Detailed Protocol: Growth Hormone Releasing Hormone Analog to Improve Nonalcoholic Fatty Liver Disease and Associated Cardiovascular Risk

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Growth Hormone Releasing Hormone Analog to Improve Nonalcoholic Fatty Liver Disease and Associated Cardiovascular Risk
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Version Date: **1/25/2023**
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I. Background and Significance

NAFLD is a growing threat to public health. Nonalcoholic fatty liver disease (NAFLD), which encompasses a spectrum from simple steatosis and steatohepatitis (NASH) to fibrosis and cirrhosis, is the leading cause of liver disease in the United States. The prevalence in U.S. adults is estimated to be 25-30% (1, 2), rising to over 50% among adults with obesity or Type 2 diabetes (T2D) (3-5). Of those with NAFLD, approximately one-fifth have NASH (6). Studies of liver transplantation since the early 2000s demonstrate several-fold increases in transplants performed for NASH (7, 8). In 2013, NASH became the second-leading cause of liver transplant, after hepatitis C (HCV) (9). With new pharmacologic treatments for HCV, NASH will likely overtake HCV as the leading indication for liver transplantation in the next decade (10).

NAFLD is a multisystem disease. NAFLD independently contributes to T2D and cardiovascular disease (CVD) (11). Mechanisms include inflammation and hepatic dysfunction as well as steatosis itself. This is an important point: although simple steatosis is thought to be benign in terms of liver outcomes, it is not metabolically benign. **Diabetes risk:** Multiple studies demonstrate that intrahepatic lipid is independently associated with peripheral insulin sensitivity in humans (12-16), and the presence of fatty liver increases the risk for incident T2D (17, 18). Further, improvements in fatty liver over time reduce diabetes risk, whereas worsening of fatty liver markedly increases the risk of incident T2D (19). Shulman and colleagues have shown that accumulation of diacylglycerol (DAG) in hepatocytes is strongly associated with activation of PKCε, which impairs insulin signaling (20). This putative mechanism by which hepatic lipid induces insulin resistance is independent of inflammation; in fact, DAG content is more strongly predictive of hepatic insulin resistance than are markers of hepatic inflammation (20). **CVD risk:** NAFLD is independently associated with CVD, the leading cause of death in NAFLD. Hepatic steatosis is associated with coronary artery calcification (21) and increased high-risk plaques (22), independent of traditional CVD risk factors and visceral fat content. Further, individuals with NAFLD have more rapid progression of CAC during follow-up compared to controls (23). Among individuals with T2D, NAFLD at baseline conveys an 84% increase in the odds of a CVD event over 5 years, independent of metabolic syndrome features, age, sex, smoking, LDL, and liver enzymes (24).

There is a significant need for highly effective treatments for NAFLD. There are currently no FDA-approved pharmacologic therapies for NAFLD and NASH, and diet and exercise are often minimally effective. Vitamin E improves histology in some patients in randomized trials (25), but concerns remain that it may increase risk of prostate cancer and all-cause mortality (26, 27). Pioglitazone also improves histological features of NASH (25, 28) but causes weight gain and may increase the risk of bladder cancer (28, 29). Both elafibranor (30) and obeticholic acid (31) have improved histological features of NASH, but neither has completed Phase 3 trials. Further, obeticholic acid increases LDL and decreases HDL (31, 32). Thus, continued investigation of strategies to treat NAFLD and NASH is greatly needed. This protocol aims to treat NAFLD and NASH by reversing the physiologic reduction in growth hormone (GH) that occurs in obesity in order to alter hepatic lipid metabolism, reduce liver fat, and ameliorate NASH.

Strong rationale exists for using GH Releasing Hormone (GHRH) to treat NAFLD in obese adults. In obese adults, we and others have demonstrated that increased adiposity, particularly abdominal adiposity, is associated with significant reductions in GH secretion (33-35). There is an inverse association between visceral adipose tissue (VAT) and GH secretion, assessed by peak GH response on provocative testing, in men and women of varying BMIs. Further, obesity-related reductions in GH are strongly associated with dyslipidemia and metabolic abnormalities (34, 36).

Patients with pituitary GH deficiency (GHD) have a high prevalence of NAFLD and NASH (37-40), and GH replacement reduces aminotransaminase levels and improves liver histology (39, 41). Further, individuals with childhood onset GHD in whom GH is stopped at attainment of final height demonstrate a high incidence of NAFLD, developing as soon as 1 year after GH discontinuation (42). Obesity-related reductions in GH also appear to be associated with increased liver fat (43). Among 142 adults who had liver biopsy during NAFLD evaluation or weight loss surgery, mean serum IGF-1 was lower in subjects with lobular inflammation ($p=0.01$), higher fibrosis stage ($p=0.005$), and NASH ($p=0.002$), with all associations persisting after controlling for age, BMI, and presence of diabetes or cirrhosis (44).

Animal models demonstrate a clear role for GH in pathogenesis of NAFLD and NASH. GH signals through STAT5 to exert multiple effects on lipid metabolism, including **(1)** suppression of hepatic *de novo* lipogenesis (DNL) (48-53), **(2)** stimulation of lipolysis (54, 55), **(3)** increased lipid utilization through β-oxidation (56, 57), and **(4)** suppression of 11βHSD1 activity, which impacts lipid metabolism by decreasing the conversion of cortisone to active cortisol at a tissue level (58, 59). The physiologic relevance of GH in lipid metabolism is demonstrated in multiple animal models, as shown in **Table 1**. Importantly, lack of GH-signaling in these models is associated not only with simple steatosis, but also with steatohepatitis.

Table 1: Evidence from rodent models linking GH insufficiency to NAFLD and NASH
Spontaneous Dwarf Rat (GH gene mutation) (45) • steatosis, fibrosis, and hepatocyte injury • elevated AST and ALT
Liver-specific GH-receptor KO mouse (46, 47)*:* • severe hepatic steatosis • increased hepatic lipid uptake • increased hepatic lipogenesis • upregulation of inflammatory cytokines • elevated AST and ALT

GHRH is a safe and effective means to augment endogenous GH secretion. GH is secreted in a pulsatile manner from the pituitary in response to positive stimulation by GHRH, counterbalanced by negative feedback from somatostatin and IGF-1. GHRH is a safe and effective means to increase endogenous GH secretion in individuals with intact pituitary function for two reasons. First, administration of GHRH preserves negative feedback by IGF-1 and somatostatin, thus decreasing the potential for supraphysiologic elevations in IGF-1. Second, GHRH preserves endogenous GH pulsatility. As is the case with multiple other hormones, pulsatile GH signals at a tissue level differently than apulsatile GH (60, 61). For example, two human studies show that pulsatile GH increases lipolysis to a greater degree than apulsatile delivery (62, 63). The study drug, tesamorelin, is a GHRH analog that has been minimally modified with c-terminal amidation to increase half-life. It has been FDA approved since 2010 for reduction of visceral fat in individuals with HIV-lipodystrophy (64-67). We have shown that tesamorelin increases endogenous GH levels by increasing both basal and pulsatile GH secretion (68). A summary of clinical safety data on tesamorelin (GHRH) is shown in **Table 2**.

Table 2: Summary of Clinical Safety Data for GHRH Analog (tesamorelin)
Known side effects: Injection site redness or itching (7-8%), peripheral edema (6%), myalgia (6%) (66) ***Effects on glycemia:*** • Phase 3 studies in HIV: average increase in HbA1c of 0.1% after 6 months, with return to baseline after 12 months; no changes in fasting insulin or glucose (66). • Our research in HIV: increase in fasting glucose by 7mg/dL at 2 weeks and decrease in insulin sensitivity by euglycemic clamp at 3 months, both of which returned to baseline by 6 months of treatment (69). • Adults with T2D: modest initial increases in fasting insulin and glucose, return to baseline at 12 weeks (70) ***Serious Adverse Events:*** Not different from placebo (64-66, 69, 71)

The purpose of this protocol is to test the effects of treatment with a GHRH analogue, tesamorelin (Theratechnologies, Inc.), in individuals with known NAFLD, with the hypotheses that augmentation of endogenous GH secretion will decrease liver fat, improve histologic features steatohepatitis, and improve cardiovascular risk, inflammation, and lipid metabolism.

