Losartan for the Treatment of Pediatric NAFLD (STOP-NAFLD): A Phase 2, Randomized, Placebo-Controlled Clinical Trial

Protocol Number: 9 IND# 139098

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Section 1.1, Synopsis:	Changed HbA1c time window to 60 days	Changed to allow 60-day time window for all laboratory measurements except ALT.
Section 1.3, Data Collection Schedule	Removed Focused Physical Examination from the data collection at the randomization visit.	A brief physical is required to determine if the patient is well on the day of randomization, but the Focused Physical Examination form is not completed.
Section 8.1.2, Screening Visits:	Protocol text changed to allow 60-day time window for all laboratory measurements except ALT	ALT measurement must be completed within 30 days of randomization; additional lab tests for screening must be completed within 60 days of randomization
Section 12.3, Blood Collection Schedule:	CBC + WBC and uric acid added to blood collection at the f36 visit, to align with the Data Collection Schedule and visit procedures specified in the protocol.	The total blood volume collected at f36 corrected from 35mL to 42mL.

Table of Contents

STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

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

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

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	**Losartan for the treatment of Pediatric NAFLD (STOP-NAFLD): A Phase 2, Randomized, Placebo-Controlled Clinical Trial**
Study Description:	This is a multicenter, randomized, double masked, placebo-controlled, parallel treatment groups phase 2 trial of losartan for pediatric NAFLD. Children ages 8-17 years will be enrolled for 24 weeks and treated with losartan (100 mg orally once per day) or matching placebo. The primary outcome of the study is improvement in alanine aminotransferase (ALT) from baseline to 24 weeks. The hypothesis is that losartan will improve ALT in children with pediatric NAFLD.
Objectives:	Primary Objective: To determine whether 24 weeks of treatment with losartan compared to treatment with placebo improves measures of nonalcoholic fatty liver disease (NAFLD) as determined by improvement in serum ALT from baseline. Secondary Objectives: To determine safety of losartan in children with pediatric NAFLD over 24 weeks and to measure other biomarkers of response to losartan.
Outcomes:	Primary Outcome Measure: • Change in serum alanine aminotransferase (ALT) from baseline to 24 weeks. • Secondary outcome measures (24 weeks of treatment with losartan

compared to treatment with placebo):

- Relative change in ALT at 24 weeks compared to baseline ALT
- Proportion of patients achieving normalization of ALT at 24 weeks
- Change in serum aspartate aminotransferase (AST) at 24 weeks compared to baseline AST
- Change in gamma-glutamyl transpeptidase (GGT) at 24 weeks compared to baseline GGT
- Change in ALT at 12 weeks compared to baseline ALT
- Change in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) at 24 weeks compared to baseline.
- Change in anthropometric measurements (weight, waist to hip ratio, waist circumference) and BMI z-score at 24 weeks compared to baseline
- Change in serum lipid profiles at 24 weeks compared to baseline
- Change in C-reactive protein (serum marker of inflammation), from screening to 24 weeks
- Change in Pediatric Quality of Life (Ped-QoL) scores at 24 weeks compared to baseline
- Change in frequency of adverse events compared to baseline

Exploratory:

- Change in peripheral proinflammatory cytokine levels (including IL-6, TNF, TGF-beta) from baseline to 24 weeks
- Change in NMR MetaboProfile Analysis (LP4) from baseline to 24 weeks.

Study Population:	The study population will be 110 children ages 8-17 years with a history of biopsy-proven NAFLD, boys and girls, located in the United States.
Phase:	Phase 2
Description of Sites/Facilities Enrolling Participants:	Participants will be enrolled at 10 pediatric NASH CRN sites located throughout the United States.
Description of Study Intervention:	The dose for patients with baseline weight ≥ 70 kg to <150 kg will be one 50 mg capsule of losartan or matching placebo per day for one week, then two capsules of 50 mg of losartan or matching placebo per day (100 mg total) for weeks 2-24. This dosing scheme is based on the recommended starting dose of 0.7 mg/kg/day and the maximum dose of 1.4 mg/kg/day.
Total Study Duration:	• Recruitment phase: 12 months • Follow-up phase: 24 months

- Expected rate of recruitment is 11 per clinical center; approximately 1 per month

Participant Duration:

- Screening phase is 60 days; randomization must occur within 730 days of liver biopsy.
- 24-week treatment period; 12-week post–treatment follow-up;
- 10 months total

Inclusion Criteria:

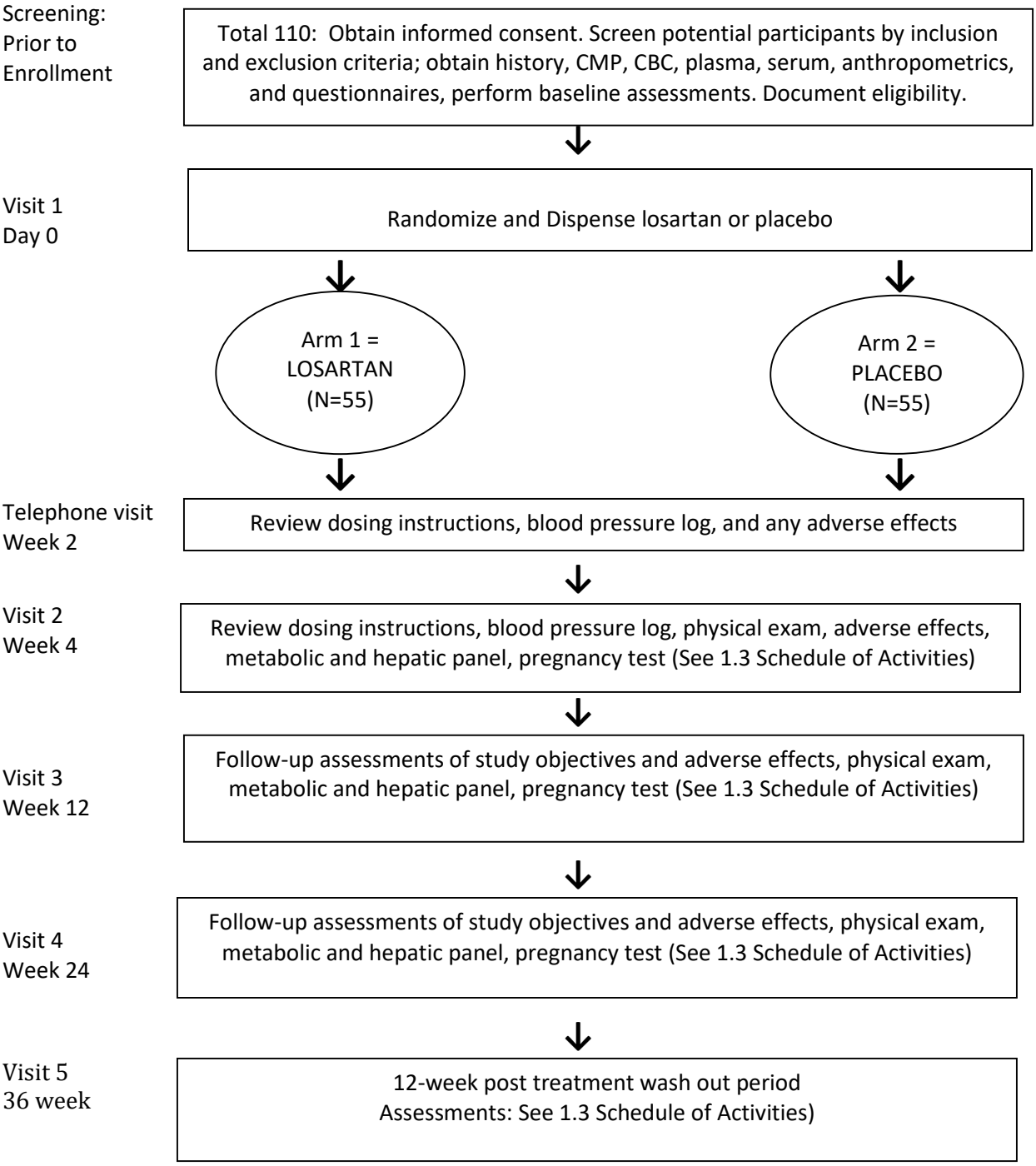
- Age 8-17 years at initial screening interview
- Histological evidence of NAFLD with or without fibrosis and a NAFLD activity score (NAS) of ≥3, on a liver biopsy obtained no more than 730 days prior to enrollment
- Serum ALT at screening ≥ 50 IU/L

Exclusion Criteria:

- Body weight less than 70 kg or greater than 150 kg at screening
- Significant alcohol consumption or inability to reliably quantify alcohol intake
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, other known hepatotoxins) for more than 2 consecutive weeks in the past year prior to randomization
- New treatment with vitamin E or metformin started in the past 90 days or plans to alter the dose or stop over the next the 24 weeks. A stable dose is acceptable.
- Prior or planned bariatric surgery
- Uncontrolled diabetes (HbA1c 9.5% or higher within 60 days prior to enrollment)
- Presence of cirrhosis on liver biopsy
- History of hypotension or history of orthostatic hypotension
- Stage 2 Hypertension or >140 systolic **or** >90 diastolic at screening
- Current treatment with any antihypertensive medications including all angiotensin converting enzyme (ACE) inhibitors or aliskiren;
- Current treatment with potassium supplements or any drug known to increase potassium
- Current daily use of nonsteroidal anti-inflammatory drugs (NSAIDs)
- Current treatment with lithium
- Platelet counts below 100,000 /mm^3
- Clinical evidence of hepatic decompensation (serum albumin < 3.2 g/dL, international normalized ratio (INR) >1.3, direct bilirubin >1.3 mg/dL, history of esophageal varices, ascites, or hepatic encephalopathy)
- Evidence of chronic liver disease other than NAFLD:
  - Biopsy consistent with histological evidence of autoimmune hepatitis
  - Serum hepatitis B surface antigen (HBsAg) positive.
  - Serum hepatitis C antibody (anti-HCV) positive.
  - Iron/total iron binding capacity (TIBC) ratio (transferrin saturation) > 45% with histological evidence of iron overload

  - Alpha-1-antitrypsin (A1AT) phenotype/genotype ZZ or SZ
  - Wilson's disease
- Serum alanine aminotransferase (ALT) greater than 300 IU/L
- History of biliary diversion
- History of kidney disease and/or estimated glomerular filtration rate (eGFR) < than 60 mL/min/1.73 m^2 using Schwartz Bedside GFR Calculator for Children isotope dilution mass spectroscopy (IDMS)-traceable
- Known Human Immunodeficiency Virus (HIV) infection
- Active, serious medical disease with life expectancy less than 5 years
- Active substance abuse including inhaled or injected drugs, in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in any clinical/investigational trial within the prior 150 days and during the STOP-NAFLD Trial.
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Inability to swallow capsules
- Known allergy to losartan potassium or other angiotensin receptor blocker
- Failure of parent or legal guardian to give informed consent or subject to give informed assent

1.2 SCHEMA


Screening: Prior to Enrollment
Total 110: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, CMP, CBC, plasma, serum, anthropometrics, and questionnaires, perform baseline assessments. Document eligibility.
Visit 1 Day 0
Randomize and Dispense losartan or placebo
Arm 1 = LOSARTAN (N=55)
Arm 2 = PLACEBO (N=55)
Telephone visit Week 2
Review dosing instructions, blood pressure log, and any adverse effects
Visit 2 Week 4
Review dosing instructions, blood pressure log, physical exam, adverse effects, metabolic and hepatic panel, pregnancy test (See 1.3 Schedule of Activities)
Visit 3 Week 12
Follow-up assessments of study objectives and adverse effects, physical exam, metabolic and hepatic panel, pregnancy test (See 1.3 Schedule of Activities)
Visit 4 Week 24
Follow-up assessments of study objectives and adverse effects, physical exam, metabolic and hepatic panel, pregnancy test (See 1.3 Schedule of Activities)
Visit 5 36 week
12-week post treatment wash out period
Assessments: See 1.3 Schedule of Activities)

1.3 SCHEDULE OF ACTIVITIES (SOA)

	Screening visits		**Follow-up visits- Weeks from Randomization**				
Assessment/Procedure		**RZ**	**f02-Phone**	**f04**	**f12**	**f24**	**f36**
Consent, HIPAA authorization, demographics	X	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.
Follow-up medical history	.	.	.	X	X	X	X
Review for adverse effects	.	.	X	X	X	X	X
Review for concomitant medications	X	X	.	X	X	X	X
Alcohol questionnaire AUDIT (A) if interim (I)	A	.	.	I	I	I	I
Detailed (D) or focused (F) physical exam	D		.	F	F	D	F
Liver biopsy review	X	A	.	A	A	A	A
Pediatric quality of life questionnaire (Peds-QL)	X	.	.	.	.	X	X
Beverage intake questionnaire (BEV-Q)	X	.	.	.	.	X	X
Standard of care materials provided	.	X	.	.	.	.	.
Eligibility confirmation	.	X	.	.	.	.	.
Study drug dispensing	.	X	.	.	.	.	.
Review of study drug adherence	.	.	X	X	X	X	.
Blood pressure log		X	X	X	.	.	.
Labs:							
Complete blood count +WBC	X	.	.	.	X	X	X
Basic metabolic panel + eGFR	X	.	.	X	X	X	X
Hepatic panel (Liver function test)	X	.	.	X	X	X	X
Uric acid	X	.	.	.	X	X	X
C-Reactive Protein (CRP)	X	.	.	.	X	X	X
Gamma glutamyltransferase -GGT	X	.	.	.	X	X	.
Prothrombin time (PT), INR	X	.	.	.	X	X	.
Fasting lipid profile	X	.	.	.	X	X	.
Fasting serum glucose	X	.	.	.	X	X	.
Fasting serum insulin	X	.	.	.	X	X	.
Fasting HbA1c	X	.	.	.	X	X	.
Etiologic tests	X	.	.	.	.	.	.
Pregnancy test	X	X	.	X	X	X	X
Banking:							
Fasting serum and plasma	X	.	.	.	X	X	X
DNA	X	.	.	.	.	.	.
Complete Case Report Forms (CRFs)	X	X	.	X	X	X	X
Closeout form							X

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

A= as available

Focused physical exam: anthropometric assessments (body weight [kg], body height [cm], waist circumference [cm], and hip circumference [cm]); vital signs (temperature, heart rate, respiratory rate, blood pressure) and liver signs

Metabolic panel: glucose, calcium, sodium, potassium, CO_2 (carbon dioxide, bicarbonate), chloride, BUN, creatinine

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

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

2 INTRODUCTION

2.1 STUDY RATIONALE

NAFLD has become the most common form of chronic liver disease in the developed world. It commonly occurs in the setting of obesity, insulin resistance and a sedentary lifestyle and it is often considered the liver manifestation of the metabolic syndrome. The primary form of treatment is optimization of lifestyle, including nutrition and exercise. In the real world, success is limited by the difficulty of diet and exercise for many children, as well as the fact that NAFLD may not always respond to these lifestyle interventions even when fully implemented. Therefore, pharmacologic treatments have been sought but none has proved universally efficacious. This may be related to the fact that the histopathological changes seen on liver biopsy currently described as NASH may be the result of multiple pathogenetic mechanisms acting in concert to varying degrees. Based on the prevalence and risk of progression of NAFLD to cirrhosis and cancer, the burden of significant disease is large, and drug therapy to prevent or treat NAFLD is needed. While the pathologist's approach to liver histology is not age-specific, histologic features differ in adults and children, therefore data from adult studies cannot be extrapolated to the pediatric population.[1]

2.1.1 DEFINITIONS

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of greater than "normal" amounts of fat in the liver. The pathologists' definition is based on observed steatotic droplets (triglyceride) exceeding 5% of surface area. This figure evolved from older studies showing that the normal liver was 5% lipid. In the largest population study of adults using MR spectroscopy, the threshold value for abnormal liver fat fraction was similar to these other assessments.[2]

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

A name for ***NAFLD that is not NASH*** has not been universally established. Terms such as nonalcoholic fatty liver (NAFL), simple steatosis, benign steatosis, bland steatosis, and isolated steatosis have been used, but each has limitations that preclude general acceptance.[4]

2.1.2 SIGNIFICANCE OF NAFLD

Prevalence

In the Study of Child and Adolescent Liver Epidemiology (SCALE) for children ages 2-19 the standardized prevalence of NAFLD was 9.6% after adjusting for age, gender, race, and ethnicity.[5] Studies evaluating the prevalence of NASH in children vary greatly by setting. In hepatology clinics in San Diego 84% of children, and in Italy 86% with biopsy proven NAFLD were reported as having NASH.[6, 7] However, in the San Diego based SCALE study, only 23% of children with NAFLD showed evidence of NASH.[5] In the NASH CRN Database study, which included American children from various geographic locations, 38% had borderline steatohepatitis and 39% were found to have definite NASH.[8] Among morbidly obese American adolescents undergoing bariatric surgery, intra-operative biopsy revealed 83% had NAFLD while only 20% had NASH.[9]

Progression to cirrhosis

In studies of children undergoing liver biopsy for suspected NAFLD, rates of cirrhosis have been reported to range from 2 to 10%.[1, 7, 10] Of those patients without evidence of cirrhosis on their initial biopsy, the risk for developing cirrhosis may vary by histology and subtype. In adults with steatohepatitis, Matteoni et al reported that one in four patients went on to develop cirrhosis.[11] Longitudinal data are needed to further elucidate the risk of disease progression in pediatric NAFLD.

