FINAL DETAILED PROTOCOL

Tesamorelin Effects on Liver Fat and Histology in HIV: A Collaborative UO1 Grant

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1. Title Page

DETAILED PROTOCOL

Full Title:

Tesamorelin Effects on Liver Fat and Histology in HIV: A Collaborative UO1 Grant

Clinical Trial Phase: N/A

Local Protocol Numbers:
MGH 2014p-001502
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NIAID Funding Mechanism and Principal Investigators:
Principal Investigators: Steven Grinspoon, MD, and Colleen Hadigan, MD, MPH
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Other Collaborators:
Takara Stanley, MD (MGH)
Martin Torriani, MD (MGH)
Wendy Henderson, CRNP, PhD (NIH)
Chia-Ying Liu, PhD (NIH)
Theo Heller MD (NIH)
David Kleiner, MD PhD (NIH)
Joseph Kovacs, MD (NIH)

Pharmaceutical and other Study Support Provided by:
Study drug and matching placebo provided at discounted cost by Theratechnologies, Inc.

IND Sponsor: Steven Grinspoon, MD

IND Number: 77,473

Protocol Chair: Steven Grinspoon, MD

DAIDS Medical Officer/Medical Monitor: Beverly Alston Smith , MD (NIH)

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2. SIGNATURE PAGE: N/A

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4. KEY ROLES

Steven Grinspoon, MD	Co-PI, Protocol Chair, and MGH Site PI. Responsibilities include (1) coordinating all progress reports to the NIH; (2) maintaining the investigator-initiated IND, including submitting annual reports and Serious Adverse Events to the FDA as required; (3) registering the trial on clinicaltrials.gov, updating the entry as required, and reporting results upon completion of the trial; (4) supervising the performance of all activities at the MGH site, including the performance of clamp and isotope studies and liver biopsy, as well as the performance of IGF-1 and insulin assays at the MGH core laboratory; (5) procuring study drug from Theratechnologies and coordinate the shipping and labeling of the drug as well as shipping of labeled drug to the NIH pharmacy. Phone: 617-724-9109; Email: sgrinspoon@partners.org
Colleen Hadigan, MD, MPH	Co-PI and NIH Site PI. Responsibilities include (1) supervising the performance of all activities at the NIH site, including recruitment, liver biopsy procedures and MRI/MRS; (2) supervising shipping of all histology specimens to Dr. Kleiner; (3) supervising the shipment of samples to MGH for assay of IGF-1; (4) working with Dr. Kleiner to ensure timely completion of histological analyses. Phone: 301-594-5754; Email: hadiganc@niaid.nih.gov
David Kleiner, MD, PhD	Central pathologist for the study. He will receive all liver biopsies and read them in a blinded manner.
Takara Stanley, MD	Co-investigator at MGH. Responsibilities collaborating with Dr. Grinspoon in the recruitment, data collection, and performance of study procedures at MGH. In addition, under the supervision of Dr. Grinspoon, Dr. Stanley will take primary responsibility for monitoring data entry and for preparing monthly reports of accrual and retention, data integrity, and adverse events for monthly study meetings.
Martin Torriani, MD	Central radiologist for the study. He will receive all liver MRS data and interpret the data in a blinded manner.
Chia-Ying Liu, PhD	NIH Special Volunteer and Investigator physicist who will oversee MRS/MRI acquisition of scans and de-identified coded data to MGH for interpretation.
Hang Lee, PhD	Study biostatistician (MGH). Will prepare randomization lists, provide statistical consultation as needed throughout the study, and oversee analysis of study data per statistical analysis plan.
Theo Heller, MD	NIH Investigator with the Liver Disease Branch of NIDDK. Will serve as the liver expert for the study, available for consultation regarding complex liver-related issues.
Wendy Henderson, PhD	NIH Investigator with the Behavioral Branch of NINR. Will

	serve as the expert in transient elastography (FibroScan) at the NIH.
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5. LIST OF ABBREVIATIONS