I. Specific Aims

The Specific Aims will be addressed in a 12-month, randomized, controlled trial of 76 individuals with NAFLD, treated with tesamorelin vs. placebo. A 6-month open-label extension phase will maximize recruitment and retention and provide data regarding longer-term use of tesamorelin.

Specific Aim 1: Tesamorelin will significantly decrease liver fat and improve histologic features of NASH in patients with biopsy-proven NASH. We hypothesize that,
a) Tesamorelin will significantly decrease hepatic fat fraction as measured by ^{1}H-MRS (1° endpoint).
b) Tesamorelin will significantly improve liver histology as assessed by increased 1) frequency of ≥ 2 point reduction in NAFLD activity score (NAS) without worsening of fibrosis and 2) reduction in individual components of the NAS including steatosis, lobular inflammation and hepatocyte ballooning.

Specific Aim 2: Tesamorelin will be associated with beneficial changes in hepatic lipid metabolism and

inflammatory milieu.
a) Tesamorelin will decrease hepatic de novo lipogenesis as measured by stable isotopes.
b) Hepatic expression of lipogenic genes (SCD1, FAS, SREBP1c) will decrease and expression of lipolytic genes (PPARα, CPT1) will increase.
c) Tesamorelin will reduce hepatic expression of pro-inflammatory (MCP1, TNFα, IL-1β, IL-6, IL-18, TLR, MyD88, CCL5, TGFβ1, NFκB) and fibrogenic (TIMP1, COL1A1, MMP1-14) genes.

Specific Aim 3: Tesamorelin will reduce markers of cardiometabolic risk in individuals with NASH. These changes will be associated with reduction in liver fat and liver inflammation.
a) Tesamorelin will improve circulating lipid (TG, HDL, TC), C-reactive protein, tissue plasminogen activator antigen and plasminogen activator inhibitor-1 antigen and adiponectin levels.

II. Subject Selection

Seventy-six obese men and women ages 18-65yo with either known hepatic steatosis on recent biopsy or with ≥5% hepatic fat fraction on ^{1}H-MRS, will be recruited, with eligibility criteria as below. A threshold of 5% hepatic fat fraction on ^{1}H-MRS is included because undiagnosed hepatic steatosis is prevalent in obesity, and hepatic fat fraction on MRS correlates extremely well with histological findings (87, 88). Further, a threshold of 5% approximates the 95th percentile of hepatic fat fraction in the normal, healthy population as reported by Szczepaniak et al. (1).

Inclusion criteria

1. Men and women 18-65yo
2. BMI ≥ 25kg/m^2
3. Hepatic steatosis as demonstrated by either a) Grade ≥1 steatosis on a liver biopsy performed within 12 months of the baseline visit, without >10% reduction in body weight or addition of medications to treat fatty liver, or b) liver fat fraction ≥5% on ^{1}H-MRS
4. Hepatitis C antibody and Hepatitis B surface antigen negative. Subjects without known history of Hepatitis C or Hepatitis C treatment who have a positive Hepatitis C antibody but a negative hepatitis C viral load will also be eligible.
5. For females ≥50yo, negative mammogram within 1 year of baseline
6. If use of vitamin E ≥400 IU, stable dose for ≥6 mos
7. Up to date with colon cancer screening recommended by the participant's primary care physician, using whatever methodology the primary physician recommends. This will be ascertained by self-report. (If a participant does not have a primary care physician, we will discuss that colon cancer screening is recommended, typically starting at age 50y, and refer the participant to primary care through Partners if s/he desires.)

Exclusion criteria

1. Heavy alcohol use defined as consumption of > 20g daily for women or > 30mg daily for men for at least 3 consecutive months over the past 5 years assessed using the Lifetime Drinking History Questionnaire (25, 89)
2. Known diagnosis of diabetes, use of any anti-diabetic medications (including thiazolidinediones or metformin), fasting glucose >126mg/dL, or HbA1c ≥6.5% Participants with stable use of metformin ≥6 months will be permitted if it is being used for pre-diabetes or another non-diabetes indication (e.g., PCOS).
3. Participants using GLP-1 receptor agonists (GLP-1 RA) for either diabetes or weight loss will not be eligible unless they have been using the GLP-1 RA at stable dose for ≥1 year and have not had any active weight loss (<10% decrease in weight over past 6 months)
4. Use of any specific pharmacological treatments for NAFLD/NASH except vitamin E within the 3 months before screening visit.
5. Known cirrhosis, Child-Pugh score ≥7, stage 4 fibrosis on biopsy, or clinical evidence of cirrhosis or portal hypertension on imaging or exam. If a subject is not known to be cirrhotic at screen but is found to be cirrhotic based on the results of liver biopsy at baseline, this subject will be referred to a hepatologist for clinical care and will be excluded from further participation in the study.

6. Chronic systemic corticosteroid use in the ≤6 months prior to the baseline visit. This includes physiologic steroid treatment in patients with adrenal insufficiency.
7. Chronic use of Actigall, methotrexate, amiodarone, tamoxifen, anticonvulsants, or cyclosporine
8. Known diagnosis of HIV-infection, alpha-1 antitrypsin deficiency, Wilson's disease, hemochromatosis, or autoimmune hepatitis
9. Use of GH or GHRH within the past 6 months
10. Change in lipid lowering or anti-hypertensive regimen within 2 months of screening
11. HgB < 10.0 g/dL or Creatinine >1.5mg/dL. If creatinine is above the upper limit of normal for the assay but <1.5mg/dL, then GFR must be >60mL/min for participant to be eligible.
12. Active malignancy
13. For men, history of prostate cancer or evidence of prostate malignancy by PSA > 5 ng/mL
14. Severe chronic illness judged by the investigator to present a contraindication to participation
15. History of hypopituitarism, head irradiation or any other condition known to affect the GH axis. Use of macimorelin.
16. Use of physiologic testosterone (men) or estrogen or progesterone (women) *unless* stable use for a year or more prior to study entry
17. Routine MRI exclusion criteria such as the presence of a pacemaker or cerebral aneurysm clip
18. Weight loss surgery within 1 year before baseline. Weight loss surgery more than 1 year prior to baseline visit is permissible as long as no active weight loss (<10% decrease in weight over past 6 months)
19. For women, positive urine hCG, trying to achieve pregnancy, or breastfeeding
20. For women able to become pregnant, unwillingness to use an acceptable form of birth control during the study. Acceptable forms of birth control include: abstinence from heterosexual vaginal intercourse, tubal ligation, male partner vasectomy, intrauterine device, etonogestrel implant (Nexplanon), or simultaneous use of TWO of the following: (a) contraceptive patch, pills, or vaginal ring, (b) diaphragm, (c) condoms.
21. Known hypersensitivity to tesamorelin or mannitol
22. Not willing or able to adhere to dose schedules and required procedures per protocol
23. Judge by the investigator to be inappropriate for the study for other reasons not detailed above.
24. Excluion criteria for undergoing Coronary Computed Tomography Angiography (CCTA) follow. These do not exclude individuals from participating in the study, only from undergoing the CCTA:
    a. Significant radiation exposure, including any history of radiation therapy, or any of the following in the 12 months prior to randomization: a) more than 2 percutaneous coronary interventions; b) more than 2 myocardial perfusion studies; 3) more than 2 CT angiograms
    b. Active consideration for a procedure or treatment that involves significant radiation exposure as defined above in the 12 months following randomization
    c. Contraindication to receiving nitroglycerin

Sources of Subjects/Recruitment Methods

Subjects will be recruited primarily from Dr. Corey's MGH NAFLD Cohort and from the MGH Liver Clinic. We will also reach out to physicians in other relevant practices, such as Weight Management Clinics and other clinics here and at other institutions to see if they may have interested patients. We will also use the Research Patient Data Registry to identify appropriate patients, and, when patients have consented, we will use the RODY system to contact potentially eligible participants. The study will also be advertised on the MGH Clinical Trials website and, depending on the pace of recruitment, ads may also be placed in the community, including potentially in newspapers and in electronic venues such as Facebook or Craigslist. One option for patients to pre-screen will be to fill out an online REDCap survey that asks preliminary questions to determine eligibility and asks patients if they are willing to be contacted. A link to this survey will be included in any ads that are placed electronically.