Development of hepatocellular carcinoma

Multiple cross-sectional studies suggest that NAFLD is a significant risk factor for the development of hepatocellular carcinoma (HCC).[12-14] It is estimated that roughly 1,000 cases of HCC in the United States each year can be attributed to NAFLD.[15] One prospective study of adult patients with NASH cirrhosis found HCC to develop in roughly 7% of patients over a 10 year period.[16] Little is known about the risk for HCC in children. However, the long duration of NASH may impact the development HCC for those who have NAFLD as children.

Comorbidities

Current data suggest that NAFLD confers an increased risk for the development of cardiovascular disease, particularly through its association with metabolic syndrome. The key components of metabolic syndrome include central obesity, impaired glucose tolerance, elevated blood pressure, and dyslipidemia.[17] A study demonstrated NAFLD to be more frequent among children with metabolic syndrome compared to children without metabolic syndrome.[18] Of the 300 children evaluated, those with biopsy-proven NAFLD had a significantly greater cardiovascular risk profile. Higher values for fasting glucose, insulin, low-density lipoprotein cholesterol, triglycerides, and systolic blood pressure were observed in the NAFLD group. In a Swedish cohort study, patients with a history of steatohepatitis had significantly higher rates of cardiovascular disease and mortality when compared to patients with isolated steatosis.[19] Identification of NAFLD should prompt consideration of cardiovascular health and relative risk reduction through lifestyle changes.

Children with NAFLD may have an increased risk for developing type 2 diabetes. In large studies of children with biopsy-proven NAFLD, 5 to 10% of children have type 2 diabetes at the time of diagnosis.[7, 20] Additionally, nearly 50% of children diagnosed with type 2 diabetes have suspected fatty liver based on ALT elevation.[21] Progression to diabetes is important to understand from both preventive and therapeutic standpoints. In adults, the risk for development of diabetes may be as high as 20 to 25% over 5 years.[22, 23]

2.1.3 PATHOGENESIS OF NASH

The majority of studies on the pathogenesis of NAFLD have been in the adult population. While pediatric and adult NAFLD share many characteristics, known differences between the two, including histological differences, indicate variation in the development of pediatric versus adult NAFLD.[24, 25] While our understanding of the pathogenesis is in its infancy, obesity, central adiposity, and insulin resistance are strongly associated with pediatric NAFLD and inflammation with progression to NASH.

Insulin resistance may be defined as the state in which a given concentration of insulin is associated with a lower than normal uptake of glucose by tissues such as muscle and liver. Insulin resistance is associated with multiple metabolic abnormalities including metabolic syndrome, abnormal glucose metabolism, reproductive abnormalities in

women, and cutaneous abnormalities including acanthosis nigricans and skin tags.[26-28] Systemic insulin resistance is thought to be critical to the pathogenesis of pediatric NAFLD and has been demonstrated to be present in a majority of children with biopsy-proven NAFLD.[7] Studies in animals provide insight to the physiologic link between insulin resistance and fatty liver; hyperinsulinemia and insulin resistance result in increased adipose tissue lipogenesis and very low density lipoproteins (VLDL) uptake resulting in increased adipocyte fat sequestration and obesity.[29] Insulin resistance in both adipose and liver tissue may be integral to the development of NAFLD.

Mechanisms of hepatocellular injury

To the extent that mitochondrial dysfunction, ATP depletion, endoplasmic reticulum stress, and oxidant stress play a role in hepatocellular injury in NASH, putative mechanisms have been proposed to tie these processes to subsequent cell death.[30, 31] Because the fatty acid metabolites responsible for initiating lipotoxic injury are not fully known, how they promote cell injury and death has yet to be resolved.[32] A major mechanism of hepatocyte death in NASH is apoptosis.[33]

Fibrosis and progression to cirrhosis

Liver fibrosis results from hepatocyte injury as demonstrated by studies showing that the production of cytokines and lipid peroxidation species from stressed or dying hepatocytes promote proliferation and activation of hepatic stellate cells. The balance of extracellular matrix deposition versus degradation is thus disrupted in favor of net accumulation of fibrosis. This pathway has been challenged by recent data indicating that epithelial progenitor cells in the liver can undergo epithelial-mesenchymal transition (EMT) when stressed.[34] The relative roles of EMT versus activation of existing stellate cells in progression of NASH fibrosis to cirrhosis has yet to be established.

2.1.4 LIMITATIONS OF CURRENT TREATMENT OF NAFLD

2.1.4.1 LIFESTYLE MODIFICATION

Because obesity, poor dietary habits, and a sedentary lifestyle predispose to the development of NAFLD, the standard of care therapeutic intervention is to address these factors through a combination of gradual and sustained weight reduction through a balanced, calorically appropriate diet composed of healthy food choices coupled with increased physical activity.[35] There are enough data to support this recommendation[36] for the typical NAFLD phenotype which includes conditions such as obesity and metabolic syndrome[37]. However, better data are needed on the histological response of children with NAFLD to standardized nutritional lifestyle interventions as recommended by the American Academy of Pediatrics.

2.1.4.2 METFORMIN

Insulin resistance is believed to be central to the development of NAFLD. Therefore, several studies have evaluated metformin as a potential treatment for NAFLD in children. In the first study to use magnetic resonance spectroscopy as a measure of hepatic steatosis, 10 non-diabetic children with biopsy-proven NASH received 500 mg of metformin by mouth twice daily for 6 months in an open-label pilot trial.[38] At the completion of the study, ALT normalized in 40% and AST normalized in 50%. Hepatic fat fraction was significantly reduced in 9 of 10 subjects, decreasing from a baseline mean of 30 ± 11% to 23 ± 9% after 24 weeks of treatment.

An open-label, 2-year observational pilot study from Rome evaluated the effect of metformin on NAFLD in children.[39] Thirty children with biopsy-proven NAFLD and mildly elevated ALT were enrolled and treated with 1,500 mg of metformin daily. All subjects also received lifestyle advice, including an individually tailored hypocaloric or isocaloric diet, physical activity recommendations, and monthly 1-hour sessions with a dietitian. Of those enrolled, 40% had a follow-up biopsy. In this subset, several histologic features, including steatosis, ballooning, and lobular inflammation were noted to have improved. However, there was no change in fibrosis.

The Treatment of NAFLD in Children (TONIC) trial was a multicenter, randomized, double-masked, placebo-controlled trial conducted by the NASH CRN[39]. A total of 173 children ages 7 to 17 years with biopsy-proven NAFLD were randomized to receive either metformin, vitamin E, or placebo for 96 weeks to assess the change in serum ALT and histology. The outcomes from the trial were published in March 2011.[40] {see also 2.1.4.4}

2.1.4.3 THIAZOLIDINEDIONES

The thiazolidinediones (TZDs, glitazones) are a class of drugs developed to treat type 2 diabetes because of their insulin sensitizing effect in states of insulin resistance. Studies have shown that the benefits of TZDs are at least partly explained by their ability to improve insulin responsiveness in adipose tissue and reduce inappropriate peripheral lipolysis.[40, 41] As ligands for the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPARγ), this class of drugs has multiple complex effects. Improved insulin signaling has been attributed to the ability of TZDs to induce adipocyte differentiation and also prevent the inhibitory effect of c-Jun N-terminal kinases (JNKs) on post-receptor insulin signaling. Pilot studies indicated that the TZDs rosiglitazone and pioglitazone might improve the histology of NASH. Placebo controlled trials have had somewhat mixed results with the French FLIRT trial showing primarily improvement in steatosis[42, 43] and other trials showing improvement in inflammation as well.[44-46] The pioglitazone treated patients in the PIVENS trial did not achieve the pre-defined histological Outcome or demonstrate improved fibrosis, but did have significant improvements in steatosis, inflammation, and the presence of steatohepatitis.[47] Similar to findings in other trials of TZDs, the improvement in ALT occurred over 3-6 months and was not sustained when the drug was discontinued. Whether the histological improvement occurs in parallel with the ALT decrease is unknown since no trial has examined serial liver biopsies in TZD treated patients. The primary side effect of using TZDs over the typical 1-2 year time course of most trials is significant weight gain in some subjects. Exacerbation of congestive heart failure, osteoporosis with distal limb fractures, and rare idiosyncratic hepatotoxicity are additional side effects known to occur with the use of TZDs, but in general they have not been observed in the relatively small NASH trials. This class of medication has not yet been tested and proven safe in children.

2.1.4.4 VITAMIN E

Trials of antioxidant agents for the treatment of NASH have been undertaken because of the proposed role of oxidant stress in the pathogenesis of steatohepatitis.[48] Vitamin E (RRR-alpha-tocopherol) has been of particular interest. Pilot studies were inconclusive but the PIVENS trial demonstrated that 43% of patients treated for two years reached the desired histological outcome compared to 19% in the placebo group ($P<0.01$).[47] Improvement in fibrosis was not observed. Similar to the pioglitazone-treated patients, the ALT improved over a time period of 3-6 months. Unlike treatment with pioglitazone, weight gain was not observed, but neither was any improvement in insulin sensitivity.

The NASH Clinical Research Network performed the Treatment of NAFLD in Children (TONIC) trial (92); a multicenter, double-blind, double-placebo, randomized clinical trial in which 173 pediatric patients received metformin (500 mg twice daily), vitamin E (400 IU twice daily), or placebo twice daily for 96 weeks. All three groups received standardized recommendations regarding lifestyle modifications, use of other medications, alcohol avoidance, and management of comorbid illnesses. The primary outcome was sustained reduction in alanine aminotransferase (ALT) level defined as reduction in serum ALT levels to below 50% of the baseline values or into the normal range (40 U/L or less) during the last 48 weeks of treatment. Secondary histologic outcomes included changes in the total NAFLD activity score and individual histological features, and the resolution of NASH. Disappointingly, neither vitamin E nor metformin was superior to placebo in achieving sustained ALT reduction or in improving steatosis, lobular inflammation or fibrosis scores. The only histologic feature of NASH that improved with both medications was ballooning. Compared to placebo, only vitamin E significantly improved the NAFLD activity score and was associated with improved resolution of NASH on the repeat liver biopsy (58% vs. 28%; P value of 0.006). The authors suggested that vitamin E should be considered in a subset of children with biopsy-proven NASH, and incorporated into guidelines by the AGA, AASLD, and ACG.

An open-label pilot study of vitamin E in 11 children with suspected NAFLD based on ultrasound showed improvement in serum aminotransferases in all subjects, without concomitant weight loss.

2.1.4.5 CYSTEAMINE

The NASH CRN recently published the results of a RCT (ClinicalTrials.gov no: NCT01529268) comparing Cysteamine bitartrate delayed release (CBDR) with placebo in 169 children. One year of CBDR did not reduce overall histologic markers of NAFLD compared with placebo in children. There was no significant difference between groups in the primary outcome (28% of children in the CBDR group vs 22% in the placebo group; RR, 1.3; 95% confidence interval [CI], 0.8–2.1; P = .34). However, children receiving CBDR had significant changes in pre-specified secondary outcomes: reduced mean levels of alanine aminotransferase (reduction, 53 ± 88 U/L vs 8 ± 77 U/L in the placebo group; P = .02) and aspartate aminotransferase (reduction, 31 ± 52 vs 4 ± 36 U/L in the placebo group; P = .008), and a larger proportion had reduced lobular inflammation (36% in the CBDR group vs 21% in the placebo group; RR, 1.8; 95% CI, 1.1–2.9; P = .03).

Summary

NAFLD is a highly prevalent disease in children with adverse clinical outcomes documented in young adults. While definitive longer term natural history data for clinical outcome data is still lacking, given the common occurrence of fibrosis in children, the high incidence of type II diabetes in children with fatty liver and the established natural history data for NAFLD in adults demonstrating early type II diabetes, cardiovascular disease and progression to cirrhosis in the setting of fibrosis, there is consensus that children with NAFLD and inflammation or fibrosis should be treated. Current standard of care treatment is lifestyle changes and consideration of vitamin E, however only a minority of children (~30%) with NAFLD have histologic improvement with lifestyle changes alone. Thus, treatments that have the potential to improve the liver and insulin resistance should be sought for children with NAFLD.

2.2 BACKGROUND

Evidence for Use of Losartan Potassium in Adult Hypertension

The antihypertensive effects of losartan potassium were demonstrated principally in 4 placebo-controlled, 6 to 12 week trials of dosages from 10 to 150 mg per day in patients with baseline diastolic blood pressures of 95 to 115 mmHg. The studies allowed comparisons of two doses (50 to 100 mg/day) as once-daily or twice-daily regimens, comparisons of

peak and trough effects, and comparisons of response by gender, age, and race. Three additional studies examined the antihypertensive effects of Losartan and hydrochlorothiazide in combination.

The 4 studies of Losartan monotherapy included a total of 1,075 patients randomized to several doses of Losartan and 334 to placebo. The 10 mg and 25 mg doses produced some effect at peak (6 hours after dosing) but small and inconsistent trough (24 hour) responses. Doses of 50, 100, and 150 mg once daily gave statistically significant systolic/diastolic mean decreases in blood pressure, compared to placebo in the range of 5.5 to 10.5/3.5 to 7.5 mmHg, with the 150 mg dose giving no greater effect than 50 to 100 mg. Twice-daily dosing at 50 to 100 mg/day gave consistently larger trough responses than once-daily dosing at the same total dose. Peak (6 hour) effects were uniformly, but moderately, larger than trough effects, with the trough-to-peak ratio for systolic and diastolic responses 50 to 95% and 60 to 90%, respectively.

Addition of a low dose of hydrochlorothiazide (12.5 mg) to Losartan 50 mg once daily resulted in placebo-adjusted blood pressure reductions of 15.5/9.2 mmHg.

Analysis of age, gender, and race subgroups of patients showed that men and women, and patients over and under 65, had generally similar responses. Losartan potassium was effective in reducing blood pressure regardless of race, although the effect was somewhat less in Black patients (usually a low-renin population).