ACC	Acetyl CoA Carboxylase
AE	Adverse Event
ALT	Alanine Aminotranferease
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CBC	Complete Blood Count
CD	Cluster of Differentiation
ChREBP	Carbohydrate Response Element Binding Protein
CK18	Cytokeratin 18
CPT-1	Carnitine Palmitoyltransferase 1
CRF	Case Report Form
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DEXA	Dual Energy X-ray Absorptiometry
DNL	De Novo Lipogenesis
DSMB	Data Safety and Monitoring Board
FAS	Fatty Acid Synthase
FATP	Fatty Acid Transport Protein
FBG	Fasting Blood Glucose
FDA	Food and Drug Administration
FFA	Free Fatty Acid
GCRC	General Clinical Research Center
GGT	Gamma-Glutamyl Transferase
GHRH	Growth Hormone Releasing Hormone
GHRLD	Liver-specific Deletion of the Growth Hormone Receptor
GPAT	Glycerol-3-Phosphate Acyltransferase
HbA1c	Glycated Hemoglobin
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HepG2	Human liver carcinoma cell line
^{1}H-MRS	Proton Magnetic Resonance Spectroscopy
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IATA	International Air Transport Authority
IGF-1	Insulin-like Growth Factor 1
IL-6	Interleukin 6
IND	Investigational New Drug
INR	International Normalized Ratio
IR	Insulin Resistance
IRB	Institutional Review Board

IU	International Units
IV	Intravenous
LDL	Low-Density Lipoprotein
MAQ	Modifiable Activity Questionnaire
MGH	Massachusetts General Hospital
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NAFLD	Non-Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non-Alcoholic Steatohepatitis
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OGTT	Oral Glucose Tolerance Test
PAI-1	Plasminogen Activator Inhibitor 1
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PO	By Mouth
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
PSA	Prostate-Specific Antigen
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QD	Every Day
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
sAg	Surface Antigen
SC	Subcutaneous
SD	Standard Deviation
SDR	Spontaneous Dwarf Rat
SREBP1c	Sterol Regulatory Element Binding Protein 1c
TG	Triglyceride
tPA	Tissue Plasminogen Activator
TNFα	Tumor Necrosis Factor Alpha
TZD	Thiazoledinedione
VAT	Visceral Adipose Tissue
VLDL	Very Low Density Lipoprotein

6. PROTOCOL SUMMARY

Full title: Tesamorelin Effects on Liver Fat and Histology in HIV: A Collaborative UO1 Grant

Short Title: TESLA

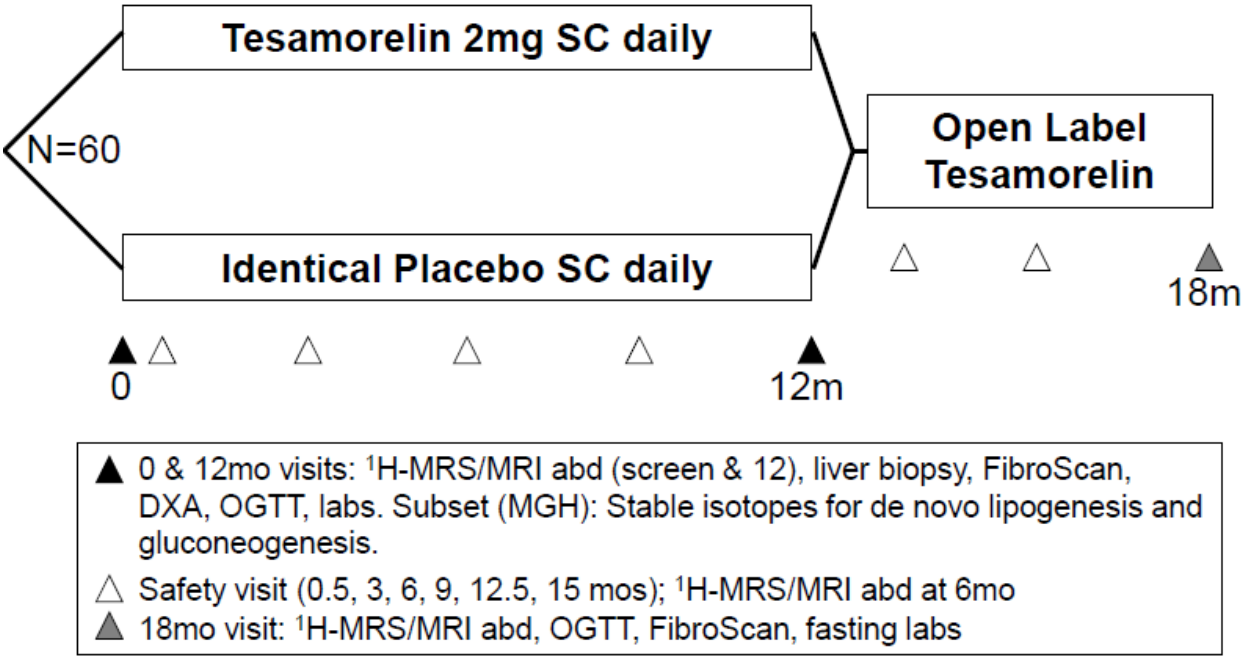
Sample Size: 60 (recruited at MGHand NIH)