III. Subject Enrollment

Methods of Enrollment

The study will be recruited primarily from the MGH NAFLD Cohort, with which Dr. Corey is involved, and the MGH Fatty Liver Clinic, in which Dr. Corey practices. In addition, the study will be advertised through other ambulatory clinics affiliated with the Massachusetts General Hospital, and we may contact physicians at relevant clinical practices (i.e., other liver clinics in the Boston area) to inform them about the study. We will also aim to recruit through the MGH Research Study Volunteer Participant database and Partners Clinical Trials website. Depending on the pace of recruitment, we may also choose to advertise through flyers and newspaper advertisements, Craigslist, and targeted advertisements on Facebook. Ads placed electronically, including in Craigslist and Facebook, will have a link to a REDCap Survey that will collect minimal information necessary to see if subjects would potentially be eligible, as well as contact information so that we can call them if they are potentially eligible. We may also perform a Partners Research Patient Data Registry (RPDR) search and/or an Epic Reporting Workbench search to identify patients who may be eligible. We will apply filters specific to our eligibility criteria, including age, BMI, NAFLD or fatty liver diagnosis. We will apply filters to exclude those who have 'opted out', and people with diabetes, malignancies, or prohibited medications listed. We may subsequently review the resulted medical records to hone in on those that meet our inclusion/exclusion criteria.. We will then send letters to potential participants through patient gateway and/or mail. We will also use Patient Gateway Targeted Research Announcements. Interested subjects will be directed to our rally website for more information and can call to speak with study staff.

Preliminary eligibility will be determined based on study staff interviews of interested subjects over the phone. Eligible subjects will then be scheduled for a screening visit. Potential participants who express interest in receiving study related materials and communications by e-mail (including a copy of the consent form) will be educated regarding the Partners HealthCare e-mail security policy. If the subject does not want to use encrypted email, they can consent to receive unencrypted e-mail, and this will be documented in the subject's record as per the subject's request.

Informed Consent

Written informed consent will be obtained by a licensed physician or NP prior to screening evaluation and testing. In all cases a licensed physician investigator will be available during the informed consent process in order to answer any further questions. When a study NP obtains consent, the NP will ask the participant if they would like to speak with a study physician about anything before signing the consent. Physician back-up will be available, usually in person and at minimum by telephone call during every consent visit performed by an NP. If the physician is only available by telephone and the participant wishes to have a video or in person conversation before signing, another time will be scheduled for this so the participant can speak with the physician before signing. Subjects will be informed that they may withdraw from participation in the study at any point. Subjects will be given a copy of the signed informed consent form.

For re-consenting purposes only, if there are changes to the consent form after a subject has initially provided consent, the subject may be called in advance of the study visit by a physician who will discuss any changes to the consent form with the subject over the phone. Provided the subject is in agreement with the changes to the consent form and has given verbal consent, the physician will then sign and date the updated consent form and document the telephone conversation (stating that the subject provided verbal consent over the phone regarding the changes in the consent form). When the subject returns for his/her next study visit, he/she will be given the consent form (signed by the physician during the aforementioned phone discussion) to sign. The subject will then be offered a copy of the signed consent form and the original will be filed in the research file.

Randomization

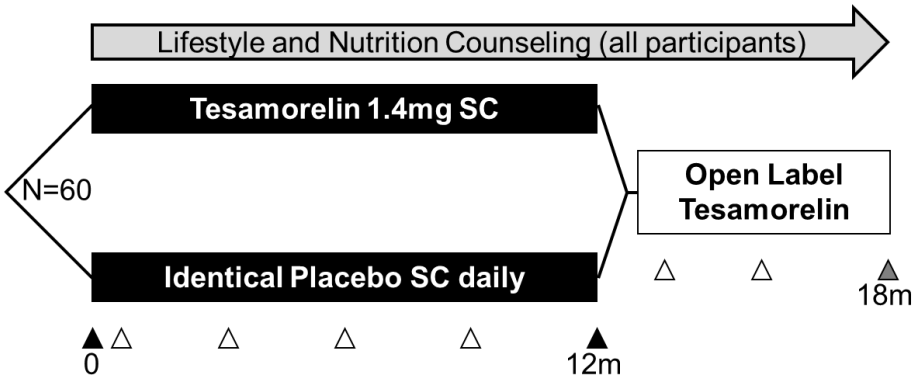
After signing consent and, prior to the baseline visit, eligible subjects will be randomized 1:1 to receive GHRH 1.4mg F4 formulation daily vs. identical placebo for 12 months (which will be followed by an open-label phase of 6 months). Randomization will be stratified by sex and vitamin E use ≥400 IU daily. A randomization list will be prepared by Dr. Lee, the study statistician, using a permuted block algorithm with randomly varying block sizes. The randomization list will be provided to the MGH Research Pharmacy. Treatment assignment will be blinded to investigators, study staff, and subjects.

IV. Study Procedures

This study will include a 12-month, double-blind, randomized, placebo-controlled treatment period followed by a 6-month open label period during which all patients receive GHRH.

After the screen visit, there are 11 more study assessments, at baseline, 2wk, 1mo, 2mo, 3mo, 6mo, 9mo, 12mo, 12.5mo, 15mo, and 18 months. The baseline and 12 month assessments may require visits on 2-3 different days depending on subject availability and logistics.

The study schema is shown in the figure.



All study visits will occur at the MGH unless extenuating circumstances require that safety visits be done remotely. Urine HCG will be performed at every visit for female subjects of child-bearing potential, except at baseline, at which a stat serum HCG will be performed (since a subset of women will also be undergoing coronary CT angiography at this visit).

Lifestyle and nutrition counseling

All subjects will be counseled in standard lifestyle modification at the baseline, 6, 12 and 18 month visits using standard recommendations for reduced dietary saturated fat (< 7%) and cholesterol intake (< 300 mg/day), and 150 minutes of activity equivalent to brisk walking a week (90, 91).

Remote Visits

In order to accommodate patient preferences, safety visits may be performed remotely if the participant is willing to go to a local Quest lab for the required lab testing using our study account and then have a phone call or video conference call with the investigator. This may only be done at the investigator's discretion and only for safety visits, NOT for the screening visit, the baseline visit, or the 12 month visit. If the visit is performed remotely the investigator will go through the CRF with the patient on the telephone or via a video visit and ask questions regarding any swelling of the hands or feet or any problems at the injection site (bruising, redness, etc.) If the patient describes physical signs that need to be seen by the investigator, the patient will be asked to come for a visit. So that a physical examination may be performed at a reasonable interval to assess for side effects, an in-person visit must be performed at least once during the first 3 months of the study (not counting the baseline visit), and at least once for the 12.5 month OR 15 month visit. The 6 month visit is typically performed in person because of the MRI and would only be offered remotely if a participant was not having an MRI at the 6 month visit for some independent reason (which would be documented as a protocol deviation). If a participant is not having MRI at 6 months, that visit may be conducted remotely. If a remote visit is performed for a safety visit at which drug is ordinarily dispensed, drug and supplies may be shipped to the participant via FedEx or UPS with signature required upon delivery. Drug will be checked to ensure identifiers match intended patient by at least 2 study staff, including at least one physician, before shipping.

Remote Consenting Visit (optional)

If there are circumstances such that a participant prefers remote consenting, we will offer a remote consenting visit as part of the screening visit. The patient will be (e)mailed a copy of the consent form prior to the visit. A study physician or NP will schedule a telephone call or video conference call to obtain informed consent, medical history, medication list, and drinking assessment. The consent will be obtained using RedCap e-consent. After consent, the patient will be allowed the option to obtain fasting labs at a local Quest site. Then, remainder of screening procedures, including bloodwork if not done previously, physical examination by study doctor, and procedures described below, will be done at MGH on a subsequent day.