Evidence for Use of Losartan potassium in Pediatric Hypertension

The antihypertensive effect of losartan was studied in one trial enrolling 177 hypertensive pediatric patients aged 6 to 16 years old. Children who weighed <50 kg received 2.5, 25, or 50 mg of losartan daily and patients who weighed ≥50 kg received 5, 50, or 100 mg of losartan daily. Children in the lowest dose group were given losartan in a suspension formulation [see Dosage and Administration (2.1)]. The majority of the children had hypertension associated with renal and urogenital disease. The sitting diastolic blood pressure (SiDBP) on entry into the study was higher than the 95th percentile level for the patient's age, gender, and height. At the end of three weeks, losartan reduced systolic and diastolic blood pressure, measured at trough, in a dose-dependent manner. Overall, the two higher doses (25 to 50 mg in patients <50 kg; 50 to 100 mg in patients ≥50 kg) reduced diastolic blood pressure by 5 to 6 mmHg more than the lowest dose used (2.5 mg in patients <50 kg; 5 mg in patients ≥50 kg). The lowest dose, corresponding to an average daily dose of 0.07 mg/kg, did not appear to offer consistent antihypertensive efficacy. When patients were randomized to continue losartan at the two higher doses or to placebo after 3 weeks of therapy, trough diastolic blood pressure rose in patients on placebo between 5 and 7 mmHg more than patients randomized to continuing losartan. When the low dose of losartan was randomly withdrawn, the rise in trough diastolic blood pressure was the same in patients receiving placebo and in those continuing losartan, again suggesting that the lowest dose did not have significant antihypertensive efficacy. Overall, no significant differences in the overall antihypertensive effect of losartan were detected when the patients were analyzed according to age (<, ≥12 years old) or gender. While blood pressure was reduced in all racial subgroups examined, too few non-White patients were enrolled to compare the dose-response of losartan in the non-White subgroup.

Animal model support for losartan potassium as a treatment for NAFLD

A wide range of animal model work has demonstrated potential of losartan as a treatment for liver diseases.[50-63] The renin-angiotensin system (RAS) is an enzymatic cascade in which renin cleaves angiotensinogen to form angiotensin I, which is then transformed to angiotensin II by angiotensin-converting enzyme (ACE). The contribution of RAS in NAFLD and NASH has been investigated extensively (reviewed in[64]) and may include effects on insulin receptors, effects on

adipogenesis, cytokine modulation and local hepatocellular regulation[64]. Both ACE inhibitors and angiotensin receptor blockers (ARBs) antagonize RAS and suppress angiotensin II. For example, in a 12 week mice model experiment, both losartan and telmisartan benefited insulin resistance and decreased plasminogen activator inhibitor-1 (PAI-1) gene expression.[57] However, the data are somewhat mixed because in a choline-deficient diet model and the carbon tetrachloride model, losartan alone did not protect against liver injury or fibrogenic events.[50, 52] A larger experiment using rats and choline deficient L-amino acid defined diet did show protection of the liver when combined with ursodeoxycholic acid.[65]

Evidence for Utilization of losartan in NAFLD in Humans

There are a number of studies and articles reporting on use of losartan in NAFLD.[61, 66-72] In humans, ARBs, including losartan, appear to have a superior effect on insulin sensitivity and two meta-analyses have found that ARBs improved insulin sensitivity and reduced incidence of type II diabetes.[73, 74] The data for liver disease are mixed. A large retrospective review of diabetic patients treated with both ACEs and ARBs demonstrated a significant association with reduced fibrosis.[75] But in an RCT treating 137 subjects with biopsy proven NASH, the combination of rosiglitazone and losartan was not superior to rosiglitazone and metformin.[72] The Fatty Liver Protection Trial by Telmisartan or Losartan Study (FANTASY) compared Telmistartan to losartan and neither improved ALT significantly. However, ALT levels were relatively low at baseline and a low dose of losartan was utilized (50 mg once a day).[68] A recently reported RCT in adults also proposed to test 50 mg of losartan daily compared to placebo however the trial was not completed due to difficulties with recruiting.[69]

Plasminogen activator inhibitor-1 (PAI-1) modulation as the possible liver relevant target of losartan potassium

Plasminogen activator inhibitor-1 (PAI-1) is an acute-phase protein that is found to be increased in insulin resistance state and inflammation and injury.[76] More recently, it has been linked to the initiation and progression of liver disease of various etiologies[77] including NAFLD. Our previous data and work by others have shown that PAI-1 is elevated in both adults and children with significant hepatic steatosis[78-80] and, furthermore, correlates with hepatic inflammation levels as well as fibrosis stages.[81] The renin-angiotensin system (RAS) has been suggested to be involved in the pathways of liver damage and might play a critical role in the pathogenesis of NAFLD.[82] Importantly, blockage of the RAS significantly inhibits the expression of PAI-1 in the liver.[57] Angiotensin receptor blockers (ARB), a class of medications that antagonize the angiotensin receptor and suppress RAS, have been proposed as a novel treatment of NAFLD in part because they decrease PAI-1 but also because they improve insulin resistance.

The underlying mechanisms of losartan in modulating lipid and insulin metabolism is only partially understood. One of the hypotheses is that the effect on insulin sensitivity is through the inhibition of PAI-1 production and consequently restoration of hepatocyte growth factor (HGF) activity. PAI-1 overexpression has been found in steatotic livers[78, 79] and upregulated PAI-1 may inhibit the maturation of pro-HGF to HGF, which fails to activate the HGF receptor (HGFR) and thus has two major downstream effects. First, disrupted HGF-HGFR interaction leads to decreased apolipoprotein (apoB) expression and microsomal triglyceride transfer protein (MTTP) activity in the liver, both of which are responsible for increased accumulation of triglyceride in the liver and a compensatory increase in very low density lipoprotein (VLDL) to shuttle the greater amount of triglyceride from hepatocytes into the circulation.[80] Second, inactivated HGF-HGFR axis is unable to interact with insulin substrate receptor thus inhibiting insulin signaling pathway and dysregulating glucose metabolism.[81] In a PAI-1 knockout mouse model, diet-induced hepatic steatosis was attenuated by ~50% and it was associated with a significant increase in HGFR activity, hepatic expression of apoB and activity of MTTP.[80] Losartan has been shown to reduce hepatic PAI-1 gene expression by ~42% and ameliorate fatty liver in an experimental model of

NAFLD.[57] Taken together, the effect of losartan on improving hypertriglyceridemia and insulin sensitivity that we found in the study participants may result from modulation of the activation of HGF through blockage of PAI-1.

Safety Data of Losartan Potassium in Pediatric NAFLD

Vos et al conducted a randomized, double-blind, placebo-controlled, crossover study (phase 1/2). It was approved by the Emory University Institutional Review Board and listed on Clinical Trials.gov (NCT01913470). Adolescents with biopsy proven NASH, who failed to normalize liver enzymes with conventional therapy (lifestyle changes), were consented and assessed for enrollment eligibility at the screening visit (week -4). After a 4-week stabilization period, subjects were randomized to losartan or placebo for 8 weeks followed by a 6-week washout and then the alternate therapy for 8 weeks. Participant's study visits were at weeks 4, 8, 12, 14, 22, and 28 for safety monitoring as well as intermediate efficacy. Fasting blood samples were collected (typically between 7-9am) at weeks 0, 8, 14, and 22. All participants continued their usual healthy diet and exercise as recommended by their NAFLD physician and were asked not to make major changes during the study. The inclusion criteria were age 11-19 years at enrollment; body weight ≥ 62.5 kg; BMI > 85th percentile for age and gender; history of definite or borderline NASH based upon histology using NASH CRN criteria[82]; ALT ≥ 3 times normal (69 U/L for girls, 78 U/L for boys) at enrollment; and at least 2 months of attempted lifestyle changes after liver biopsy. Exclusion criteria were history of cirrhosis and liver synthetic dysfunction (INR ≥ 1.5); history of hypotension; diagnosis of diabetes (or fasting glucose > 125 mg/dl); renal insufficiency (GFR < 30); any other chronic disease requiring daily medication (except medications for acid reflux, allergies or asthma); acute illness within past 2 weeks prior to enrollment (fever > 100.4°F); and anti-oxidant therapy or supplement within past 4 weeks before enrollment. Patients started losartan or identical placebo pills at 25 mg per day for 1 week and 50 mg for 7 weeks, and then the alternate therapy for 8 weeks (25 mg daily for 1 week and 50 mg daily for 7 weeks) after a 6-week washout. The primary side effect associated with losartan is reduction of blood pressure. To ensure safety, parents were provided and taught to use an automated blood pressure cuff for home monitoring of blood pressure.

The results of this pilot study were reported in abstract form at DDW 2016.[88] In brief, the findings are as follows. Twelve children were enrolled with 9 subjects completing all study visits. One participant was lost to follow-up, one withdrew, and one was withdrawn due to noncompliance. 67% of subjects were Hispanic with mean age of 14 years and BMI z-score of 2.32. There were no serious adverse events reported and other adverse events are reported in Table 1.

Table 1. Descriptive list of adverse events during study.

	Number of events		
	Losartan treatment	**Placebo Treatment**	**Screening, washout, follow-up**
Respiratory related	9	8	5
Abdominal Pain	1	3	2
Nausea	1	3	1
Dizziness	4	2	2
Chest Pains	2	1	0
Muscle Pain	3	2	2

Respiratory related events include, upper respiratory infections, nasal congestions, sinus infection and cough.
Number of events shows number of total events, some of which may have occurred in one subject or multiple subjects.
Placebo treatment only includes subjects that did not have carry-over effects from losartan (starting losartan treatment first).

Analysis of this pilot study demonstrated that there was an unexpected, significant carry-over effect of losartan in the group that was randomized to losartan treatment first. However, importantly losartan had no safety issues and there were no changes in blood pressure observed within and between groups for the duration of the study (Table 2).

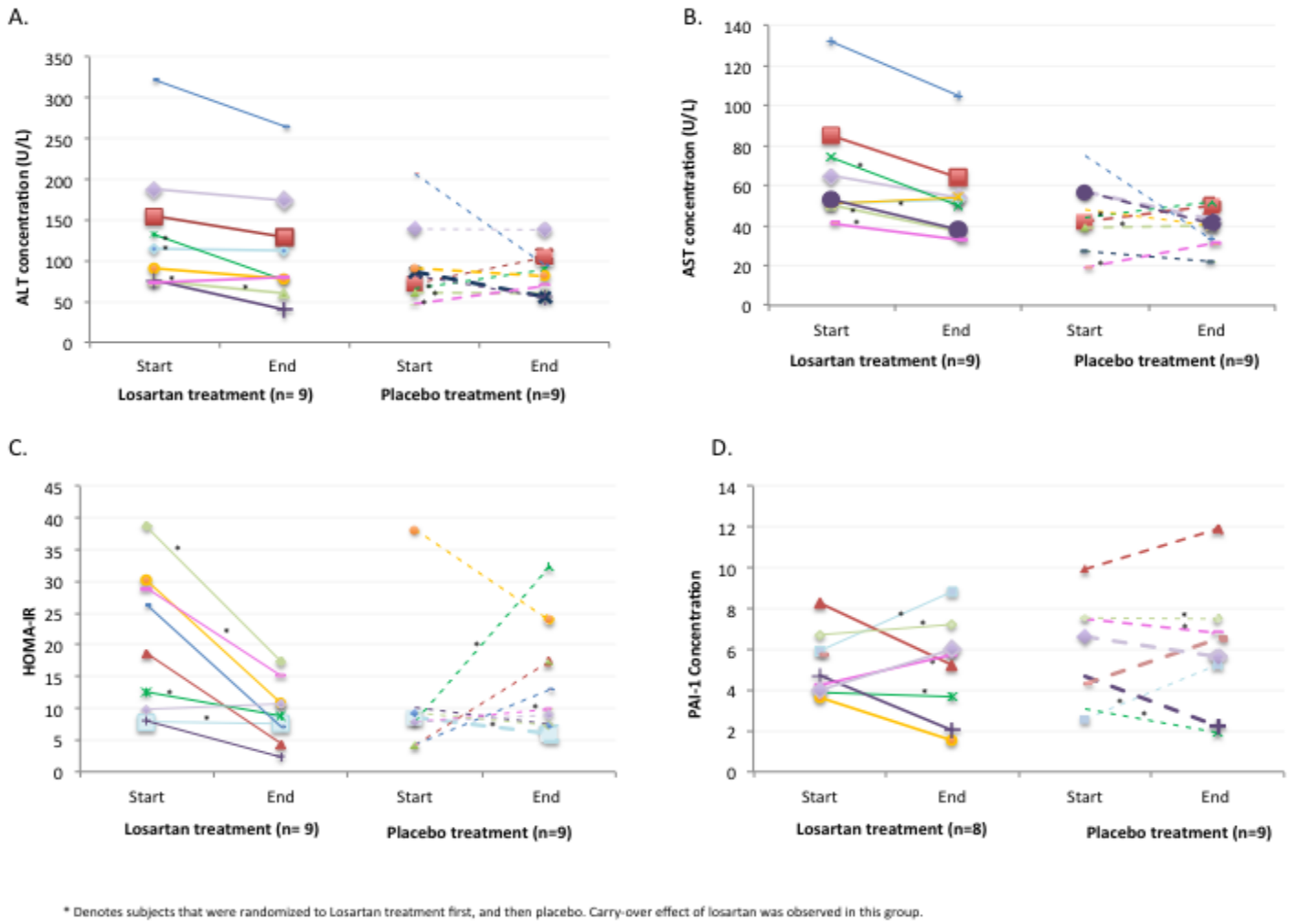
Table 2. Blood Pressure monitoring

Arm/Group Title	**Losartan treatment**	**Placebo treatment**
Baseline SBP	122.8 ± 11.58	118.6 ± 6.32
End of treatment SBP	117.0 ± 16.73	118.2 ± 3.37
Baseline DBP	71.6 ± 7.84	71.6 ± 5.97
End of treatment DBP	71.4 ± 10.37	68.7 ±6.62

Placebo treatment only includes subjects that did not have carry-over effects from losartan (starting losartan treatment first). Data is presented as mean ± S.D., unless indicated otherwise.

SBP, Systolic Blood Pressure; **DBP**, diastolic Blood Pressure

Changes in ALT, AST and HOMA-IR over 8 weeks of treatment are shown for individual subjects with baseline and end values visualized in Figure 1.



* Denotes subjects that were randomized to Losartan treatment first, and then placebo. Carry-over effect of losartan was observed in this group.

Figure 1 (A-D): During losartan treatment, more participants decreased ALT compared to during the placebo time period. A similar pattern was seen in HOMA-IR. The change in PAI-1 from baseline to end of treatment was not different between treatment periods. In conclusion, this pilot study of losartan potassium in children with NAFLD using a dose of 50 mg demonstrated safety and some signals of preliminary efficacy, however we suspect the dose was insufficient given the mean BMI z-score of 2.32.

Importance of this Trial and Controversies in Pediatric NAFLD Treatment

NAFLD has quickly become the most common liver disease in children and it is apparent that treatment with lifestyle alone is insufficient to resolve the condition in children with advanced disease.[37] Thus, efficacious and safe medications are needed. Losartan is a relatively inexpensive medication, widely available that has an established safety record in children and adults. Further, there are preliminary safety data for losartan in children with NAFLD.

There remains controversy regarding which children with NAFLD will require medications in addition to the standard of care treatment of healthy lifestyle. It is uncertain at this point, due to a lack of long term data, which children are at higher risk of advancing to clinical outcomes such as type II diabetes, portal hypertension, cirrhosis and hepatocellular carcinoma. In adults, it appears that fibrosis on liver biopsy is the most consistent risk factor for future liver related outcomes.[89] However, in children this has not been demonstrated. In children, the most rapid clinical outcome is likely to be progression to type II diabetes which has been shown to be strongly associated with NAFLD.[90] Thus, therapeutics that improve both insulin resistance and liver histologic damage are highly desirable.