Study Population: HIV infected men and women, ages 18-70y, judged to have nonalcoholic fatty liver disease (NAFLD) on the basis of liver fat fraction ≥5% on ^{1}H-MRS.

Participating Sites: Massachusetts General Hospital and National Institutes of Health Clinical Center

Study Design: 12 month double-blind, randomized, placebo-controlled phase followed by 6 month open-label phase.

Study Schema:



Schedule of Procedures/Evaluations

- Screen visit for eligibility assessment, including ^{1}H-MRS/MRI for quantification of liver fat and visceral and subcutaneous abdominal fat area
- Baseline visit for laboratory evaluation, liver biopsy, DEXA, transient elastography (FibroScan), and, at MGH, stable isotopes for assessment of de novo lipogenesis and gluconeogenesis. Study drug started at conclusion of baseline visit.
- Safety visits at 0.5, 3, 6, 9 months for measurement of IGF-1, fasting glucose, AST, and ALT. ^{1}H-MRS/MRI also performed at 6 month visit.
- 12 month visit identical to baseline, with addition of ^{1}H-MRS/MRI. Open label tesamorelin started at conclusion of 12 month visit.

- Safety visits at 12.5 and 15 months for measurement of IGF-1, fasting glucose, AST, and ALT.
- 18 month visit to consist of repeat ^{1}H-MRS/MRI as well as laboratory evaluation and transient elastography.

Study Duration: 1 month screening period, 12 month double-blind phase, 6 month open-label phase. Total subject participation anticipated at 19 months from screen visit to conclusion of 18 month visit.

Study Regimen/Intervention: Lifestyle counseling throughout for all participants. First 12 months: tesamorelin vs. identical placebo, 2mg subcutaneously daily. Final 6 months: open-label tesamorelin 2mg subcutaneously daily.

Primary Objective: To determine the effects of tesamorelin (vs. placebo) on liver fat quantity in HIV-infected individuals with NAFLD.

Primary Endpoint: Change in hepatic fat fraction as measured by ^{1}H-MRS after 1 year of randomized, double-blind treatment with study drug.

7. INTRODUCTION

7.1 Background Information

Liver disease is one of the leading co-morbidities of HIV-infection, highly prevalent even in those without hepatitis B or C co-infection. Studies of hepatitis B- and C-negative adults with treated HIV demonstrate 20-60% prevalence of elevated liver enzymes [1-4]. Among this group, 60-70% of HIV-monoinfected individuals with elevated liver enzymes have non-alcoholic fatty liver disease (NAFLD) [5, 6]. NAFLD may also be present in the absence of liver enzyme elevation, and, overall, it is estimated to occur in up to 30-40% of patients with HIV [7, 8].

NAFLD is of significant concern in the context of HIV-infection for two reasons. First, the disease spectrum of NAFLD encompasses not only steatosis but also inflammation, hepatocellular damage, and fibrosis, which are hallmarks of non-alcoholic steatohepatitis (NASH). NASH, in turn, may progress to cirrhosis and eventual liver failure. NASH is also a significant risk factor for hepatocellular carcinoma [9]. Second, NAFLD is strongly associated with visceral adiposity and other cardiometabolic abnormalities, including insulin resistance (IR) and cardiovascular disease (CVD) [7]. Abundant evidence suggests a causal link between NAFLD and IR, and emerging data suggest that NAFLD may also be causal in CVD. Thus, understanding NAFLD in HIV and developing strategies to treat it are critical to reduce liver disease and ameliorate cardiometabolic risk in this population.