Screen Visit (to determine eligibility)

1. Informed consent
2. Detailed H&P and medications (past and current), Lifetime Drinking History assessment
3. Fasting blood: AST, ALT, GGT, creatinine, albumin, total bilirubin, direct bilirubin, alkaline phosphatase, PT/INR and PTT, CBC, glucose, HbA1c, Hepatitis B sAg, Hepatitis C antibody, PSA (male subjects).
4. ^{1}H-MRS/MRI of abdomen for quantification of liver fat and to exclude abnormal liver lesions. This scan will also be used to assess VAT for those eligible subjects participating in the baseline visit.
5. Height and weight
6. Liver ultrasound ("Sonosite") may be performed if subjects do not have a diagnosis NAFLD or have had significant changes in health since NAFLD diagnosis. This is an optional procedure intended to provide an easier alternative to MRI for determining if a study candidate has fatty liver. Subjects may opt to have this test, and then could forego MRI and/or blood sampling if they do not have fatty liver.

Baseline Visit

The following procedures will be performed. Depending on availability of appointments for the various procedures as well as subject availability, subjects may be asked to come on 2-3 different days in order to complete all procedures. (For example, the liver biopsy will usually be performed on a different day than other procedures.)

1. H&P
2. Fasting labs: IGF-I, glucose, AST, ALT, GGT, total bilirubin, direct bilirubin, alkaline phosphatase, albumin, lipid panel, CBC, PT/INR, HbA1c, frozen serum for adiponectin, IL-6, CRP, CK18, and other relevant inflammatory biomarkers, as guided by the histological results
3. Height, metabolic weight, anthropometrics
4. Whole body DXA scan for regional fat
5. 24-hour Food Recall and Modifiable Activity Questionnaire by CRC bionutrition
6. Transient elastography (FibroScan)
7. Liver biopsy. Biopsy will not be done if performed in the 12 months prior to baseline and, in the interval since biopsy, no new treatment initiated and no weight change >10%
8. Stable isotopes for assessment of hepatic de novo lipogenesis
9. Coronary CT angiography (CCTA) performed in individuals who meet the following criteria: BMI <40kg/m2, age ≥ 40y for Males or ≥ 50y for Females. Individuals with any of the criteria detailed in "Exclusion Criteria (#22)" above will not undergo CCTA.
10. Peripheral blood mononuclear cells (PBMCs) will be processed for flow cytometry of immune cells and related immune studies at baseline and 12 months, as well as processed and stored for DNA at baseline.

Start of Study Drug

At the conclusion of the baseline visit, subjects will be instructed in reconstituting and administering tesamorelin or identical placebo, and they will self-administer their first injection at the conclusion of the visit. After administration, they will be observed for 30 minutes to ensure no allergic reaction.

Safety Visits (0.5, 1, 2, 3, 6, 9, 12.5, 15, months)

1. Interval H&P, weight
2. Fasting glucose, AST, ALT, GGT, albumin, alkaline phosphatase, total bilirubin, direct bilirubin, PT/INR, IGF-1
3. At 3, 6, 9, and 15 months, HbA1c
4. At 6 month visit only: ^{1}H-MRS/MRI of abdomen for quantification of liver fat and VAT, fibroscan, lipid panel

12 Month Visit

Identical to baseline visit except: ^{1}H-MRS/MRI of abdomen for quantification of liver fat and VAT will be performed; CCTA will not be performed. At the conclusion of the 12 Month Visit, subjects will self-administer

their first open-label injection, after which they will be observed for 30 minutes to ensure no allergic reaction.

18 Month Visit

1. Interval history, H&P
2. Fasting labs: glucose, IGF-I, AST, ALT, GGT, alkaline phosphatase, total bilirubin, lipid panel, HbA1c
3. Height, weight, anthropometrics
4. Whole body DXA scan for regional fat
5. 1H-MRS for quantification of liver fat, MRI of the abdomen for quantification of VAT
6. Transient elastography (FibroScan)
7. 24-hour Food Recall and Modifiable Activity Questionnaire by CRC bionutrition

Early Termination Visit

If a subject wants to discontinue the study, or if discontinuation is required due to an adverse event and/or one of the stopping rules, we may ask the subject to return for an Early Termination Visit that includes elements of the 18 month visit. This will be at the discretion of the Co-PIs and will be based on how much of the study the subject has already completed and which tests have already been performed.

Telephone Calls

In order to enhance subject retention, study staff or study physicians may call subjects periodically to ensure that study drug administration is going well and that they do not have any questions. Whenever possible, these calls will typically take place about midway between visits. Up to 6 of these phone calls may be made over 18 months.

Study Drug

The study drug is tesamorelin, a GHRH agonist (Theratechnologies, Inc., Montreal, Canada). Tesamorelin is FDA approved for use in HIV-infected patients with visceral adiposity. Tesamorelin will be used in this study under an IND (#138045, Sponsor-Investigator T.Stanley). The dose of tesamorelin is 1.4mg of F4 formulation subcutaneously daily, consistent with the FDA approved dose and equivalent to the dose used in prior studies.

IGF-1 levels will be checked at each visit and monitored by an independent physician not otherwise involved in the study. If a subject's IGF-1 z-score is ≥3, this physician will instruct investigators to perform a dose reduction to 0.7mg daily, along with a dummy dose reduction in a placebo patient if the subject with elevated IGF-1 is in the double-blind phase of the study. IGF-1 will be rechecked in 1 month (window 3-5 weeks), following the dose reduction, and the subject will be discontinued from the study if z-score remains ≥ 3. Subjects who are discontinued for persistent IGF-1 Z-score >3 SD will be asked to come back in 1-2 months for a final IGF-1 recheck to ensure that it has normalized; if it has not normalized, the subject may have an underlying endocrine disease and will be referred to endocrinology.

Subjects who have symptoms that may be consistent with growth hormone excess, including myalgia, arthralgia, paresthesia, or edema, will be offered a dose decrease to 1mg daily or the option to discontinue the study. Subjects who opt for a dose reduction and still have these symptoms will be discontinued from the study if the investigator judges the symptoms to be greater than mild in nature.

With regard to injection site reactions, subjects will be informed to notify a study investigator of any sign of erythema, swelling, or pruritis. Subjects who notify us of such reactions will be asked to stop the study drug and come in for a supervised injection with a study investigator. If the reaction is not present during the supervised injection, and the subject feels comfortable continuing, s/he may restart drug with careful instructions to call again if they experience any erythema, swelling, or pruritis. Subjects with reports of these symptoms on two separate occasions, or any subjects with erythema and/or pruritis extending beyond the immediate injection site will be discontinued from the study. Subjects will be informed to seek emergency medical care immediately if they are experiencing any signs of allergic reaction such as hives, generalized urticaria, nausea, abdominal pain, vomiting, dizziness, syncope, difficulty breathing, incontinence, or general feeling of unwellness. Any subject who has any one of these symptoms in temporal combination with urticaria, redness, or swelling at the injection site will also be discontinued from the study.

Subjects with fasting glucose of >126mg/dL or HbA1c >6.5% at any visit, confirmed by a re-draw, will be discontinued from the study. Although there are not plans to check random glucose levels, if any subject

has a random glucose level checked for other reasons that is ≥200mg/dL, we will perform a fasting glucose. If this fasting glucose is ≥126mg/dL, verified on repeat, this subject will be discontinued from the study.

Any subject with an evolving medical condition (e.g., progressive liver disease) such that continued participation in the study may incur risk or preclude appropriate clinical management will also be discontinued. Subjects with cirrhosis or decompensation during the trial will be discontinued. A physician or NP will be available 24/7 to subjects for any questions or concerns about the study drug.

Abnormal liver function tests will be managed per the algorithm below. If AST or ALT are ≥8x ULN or 600 IU/L, we will ask patients to return within 1 week for CPK and LDH as needed for the algorithm.