A controversy in NAFLD clinical trials is the selection of the primary outcome because no surrogate markers are validated to predict future clinical outcomes of feeling, function, or survival. Typically, either ALT or histology is used as the primary outcome and for shorter phase 2 trials, ALT is well accepted as a surrogate marker of improvement of liver inflammation in children with NAFLD.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

- Hypotension – Losartan is an antihypertensive approved in children and the children in the trial may or may not have elevated blood pressures. This side effect is worse with co-treatment of other antihypertensives.
- Hyperkalemia – is a rare reported side effect of losartan. This side effect may be worse with co-treatment of other antihypertensives, with other drugs affecting renal function, or with use of potassium supplementation.
- Acidosis – is a rare side effect of losartan also worse in the setting of co-treatment.
- Renal function decline – is a rare reported side effect of losartan. This is worse with co-treatment with other drugs affecting renal function.
- Harm to unborn child
- Blood draws

2.3.2 KNOWN POTENTIAL BENEFITS

Immediate potential benefits to individual participants in this trial are twofold. First, all participants will receive lifestyle counseling at baseline which is the standard of care for pediatric NAFLD. Lifestyle counseling will be standardized and based upon the established protocols of the NASH CRN. Second, losartan is well established as a medication that improves insulin resistance in children, and insulin resistance is strongly associated with pediatric NAFLD; perhaps even fundamental in the mechanisms perpetuating the dysregulated lipid and inflammatory physiology driving NASH. If losartan reduces insulin resistance, inflammation and PAI-1, this will improve the histologic injury and potentially reduce progression to type II diabetes in children with NAFLD.

Long range potential benefits include to reduce progression to type II diabetes, cirrhosis, cardiovascular disease, and hepatocellular carcinoma in children with pediatric NAFLD. If losartan is effective in improving ALT and insulin resistance, a longer term, histology-based trial will be planned to test effectiveness on histologic improvement. If it is proven to be efficacious, this will benefit children across the world because losartan is a relatively inexpensive medication, available as a generic that is known to be safe for long term use in children with hypertension as well as other pediatric disorders. This medication could be used to decrease the prevalence of progression to clinical outcomes in children with NAFLD.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Rationale for necessity of exposing participants to risk and minimization of risks: Risks to the patients in this study include that losartan potassium can rarely cause hyperkalemia, hypotension, acidosis, and renal function deterioration. Risks are minimized by avoiding other medications that increase risk of hyperkalemia, acidosis and renal deterioration, and hypotension and by close monitoring. Risks are also minimized in this trial by using ALT as a surrogate marker of histologic response, thus avoiding the need for liver biopsies in this phase 2 study. The number of time points for blood

draws was minimized to decrease potential harm from blood draws and inconvenience to the families regarding missing work and school.

- **Hypotension:** All children will be monitored using blood pressure at the screening visit and randomization visit. Each participant will be given a home blood pressure cuff and blood pressure log at the randomization visit. They will be asked to check blood pressure each morning for the first 14 days (7 days after starting the initial dose and 7 days after increase to maximum dose. The logs will be reviewed at each site and checked for hypotension. Participants will be instructed to call for any blood pressures under 90/60 and for symptoms of hypotension (dizziness, fainting, lightheadedness).

- **Hyperkalemia**: The risk of harm has been minimized by treating children with an established dose known to be safe in children with Marfan Syndrome, renal disorders and in children with hypertension. Further, the protocol has been designed to assess potassium levels at 1 month, 3 months and at completion of the treatment phase (6 months). Use of other medications that also increase potassium are prohibited in the trial. Finally, the DSMB will review the potassium levels at each time point to assess differences between the treatment and placebo group to detect trends.

- **Acidosis**: The risk of harm has been minimized by treating children with an established dose known to be safe in children with Marfan Syndrome, renal disorders, and in children with hypertension. Further, the protocol has been designed to assess CO_2 levels at 1 month, 3 months and at completion (6 months). Use of other medications that also enhance acidosis are prohibited in the trial. Finally, the DSMB will review the CO_2 at each time point to assess differences between the treatment and placebo group to detect trends.

- **Renal function deterioration:** All children will be monitored closely using labs to follow renal function (using creatinine). Methods to decrease risk include only enrolling children with normal renal function at baseline, excluding usage of other medications that can harm the kidneys, and encouraging good hydration.

- **Blood draws**: All children will be enrolled at experienced pediatric sites and established techniques will be used to minimize bleeding, infection, and pain/discomfort with blood draws.

Justification of the risks: All children will receive standard of care as part of the trial and will potentially benefit if they are in the treatment group (losartan) by improvement in ALT and insulin resistance. The value of the information is high because all of the children in the study have confirmed NAFLD and are in need of treatment beyond lifestyle changes. By conducting the trial and furthering the understanding of losartan for pediatric NAFLD, all of the children in the study stand to benefit in the future if losartan is proven to be effective.

3 OBJECTIVES AND OUTCOMES

OBJECTIVES	OUTCOMES	JUSTIFICATION FOR OUTCOMES
Primary		
To determine whether 24 weeks of treatment with losartan compared to treatment with placebo improves measures of nonalcoholic fatty liver disease (NAFLD)	*Change in ALT from screening to 24 weeks*	*ALT is the most accepted short term (<12 months) surrogate marker for histologic improvement in NAFLD*
Secondary		
The secondary objective(s) have been selected to provide further information on whether 24 weeks of treatment with losartan compared to treatment with placebo improves measures of liver indices, insulin resistance, dyslipidemia and body size.	*Relative change in ALT from screening to 24 weeks* *Proportion of patients achieving normalization of ALT at 24 weeks* *Change in AST and GGT from screening to 24 weeks* *Mean ALT (12 and 24 weeks) compared to baseline* *Change in fasting markers of insulin resistance (HOMA-IR) from screening to 24 weeks* *Change in anthropometric measurements (weight, BMI z-score, waist to hip ratio, waist circumference) from screening to 24 weeks* *Change in serum lipid profiles from screening to 24 weeks* *Change in C-reactive protein (serum marker of inflammation) from screening to 24 weeks* *Change in Health-related Quality of Life (HR-QoL) scores from screening to 24 weeks* *Change in frequency of adverse events from screening to 24 weeks*	*These outcomes were selected because they measure important co-morbidities of NAFLD and are useful for determining if losartan improves other features commonly associated with NAFLD. Safety and quality of life outcomes are also included.*
Tertiary/Exploratory	Change from screening to 24 weeks:	
Tertiary/exploratory objective(s) were selected to serve as a basis for explaining or supporting findings of primary analyses and for suggesting further hypotheses for later research.	*Change in peripheral proinflammatory cytokine levels (including IL-6, TNF, TGF-beta) from baseline to 24 weeks.* *Change in NMR MetaboProfile Analysis (LP4) from baseline to 24 weeks.*	*Inflammation and oxidative stress are considered major drivers of NAFLD and improvement in these would support further development of losartan for NAFLD.*

4 STUDY DESIGN

4.1 OVERALL DESIGN

- The hypothesis of the trial is that 24 weeks of treatment with losartan potassium in children with NAFLD will improve the ALT compared to screening values.
- Phase 2a
- A Multicenter, randomized, double-masked, placebo-controlled, parallel treatment groups phase 2a trial with 24-week change from baseline in serum ALT as the primary outcome.
- Methods to decrease bias will include placebo control, double masking and randomization.
- Two study arms
- Multisite
- Study intervention is losartan potassium (100 mg orally once per day) or matching placebo.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

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

Rationale for a Placebo Group: NAFLD is a highly variable disease with a high placebo response rate of 10-30%[91, 92], likely in part because of the ongoing standard of care treatment of diet and exercise during all trials. Because of this, it is important to have a placebo control group to allow discernment of treatment effect over and above lifestyle changes. Lifestyle interventions are considered the standard treatment for NAFLD, but are often not available to children with NAFLD due to both the lack of available care and limitations of insurance coverage for lifestyle interventions. In this study we will provide all participants, including those receiving placebo with a standardized nutrition and exercise intervention consistent with the recommendations of the American Academy of Pediatrics. Thus, all children will receive treatment.

Currently, there are no FDA approved therapies for NAFLD in children. Medications that are being investigated for the treatment of NAFLD cannot be compared with an active alternative treatment arm. In order to assess the efficacy of an agent in NAFLD, a placebo-arm is needed to determine its relative efficacy in improving liver histology beyond that achieved with a placebo. Previous non-randomized and pilot studies have shown the efficacy of several agents such as ursodiol and betaine in the treatment of NAFLD, but follow-up randomized-placebo-controlled studies failed to show improvement in liver histology beyond that observed in placebo groups.[85, 86] In order to have the highest quality of evidence to test our hypothesis, the STOP-NAFLD trial utilizes a randomized, double-masked, placebo-controlled study design (*http://www.ahrq.gov/clinic/uspstf/grades.htm*). As there is no proven pharmacologic therapy for NAFLD in children, using a placebo for comparative purposes is justified.

The trial is double masked to prevent bias by investigators and participants. The trial is 24 weeks because significant differences in ALT have been seen at 24 weeks in patients who go on to have histologic improvement[84], and thus ALT change at 24 weeks is widely accepted as a surrogate marker of future histologic benefit and future clinical benefit for patients.[91, 92]

The CyNCh trial data below show improvements in ALT were evident as soon as 12 weeks and were even greater at 24 weeks of treatment.

CyNCh Trial: Changes in ALT (U/L) at 12 and 24 weeks by treatment group

Change in ALT (U/L) from baseline – mean (SD)	Cysteamine (N=88)	Placebo (N=81)	Total (N=169)	P
12 weeks	-54 (88)	-9 (48)	-30 (73)	0.008
24 weeks	-55 (90)	-10 (54)	-32 (77)	0.03

Rationale for a two year (730 days) maximum duration between liver biopsy and enrollment: Use of a historical liver biopsy (up to 730 days prior to enrollment) ensures that all patients have a liver biopsy based diagnosis. The primary outcome is ALT, and this will be obtained at screening to ensure that it is elevated, suggesting ongoing inflammation in the liver.

Treatment duration-rationale for 24 weeks: An ideal duration of a treatment should only expose participants to a study drug long enough to show meaningful improvement in ALT if it is going to occur. This enables any positive findings to be reported as soon as possible.

4.3 JUSTIFICATION FOR DOSE

The usual dose of losartan potassium in children is orally administered and a starting dose of 0.7 mg per kg daily (up to 50 mg total for starting dose). The upper limit is 1.4 mg/kg or 100 mg daily. The doses proposed in this trial will range from 0.7 mg/kg/day to 1.4 mg/kg/day. The previous Vos pilot study of losartan potassium in children with NAFLD using a dose of 50 mg demonstrated safety and some signals of preliminary efficacy, however we suspect the dose was insufficient given the mean BMI z-score of 2.32.

The table below illustrates the dose ranges proposed and the distribution of weights in patients who meet the biopsy eligibility criteria in the current NASH CRN Pediatric Database 2 study.

Participant weight (kg)	50 mg Dose	100 mg Dose	Eligibility Criteria	Current biopsy eligible Pediatric DB2 participants N=79
	mg/kg/day	mg/kg/day		
<40			**Exclude if <70 kg** **50 mg dose (0.8 mg/kg-1.3 mg/kg)**	N=25
40	1.3			
45	1.1			
50	1.0			
55	0.9			
60	0.8	1.7		
65	0.8	1.5		
70	0.7	1.4		
75		1.3		
80		1.3		
85		1.2		
90		1.1		
95		1.1		

100		1.0	**≥70 kg to <150 kg weight** **100 mg dose (0.7-1.4mg/kg)**	**N=53**
105		1.0		
110		0.9		
115		0.9		
120		0.8		
125		0.8		
130		0.8		
135		0.7		
140		0.7		
145		0.7		
150		0.7		
>150			**Exclude if kg >=150**	**N=1**

4.4 END OF STUDY DEFINITION

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

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

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

- Age 8-17 years at initial screening interview
- Histological evidence of NAFLD with or without fibrosis and a NAFLD activity score (NAS) of ≥3, on a liver biopsy obtained no more than 730 days prior to enrollment.
- Serum ALT at screening ≥ 50 IU/L

5.2 EXCLUSION CRITERIA

- Body weight less than 70 kg or greater than 150 kg at screening
- Significant alcohol consumption or inability to reliably quantify alcohol intake
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, other known hepatotoxins) for more than 2 consecutive weeks in the past year prior to randomization
- New treatment with vitamin E or metformin started in the past 90 days or plans to alter the dose or stop over the next the 24 weeks. A stable dose is acceptable.
- Prior or planned bariatric surgery
- Uncontrolled diabetes (HbA1c 9.5% or higher)
- Presence of cirrhosis on liver biopsy
- History of hypotension or history of orthostatic hypotension
- Stage 2 Hypertension or >140 systolic **or** >90 diastolic at screening
- Current treatment with any antihypertensive medications including all angiotensin converting enzyme (ACE) inhibitors or aliskiren
- Current treatment with potassium supplements or any drug known to increase potassium

- Current daily use of NSAIDs
- Current treatment with lithium
- Platelet counts below 100,000 /mm^3
- Clinical evidence of hepatic decompensation (serum albumin < 3.2 g/dL, INR >1.3, direct bilirubin >1.3 mg/dL, history of esophageal varices, ascites, or hepatic encephalopathy)
- Evidence of chronic liver disease other than NAFLD:
  - Biopsy consistent with histological evidence of autoimmune hepatitis
  - Serum hepatitis B surface antigen (HBsAg) positive.
  - Serum hepatitis C antibody (anti-HCV) positive.
  - Iron/total iron binding capacity (TIBC) ratio (transferrin saturation) > 45% with histological evidence of iron overload
  - Alpha-1-antitrypsin (A1AT) phenotype/genotype ZZ or SZ
  - Wilson's disease
- Serum alanine aminotransferase (ALT) greater than 300 IU/L
- History of biliary diversion
- History of kidney disease and/or eGFR < than 60 mL/min/1.73 m^2 using Schwartz Bedside GFR Calculator for Children IDMS-traceable (link)
- Known Human Immunodeficiency Virus infection
- Active, serious medical disease with life expectancy less than 5 years
- Active substance abuse including inhaled or injected drugs, in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in any clinical/investigational trial within the prior 150 days and during the STOP-NAFLD Trial.
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Inability to swallow capsules
- Known allergy to losartan potassium or other angiotensin receptor blocker
- Failure of parent or legal guardian to give informed consent or subject to give informed assent

5.3 LIFESTYLE CONSIDERATIONS

5.3.1 STANDARD TREATMENT RECOMMENDATIONS

The use of prescription or non-prescription medicines or herbal remedies or dietary supplements, consumption of alcohol, and management of various co-morbid illnesses will be discussed with the patients. These recommendations have been prepared by the NASH CRN Pediatric Standard of Care Committee and are approved by the NASH CRN Steering Committee to be applied across all study sites. This will help ensure that the patients in both groups receive the same standard of care treatment for NAFLD.

Enhanced Lifestyle Intervention

All children, including those in the placebo group will receive an intervention in the form of standardized lifestyle intervention recommendations consistent with the latest recommendations from the American Academy of Pediatrics (AAP). Assessing the impact of such an intervention on NAFLD will be important. Attaining a healthy weight is the

cornerstone of current treatment of pediatric NAFLD, given the lack of proven pharmaceutical therapy in this age group and the strong association of NAFLD with excess adiposity, in particular central adiposity.[87] Weight loss has been associated with improvements in liver enzymes and histology in adults with NAFLD, while weight loss in children with NAFLD has been shown to improve serum aminotransferase levels in small pilot studies.[36, 88, 89] Therefore, lifestyle intervention through changes in diet and exercise will be encouraged for participants in both the placebo and active study drug treatment group of the STOP-NAFLD trial, as this represents the current standard of care for children.