Accumulation of triglyceride (TG) in the cytoplasm of hepatocytes is the sine qua non of NAFLD. Sources of hepatic TG are (i) dietary fats; (ii) uptake of circulating free fatty acid (FFA), mediated by fatty acid transport proteins (primarily FATP2 and FATP5); and (iii) hepatic DNL whereby palmitate is synthesized from glucose, particularly during conditions of carbohydrate excess [10, 11]. Investigation with stable isotope tracers suggests that approximately 59% of hepatic TG are from uptake of circulating FFA, 25% from hepatic DNL, and 15% from the diet [10]. We hypothesize that both hepatic FFA flux – and therefore FFA uptake – and hepatic DNL are increased in HIV-infected individuals with NAFLD, and, further, that GHRH treatment may decrease these two processes and thereby reduce hepatic fat.

Hepatic DNL is stimulated by glucose, acting through carbohydrate response element binding protein (ChREBP), and by insulin, acting through sterol regulatory element binding protein 1c (SREBP1c). Activation of these transcription factors increases the expression of lipogenic genes, including acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS), both of which encode enzymes in the pathway through which glucose is used as a precursor to produce palmitate. Importantly, even in the context of significant hepatic IR, insulin retains the ability to promote lipogenesis in the liver [12-14]. This paradoxical preservation of hepatic DNL in the context of hepatic and whole body IR is a critical factor promoting NAFLD.

DNL is increased in HIV-infection [15] and in NAFLD [16, 17]. Lambert et al. have recently shown that DNL is more than twice as high in those with high liver fat compared to controls [16]. Moreover, DNL is significantly positively associated with quantity of intrahepatic TG [16]. Importantly, GH administration suppresses hepatic DNL both in animal models [18-22] and in individuals with HIV-associated abdominal obesity [23]. The effects of tesamorelin per se on DNL have never been investigated. We hypothesize that one of the mechanisms by which GHRH analogue will reduce liver fat is through reduction in hepatic DNL.

Free fatty acids from the circulation comprise approximately 60% of hepatic TG accumulation in NAFLD [10], and increased FFA flux plays an important role in the development of NAFLD, because of both increased delivery of lipid substrate and the resulting increases in oxidative stress and mitochondrial dysfunction due to lipotoxicity [24-29]. Stable isotope studies show strong associations between increased adipocyte lipolysis – resulting from impaired insulin suppression of lipolysis due to IR – and increased liver TG content [25, 26]. Visceral adipose tissue (VAT), which is highly lipolytic and releases FFA directly to the portal circulation, may be particularly pathogenic. Quantity of VAT is strongly associated with NAFLD and NASH

in both the general population [30, 31] and HIV-infected individuals [7, 8]. In vitro lipolytic activity of visceral but not subcutaneous adipocytes is significantly associated with increased liver fat [32, 33]. Further, fatty acids derived from visceral fat comprise a significantly greater percent of very low density lipoprotein (VLDL) in individuals with fatty liver and IR than in controls, suggesting significantly greater hepatic flux of FFA derived from visceral fat in NAFLD [26, 34]. We have previously demonstrated that reductions in liver fat were significantly associated with reductions in VAT, and we hypothesize that reduction in visceral fat, resulting in decreased FFA flux to the liver, is another mechanism whereby GHRH reduces liver fat.

The pathophysiological mechanisms distinguishing simple steatosis from NASH are not yet well understood, but one mechanism thought to play a central role in the development of steatohepatitis is alteration in adipokines and inflammatory cytokines consistent with a pro-inflammatory milieu [27, 35, 36]. In particular, NASH appears to be closely linked to changes in adipose tissue production of adipokines and inflammatory cytokines. In rodent models, a high fat diet leads to increased adipose tissue macrophage activation and pro-inflammatory gene expression within a few weeks, and the magnitude of these inflammatory changes in adipose tissue portends subsequent inflammatory changes in the liver and development of NASH several weeks later [37, 38]. In humans, adipose tissue expression of macrophage markers and inflammatory cytokines is increased in those with NAFLD, independent of obesity [39, 40]. Conversely, expression of the insulin sensitizing hormone adiponectin is decreased in NAFLD, independent of obesity [40], and adiponectin levels are significantly inversely associated with steatosis and features of steatohepatitis [41, 42]. Measurement of circulating adipocytokines shows similar results, with elevated TNFα [43] and CRP [44] and decreased serum adiponectin in NAFLD and NASH. In a cohort of 80 individuals with NASH and 29 with simple steatosis, adiponectin was significantly reduced in NASH as compared to simple steatosis, suggesting a role for adiponectin in preventing steatohepatitis as well as steatosis [43]. Further suggesting a role for adiponectin, a model of C57BL/6 mice fed high fat diet showed that treatment with adiponectin reverses steatosis and significantly improves inflammation [45].

