ p.m.	
52		____:____ ☐ a.m. / ☐ p.m.	
53		____:____ ☐ a.m. / ☐ p.m.	
54		____:____ ☐ a.m. / ☐ p.m.	
55		____:____ ☐ a.m. / ☐ p.m.	
56		____:____ ☐ a.m. / ☐ p.m.	
57		____:____ ☐ a.m. / ☐ p.m.	
58		____:____ ☐ a.m. / ☐ p.m.	
59		____:____ ☐ a.m. / ☐ p.m.	
60		____:____ ☐ a.m. / ☐ p.m.	
61		____:____ ☐ a.m. / ☐ p.m.	
62		____:____ ☐ a.m. / ☐ p.m.	
63		____:____ ☐ a.m. / ☐ p.m.	
64		____:____ ☐ a.m. / ☐ p.m.	
65		____:____ ☐ a.m. / ☐ p.m.	
66		____:____ ☐ a.m. / ☐ p.m.	

67		____:____ ☐ a.m. / ☐ p.m.	
68		____:____ ☐ a.m. / ☐ p.m.	
69		____:____ ☐ a.m. / ☐ p.m.	
70		____:____ ☐ a.m. / ☐ p.m.	
71		____:____ ☐ a.m. / ☐ p.m.	
72		____:____ ☐ a.m. / ☐ p.m.	
73		____:____ ☐ a.m. / ☐ p.m.	
74		____:____ ☐ a.m. / ☐ p.m.	
75		____:____ ☐ a.m. / ☐ p.m.	
76		____:____ ☐ a.m. / ☐ p.m.	
77		____:____ ☐ a.m. / ☐ p.m.	
78		____:____ ☐ a.m. / ☐ p.m.	
79		____:____ ☐ a.m. / ☐ p.m.	
80		____:____ ☐ a.m. / ☐ p.m.	
81		____:____ ☐ a.m. / ☐ p.m.	
82		____:____ ☐ a.m. / ☐ p.m.	
83		____:____ ☐ a.m. / ☐ p.m.	
84		____:____ ☐ a.m. / ☐ p.m.	
85		____: ____ ☐ a.m. / ☐ p.m.	
86		____:____ ☐ a.m. / ☐ p.m.	
87		____:____ ☐ a.m. / ☐ p.m.	
88		____:____ ☐ a.m. / ☐ p.m.	

FOR STUDY TEAM USE ONLY	
Date study drug dispensed:	Date study drug returned:
Number of tablets dispensed:	Number of tablets returned:
Number of tablets that should have been taken:	
Discrepancy/notes: Staff Signature and Date Study Diary Reviewed with Participant:	

APPENDIX F SUGGESTED LANGUAGE FOR PARTICIPANT COMMUNICATION

Reminder to be sent via Text Message or Email

Text Message:
This is a reminder to take your medication for the **RELIEF-NAFLD** research study this morning before breakfast. Please swallow the capsule whole, and please remember to fill out your study diary. Thank you!

Email:
This is a friendly reminder to take your medication for the **RELIEF-NAFLD** research study this morning before breakfast. Please swallow the capsule whole and do not chew, crush, or open it. Please also remember to fill out your study diary, noting the date and time when you took the medication. Let us know if you have any questions or concerns. Thank you!

Emails or Telephone calls to assess compliance, toxicity, and changes in medication:

Hello my name is [insert name], Research Coordinator from [site name/department]. I wanted to check in with you briefly about the NWU20-01-03 RELIEF-NAFLD research study. Is this a good time to talk?

If no: "When would be a better time for me to call you?" If yes: "As part of your study follow up, I am calling you to briefly assess your study compliance and symptoms. Since we last spoke on < >, have you missed your study medication on any days?"

If yes: "How many days did you miss your study medication? On what dates did you miss your study medication? Since we last spoke, have there been any changes to your current medications? For example, a change in dose, stopping a medication, or starting a new medication.

If yes: "Please tell me what has changed.

Since we last spoke on < >, have you experience any new symptoms?

Please remember to take your study medication daily in the morning prior to breakfast. Swallow the capsule whole and do not chew, crush, or open it. Please also remember to fill out your study diary, noting the date and time when you took the medication. Your next study visit is scheduled for < >, please bring your pill bottle and study diary. Let us know if you have any questions or concerns! Thank you!

APPENDIX G STOOL COLLECTION INSTRUCTIONS

1. Empty your bladder before beginning the collection.
2. Lift the toilet seat.
3. Place the toilet hat provided to you onto the toilet bowl, then lower the seat (see Image 1 below).
4. Open small Ziploc bags and place them on a clean surface
5. Sit down and pass a bowel specimen into the toilet hat. ***Stool may not be used if it comes into contact with toilet water or urine***.
6. Put on gloves and open the small collection cup.
7. Use the spoon attached to the lid to transfer one spoonful of stool into the liquid in the cup (see Image 2 below).
8. Screw the cap to close the cup and place the cup in the small Ziploc bag with the paper towels (see Image 3 below).
9. Remove your gloves and wash your hands.
10. Seal the Ziploc bag. Place the bag with your sample in the self-addressed pre-paid envelope.
11. Place Ziploc bag into the FedEx envelope and mail.

Image 1

Image 2

Image 3