The standard of care lifestyle intervention designed for this trial will incorporate components of the AAP's 2007 Expert Committee Recommendations Regarding the Prevention, Assessment, and Treatment of Child and Adolescent Overweight and Obesity that can be reproduced across the study sites.[90] STOP-NAFLD Trial participants will be given written materials that will include evidence-based strategies to achieve a healthier diet and increase physical activity, as endorsed by the American Academy of Pediatrics as well as the CDC and NIH.[90-92] A family-based, patient-centered and stepped approach to making lifestyle changes will be employed as recommended by the AAP.[90] Accordingly, the lifestyle materials will be reviewed with trial participants and their family members at each study visit and they will be encouraged to select 1-2 goals which they consider personally obtainable and that they can commit to pursuing in the interval until their next study visit.

Topics to be covered in the materials will include:

1) Reduce fat intake and sugar intake to 0 servings per day
2) Reduce screen time to 2 hours or less per day
3) Increase physical activity to 1 hour or more per day
4) Increase fruits and vegetable intake to 5 or more servings per day
5) Reduce fast food intake and make healthier choices when eating out

These topics and the specific strategies to be included in the lifestyle intervention materials are in accordance with healthy weight strategies currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health National Heart Lung and Blood Institute's "We Can" program.[91-94] Therefore, we will also include references in the study's lifestyle intervention materials to these freely accessible, federal government sponsored websites so that participants and their families can easily access additional information on these lifestyle changes and strategies to achieve them.

5.4 SCREEN FAILURES

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

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a low ALT may be rescreened once. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

5.5.1 RECRUITMENT

Approximately 110 participants in 2 groups of equal size (55 per group) will be recruited at the ten participating clinical centers of the NASH CRN (averaging 11 patients per center) over a 12-month period.

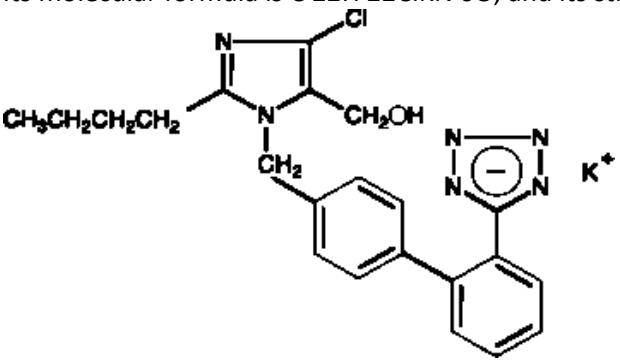
Eligible patients will be identified and recruited at the participating clinical centers subject to the inclusion and exclusion criteria. Clinics will be expected to recruit sufficient overall numbers of minorities and females so that results can be generalized to these populations. Each clinic will develop a recruitment plan. These plans will vary from clinic to clinic depending on the available pools of patients and local recruitment resources and referral patterns.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION FROM FDA PACKAGE INSERT

Losartan potassium tablets USP is an angiotensin II receptor blocker acting on the AT 1 receptor subtype. Losartan potassium, a non-peptide molecule, is chemically described as 2-butyl-4-chloro-1-[p-(o-1 H--tetrazol-5-ylphenyl) benzyl]imidazole-5-methanol monopotassium salt.
Its molecular formula is C 22H 22ClKN 6O, and its structural formula is:



Losartan potassium USP is a white to off-white free-flowing crystalline powder with a molecular weight of 461.01. It is freely soluble in water, soluble in alcohols, and slightly soluble in common organic solvents, such as acetonitrile and methyl ethyl ketone. Oxidation of the 5-hydroxymethyl group on the imidazole ring results in the active metabolite of Losartan.

Losartan potassium is available as tablets for oral administration containing either 25 mg, 50 mg or 100 mg of Losartan potassium USP and the following inactive ingredients: Microcrystalline cellulose, lactose monohydrate, pregelatinized starch, magnesium stearate, Opadry white (hydroxypropyl cellulose, hypromellose, titanium dioxide).

Losartan potassium 25 mg, 50 mg and 100 mg tablets contain potassium in the following amounts: 2.12 mg (0.054 mEq), 4.24 mg (0.108 mEq) and 8.48 mg (0.216 mEq), respectively.

Losartan - Clinical Pharmacology
Mechanism of Action
Angiotensin II [formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II)] is a potent vasoconstrictor, the primary vasoactive hormone of the renin-angiotensin system, and an important component

in the pathophysiology of hypertension. It also stimulates aldosterone secretion by the adrenal cortex. Losartan and its principal active metabolite block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT 1 receptor found in many tissues, (e.g., vascular smooth muscle, adrenal gland). There is also an AT 2 receptor found in many tissues but it is not known to be associated with cardiovascular homeostasis. Neither Losartan nor its principal active metabolite exhibit any partial agonist activity at the AT 1 receptor, and both have much greater affinity (about 1000-fold) for the AT 1 receptor than for the AT 2 receptor. In vitro binding studies indicate that Losartan is a reversible, competitive inhibitor of the AT 1 receptor. The active metabolite is 10 to 40 times more potent by weight than Losartan and appears to be a reversible, non-competitive inhibitor of the AT 1 receptor.

Neither Losartan nor its active metabolite inhibits ACE (kininase II, the enzyme that converts angiotensin I to angiotensin II and degrades bradykinin), nor do they bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation.

Pharmacodynamics
Losartan inhibits the pressor effect of angiotensin II (as well as angiotensin I) infusions. A dose of 100 mg inhibits the pressor effect by about 85% at peak with 25 to 40% inhibition persisting for 24 hours. Removal of the negative feedback of angiotensin II causes a doubling to tripling in plasma renin activity and consequent rise in angiotensin II plasma concentration in hypertensive patients. Losartan does not affect the response to bradykinin, whereas ACE inhibitors increase the response to bradykinin. Aldosterone plasma concentrations fall following Losartan administration. In spite of the effect of Losartan on aldosterone secretion, very little effect on serum potassium was observed.

The effect of Losartan is substantially present within one week but in some studies the maximal effect occurred in 3 to 6 weeks. In long-term follow-up studies (without placebo control) the effect of Losartan appeared to be maintained for up to a year. There is no apparent rebound effect after abrupt withdrawal of Losartan. There was essentially no change in average heart rate in Losartan-treated patients in controlled trials.

Pharmacokinetics
Absorption: Following oral administration, Losartan is well absorbed and undergoes substantial first-pass metabolism. The systemic bioavailability of Losartan is approximately 33%. Mean peak concentrations of Losartan and its active metabolite are reached in 1 hour and in 3 to 4 hours, respectively. While maximum plasma concentrations of Losartan and its active metabolite are approximately equal, the AUC (area under the curve) of the metabolite is about 4 times as great as that of Losartan. A meal slows absorption of Losartan and decreases its C max but has only minor effects on Losartan AUC or on the AUC of the metabolite (~10% decrease). The pharmacokinetics of Losartan and its active metabolite are linear with oral Losartan doses up to 200 mg and do not change over time.

Distribution: The volume of distribution of Losartan and the active metabolite is about 34 liters and 12 liters, respectively. Both Losartan and its active metabolite are highly bound to plasma proteins, primarily albumin, with plasma free fractions of 1.3% and 0.2%, respectively. Plasma protein binding is constant over the concentration range achieved with recommended doses. Studies in rats indicate that Losartan crosses the blood-brain barrier poorly, if at all.

Metabolism: Losartan is an orally active agent that undergoes substantial first-pass metabolism by cytochrome P450 enzymes. It is converted, in part, to an active carboxylic acid metabolite that is responsible for most of the angiotensin II receptor antagonism that follows Losartan treatment. About 14% of an orally-administered dose of Losartan is converted to the active metabolite. In addition to the active carboxylic acid metabolite, several inactive metabolites are formed. In vitro studies indicate that cytochrome P450 2C9 and 3A4 are involved in the biotransformation of Losartan to its metabolites.

Elimination: Total plasma clearance of Losartan and the active metabolite is about 600 mL/min and 50 mL/min, respectively, with renal clearance of about 75 mL/min and 25 mL/min, respectively. The terminal half-life of Losartan is

about 2 hours and of the metabolite is about 6 to 9 hours. After single doses of Losartan administered orally, about 4% of the dose is excreted unchanged in the urine and about 6% is excreted in urine as active metabolite. Biliary excretion contributes to the elimination of Losartan and its metabolites. Following oral 14C-labeled Losartan, about 35% of radioactivity is recovered in the urine and about 60% in the feces. Following an intravenous dose of 14C-labeled Losartan, about 45% of radioactivity is recovered in the urine and 50% in the feces. Neither Losartan nor its metabolite accumulates in plasma upon repeated once-daily dosing.

Special Populations
Pediatric: Pharmacokinetic parameters after multiple doses of Losartan (average dose 0.7 mg/kg, range 0.36 to 0.97 mg/kg) as a tablet to 25 hypertensive patients aged 6 to 16 years are shown in Table 4 below. Pharmacokinetics of Losartan and its active metabolite were generally similar across the studied age groups and similar to historical pharmacokinetic data in adults. The principal pharmacokinetic parameters in adults and children are shown in the table below.

Table 2: Pharmacokinetic Parameters in Hypertensive Adults and Children Age 6 to 16 Following Multiple Dosing

	Adults given 50 mg once daily for 7 days N=12		**Age 6 to 16 given 0.7 mg/kg once daily for 7 days N=25**	
	Parent	**Active Metabolite**	**Parent**	**Active Metabolite**
$AUC_{0\ to\ 24}$ (ng•hr/mL) *	442 ± 173	1685 ± 452	368 ± 169	1866 ± 1076
C_{MAX} (ng/mL) *	224 ± 82	212 ± 73	141 ± 88	222 ± 127
$T_{1/2}$ (h) †	2.1 ± 0.70	7.4 ± 2.4	2.3 ± 0.8	5.6 ± 1.2
T_{PEAK} (h) ‡	0.9	3.5	2	4.1
CL_{REN} (mL/min) *	56 ± 23	20 ± 3	53 ± 33	17 ± 8

* Mean ± standard deviation

† Harmonic mean and standard deviation

‡ Median

The bioavailability of the suspension formulation was compared with Losartan tablets in healthy adults. The suspension and tablet are similar in their bioavailability with respect to both Losartan and the active metabolite.

Geriatric and Sex: Losartan pharmacokinetics have been investigated in the elderly (65 to 75 years) and in both genders. Plasma concentrations of Losartan and its active metabolite are similar in elderly and young hypertensives. Plasma concentrations of Losartan were about twice as high in female hypertensives as male hypertensives, but concentrations of the active metabolite were similar in males and females. No dosage adjustment is necessary.

Race: Pharmacokinetic differences due to race have not been studied.

Renal Insufficiency: Following oral administration, plasma concentrations and AUCs of Losartan and its active metabolite are increased by 50 to 90% in patients with mild (creatinine clearance of 50 to 74 mL/min) or moderate (creatinine

clearance 30 to 49 mL/min) renal insufficiency. In this study, renal clearance was reduced by 55 to 85% for both Losartan and its active metabolite in patients with mild or moderate renal insufficiency. Neither Losartan nor its active metabolite can be removed by hemodialysis.

Hepatic Insufficiency: Following oral administration in patients with mild to moderate alcoholic cirrhosis of the liver, plasma concentrations of Losartan and its active metabolite were, respectively, 5-times and about 1.7-times those in young male volunteers. Compared to normal subjects the total plasma clearance of Losartan in patients with hepatic insufficiency was about 50% lower and the oral bioavailability was about doubled. Use a starting dose of 25 mg for patients with mild to moderate hepatic impairment. Losartan potassium has not been studied in patients with severe hepatic impairment.

Drug Interactions
No clinically significant drug interactions have been found in studies of Losartan potassium with hydrochlorothiazide, digoxin, warfarin, cimetidine and phenobarbital. However, rifampin has been shown to decrease the AUC of Losartan and its active metabolite by 30% and 40%, respectively. Fluconazole, an inhibitor of cytochrome P450 2C9, decreased the AUC of the active metabolite by approximately 40%, but increased the AUC of Losartan by approximately 70% following multiple doses. Conversion of Losartan to its active metabolite after intravenous administration is not affected by ketoconazole, an inhibitor of P450 3A4. The AUC of active metabolite following oral Losartan was not affected by erythromycin, an inhibitor of P450 3A4, but the AUC of Losartan was increased by 30%.

The pharmacodynamic consequences of concomitant use of Losartan and inhibitors of P450 2C9 have not been examined. Subjects who do not metabolize Losartan to active metabolite have been shown to have a specific, rare defect in cytochrome P450 2C9. These data suggest that the conversion of Losartan to its active metabolite is mediated primarily by P450 2C9 and not P450 3A4.

Nonclinical Toxicology
Carcinogenesis, Mutagenesis, Impairment of Fertility
Losartan potassium was not carcinogenic when administered at maximally tolerated dosages to rats and mice for 105 and 92 weeks, respectively. Female rats given the highest dose (270 mg/kg/day) had a slightly higher incidence of pancreatic acinar adenoma. The maximally tolerated dosages (270 mg/kg/day in rats, 200 mg/kg/day in mice) provided systemic exposures for Losartan and its pharmacologically active metabolite that were approximately 160 and 90 times (rats) and 30 and 15 times (mice) the exposure of a 50 kg human given 100 mg per day.

Losartan potassium was negative in the microbial mutagenesis and V-79 mammalian cell mutagenesis assays and in the in vitro alkaline elution and in vitro and in vivo chromosomal aberration assays. In addition, the active metabolite showed no evidence of genotoxicity in the microbial mutagenesis, in vitro alkaline elution, and in vitro chromosomal aberration assays.

Fertility and reproductive performance were not affected in studies with male rats given oral doses of Losartan potassium up to approximately 150 mg/kg/day. The administration of toxic dosage levels in females (300/200 mg/kg/day) was associated with a significant ($p<0.05$) decrease in the number of corpora lutea/female, implants/female, and live fetuses/female at C-section. At 100 mg/kg/day only a decrease in the number of corpora lutea/female was observed. The relationship of these findings to drug-treatment is uncertain since there was no effect at these dosage levels on implants/pregnant female, percent post-implantation loss, or live animals/litter at parturition. In nonpregnant rats dosed at 135 mg/kg/day for 7 days, systemic exposure (AUCs) for Losartan and its active metabolite were approximately 66 and 26 times the exposure achieved in man at the maximum recommended human daily dosage (100 mg).

Contraindications
Losartan potassium is contraindicated:

- In patients who are hypersensitive to any component of this product.
- For co-administration with aliskiren in patients with diabetes.

Warnings and Precautions

Fetal Toxicity

Use of drugs that act on the renin-angiotensin system during the second and third trimesters of pregnancy reduces fetal renal function and increases fetal and neonatal morbidity and death. Resulting oligohydramnios can be associated with fetal lung hypoplasia and skeletal deformations. Potential neonatal adverse effects include skull hypoplasia, anuria, hypotension, renal failure, and death. When pregnancy is detected, discontinue Losartan potassium as soon as possible.

Hypotension in Volume- or Salt-Depleted Patients

In patients with an activated renin-angiotensin system, such as volume- or salt-depleted patients (e.g., those being treated with high doses of diuretics), symptomatic hypotension may occur after initiation of treatment with Losartan potassium. Correct volume or salt depletion prior to administration of Losartan potassium.

Renal Function Deterioration

Changes in renal function including acute renal failure can be caused by drugs that inhibit the renin--angiotensin system and by diuretics. Patients whose renal function may depend in part on the activity of the renin-angiotensin system (e.g., patients with renal artery stenosis, chronic kidney disease, severe congestive heart failure, or volume depletion) may be at particular risk of developing acute renal failure on Losartan potassium. Monitor renal function periodically in these patients. Consider withholding or discontinuing therapy in patients who develop a clinically significant decrease in renal function on Losartan potassium.

Hyperkalemia

Monitor serum potassium periodically and treat appropriately. Dosage reduction or discontinuation of Losartan potassium may be required.