Its contribution to a pro-inflammatory milieu is another respect in which visceral fat is likely central in the etiology of NASH. Visceral adipose tissue shows significantly higher monocyte/macrophage infiltration compared to subcutaneous adipose tissue [46-48], as well as significantly increased expression of pro-inflammatory cytokines and reduced expression of adiponectin [49-51]. As an example of the contribution of visceral cytokines to hepatic and systemic inflammation, Fontana et al. found that portal vein concentrations of IL-6 are 50% higher than those in the systemic circulation, presumably due to elevated visceral production of IL-6 [52]. In individuals with NAFLD, visceral adipose tissue volume is significantly associated with the extent of hepatic inflammation and fibrosis, even after adjusting for both IR and hepatic lipid content, suggesting a strong contribution of visceral fat to the pathogenesis of NAFLD [31]. Thus we anticipate that VAT reduction achieved with GHRH analogue will be accompanied by improvements in circulating adipokines. We have previously shown that VAT reductions achieved with GHRH analogue are significantly associated with increases in adiponectin and improvements in fibrinolytic markers tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) [53, 54]. In the current proposal, we will perform more comprehensive analysis of changes in circulating inflammatory cytokines as well as hepatic expression of pro-inflammatory genes to better assess changes in inflammation achieved with GHRH treatment and associated VAT reduction in a specific HIV population with NAFLD.

Independent of its effects to decrease VAT, we expect that GHRH analogue will have direct effects on hepatic lipid metabolism and inflammation. Indeed, in the large Phase III studies leading to FDA approval of tesamorelin to reduce visceral fat, triglycerides levels significantly decreased in the tesamorelin vs. placebo-treated subjects (-37±139 vs. +6±112 mg/dl, P<0.001; net difference 43mg/dL). Abundant data show the importance of the GH-IGF-1 axis in hepatic lipid metabolism and suggest that deficiencies in this axis increase the risk of

hepatosteatosis. IGF-1 levels are lower in individuals with NAFLD compared to controls [55], and, among those with NAFLD, IGF-1 levels are inversely associated with degree of steatosis and fibrosis [56-58]. Further, liver IGF-1 mRNA expression is inversely associated with histological Kleiner score [56]. Supporting these findings, adults with GH deficiency due to hypopituitarism have a significantly higher prevalence of NAFLD than controls, and GH replacement improves histological findings associated with NAFLD [59-61].

In vitro and animal models also highlight the significance of the GH-IGF-1 axis in hepatic metabolism. As discussed above, GH suppresses hepatic DNL, and GH and IGF-1 signaling may also affect hepatic lipid metabolism and inflammation in other respects. Mice with liver-specific GH-receptor knock-out (GHRLD) demonstrate severe hepatic steatosis, in conjunction with increased hepatic lipogenesis [62]. These changes are accompanied by upregulation of SREBP1c, peroxisome proliferator-activated receptor-γ (PPARγ), and CD36 fatty acid transporter, all of which promote hepatic lipogenesis [62]. Adenovirus-mediated rescue of GHR expression reverses these changes and normalizes hepatic triglyceride output [62]. In this model, IGF-1 treatment did not reverse the phenotype, suggesting important metabolic effects of GH independent of IGF-1. Other studies, however, demonstrate that IGF-1 also plays a significant role in hepatic lipid metabolism. The spontaneous dwarf rat (SDR), which has a truncated GH gene resulting in severe GH deficiency, also demonstrates hepatic steatosis and fibrosis. In the SDR model, contrasting with the GHRLD model, treatment with either GH *or* IGF-1 reverses steatosis and fibrosis [63]. On a cellular level, hepatocyte mitochondria in the SDR rat are small and irregularly shaped, and mRNA expression of the rate-limiting enzyme for β-oxidation, carnitine palmitoyltransferase I (CPT-1), is significantly decreased, whereas hepatic expression of glycerol-3-phosphate acyltransferase (GPAT), which catalyzes the first step in TG synthesis, is increased, consistent with increased hepatic lipogenesis [63]. Treatment with either GH or IGF-1 restores normal mitochondrial morphology and reverses the observed changes in gene expression [63]. Further, in vitro data show that treatment of HepG2 cells with IGF-1 attenuates the pro-inflammatory effects of IL-6 exposure [56], and gene transfer of IGF-1 to cirrhotic hepatic tissue decreases fibrosis and improves liver function [64]. Taken together, these data demonstrate potentially critical effects of GH and IGF-1 in hepatic metabolism and inflammation. Whereas some of these effects appear to be unique to GH, acting independently of IGF-1, others may be IGF-1 mediated.