Monitoring and Algorithm for Drug Induced Liver Injury

A. If isolated transaminase (AST or ALT) elevations are observed, defined as:

Normal bilirubin and absence of clinical hepatitis, AND

- ALT or AST ≥ 5 times the ULN if normal at baseline, OR
- ALT or AST ≥ 3 times the baseline or 400 U/L if abnormal at baseline

THEN

- Repeat liver profile (ALT, AST, bilirubin and PT/INR) within 2 to 3 days and
- Monitor the patient with laboratory testing and physical examination 2 to 3 times per week as per the "close observation" definition in the DILI guidance (https://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf).
- We will be in close communication with the patient's hepatologist and, if the abnormalities are thought to be related to study drug or do not have another reasonable explanation, we will permanently discontinue study drug

B. If any ONE of the following criteria is met:

- Transaminases (ALT or AST ≥ 3 x baseline) AND bilirubin (total bilirubin > 2 x ULN);
- Cholestatic markers (ALP or GGT > 2 x baseline);
- Isolated ALT/AST ≥8x ULN or 600 U/L in presence of normal CPK and LDH and not secondary to cholangitis
- INR increase >1.5
- OR: if any ONE of the following criteria is met for total bilirubin unrelated to hemolysis:
  - Doubling of total bilirubin
  - OR: any increase in total bilirubin if: there are symptoms of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain)
  - OR: there are immunological reaction (rash or >5% eosinophilia),

THEN

1. study medication will be permanently discontinued
2. a drug-induced liver injury workup must be initiated for alternate etiologies;
3. repeat liver profile (ALT, AST, total bilirubin, direct bilirubin, ALP) and PT/INR within 48 to 72 hours;
4. monitor the subject with further laboratory testing and physical examination 2 to 3 times per week as per the "close observation" definition in the DILI guidance (https://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf.
5. If a subject lives in a remote area, they can be tested locally and the results communicated to the investigator promptly.
6. Subjects will be followed by the study investigator until changes are resolved or stabilized but will not come for further study visits apart from those related to safety monitoring of liver injury.

Individual stopping rules

Subjects will be discontinued from the study for any of the following:

- Fasting glucose >126mg/dL confirmed on a re-draw
- HbA1c > 6.5% confirmed on a re-draw if participant wishes
- IGF-1 Z-score persistently ≥3 after dose reduction
- Erythema or pruritis extending beyond the immediate injection site, indicating allergic reaction
- Pregnancy

- Development/diagnosis of active malignancy
- Significant symptoms of GH excess felt to be related to the study drug
- Clinical conditions, which in the best judgment of the investigator are believed to be harmful or potentially life-threatening to the participant, even if not felt to be related to the study drug and/or not addressed in the AE management section of the protocol.
- Request by participant to terminate study product(s)/intervention(s)

Because some of the follow-up study procedures, including liver biopsy and cardiac CT scan, have associated risks, there are no plans to continue following subjects who have discontinued study drug unless otherwise specified above, apart from the possibility of conducting an early termination visit as described above and/or ensuring return to baseline health for subjects who have had an adverse event.

Concomitant Medications

Concomitant medications will be documented at the screening visit and any change in medications will be carefully documented at each subsequent visit. All concomitant medications are allowed unless specifically prohibited in the eligibility criteria above. Given the possibility for growth hormone to affect CYP450, we have excluded patients receiving anticonvulsants or cyclosporine, as well as subjects who require corticosteroids for adrenal insufficiency. (Systemic corticosteroid use within 6 months of the study is also an exclusion criterion.) Subjects who are taking sex steroids or using other forms (e.g., topical) of corticosteroids will be warned that there may be an effect of the study drug to reduce the systemic concentration of these medications, and that we will speak with their prescribing physicians about this if requested.

Study stopping/pausing rules

- If one subject experiences a Grade 5 toxicity (based on National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] version 5), the trial will be paused and information will be submitted to the FDA for review. The trial will not be resumed without the okay from the FDA.
- If either of the following occurs, a special meeting of the DSMB will be convened within 2 weeks to determine if the trial may be continued. In this event, the trial will be continued pending DSMB review and determination:
  - More than 2 patients develop a CTCAE grade 3 or higher in the same category
  - More than 2 patients meet the individual stopping rules

Methods

^{1}H Magnetic Resonance Spectroscopy (MRS) & MRI: After at least 8-hour overnight fast subjects will undergo ^{1}H -MRS of the liver. A breath-hold true fast imaging with steady precession sequence will be obtained. ^{1}H -MRS data will be acquired using point-resolved spatially localized spectroscopy pulse sequence without water suppression. For abdominal visceral and subcutaneous fat volumes, conventional MR images will be acquired and will serve as anatomic reference of 1H-MRS overlays. Proton density fat fraction will be calculated from integral lipid and water peak areas as previously described (88, 92).

Subjects undergoing MRI/MRS may develop claustrophobia during the scan. Patients will be queried regarding a history of claustrophobia and their ability to tolerate other MRIs during their screening history and phone screen. If they report prior claustrophobia or develop distress during the study MRI, they will be offered a low-dose benzodiazepine (1 mg lorazepam orally) at the time of their scan. Patients who receive anxiolytics will be monitored for a return to baseline mental status following the scan, and will be provided with a ride home.

Liver Biopsy: Percutaneous liver biopsy will be performed under ultrasound guidance using a 16-gauge needle by experienced interventional radiologists. Participants with any contraindication to liver biopsy, including platelets < 75,000, elevated PT or PTT, or chronic anticoagulation use will not undergo biopsy. Individuals on aspirin for primary prevention of CVD may undergo biopsy following discontinuation of aspirin for ≥5 days. One biopsy fragment will be fixed in 10% formalin for histopathological analysis. Histologic scoring will use validated scoring systems NASH Clinical Research Network scoring system (93) and Brunt Fibrosis staging. NASH will be defined by the presence of: steatosis ≥1, lobular inflammation ≥1 and hepatocyte ballooning ≥1. Steatosis will be defined the presence of steatosis grade ≥1, not meeting criteria for NASH.

Fibrosis stage will be scored from 0-4 following the Brunt staging system. A second fragment will be placed in an RNA stabilization reagent (RNAlater, Qiagen) for gene expression analysis. Following RNA extraction, tissue RNA will be subjected to Counter digital transcript counting (Nanostring) analysis in Dr. Chung's laboratory using customized codesets and probes specifically designed for key inflammation, fibrosis, and metabolism-related genes. This technique has been used successfully by his group to perform expression analysis of specific gene sets in peripheral blood and liver tissue from large numbers of patients.

Contrast-Enhanced Coronary Computed Tomography Coronary Angiography (CCTA): This study is being performed in a subset of individuals in order to maximize image quality and the probability of finding plaque. Individuals with BMI≥40kg/m^2 will not have a scan because scan quality would be poor. Men <40yo and women <50yo will not be scanned because of the low likelihood of these individuals having plaque. Subjects must have a GFR of ≥ 60 for participation in the study. Participants on metformin will be asked to hold metformin 48 hours prior to CT scan as well as 48 hours after the scan. If the participant and/or his/her physician feel that holding metformin is not safe, the participant may participate in the study but will not have a the CT scan. Additional criteria for undergoing CCTA are detailed in Exclusion Criteria #22 above. Image acquisition will take place at MGH using a standardized protocol that employs several state-of-the-art technologies to minimize radiation dose (94). The CCTA protocol is based on the standard clinical CCTA protocol at MGH: Sublingual nitroglycerin (0.3-0.9 mg, typically 0.6 mg) will also be administered as per standard protocol for cardiac CT scans. The supervising physician will carefully screen for contraindications to this medication prior to administration. Prior to scans, subjects will be asked not to use medications for erectile dysfunction (e.g., Cialis, Viagra) for 3 days. A prospectively-ECG triggered noncontrast CT of the heart is obtained to measure coronary calcium. Contrast-enhanced CT acquisition will then take place. Based on subject size, 60-100 ml of nonionic iodinated contrast (iopamidol 370 g/cm^3, Isovue 370, Bracco Diagnostics, Princeton, NJ) will be injected, followed by a flush of 40 ml of saline solution. After a delay based on the test bolus, a CT acquisition will be performed of the heart. A number of high-resolution images of the heart at slightly different time-positions within the cardiac cycle will be reconstructed, of which the best phase will be used to evaluate the coronary artery lumen, plaque distribution, and plaque morphology.