Adverse Reactions

Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Hypertension

Losartan potassium has been evaluated for safety in more than 3300 adult patients treated for essential hypertension and 4058 patients/subjects overall. Over 1200 patients were treated for over 6 months and more than 800 for over one year.

Treatment with Losartan potassium was well-tolerated with an overall incidence of adverse events similar to that of placebo. In controlled clinical trials, discontinuation of therapy for adverse events occurred in 2.3% of patients treated with Losartan potassium and 3.7% of patients given placebo. In 4 clinical trials involving over 1000 patients on various doses (10 to 150 mg) of Losartan potassium and over 300 patients given placebo, the adverse events that occurred in ≥2% of patients treated with Losartan potassium and more commonly than placebo were: dizziness (3% vs. 2%), upper respiratory infection (8% vs. 7%), nasal congestion (2% vs. 1%), and back pain (2% vs. 1%).

The following less common adverse reactions have been reported:

Blood and lymphatic system disorders: Anemia.
Psychiatric disorders: Depression.

Nervous system disorders: Somnolence, headache, sleep disorders, paresthesia, migraine.
Ear and labyrinth disorders: Vertigo, tinnitus.
Cardiac disorders: Palpitations, syncope, atrial fibrillation, CVA.
Respiratory, thoracic and mediastinal disorders: Dyspnea.
Gastrointestinal disorders: Abdominal pain, constipation, nausea, vomiting.
Skin and subcutaneous tissue disorders: Urticaria, pruritus, rash, photosensitivity.
Musculoskeletal and connective tissue disorders: Myalgia, arthralgia.
Reproductive system and breast disorders: Impotence.
General disorders and administration site conditions: Edema.

Cough
Persistent dry cough (with an incidence of a few percent) has been associated with ACE-inhibitor use and in practice can be a cause of discontinuation of ACE-inhibitor therapy. Two prospective, parallel-group, double-blind, randomized, controlled trials were conducted to assess the effects of Losartan on the incidence of cough in hypertensive patients who had experienced cough while receiving ACE-inhibitor therapy. Patients who had typical ACE-inhibitor cough when challenged with lisinopril, whose cough disappeared on placebo, were randomized to Losartan 50 mg, lisinopril 20 mg, or either placebo (one study, n=97) or 25 mg hydrochlorothiazide (n=135). The double-blind treatment period lasted up to 8 weeks. These studies demonstrate that the incidence of cough associated with Losartan therapy, in a population that all had cough associated with ACE-inhibitor therapy, is similar to that associated with hydrochlorothiazide or placebo therapy. Cases of cough, including positive re-challenges, have been reported with the use of Losartan in postmarketing experience.

Hypertensive Patients with Left Ventricular Hypertrophy
In the Losartan Intervention for Endpoint (LIFE) study, adverse reactions with Losartan potassium were similar to those reported previously for patients with hypertension.

Nephropathy in Type 2 Diabetic Patients
In the Reduction of Endpoints in NIDDM with the Angiotensin II Receptor Antagonist Losartan (RENAAL) study involving 1513 patients treated with Losartan potassium or placebo, the overall incidences of reported adverse events were similar for the two groups. Discontinuations of Losartan potassium because of side effects were similar to placebo (19% for Losartan potassium, 24% for placebo). The adverse events, regardless of drug relationship, reported with an incidence of ≥4% of patients treated with Losartan potassium and occurring with ≥2% difference in the Losartan group vs. placebo on a background of conventional antihypertensive therapy, were asthenia/fatigue, chest pain, hypotension, orthostatic hypotension, diarrhea, anemia, hyperkalemia, hypoglycemia, back pain, muscular weakness, and urinary tract infection.

Postmarketing Experience
The following additional adverse reactions have been reported in postmarketing experience with Losartan potassium. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to estimate their frequency reliably or to establish a causal relationship to drug exposure:

Digestive: Hepatitis.
General Disorders and Administration Site Conditions: Malaise.
Hematologic: Thrombocytopenia.
Hypersensitivity: Angioedema, including swelling of the larynx and glottis, causing airway obstruction and/or swelling of the face, lips, pharynx, and/or tongue has been reported rarely in patients treated with Losartan; some of these patients previously experienced angioedema with other drugs including ACE inhibitors. Vasculitis, including Henoch-Schönlein purpura, has been reported. Anaphylactic reactions have been reported.
Metabolic and Nutrition: Hyponatremia.
Musculoskeletal: Rhabdomyolysis.

Nervous system disorders: Dysgeusia.
Skin: Erythroderma.

Drug Interactions
Agents Increasing Serum Potassium
Coadministration of Losartan with other drugs that raise serum potassium levels may result in hyperkalemia. Monitor serum potassium in such patients.

Lithium
Increases in serum lithium concentrations and lithium toxicity have been reported during concomitant administration of lithium with angiotensin II receptor antagonists. Monitor serum lithium levels during concomitant use.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Including Selective Cyclooxygenase-2 Inhibitors (COX-2 Inhibitors)
In patients who are elderly, volume-depleted (including those on diuretic therapy), or with compromised renal function, coadministration of NSAIDs, including selective COX-2 inhibitors, with angiotensin II receptor antagonists (including Losartan) may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible. Monitor renal function periodically in patients receiving Losartan and NSAID therapy.

The antihypertensive effect of angiotensin II receptor antagonists, including Losartan, may be attenuated by NSAIDs, including selective COX-2 inhibitors.
Dual Blockade of the Renin-Angiotensin System (RAS)
Dual blockade of the RAS with angiotensin receptor blockers, ACE inhibitors, or aliskiren is associated with increased risks of hypotension, syncope, hyperkalemia, and changes in renal function (including acute renal failure) compared to monotherapy.

The Veterans Affairs Nephropathy in Diabetes (VA NEPHRON-D) trial enrolled 1448 patients with type 2 diabetes, elevated urinary-albumin-to-creatinine ratio, and decreased estimated glomerular filtration rate (GFR 30 to 89.9 mL/min), randomized them to lisinopril or placebo on a background of Losartan therapy and followed them for a median of 2.2 years. Patients receiving the combination of Losartan and lisinopril did not obtain any additional benefit compared to monotherapy for the combined endpoint of decline in GFR, end stage renal disease, or death, but experienced an increased incidence of hyperkalemia and acute kidney injury compared with the monotherapy group.
In most patients no benefit has been associated with using two RAS inhibitors concomitantly. In general, avoid combined use of RAS inhibitors. Closely monitor blood pressure, renal function, and electrolytes in patients on Losartan potassium and other agents that affect the RAS.

Do not co-administer aliskiren with Losartan potassium in patients with diabetes. Avoid use of aliskiren with Losartan potassium in patients with renal impairment (GFR <60 mL/min).

6.1.2 DOSING AND ADMINISTRATION

Treatment administration

- For patients with baseline weight ≥ 70 kg and < 150 kg, start treatment with one capsule of 50 mg of losartan or matching placebo in the morning for 1 week, then increase to two 50 mg capsules in the morning (100 mg total) losartan or matching placebo starting in week 2.

Dose adjustment: If patient is currently taking the higher dose (100 mg losartan or matching placebo), and he/she develops a treatment related CTCAE v5.0 -defined adverse event grade 3 or above, or low blood pressure defined as

either SBP < 90 mmHg **or** DBP < 60 mmHg, reduce dosage to a 50 mg dose (losartan or matching placebo, one capsule each morning) for one week. If the event resolves, an attempt to reach the higher dose may be made.
If the event did not resolve at the lower 50 mg dose (losartan or matching placebo), the study medication will be stopped and the patient will no longer receive the study medication, but will continue to be followed in the study according to the protocol, in keeping with the "intention-to-treat" paradigm.

Holding medication: If patient is currently taking the lower dose (50mg losartan or matching placebo), and he/she develops a treatment related CTCAE v5.0-defined adverse event grade 3 or above, or low blood pressure defined as **either** SBP < 90 mmHg **or** DBP < 60 mmHg, the medication will be stopped and the patient assessed by the site study investigator who is a licensed physician. *See section 7.1 for additional stopping guidelines.* If the low blood pressure was mild and short in duration, interventions recommended including 1) drinking more water 2) increasing salt intake and the low dose may be re-attempted giving the first dose in a controlled setting (pediatric research center) with 4 hours of post dose observation.

If the low blood pressure was severe, prolonged, or in the view of the investigator the subject is intolerant of the medication, the study medication will be stopped and the patient will no longer receive the study medication, but will continue to be followed in the study according to the protocol, in keeping with the "intention-to-treat" paradigm.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

An investigator may not administer an investigational new drug to human subjects until the IND goes into effect (30 days after IND receipt by FDA) or sooner if notified. And investigational drug under IND may only be used by an investigator in compliance with 21 CFR Part 50 and 21 CFR Part 56. All drug and placebo will be distributed to the individual sites by the Drug Distribution Center (DDC) and received by the research pharmacist at each site. Expired or unused drug at the end of the study will be disposed of by each site.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

FROM FDA PACKAGE INSERT:
How Supplied/Storage and Handling
Losartan Potassium USP 50 mg tablets are White to off-white, oval shaped, film-coated tablets with "A50" engraved on one side and break line on the other side. They are supplied as follows:

NDC 42571-111-30 unit of use bottles of 30
NDC 42571-111-90 unit of use bottles of 90
NDC 42571-111-10 unit dose packages of 1000

For this study, all losartan and placebo will be over-encapsulated to ensure the losartan and placebo capsules look identical and will be labeled as "losartan or placebo for investigational use only".

6.2.3 PRODUCT STORAGE AND STABILITY

Store at 25°C (77°F); excursions permitted to 15 to 30°C (59 to 86°F) [see USP Controlled Room Temperature]. Keep container tightly closed. Protect from light.

6.2.4 PREPARATION

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

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization: The randomization scheme will assign patients into two groups to receive either losartan potassium or placebo. The randomization design will be stratified by clinical center with assignments in permuted blocks of random length within each clinic. This scheme will ensure that the two groups will be balanced by calendar time of enrollment (to minimize secular effects) and by clinic (to minimize clinic-specific effects of differences in patient populations and management).

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

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

Unmasking of study medication may occur under the following conditions:

- **Severe allergic reaction (Stevens-Johnson Syndrome)**: Study medication is stopped indefinitely. The patient, primary care provider (PCP), and the investigator may be unmasked.
- **Pregnancy during the study:** Study medication will be stopped indefinitely, and the coded medication may be unmasked. The patient, PCP, and investigator will be notified of the associated risks of teratogenicity.
- **Development of hepatotoxicity:** Hepatotoxicity will be defined as 1) the development of jaundice with a serum direct bilirubin of > 1.0 mg/dL, 2) an increase in the baseline ALT and AST value that is two-fold, or 3) ALT and AST value > 400 U/L during treatment. Study medication will be discontinued; however, there will be no unmasking until study conclusion.
- **Development of hypotension:** Drug will be stopped and participant evaluated. Drug may be unmasked if the hypotension is severe.

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

6.4 STUDY INTERVENTION COMPLIANCE

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

- Counts of capsules in the patient's returned study drug bottles

- Conducting a brief, structured interview, in which the study coordinator will assist the patients in identifying problems in taking the study drug and in estimating adherence to the prescribed medicine since their previous visit.

These assessments will guide the consideration of strategies to improve adherence. Resources will be provided to remove barriers to participation such as child care, transportation, and parking expenses. These resources can be provided as cash, transportation vouchers, or parking passes. An honorarium (up to $50 per visit) may be paid to participants in recognition of their time and effort when scheduled visits and procedures are completed successfully. Certificates of appreciation may be given at enrollment and at conclusion of the STOP-NAFLD trial as an incentive.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements. Certain medications known to interact with losartan potassium are excluded from use during the trial. These include:

- ACE inhibitors
- Any anti-hypertensive drug
- Potassium supplements
- Any drug known to increase potassium
- NSAIDs

Participants will be questioned regarding any new medication at each visit and informed of the above exclusions.

6.5.1 RESCUE MEDICINE

N/A

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

If patient develops a treatment related CTCAE v5.0-defined adverse event (grade 3 or above) or low blood pressure defined as **either** SBP < 90 mmHg **or** DBP < 60 mmHg and he/she is on the starting dose (50 mg losartan or matching placebo), the medication will be held and the patient assessed by the site a study investigator who is a licensed physician. If the low blood pressure was mild and short in duration, interventions recommended include 1) drinking more water 2) increasing salt intake; and the dose may be re-attempted giving the first dose in a controlled setting (pediatric research center) with 4 hours of post dose observation. If the low blood pressure was severe, prolonged, or in the view of the investigator the subject is intolerant of the medication, the participant will be withdrawn from the study intervention but will continue to be followed. *See 6.1.2.*

Study medication will be STOPPED if participant is determined to be pregnant.

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

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
ALT	BSL nl: 3x ULN BSL elevated: 3x BSL	BSL nl: >3-5x ULN BSL elevated: >3-5x BSL	BSL nl: >5-20x ULN BSL elevated: >5 -20x BSL	BSL nl: >20x ULN BSL elevated: >20x BSL

AST	BSL nl: 3x ULN BSL elevated: 3x BSL	BSL nl:>3-5x ULN BSL elevated: >3-5x BSL	BSL nl:>5-20x ULN BSL elevated: >5-20x BSL	BSL nl:>20x ULN BSL elevated: >20x BSL
T Bilirubin	1.5x ULN	>1.5-3.0x ULN	>3.0-10.0x ULN	>10.0x ULN

In addition, patients will have the study medication stopped if ANY of the following occur:

If normal baseline AST:

- AST value >5x ULN in two repeat tests; OR
- AST value is >x8 ULN; **OR**
- AST value >5x ULN AND total bilirubin >2x ULN* OR presence of symptoms suggesting liver injury (severe fatigue, nausea, vomiting or right upper quadrant pain),

If elevated baseline AST or ALT:

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

* For patients with suspected Gilbert's Syndrome at baseline: doubling of direct bilirubin. Patients will be monitored for hyperkalemia and will use the CTCAE 5.0 based scale below to grade severity and determine course of action:

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Potassium	>ULN - 5.5 mmol/L	>5.5 - 6.0 mmol/L; intervention initiated	>6.0 - 7.0 mmol/L; hospitalization indicated	>7.0 mmol/L; life-threatening consequences

Study medication **will be stopped** if hyperkalemia reaches Grade 2.

In addition, patients will be monitored for renal disease, and we will use the following CTCAE based scale to grade severity:

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Chronic Kidney Disease	eGFR<LLN-60 ml/min/1.73 m^2	eGFR 59-30 ml/min/1.73 m^2	eGFR 29-15 ml/min/1.73 m^2	eGFR <15 ml/min/1.73 $m^{2;}$ dialysis or renal transplant indicated
Creatinine Increased	Creatinine 1.5x ULN	Creatinine 1.5-3.0x baseline; >1.5 -3.0x ULN	Creatinine >3.0x baseline; >3.0 -6.0x ULN	Creatinine >6.0x ULN

Study medication **will be stopped** if eGFR drops to 59 - 30 ml/min/1.73 m^2, OR if creatinine becomes elevated > 1.5x ULN.

Discontinuation from study medication (losartan or matching placebo) does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding

is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Labs and anthropometrics
- Study medication bottles and documentation of capsule counts

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

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

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

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 1 scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant does not return to the clinic for a required study visit:

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

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

8.1.1 VISIT SCHEDULE OVERVIEW

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

- Screening for eligibility for enrollment (60 days)
- Randomization to treatment (1 visit)
- Treatment phase (3 visits over 24 weeks)
- Post-treatment washout phase (1 visit at 36 weeks)

8.1.2 SCREENING AND BASELINE DATA COLLECTION

Patients who appear to be eligible after chart review will be invited to undergo screening. Recording of screening data on NASH CRN forms may not start until the parent or legal guardian has signed the consent statement and the patient has signed the assent statement. Screening and baseline data collection procedures will include questionnaires, physical examination, measurement of fasting serum glucose and insulin, standard of care liver biopsy, lipid and metabolic tests, etiologic tests, urine analysis, and blood collection for DNA and serum and plasma banking. Prior therapy for NAFLD will be reviewed as outlined in the inclusion and exclusion criteria. Patient charts will be reviewed for historical information and previous liver biopsy findings.