7.2 Rationale

There are currently no highly effective strategies to decrease liver fat in HIV-infected patients with NAFLD. In previous work, treatment with a growth hormone releasing hormone (GHRH) analogue in HIV-infected individuals with abdominal adiposity reduces liver fat as measured by ^{1}H-MRS spectroscopy [65]. The current protocol builds upon these data, investigating GHRH analogue in a cohort specifically chosen for NAFLD. The current protocol also investigates mechanisms by which GHRH may reduce liver fat. Mechanistic study is particularly critical in the HIV-infected population, as metabolic changes caused by HIV and its treatment may mediate the development of NAFLD and NASH in this population. For example, hepatic de novo lipogenesis (DNL) is known to be increased in HIV infection [15] and is also a critical pathway underlying NAFLD. Further, many of the mechanisms thought to cause progression from NAFLD to NASH – including increased expression of inflammatory cytokines and impaired mitochondrial function – are also characteristics of HIV infection, such that HIV-infection may alter the natural history of NAFLD and increase the risk of progression to NASH. The current protocol assesses the effects of GHRH on steatosis, inflammation, and fibrosis at a tissue level also assesses effects on mRNA expression of genes involved in substrate metabolism.

7.3 Study Hypotheses

- We hypothesize that GHRH analogue will reduce liver fat in patients with NAFLD and NASH (Specific Aim 1), and, in conjunction, will reduce inflammation and may improve indices of hepatocellular damage and fibrosis (Specific Aim 2).
- We hypothesize that GHRH analogue will reduce hepatic de novo lipogenesis and expression of pro-inflammatory and pro-lipogenic genes (Specific Aim 3a and 3c). We further hypothesize that reductions in liver fat will result in improved hepatic and peripheral insulin sensitivity (Specific Aim 3b).

8. OBJECTIVES & STUDY ENDPOINTS

8.1 Primary Objective: To determine the whether tesamorelin treatment for 1 year reduces liver fat quantity compared to placebo treatment for 1 year.

8.2 ***Secondary Objectives:*** *To compare the effects of tesamorelin treatment for 1 year compared to identical placebo on the following*

a) Hepatic enzymes (AST, ALT, GGT)
b) histologic features of steatohepatitis (steatosis, inflammation, hepatocellular ballooning, and fibrosis)
c) serum CK-18, a marker of hepatocellular inflammation
d) hepatic glucose metabolism
e) hepatic lipid metabolism

8.3 Exploratory Objectives: *N/A*

8.4 Primary Endpoint: Change in hepatic fat fraction, as measured by ^{1}H-MRS, between baseline and 12 months.

This primary endpoint is chosen based on known effects of growth hormone to increase lipolysis and reduce hepatic de novo lipogenesis and on previous data demonstrating an effect of tesamorelin to decrease liver fat as compared to placebo over 6 months of treatment. We hypothesize that changes in liver fat may be associated with improvements in steatohepatitis and hepatic substrate metabolism, which will be assessed as secondary endpoints.

8.5 Secondary Endpoints:

a) Change in ALT, AST, and GGT over 12 months

These are chosen as clinically relevant markers of hepatocellular inflammation, which we hypothesize will decrease with tesamorelin treatment as compared to placebo.

b) Change in histologic scores for steatosis, inflammation, and hepatocellular ballooing, and change in histologic staging of fibrosis after 12 months

Examination of histologic features of steatohepatitis is critical to determine the effects of tesamorelin on hepatocellular inflammation and fibrosis, beyond its effects to reduce liver fat per se. NAFLD activity score (NAS) will be assessed using the sum of subscores for steatosis, inflammation, and hepatocyte ballooning. Fibrosis stage will also be assessed. These assessments are standard in studies of NAFLD and have been developed by Dr. Kleiner and others as tools to assess the effect of interventions for NAFLD.

c) Change in CK-18 after 12 months

CK-18 is expressed by epithelial cells, including hepatocytes, and CK-18 cleavage fragments are released during apoptosis. CK-18 is a well-validated marker of nonalcoholic steatohepatitis and will be assessed as a measure of apoptotic activity and NAFLD severity.