Stable Isotope Assessment of Hepatic DNL: Subjects will come to the CRC the evening before their baseline visit and will have 2 baseline samples drawn for baseline concentrations of stable isotope. Following this, they will consume 1g/kg of deuterated water along with a meal that contains caloric content equal to 40% of the estimated daily caloric needs (±100kcal) based on the Mifflin-St. Joer Equation and macronutrient content as follows: 55% (±5%) carbohydrate, 30% (±5%) fat, and 15% (±5%) protein. Upon completion of the standardized meal, subjects will be discharged with a snack they can eat that evening, and otherwise will be instructed to remain fasting. They will arrive to the CRC in the morning following a 12-hour fast. Before breakfast, they will have fasting blood samples drawn at two timepoints, a few minutes apart, to assess deuterium incorporation into plasma TG-palmitate for calculation of fasting hepatic de novo lipogenesis. They will then have a standardized breakfast, with the same caloric and macronutrient content as the meal the night before. All water consumed by the subject until the completion of testing will contain 5g/L D_2O in order to maintain a plasma enrichment of 0.3%. Four hours after the standardized breakfast, at +230 and +240 minutes from completion of breakfast, blood samples will be drawn to assess post-prandial hepatic de novo lipogenesis. Use of deuterated water ingestion to measure enriched palmitate incorporation into triglyceride (TG) is now a well established method for assessment of hepatic DNL (95). Deuterated water will be obtained from Cambridge Isotope Laboratories, and assays for plasma TG-palmitate enrichment will be performed by Dr. David Wagner of Metabolic Solutions (Nashua, NH). Of note, subjects with body weight ≥100kg will be offered the option to skip the stable isotope testing because of the risk of side effects (see VI. Risks and Discomforts).

Transient Elastography: Liver stiffness will be determined using transient elastography (FibroScan®, Echosens, Paris, France) (96). Probe size (M or XL) will be selected based on the subject's body habitus, and the same probe will be used at all timepoints. At least ten measurements will be made and the median will be expressed in kilopascal (kPa) units.

Liver Ultrasound: A liver ultrasound ("Sonosite") may be used to determine the presence of fatty liver in

subjects without a known diagnosis. This will be performed by a trained technician and read by an experienced radiologist. This is not formally used for study eligibility but may be used to inform subjects that they do not seem to have fatty liver and may not want to undergo the remainder of screening procedures.

Whole Body DXA, Assessment of Nutrition and Activity: CRC bionutrition staff will perform the following assessments: Whole body DXA will be used to determine whole body and regional fat. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass (97). 24-hour food recall will be administered to assess micronutrient and macronutrient intake, and Modifiable Activity Questionnaire (MAQ) will be administered to assess physical activity (98).

Medication adherence will be assessed by vial count and medication diary.

V. Biostatistical Analysis

Endpoints

Aim 1: Change in hepatic fat fraction as measured by ^{1}H-MRS after 1 year of randomized treatment **(Primary endpoint, Aim 1a)**. Histological features of NASH, assessed by histopathological analysis of liver biopsy, including frequency of ≥2 point reduction in NAFLD activity score (NAS score) with no worsening of fibrosis stage and reduction in individual components of the NAS **(Aim 1b)**.
Aim 2: Change in post-prandial hepatic DNL by stable isotope methods (**Aim 2a**). Change in mRNA expression of genes related to hepatic lipid metabolism in liver tissue obtained by biopsy. Target genes will include SCD1, FAS, SREBP1c, CD36, PPARα, PPARγ, CPT1, LDLR (**Aim 2b**). Change in mRNA expression of genes related to inflammation/oxidative stress and fibrosis/fibrogenesis in, liver tissue. Target genes include: MCP1, TNFα, IL-1β, IL-6, IL-18, TLR, MyD88, CCL5, TGFβ1, NFκB, TIMP1, COL1A1, MMP 1-14 (**Aim 2c**). Exploratory endpoint: change in immune cell phenotypes assessed by flow cytometry
Aim 3: Change in circulating fasting lipids, c-reactive protein, tissue plasminogen activator antigen, plasminogen activator inhibitor-1 antigen, adiponectin, and potentially other circulating markers as guided by emerging science regarding noninvasive CVD risk measures (**Aim 3a**).
Supportive endpoints of interest include change in liver stiffness measured by fibroscan, change in AST, ALT, and GGT, and change in fasting glucose, insulin, and HOMA-IR.

Planned Data Analysis

No interim data analyses are planned. Primary data analysis will be performed after the last patient finishes the 12-month visit, at which time the double-blind portion of the study will be completed. Secondary analyses will be performed for the open-label phase as below.

Analysis will be based on intention to treat population using all available data, including interim data for subjects not completing the study. Baseline variables (including age, gender, race, ethnicity, body mass index, fasting glucose, insulin, HOMA-IR, ALT, AST, lipid panel, liver histology parameters, MRS parameters, comorbidities) will be compared between treatment groups, and any baseline variable that is different between treatment groups will be adjusted for in subsequent analysis. For variables measured at baseline, 6 and 12 months in the study, including the primary endpoint of liver fat by MRS, we will analyze the longitudinal data including 0, 6, and 12 month data using general linear mixed effects modeling for which the subject level intercept will be random, and effects of treatment group, time, time x treatment group will be random, and a compound symmetry error covariance structure will be considered. The longitudinal treatment effect between tesamorelin and placebo will be examined by testing for time x treatment group interaction. The same statistical methods will be applied to the analyses of other endpoints performed at repeated time points in the study. If there is evidence that any of the outcomes is not normally distributed, we will choose a proper transformation for normalization before the mixed effects model analyses. For variables assessed at baseline and 1 year in the study, including changes in DNL and histological features of NAFLD, the effect of tesamorelin vs. placebo will be assessed first using a two-sample t-test to compare the mean changes or Wilcoxon Rank Sum test if not normally distributed. If necessary, adjusted analyses will also be performed for the differences in any baseline characteristics between treatment groups. We will carefully assess changes in physical activity and dietary intake in terms of overall caloric intake, micronutrient intake, including Vit E, and macronutrient composition between the groups, and we will determine if changes in body weight, physical activity or dietary

intake are contributing to the observed treatment effect on hepatic fat, histologic and metabolic variables. If so, we will include these variables in the analyses and modeling as adjusted covariates. If any other variables are different between groups, such as medication adherence, these will also be included as covariates.

Genetic analyses: Hepatic mRNA expression for the targeted genes listed above will be quantified at baseline and 12 months. Our statistician, Dr. Lee, has designed a stepwise analysis that will serve as an appropriate approach for this sample size and the discrete set of genes that we propose to measure. (1) We will first assess whether there are significant changes in expression between baseline and 12 months in the GHRH-treated subjects, using paired samples t-test. (2) For genes that show a significant change in expression in this analysis, we will then compare fold-changes in expression between the tesamorelin and placebo groups. False discovery rate based type-1 error adjustment will be applied for multiple comparisons. (3) Genes with differential changes in the tesamorelin vs. placebo groups will be further investigated by assessing associations between mRNA expression and histological and biochemical parameters. For example, if SREBP1c is found to be downregulated in tesamorelin compared to placebo groups, we will assess correlations between mRNA expression of SREBP1c and histological steatosis score as well as rate of *de novo* lipogenesis quantified using stable isotopes.

Missing data: We expect that the pattern of any missing data will be at random (MAR), and the longitudinal mixed effects model approach utilizing all available observation will provide unbiased effect size estimates. However, we will perform sensitivity analyses if the dropout rate is higher than expected or differs between groups. We will initially consider applying the last observation carried forward (LOCF) as a conservative approach. We will also consider multiple imputations (MI) based analysis.