All participants who sign the consent/assent documents will be registered in the trial database. Each participant who starts screening will be accounted for at the end of screening, as either a screening success (enrolling in the trial) or a screening failure. A screening failure is defined as a participant who signed the consent/assent form, but is found to be ineligible prior to randomization. Screening failures include patients who meet medical eligibility criteria but who refuse enrollment in the trial. Reasons for screening failure will be recorded in the trial database. Screening data collection will be conducted over two clinic visits usually completed on separate calendar days. The goal of the first screening visit is to obtain consent and record data regarding the trial's inclusion and exclusion criteria; the goal of the second screening visit is to complete collection of baseline data on patients who appear eligible. This separation of procedures between two visits is provided as a practical guideline. Screening procedures and data collection can be organized as appropriate at each clinical center. The procedures completed during screening are described below.

Screening visits

Determination of eligibility will be based mostly on chart review of standard of care tests and procedures that were completed before the first screening visit. The parent or legal guardian and child will sign the consent and assent at the first screening visit (or before) to obtain any tests and procedures needed to finalize eligibility after chart review. Participants will undergo a medical history and detailed physical examination including anthropometric assessments (body weight [kg], body height [m], body mass index [BMI], waist circumference [cm], and hip circumference [cm]) to identify other illnesses and contraindications for participation, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor, or stage 2 hypertension for their age and height based on the AAP Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents[104]. History of prior liver biopsies will be obtained and recorded. Laboratory test results that need to be recorded from chart review or obtained as part of the screening visits include: tests for hepatitis B (HBsAg) and hepatitis C, anti-nuclear antibody (ANA), anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA), ceruloplasmin, A1AT concentration, iron overload (iron, TIBC, and ferritin). **The ALT measurement must be completed within 30 days of randomization. The additional lab tests for screening must be completed within 60 days of randomization:** fasting serum glucose, insulin, HgbA1C, complete blood count (CBC) with white blood cell differential, hepatic panel (total and direct bilirubin, AST, alkaline phosphatase, albumin, total protein, GGT, PT, INR), metabolic panel (sodium, potassium, chloride, carbon

dioxide, calcium, BUN, creatinine, uric acid), eGFR (*using Schwartz Bedside GFR Calculator for Children IDMS-traceable)*, C-reactive protein and lipid panel (total cholesterol, triglyceride, LDL, HDL).

Females of childbearing potential must have a negative pregnancy test. Frequency and amount of alcohol intake will be obtained using the Alcohol Use Disorders Identification Test (AUDIT). Patients and parents will complete the health-related quality of life questionnaire (PedsQL) and the BEV-Q questionnaire to document habitual beverage intake (grams, energy). A blood sample will be drawn to obtain DNA for banking and serum/plasma for banking for future analysis of markers of exploratory outcomes including cytokines and NMR based lipids.

Baseline liver biopsy

Eligibility requires a liver biopsy within 730 days prior to enrollment). The baseline liver biopsy is not performed as a procedure in this study but as standard of care done for clinical care. Usually, this will have been done prior to consideration for this study. If a child's hepatologist believes that the child has NAFLD, has scheduled him or her for a clinical liver biopsy, and they meet all other inclusion and exclusion criteria, then the child may be registered prior to the liver biopsy. Regardless of whether the biopsy is done before or after registration, the liver histology must be determined to be NAFLD with a NAS of at least 3. The NASH CRN study physician should check if tissue blocks and/or additional slides can be obtained from the original biopsy. A NASH CRN certified pathologist will read and score the biopsy. For participants who have had a prior biopsy that was centrally reviewed by the NASH CRN pathologists, those scores will be used.

Clinic staff should note that the date of the biopsy establishes a hard window for completion of screening procedures prior to randomization – **randomization must take place within 730 days of the date of biopsy**. Clinic staff will have to monitor completion of screening procedures in order to assure adherence to the allowable time window.

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

8.1.3 RANDOMIZATION VISIT

The randomization visit is the visit at which randomization takes place and the patient is issued the study medication randomly assigned to the patient. Randomization is the act of generating the random study medication assignment and is the procedure which defines a patient's enrollment into the trial. Randomization can only occur after eligibility has been fully checked and all data collected at the screening visits have been keyed to the trial database. Since these processes take time, randomization cannot be done at a screening visit, and since study medication needs to be issued to the patient, the randomization visit must be completed in person with the patient. Therefore, a visit separate from the screening visit is necessary. Since this will be a visit on a different calendar day and medication will be started at this visit, good clinical practice requires that a few basic checks of the patient's well-being be completed at the randomization visit.

The procedures completed at the randomization visit are: pregnancy test for females of child bearing potential; verification that the patient is feeling well; affirmation of consent; review of concomitant drugs and vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature). All patients will be given information on a healthy life style and diet appropriate for their weight and other factors.

Generation of the random treatment assignment will occur at this visit. The randomization process includes the same electronic check on eligibility that the staff may run prior to the randomization visit. The medication assignment will not be generated unless the check finds that the patient is eligible, and the clinic staff indicates that they want to randomize the patient. The random treatment assignment will consist of medication bottle numbers; these numbers will be unique and will be specific to the particular patient it was generated for. They will correspond to numbered bottles of medications which have been sent to the clinical center's research pharmacy (or clinical coordinator if not using a pharmacy) by the NASH CRN Drug Distribution Center. The research pharmacy (or clinical coordinator) will issue the specific numbered bottles to the patient. Each patient's random treatment assignment will be generated for that specific patient and will not be transferable to another patient. Once the assignment has been generated, the patient should be issued the assigned study drug (in person) and instructed about starting the drug and monitoring for adverse effects and completion of the blood pressure log. Patients and their parents will be taught to use an automated blood pressure monitor. Patients will be asked to take their blood pressure each morning for the first 14 days and bring the log to their next clinic visit. **Patients and parents will be instructed to call the clinic if blood pressure drops below < 90 mmHg systolic or < 60 mmHg diastolic.** The logs will be reviewed at the first follow-up visit and checked for hypotension. The date of randomization is the 0 time for reckoning all follow-up visits (i.e., all follow-up visits are scheduled at specific times measured from the date of randomization). The randomization computer program will generate a personalized appointment schedule for the patient; this schedule will indicate the ideal date for each follow-up visit, as well as the time window around the ideal date during which the follow-up visit may be done. This will ensure that the data collected at the follow-up visit may be used in the trial.

8.1.4 FOLLOW-UP VISITS

Patients will be called by study staff during **Week 2** after they have started on the higher dose (100 mg losartan or matching placebo). The blood pressure log will be reviewed on the telephone call and parents/patients will be asked about any adverse effects they (their child) may be experiencing.

Patients will return to the clinical center for follow-up visits at 4, 12, 24 weeks after randomization. Patients will be seen for one visit at four weeks after randomization, then at 12 weeks after randomization, and 24 weeks after randomization (end of treatment visit). A post-treatment washout phase visit will occur at week 36. Each visit will have an interval of time surrounding the ideal date for the visit during which the visit may be done and the data included in the trial database. The ideal date for a visit is the exact anniversary date from randomization. Visit windows will be constructed to be contiguous, so that at any point in time, some visit window is open, subject to a check on the minimum separation of at least two weeks is required between consecutive visits. The specific procedures to be completed at each of the follow-up visits are:

- **Week 4 visit**: Review of blood pressure log, medications, adverse effects, study drug adherence, pregnancy test, blood draw for metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, eGFR), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein); focused physical examination including height, weight, waist and hip measurements, vital signs

(temperature, heart rate, respiratory rate, blood pressure), and liver signs; and standardized nutrition and exercise prescription and counseling. Review study drug adherence with patient.

- **Week 12 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination including height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver signs; blood draw for CBC, metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, eGFR), uric acid, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), GGT, PT, INR, C-reactive protein; fasting glucose, insulin, HbA1c, lipid profile; plasma and serum for banking at a central repository; pregnancy test (for females of child-bearing potential); standardized nutrition and exercise prescription. Review study drug adherence with patient.

- **Week 24 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; a health-related quality of life questionnaire (PedsQL); BEV-Q questionnaire; detailed physical examination, including height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), organ systems and liver signs; blood draw for CBC, comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, eGFR), uric acid, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), GGT, PT, INR, C-reactive protein; fasting glucose, insulin, HbA1c, lipid profile; blood draw for plasma and serum banking at a central repository; pregnancy test (for females of child-bearing potential); standardized nutrition and exercise prescription and counseling. Review study drug adherence with patient. Collect all study drug bottles.

- **Week 36 visit**: Follow-up visit 12 weeks after discontinuation of study drug. Follow-up medical history including review of medications, adverse effects, and interim drinking history; a health-related quality of life questionnaire (PedsQL); BEV-Q questionnaire; focused physical examination including height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver signs; blood draw for CBC, metabolic panel (sodium, potassium, chloride, carbon dioxide, calcium, BUN, creatinine, eGFR), C-reactive protein; uric acid, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase, albumin, total protein), and for plasma and serum banking at a central repository.

8.1.5 STANDARDIZED QUESTIONNAIRES

Standardized questionnaires will be administered to patients enrolled in the STOP-NAFLD trial. Questionnaires will be administered at screening (prior to randomization) and during follow-up at specified intervals (see 1.3) for the data collection schedule). The purpose of the questionnaires is to obtain important information regarding alcohol intake, beverage intake, and health-related quality of life.

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

The **Pediatric Quality of Life (PedsQL)** questionnaire will be administered during screening, after 24 weeks of treatment, and at the week 36 visit.

Bev-Q: The Beverage questionnaire will be administered during screening, after 24 weeks of treatment and at the week 36 visit.

Specimen repositories

Specimens will be collected and stored in the NIDDK central repositories for use as approved by the Steering Committee of the NASH CRN. Specimens include serum, plasma, and DNA. The blood collected during screening, and at the 12, 24, and 36 week follow-up visits will be separated into plasma and serum, and divided into 0.5 mL aliquots. Aliquots will be kept in a storage facility at -70 degrees C until they are shipped to the NIDDK Biosample Repository on dry ice. If the patient provided additional consent, blood will be collected during screening and sent to a laboratory where DNA will be extracted, and the DNA sample will be shipped to the NIDDK Genetics Repository for banking.

8.2 SAFETY AND OTHER ASSESSMENTS

Assessments for Safety

- **Physical examination** (e.g., height, weight, waist and hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms.
- **Laboratory results –** All labs including creatinine (and calculated GFR) potassium levels and bicarbonate will be monitored at sites and in the central database and all abnormal values will be managed by the site investigator. If the potassium or creatinine is elevated (or GFR decreases by more than 30%), it will be repeated within 5 days See section 7.1 for discontinuation of the study medication.

Biological specimen collection and laboratory evaluations – There will be consistent methods throughout the study to ensure comparison. ALT will be measured at each site and the same lab used for the measurement throughout the study. NMR lipids will be measured by Labcorp, a CLIA compliant entity.

Management of Comorbid Conditions

Elevated blood pressures, hyperlipidemia, and diabetes will be managed according to the guidance described in the Standard of Care document prepared by the Pediatric Standard of Care Committee of NASH CRN and in conjunction with the patient's primary care physician or other specialist. If a participant develops hypertension during the patient's enrollment in the trial, and the physician determines the need of an anti-hypertensive medication (e.g. because lack of response to life style changes), the use of diuretics, beta-blockers or calcium channel blocker will be recommended for the specialist to consider.

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

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

Follow up of Ongoing Adverse Events

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

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

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

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used to classify Adverse Events when possible.

8.3.3.1 SEVERITY OF EVENT

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

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

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

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

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to

the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

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

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

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

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

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

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

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

8.3.5 ADVERSE EVENT REPORTING

Adverse events will be recorded on study data forms whether or not they are thought to be associated with the study or with the study drug. Adverse events may be discovered during regularly scheduled visits or through unscheduled patient contacts between visits.

Summary data on adverse events will be monitored by the DSMB quarterly and at its semiannual meetings or more frequently, as needed. These summaries will include analyses comparing rates of adverse events by treatment group, by clinic, or in other subgroups requested by the DSMB. Where applicable, signs and symptoms associated with the adverse

event will be graded as to severity by the clinical site staff as mild, moderate, or severe using Version 5.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE). [103]

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

8.3.6 SERIOUS ADVERSE EVENT REPORTING

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

If the SAE is **unexpected AND there is a reasonable possibility that the study drug caused the SAE,** then the clinical center must complete a data form for an IND Safety Report and submit it along with a narrative and a copy of the IRB report to the DCC. The DCC will submit a preliminary report to the NIDDK for review within three business days of receiving the SAE data form. The pharmaceutical manufacturer will also be notified within 7 days of the serious adverse event, if applicable. If NIDDK determines that the SAE requires an expedited IND Safety Report, the NIDDK program official or the NIDDK Regulatory Affairs Specialist will notify the FDA no more than 15 calendar days from the initial receipt of the SAE by the DCC (no later than 7 calendar days if the SAE is fatal or life threatening), if applicable. The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form and additional information for a follow-up SAE report as information becomes available. If the FDA determines that a change to the investigators brochure, IND or protocol is needed, the Data Coordinating Center will send a copy of the IND Safety Report to all clinical centers, with instructions to forward the report to their IRB.