Open label analysis: For the 6-month open-label extension, hepatic fat and relevant secondary endpoints including fibrosis as assessed by Fibroscan, and AST and ALT will be determined. We will first examine the 18 month effects by comparing 0-18 months mean change of Group 1 (initially randomized to tesamorelin and maintaining active treatment from month 12-18) to that of Group 2 (initially randomized to placebo and crossed over to tesamorelin from months 12-18) using independent samples t-test, and then compare mean change over 1 year to that over the later 6 months within Group 1 using paired samples t-test. Although the primary purpose of the 6-month open label extension is to increase recruitment and retention, analysis of data from the group receiving tesamorelin for 18 months will provide information regarding whether reductions in liver fat translate into sustained longer-term improvements in transaminases and liver fibrosis as measured by FibroScan and will provide longer-term safety data in this group.

Sample Size and Power Calculations

Aim 1a (Primary endpoint): In our previous study of tesamorelin in HIV (see Preliminary Data), the change in liver fat over 6 months among individuals who started with hepatic fat fraction >5% was -2.5 ± 11.9 lipid-to-water percent in placebo vs. -9.1 ± 7.6 lipid-to-water percent in tesamorelin. Based on the prior data, we assumed that the pooled standard deviation of the 1 year change is 8% and the tesamorelin treatment effect over 1 year (difference in tesamorelin vs. placebo changes) is at least a 7 point change in the lipid to water percentage. Using this data we estimate that an initial sample size of 76 will provide 91% power to detect a clinically relevant treatment difference of 7% or larger 1 year mean changes in liver fat (lipid to water %) at $\alpha = 0.05$ if the discontinuation rate is 20% (61 evaluable patients), or 80% power if the discontinuation rate is 25%. In the prior study of tesamorelin led by Dr. Stanley, discontinuation rate was 14% after 6 months (69). These assumptions are conservative as we expect that the 1 year mean change in the current proposed trial among the patients on tesamorelin will be greater than that was observed above in our 6 month preliminary studies. We also anticipate the variability over time will be less in the placebo group assessed by repeated measures in the proposed study rather than a single point in time as in our prior study. **Aim 1b:** Based on baseline data from PIVENS (25), in which the standard deviation in NAS is 1.4, we will have >90% power to detect a change of 2 in NAS. **Aims 2-3**: For analysis of the variables in Aims 2-3, it is important to note that the purpose of the study is to interrogate physiology, and the sample size is relatively limited due to the detailed assessments performed. Thus, these variables will be largely viewed as hypothesis generating, and statistical analyses will be interpreted in such a context. (For example, if there is a marginally significant change in one lipogenic gene but no signal in other genes, this will not be considered meaningful, whereas a trend toward change in all

lipogenic genes in the same direction may be meaningful). Thus we do not plan to correct Type 1 error for multiple comparisons, which would limit power and increase the probability of Type 2 error (99, 100). For the variables in Aims 2-3, the sample size will provide 80% power to determine a 0.73 SD difference in 1 year mean changes between treatment groups at $\alpha = 0.05$ with 61 evaluable patients. Although changes of <0.7 SD in some of these variables may be clinically relevant, most clinically meaningful effects are expected to be 1SD or more, a magnitude for which the study is sufficiently powered.

VI. Risks and Discomforts

GHRH (Tesamorelin)

In previous large, placebo-controlled studies of tesamorelin in HIV-infected individuals, adverse events that were more common in the treatment vs. placebo group were injection site erythema (8.5% in treated vs. 2.7% in placebo), injection site pruritis (7.6% of treated vs. 0.8% placebo), and peripheral edema (6.1% in treated vs. 2.3% in placebo). With respect to serious adverse events (SAEs), prevalence was not statistically different (3.7% in treated vs. 4.2% in placebo). There is a risk of hyperglycemia with GHRH, particularly in the few weeks following initiation of treatment, but glycemia returns to baseline after several months. Clinically significant changes have not been observed for liver function (alanine aminotransferase or aspartate aminotransferase), kidney function (creatinine), or blood pressure (diastolic and systolic).

With regard to injection site reactions, subjects will be informed to notify a study investigator of any sign of erythema, swelling, or pruritis, and subjects with erythema and/or pruritis extending beyond the immediate injection site will be discontinued from the study. Subjects will also be informed of risks associated with excess GH, including arthralgia, paresthesia, and hyperglycemia. Although there is a theoretical risk of neoplasm with an agent that increases GH and IGF-I, there is no evidence of increased malignancy with tesamorelin. This risk is further minimized based on the data from previous studies showing achievement of physiologic, rather than pharmacologic, increases in IGF-1 SD scores. Nonetheless, subjects with active malignancy will be excluded from the study. Further, men with history of prostate cancer or PSA >5 ng/mL, or women 50 years or older without documentation of negative mammogram in the past year will be excluded from the study. To minimize the risk of hyperglycemia, subjects with known diabetes or with fasting glucose >126mg/dL or HbA1c ≥6.5% will be excluded from the study.

Liver biopsy

Ultrasound-guided percutaneous liver biopsy will be performed by trained interventional radiologists who specialize in gastrointestinal procedures at the MGH. Subjects will be observed as outpatients following liver biopsy per standard interventional radiology procedure. The risks associated with liver biopsy include pain, transient hypotension (vasovagal response), bleeding, and transient bacteremia. Participants with any contraindication to liver biopsy, including platelets < 75,000, elevated PT or PTT, or chronic anticoagulation use will not undergo biopsy. Individuals on aspirin for primary prevention of MI may undergo biopsy following discontinuation of aspirin for ≥5 days. After discharge from interventional radiology following the procedure and outpatient observation, subjects will also be called the next day by study staff to ensure that any post-procedure pain has resolved and that no new symptoms are present. Subjects with persistent pain or other concerning symptoms will be asked to return for evaluation. Drs. Stanley and Corey have successfully conducted previous studies with liver biopsies performed as described above.

Blood drawing

The total volume of blood sampling during the 18 months of the study is approximately 700cc. The maximum amount of blood drawn during any single visit will be 155cc, drawn at the baseline and 12 month visits. This amount of blood drawing is well within with our Human Research Committee guidelines, which urge precaution for blood sampling of more than 200cc on any given occasion and prohibit blood sampling of more than 550 cc in an 8-week period. There will be a minimum amount of discomfort associated with blood drawing and IV placement, with risks including minor bruising or bleeding or, much more rarely, infection, lightheadedness, or syncope.

Ionizing Radiation

Subjects will have 3 total body DXA scans (0, 12, and 18 months, 0.5 μSv each) and 1 coronary CT, with a median of 6mSv, with modest variation in dose for each individual since each exam is tailored to subject size, heart rate, and heart rhythm. The 95th percentile range for the cohort is estimated to be 2.0 – 12.7 mSv for the CCTA. Because subjects with BMI ≥40kg/m^2 will not undergo CCTA, this will limit the upper level of radiation received by subjects from the CCTA. Thus, the total median radiation for the study will be 6.002mSv. For comparison, the average person in the United States receives a radiation exposure of 3.1mSv each year from natural background sources; thus the total radiation in the study will be equivalent to a little less than 2 years of natural background radiation. Scientists disagree regarding whether radiation at these low levels are harmful. In order to ensure that exposure to radiation from the study does not compound concurrent radiation exposure, we will exclude patients who report (1) any history of radiation therapy, (2) any significant radiation exposure over the course of the year prior to randomization, or (3) being under active consideration for a procedure or treatment that includes significant radiation exposure in the year following randomization. Please see the Research Strategy for more details.

Magnetic Resonance Imaging/Magnetic Resonance Spectroscopy

Subjects will be carefully screened for metal implants such as surgical clips or pacemakers prior to MRI scanning. Subjects will be given earplugs due to the loud noises during the test. Subjects who feel uncomfortable in confined spaces may have difficulty in the narrow cylinder of the MRI, and the MRI can be stopped at any time at the patient's request. A low dose of lorazepam may be administered at the time of MRI/MRS for patients who have a history of claustrophobia. Lorazepam is a benzodiazepine used to treat anxiety. It may cause drowsiness, dizziness, or unsteadiness. Patients will be cautioned to not combine this medication with other sedating medications or alcohol. We will also ensure that the patient is driven home by someone else.