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

8.3.7 REPORTING EVENTS TO PARTICIPANTS

N/A

8.3.8 EVENTS OF SPECIAL INTEREST

N/A

8.3.9 REPORTING OF PREGNANCY

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

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

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

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

8.4.2 UNANTICIPATED PROBLEM REPORTING

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

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

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

- UPs that are serious adverse events (SAEs) must be reported to the IRB and to the DCC/study sponsor promptly but no later than 10 days from the date the investigator was aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 10 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 10 days of the IRB's receipt of the report of the problem from the investigator.
- Reports should be sent to: *IRPT.OS@hhs.gov*

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

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

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Outcomes(s): That losartan potassium at 100 mg orally daily for 24 weeks is better than placebo in improving ALT in children with NAFLD.
- Secondary Efficacy Outcome(s): That losartan potassium at 100 mg orally daily for 24 weeks is better than placebo in improving:
    - Relative change in ALT at 24 weeks compared to baseline ALT
    - Proportion of patients achieving normalization of ALT at 24 weeks
    - Change in AST at 24 weeks compared to baseline AST
    - Change in GGT at 24 weeks compared to baseline GGT

- Change in ALT at 12 weeks compared to baseline ALT
- Change in fasting markers of insulin resistance (HOMA-IR) at 24 weeks compared to baseline.
- Change in anthropometric measurements (weight, BMI z-score, waist to hip ratio, waist circumference) at 24 weeks compared to baseline
- Change in serum lipid profiles at 24 weeks compared to baseline
- Change in Health-related Quality of Life (HR-QoL) scores at 24 weeks compared to baseline
- Change in frequency of adverse events compared to baseline

9.2 SAMPLE SIZE DETERMINATION

Sample size considerations

- 2 equal size groups
- Type 1 error (2-sided) = 0.05
- Power = 90%
- Loss to follow-up = 10%, assumed to be missing completely at random
- Primary outcome = change (FU-BL) in ALT at 24 weeks
- Assumptions based on CYNCH Placebo group
  - Mean (SD) change (FU-BL) in ALT at 24 weeks = -12 (55) U/L
  - Mean change (FU-BL) in ALT at 24 weeks = -12 U/L
  - SD change (FU-BL) in ALT at 24 weeks = 55 U/L (losartan and placebo)
  - Correlation of ALT at baseline and 24 weeks = 0.64
- Method of analysis = ANCOVA (analysis of covariance) of ALT changes (FU-BL) regression
- Detectable difference in change (FU-BL) in ALT at 24 weeks
  - 0.5 SD = 28 U/L (i.e., Losartan vs placebo = -40 vs. -12 U/L)
- **Sample size = 55 per group; 110 total**

Sample size and power estimation varying minimum clinically important difference (MCID) and correlation between baseline and follow-up

	Minimum clinically important difference (MCID)			
	SDs	**ALT (U/L)**	**Correlation between ALT at baseline and 24 weeks**	**Power**
In protocol	0.50	28	0.64	0.90
Varying MCID	0.45	25	0.64	0.83
	0.40	22	0.64	0.74
	0.35	19	0.64	0.62
Varying correlation	0.50	28	0.55	0.85
	0.50	28	0.50	0.82
	0.50	28	0.45	0.80

Assumptions:

- Sample size is 55 per group (110 total)
- 2-sided Type I error = 5%
- Increase in sample size due to missing data = 10%
- Method of calculation = ANCOVA

9.3 POPULATIONS FOR ANALYSES

Analysis dataset will include:

a.) Intention-to-Treat dataset for efficacy
b.) Safety Analysis Dataset
c.) Other Datasets for Sensitivity analyses (e.g. subgroups by dose, sex, age, baseline labs, adherence)

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The primary analysis is an intention-to-treat analysis of the change in ALT from baseline over 24 weeks of treatment. The statistical model for change in ALT at 24 weeks will be an ANCOVA model with an indicator variable for treatment group adjusted for ALT at baseline. If the percentage of patients with missing data on the primary outcome is greater than 10%, multiple imputation modeling for missing data will be used; otherwise complete-case analysis will be used, excluding patients missing ALT over all 24 weeks of follow-up.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY OUTCOME(S)

- Intention-to-Treat (ITT) Analysis Dataset

The primary outcome is 24-week change in serum ALT, defined as the ALT obtained at 24 week visit minus ALT at the baseline visit. The change in serum ALT is measured on a continuous scale and is a single endpoint. The treatment effect will be estimated using an analysis of covariance (ANCOVA) model, modeling the change in ALT on an indicator variable for treatment group adjusting for the baseline value of ALT. The treatment group difference in adjusted mean change at 24 weeks along with 95% confidence interval on the difference and p-value from the ANCOVA model will be presented. Sensitivity analyses include use of robust regression to down-weight the effect of influential outliers as well as to adjust for additional baseline variables.

9.4.3 ANALYSIS OF THE SECONDARY OUTCOME(S)

For each secondary outcome:

- Analysis of secondary outcomes are not dependent on findings of primary endpoint
- Intention-to-Treat (ITT) Analysis Dataset (i.e., all randomized participants)

The analysis of secondary outcomes will use the ANCOVA model for continuous outcomes, logistic regression for binary outcomes, and Poisson regression for counts. Complete case analyses will be used if missing data < 10%, and multiple imputation will be used if missing data is 10% or greater. Sensitivity analyses include use of non-parametric regression for continuous data (lowess plots) and scale factor estimation to account for over-dispersion in generalized linear models for binary outcome data (dispersion-corrected logistic regression) and count outcome data (dispersion-corrected negative binomial regression).

9.4.4 SAFETY ANALYSES

Safety data will be presented by treatment group to the Data and Safety Monitoring Board during the course of the trial. Each adverse event will be displayed showing AE name per the CTCAE v5.0 when applicable, class, severity, patient ID, clinic, treatment group, time since randomization, resolution status and physician's assessment of relatedness to treatment. Patient-specific summary tables including any adverse event (yes vs no), frequency of multiple adverse

events (i.e., 0, 1, 2, 3+) and highest severity of adverse event (none, mild, moderate, severe, life-threatening) will be displayed by treatment group along with p-values from Fisher's exact tests.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

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

9.4.6 PLANNED INTERIM ANALYSES

There are no planned interim analyses.

9.4.7 SUB-GROUP ANALYSES

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

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

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

9.4.9 EXPLORATORY ANALYSES

Similar to the statistical methods used to compare continuous outcomes between the treatment groups for both the primary and secondary analyses, ANCOVA analysis (linear regression of 24-week change among treatment groups adjusted for baseline value of outcome) will be used for exploratory analyses.
Exploratory outcomes:

- Change in peripheral pro-inflammatory cytokine levels (including IL-6, TNF, TGF-beta) from baseline to 24 weeks
- Change in NMR MetaboProfile Analysis (LP4) from baseline to 24 weeks.

In addition, the exploratory outcomes above will be compared by treatment group within subgroups of patients defined by response to treatment at the end of the trial. Response to treatment is defined as ≥ 28 U/L decrease in ALT at 24 weeks from baseline. Analysis 1 will compare treatment groups with respect to each of the exploratory outcomes above using ANCOVA analyses. Analysis 2 will determine whether the treatment group differences for each of the exploratory outcomes will differ depending on whether a patient is a responder or not. This requires 2 ANCOVA models (one for each exploratory outcome) with an indicator variable for treatment group (losartan vs. placebo), an indicator variable for treatment response (yes vs. no), and an interaction term that is the product of these two indicator variables. If the interaction term is statistically significant ($p<0.01$), we will conclude that the response to losartan varied by responder status and we will present the treatment group comparisons and ANCOVAs within subgroups of responders and non-responders.
Losartan is known to improve insulin sensitivity through inhibition of PAI-1 production and restoration of hepatocyte growth factor activity. We hypothesize that the MetaboAnalysis panel ("deep lipids panel") performed by Labcorp will show improvement in the risk prediction panel for diabetes and will show improvement in the lipoprotein particle profile (specifically smaller VLDL particle sizes and fewer atherogenic small LDL particles after treatment with losartan.

Cytokines, including IL-6 and TNF-alpha are markers of the inflammatory state in NAFLD. Modulation of the angiotensin system by ARB's such as losartan are likely to decrease the chronic inflammation of NAFLD. We hypothesize that IL-6 and TNF-alpha will decrease after treatment with losartan.

These exploratory endpoints are research measurements and will be conducted using batched samples after the final 24 weeks visit of the last patient. The MetaboAnalysis panel frozen plasma samples will be shipped to Labcorp. The IL-6 and TNF-alpha can be measured in frozen serum and will be performed at a core research lab.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the parent or legal guardian and participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The draft consent materials are submitted with this protocol.

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

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

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

10.1.2 STUDY DISCONTINUATION AND CLOSURE

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

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary outcome has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

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

All laboratory specimens, study forms, reports, and other records that are part of the study data collection materials will be identified by coded number to maintain patient confidentiality. All records will be kept in locked file cabinets. All electronic records of study data will be identified by coded number. Clinical information will not be released without written permission of the patient, except as necessary for monitoring by the IRB. Consent procedures and forms, and the communication, transmission and storage of patient data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA).

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

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

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NASH CRN DCC. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NASH CRN DCC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at NASH CRN DCC.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

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

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

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

When the study is completed, access to study data and/or samples will be provided through the NIDDK Central Repository.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Principal Investigator**	**Medical Monitors**
Miriam B. Vos, MD, MSPH	Joel Lavine, MD, PhD	Mariana Lazo, MD and Jeanne Clark, MD, MPH
Emory University	Columbia University	Johns Hopkins University
1760 Haygood Dr, Decatur, GA	New York, New York	2024 E Monument St Rm 2-600, Baltimore, MD 21205
404-803-7733		410-614-4096
mvos@emory.edu	jl3553@columbia.edu	mlazo@jhu.edu and jmclark@jhmi.edu

NASH CRN Executive Committee (EC)- Consists of NASH-CRN co-chairpersons, SC representatives, principal investigator of the Data Coordinating Center, safety officer, NIDDK program official, and NIDDK project scientist. The NASH CRN EC discusses directions and strategic issues related to the scientific aims of the NASH CRN; organizes and sets agenda for Steering Committee meetings and provides oversight of the study.

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

10.1.6 SAFETY OVERSIGHT: DATA AND SAFETY MONITORING BOARD

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

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

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

The DSMB will review quarterly reports by masked treatment groups of incident hepatotoxicities, as well as counts of patients who required more frequent liver function testing due to rises in ALT levels of more than 2 times baseline ALT or beyond 300 U/L. The DSMB will also examine the trends in ALT or AST levels for each patient who experiences a rise in ALT.

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

10.1.7 CLINICAL MONITORING

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

- Monitoring for this study will be performed by the DCC of the NASH CRN.
- To ensure data quality and reduce data entry error, double data entry of all data collection forms is required. The DCC will incorporate into the data entry programs a series of edit checks, including range checks, logic checks (e.g., skip patterns), and consistency checks, both within a data form and across forms. These integral checks are designed to detect deficiencies in the data before they are entered into the study database, so that the deficiencies may be easily and accurately corrected. In addition, the DCC will perform frequent computerized edit checks on entered data. The DCC will also perform ongoing comparisons of copies of entered data forms with the databases to ensure that data entered into the data system reflect those recorded on the forms. Such audits will help to pinpoint problems that cannot be detected by computer editing and may be used to guide increased scrutiny when needed. Any data queries identified will be flagged for action by the clinical

site until resolved.

- The data system will maintain a clear and complete audit trail of all changes to the study databases. All changes to data forms will be documented appropriately on the paper form and entered into NASH CRN data system. All forms entered and/or edited in the data system will be identified by the PIN of the operator, the network address (the "IP" address) of the computer being used, and the date and time of the operation. Records will not be deleted but rather marked as having been superseded. The DCC will implement various procedures to ensure the quality of its internal operations. These will include the development and maintenance of documentation regarding procedures for receiving, processing, and analyzing data, and duplicated programming for selected procedures to check for errors in software database and analysis systems.
- Performance monitoring: The web-based data system will allow for real-time reporting of most recruitment and data management activities. However, additional performance reports will be generated and circulated monthly and typically reviewed at each Steering Committee (SC) meeting. These reports include several key indicators of study performance, including counts of patients screened and randomized, of completed visits and of missing key data, of missed visits, and statistics summarizing performance with respect to timeliness of data entry and response to data queries.

  The SC and the DSMB will conduct a formal review of the study by conference call or in-person meeting twice a year and will address quality assurance as part of their agenda. These committees will have responsibility for recommending corrective actions based on the performance data. It is anticipated that the primary responsibility for formulating and implementing these actions will reside in the SC. Potential actions might include specific recommendations for training, redistribution of study resources, or possibly termination of support for a center.
- Independent audits outside the DCC will not be conducted.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

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

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

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

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

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

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

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

10.1.9.2 STUDY RECORDS RETENTION

The NIDDK records retention policy is to maintain clinical trial records for 3 years after the granting period ends or the trial ends, whichever is later.

10.1.10 PROTOCOL DEVIATIONS

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

These practices are consistent with ICH GCP:

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

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

10.1.11 PUBLICATION AND DATA SHARING POLICY

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

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

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

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-

scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 CONFLICT OF INTEREST POLICY

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

10.2 ADDITIONAL CONSIDERATIONS

n/a

10.3 ABBREVIATIONS

A1AT	alpha-1-antitrypsin
AE	Adverse Event
ALT	alanine aminotransferase
AMA	antimitochondrial antibody
ANA	antinuclear antibody
ANCOVA	Analysis of Covariance
Anti-HCV	hepatitis C antibody
ASMA	anti-smooth muscle antibody
AST	aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
BMI	body mass index (kg/m2)
BUN	blood urea nitrogen
CC	Clinical Center
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRN	Clinical Research Network
CRP	C reactive protein
CTCAE	Common Terminology Criteria for Adverse Event
DCC	Data Coordinating Center
DDC	Drug Distribution Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Executive Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice

GFR	glomerular filtration rate
GGT	gamma glutamyltransferase
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HbA1c	hemoglobin A1c
HBc	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HCC	hepatocellular carcinoma
HCV	Hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IL-6	Interleukin-6
IND	Investigational New Drug Application
INR	international normalized ratio
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MCV	mean corpuscular volume
MSDS	Material Safety Data Sheet
NAFL	nonalcoholic fatty liver
NAFLD	nonalcoholic fatty liver disease
NAS	nonalcoholic fatty liver disease activity score
NASH	nonalcoholic steatohepatitis
NCT	National Clinical Trial
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NMR Lipo	Nuclear magnetic resonance lipid profile
NSAID	Nonsteroidal anti-inflammatory drugs
OHRP	Office for Human Research Protections
PAI-1	Plasminogen activator inhibitor-1
PI	Principal Investigator
PPARγ	peroxisome proliferator-activated receptor-gamma
PT	prothrombin time
QA	Quality Assurance
QC	Quality Control
ROC	reactive oxidative species
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	Standard of Care
SOP	Standard Operating Procedures
TIBC	Total iron binding capacity
TNFα	Tumor necrosis factor alpha

UP	Unanticipated Problem
US	United States
WBC	White blood cell count

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.3	18 Feb 19	Section 1.1, Synopsis: Changed HbA1c time window to 60 days.	Changed to allow 60-day time window for all laboratory measurements except ALT.
1.3	18 Feb 19	Section 1.3, Data Collection Schedule: Removed Focused Physical Examination from the data collection at the randomization visit.	A brief physical is required to determine if the patient is feeling well on the day of randomization, but the Focused Physical Examination form is not completed.
1.3	18 Feb 19	Section 8.1.2, Screening Visits: Protocol text changed to allow 60-day time window for all laboratory measurements except ALT.	ALT measurement must be completed within 30 days of randomization; additional lab tests for screening must be completed within 60 days of randomization.
1.3	18 Feb 19	Section 12.3, Blood Collection Schedule: CBC + WBC and uric acid added to blood collection at f36 visit, to align with the Data Collection Schedule and visit procedures specified in the protocol.	The total blood volume collected at f36 corrected from 35mL to 42mL.

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12 APPENDIX

12.1 PARTICIPATING CENTERS

Clinical Centers

Northwestern University, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL

Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Columbia University, New York, NY

Emory University, Atlanta, GA

Indiana University, Indianapolis, IN

Saint Louis University, St. Louis, MO

Texas Children's Hospital, Houston, TX

University of California, San Diego, CA

University of California, San Francisco, CA

University of Washington, Seattle Children's Hospital, Seattle, WA

Data Coordinating Center:

Johns Hopkins University, Baltimore, MD

National Institutes of Health:

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Central Repositories:

Biosample repository: Precision for Medicine, Frederick, MD

Genetics repository: RUCDR Infinite Biologics, Piscataway, NJ

Data repository: Information Management Services (IMS), Calverton, MD

12.2 BLOOD COLLECTION SCHEDULE (amounts in mL)

	Screening visits		**Follow-up visits- Weeks from Randomization**				**mL**
Labs		**RZ**	**f04**	**f12**	**f24**	**f36**	**Total mL**
Complete blood count + WBC	5			5	5	5	20
Basic metabolic panel + eGFR	2	.	2	2	2	2	10
Uric acid	2			2	2	2	8
Hepatic panel (Liver function test)	2	.	2	2	2	2	10
Gamma glutamyltransferase -GGT	2	.		2	2		6
Prothrombin time (PT), INR	5	.		5	5		15
C-Reactive Protein (CRP)	1			1	1	1	4
Fasting lipid profile	2	.	.	2	2		6
Fasting serum glucose	2	.	.	2	2		6
Fasting serum insulin HbA1c	2	.	.	2	2		6
Fasting HbA1c	5	.	.	5	5		15
Etiologic tests (as needed)	20	.	.	.	.		20
Banking:							
Fasting plasma	10	.	.	10	10	10	40
Fasting serum	20			20	20	20	80
DNA	10	.	.	.	.	.	10
Total (not including optional DNA)	80		4	60	60	42	246
Total (Including optional DNA)	90		4	60	60	42	256

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

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