Contrast-enhanced Coronary Computed Tomography Angiography (CCTA)

CCTA will involve the use of contrast as well as nitroglycerin, with risks as below.

CT Contrast Medium

Coronary CT angiography requires the injection of an iodinated contrast medium. These contrast media have been known to cause renal failure, particularly in patients with a pre-existing renal impairment. An eGFR will be reviewed for all participants prior to their undergoing contrast administration. Subjects with GFR < 60 will be excluded. Participants on metformin will be asked to hold metformin 48 hours prior to CT scan as well as 48 hours after the scan. We will check with the participant's physician to ensure this is safe; if the physican feels that holding metformin is not safe, the participant may participate in the study but will not have the CT scan. Adequate hydration will also be emphasized to each study participant. The contrast dose in cardiac CT has decreased with the introduction of faster scanners. The dose required will be between 75 to 100 ml. Allergic reactions to iodinated contrast media may cause skin reactions or in very rare occasions result in breathing difficulty and hypotension. Subjects will be closely monitored for the development of any allergic reactions.

Nitroglycerin:

Nitroglycerin is commonly used in patients with angina pectoris and is generally considered safe. The side-effects and risks of nitroglycerin are generally mild and of short duration and include hypotension, tachyarrhythmia, headache, lightheadedness, and visual disturbance. Subjects who are on sildenafil, tadalafil, or vardenafil will need to stop these drugs at least 3 days prior to receiving nitroglycerin on the day of the cardiac CT scan. Nitroglycerin will not be administered to subjects who have taken sildenafil, tadalafil, or vardenafil within 3 days of the cardiac CT scan. If baseline systolic blood pressure is below 90, nitroglycerin will not be given. If a participant's blood pressure were to drop precipitously or if the participant develops symptoms, standard clinical care will be provided.

Stable Isotope Test

Drinking deuterated water may cause transient dizziness, nausea, or – less commonly – vomiting. Literature suggests that this occurs with ingestion of larger amounts of 100-200cc at one time (Miller et al., J Appl Physiol

128: 1163–1176, 2020). Because of this, we will offer those with weight ≥100kg the option to forego the test if they are worried about the risk of these transient side effects.

Incidental Findings

Non-cardiac incidental findings may be discovered on the MRI of the liver and abdomen or on the CCTA. Additionally, clinically actionable cardiac findings will be reported from the CCTA in real-time. These findings will be communicated to the subject and, with the subject's permission, his/her regular care providers. When necessary, we will help facilitate follow-up for findings that require timely evaluation or treatment. Incidental findings may cause worry and additional diagnostic clinical procedures for subjects. Of note, non-actionable cardiac findings on the CCTA will not be reported to subjects, who will be informed in the consent process that the scan is for research, is not meant to replace their routine care or provide cardiovascular screening, and will not be available to them. As above, findings such as severe stenosis of a vessel that are clinically actionable will be reported and communicated to subjects.

Fibroscan and Liver Ultrasound do not have direct risks, although either may cause discomfort from lying supine, temporary soreness at the site of the scan, or a skin reaction to the ultrasound gel used.

VII. Potential Benefits

Potential Benefits of the Proposed Research to Human Subjects and Others

All participants will receive nutrition counseling from Registered Dietitians, and will receive information about their metabolic and nutritional health. Our preliminary data suggest that the study drug results in a reduction in visceral fat in a majority of patients and, on average, reduces systemic inflammation, triglyceride levels, and carotid intima-medial thickness; these are potential benefits for subjects randomized to study drug during the double-blind phase and for all subjects in the open-label phase. The moderate level of risk involved in study participation is thus thought to be balanced by the possible benefits to participants. This study investigates a novel therapy for NAFLD and, if the study hypotheses are correct, has the potential to lead to a treatment for NAFLD that would be of benefit to the larger population.

Importance of the Knowledge to be Gained

NAFLD is a prevalent disease that poses significant health consequences. Further, there is currently a lack of effective pharmacological therapies, and the development of strategies for treatment is urgent. The current proposal will investigate a novel strategy to address underlying pathophysiology of NAFLD, and may result in significant progress toward development of a therapy. Even if the hypotheses are proven incorrect, the proposed research will provide data that allow for significant insight into the pathophysiology of NAFLD as well as the metabolic and endocrine processes related to NAFLD. Thus the potential benefits to the study population outweigh the risks of participation.

XI. Monitoring and Quality Assurance

Data Quality and Monitoring

Data will be collected on written Case Report Forms that are maintained in each subject's binder along with source documentation. Data will be entered into REDCap; double-data-entry will be used to ensure correctness of data.

The study investigators will monitor all data collected for the studies, and will ensure completeness and timeliness of data collection and entry. Data will be stored securely, with access restricted to co-investigators and study staff. Binders with subject information will be labeled with coded enrollment number to protect confidentiality. Electronic databases will be locked, and password-protected, with access available only to study staff. Data will not be saved on the hard drive of any laptop or desktop computers or on any removable data storage devices such as flash drives or CDs.

Safety Monitoring

The Co-PIs of the study will monitor adverse events in real time. There are individual stopping rules as above. There are no study stopping rules, but the study will have ongoing monitoring by a Data Safety Monitoring Board (DSMB).

A DSMB will be established by the Co-PIs and that will consist of a statistician as well as 2 physicians with expertise in endocrinology and hepatology. The DSMB will meet every 6 months by telephone or in person to review all adverse events as well as recruitment and retention information, protocol deviations, and any unanticipated problems.

Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), significant symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse events will be judged by the Co-PIs or Co-Investigators based on seriousness, causality, and expectedness. Adverse events will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 grading system (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50)

A **serious adverse event** is one that results in one or more of the following:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Causality will be defined as follows and will be judged by a Co-PI or Co-Investigator:
Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

OR

- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Expectedness will be determined based on the information in the investigator's brochure (IB) or package insert (PI), as well as the known side effects of growth hormone excess, and the known risks of study procedures. Adverse events that are not listed in the IB or PI, and are not known side effects of GH excess or possible side effects of study procedures will be judged as unexpected. The determination of expectedness will be made by a Co-PI or Co-Investigator.

AEs will be reported to the Partners IRB per IRB guidelines. Serious adverse events that are unexpected and thought to be related to the study will be reported to the IRB per IRB guidelines and to the FDA per FDA guidelines.

Subjects will be instructed to report immediately any adverse events. A physician or nurse practitioner will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

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Appendix 1: Summary of Procedures

	Scr	BL	2wk	1m	2m	3m	6m	9m	12m	12.5m	15m	18m	ETV
Informed Consent	X												
H&P	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X												
Liver panel*	X	X	X (no GGT)	X	X	X	X	X	X	X	X	X	X
CBC	X	X							X				
Creatinine	X												
Fasting glucose	X	X	X	X	X	X	X	X	X	X	X	X	X
HbA1c	X	X				X	X	X	X		X	X	X
Hepatitis B sAg	X												
Hepatitis C Ab	X												
PSA (M only)	X												
IGF-1		X	X	X	X	X	X	X	X	X	X	X	X
Lipid panel		X					X		X			X	X
Adiponectin		X							X				
IL-6		X							X				
CRP		X							X				
CK18		X							X				
1H-MRS	X						X		X			X	X
24h Food Recall	X								X			X	X
Modifiable Activity Questionnaire		X							X			X	X
Fibroscan		X					X		X			X	X
DXA scan		X							X			X	X
Liver Biopsy		X							X				
Stable Isotopes – de novo lipogenesis		X							X				
CCTA**		X											
PBMCs		X											

*Liver panel includes AST, ALT, GGT, albumin, total bilirubin, direct bilirubin, PT/INR, alkaline phosphatase.
**CCTA will be done only in individuals who meet the criteria described in the protocol