d) Change in hepatic glucose metabolism as measured by endogenous glucose production (EGP) during the fasting state and suppressibility of EGP during low-dose insulin clamp (assessed at MGH only)

NAFLD is strongly associated with insulin resistance, and we hypothesize that improvements in NAFLD may improve hepatic glucose metabolism, i.e., decrease hepatic insulin resistance. This will be assessed through two measures, namely measurement of EGP in the fasting state using stable isotopes and subsequent assessment of the suppressibility of EGP in response to "low-dose" insulin clamp.

e) Change in hepatic lipid metabolism as measured by stable isotope measurement of de novo lipogenesis, using deuterium incorporation into palmitate (assessed at MGH only)

An etiologic contributor to NAFLD is increased hepatic de novo lipogenesis, which is increased in HIV infection and thought to be reduced by growth hormone. We will assess whether treatment with tesamorelin decreases hepatic de novo lipogenesis using stable isotopes (deuterated water consumption, followed by assessment of deuterium enrichment of palmitate).

9. STUDY DESIGN

- **Overall design:** Randomized, placebo-controlled, double blind during first 12 months ("double-blind" phase), followed by a 6 month "open-label" phase during which all study participants receive tesamorelin. Double-blind phase has 2 arms: tesamorelin and placebo, with participants allocated in 1:1 ration
- **Intervention:** tesamorelin 2mg subcutaneously daily vs. identical placebo. One-time dose-reduction to 1mg built in for subjects whose IGF-1 level exceeds 3 standard deviations above the mean. This will be determined by an independent endocrinologist who monitors IGF-1 levels of all participants and requests dose reduction if needed. A "dummy" dose-adjustment will be requested for a placebo patient to maintain blinding.
- **Randomization** for double-blind phase is 1:1 allocation, using a permuted block algorithm with randomly varying block sizes. Randomization will be stratified by site and use of Vitamin E ≥400 IU daily
- **Sample size**: 60 participants randomized
- **Duration of each subject's participation:** 18 months
- **Duration of study** (study initiation until last patient, last visit)**:** 36 months
- **Number of sites:** 2

10. STUDY POPULATION

10.1 Inclusion/Exclusion Criteria

10.1.1 60 HIV-infected subjects will be enrolled in this 18 month study. Inclusion criteria:

- Men and women 18-70yo
- HIV-infection and treatment with a stable antiretroviral regimen for ≥ 3 months. HIV-status will be documented with any one of the following:
    - Documentation of HIV diagnosis in the medical record by a licensed health care provider;
    - OR HIV-1 RNA detection by a licensed HIV-1 RNA assay demonstrating > 1000 RNA copies/mL;
    - OR any licensed HIV screening antibody and/or HIV antibody/antigen combination assay confirmed by a second licensed HIV assay such as a HIV-1 Western blot confirmation or HIV rapid multispot antibody differentiation assay.

      NOTE: A "licensed" assay refers to a US FDA-approved assay, which is required for all IND studies.
- Hepatic steatosis as demonstrated by liver fat fraction ≥5% on ^{1}H-MRS
- Hepatitis C antibody negative, or, if Hepatitis C antibody positive, either: a) known clinical disease, successful therapy ≥1 year prior to baseline and undetectable HCV RNA, or b) HCV resolved spontaneously and undetectable HCV RNA. Hepatitis B surface antigen negative at screen visit
- For females ≥50yo, negative mammogram within 1 year of baseline visit
- If use of Vitamin E ≥400 IU daily (in any formulation), stable dose for ≥6 months prior to study.

10.1.2 Exclusion criteria:

- Heavy alcohol use defined as consumption of more than 20g daily for women or more than 30g daily for men for at least 3 consecutive months over the past 5 years assessed using the Lifetime Drinking History Questionnaire [66, 67];
- Use of insulin or thiazoledinediones (TZDs), or HbA1c ≥7%. Individuals with mild diabetes that is well-controlled with diet and/or oral anti-diabetic agents besides TZDs will be included. Use of oral anti-diabetics must have been stable for ≥6 months prior to study entry.
- Known diabetic retinopathy.
- Known cirrhosis, or 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.
- Chronic corticosteroid use except intermittent use of topical steroid creams and/or prior short-term physiologic corticosteroid use in the ≤ 6 months prior to baseline visit
- Chronic use of methotrexate, amiodarone, or tamoxifen
- Known diagnosis of Alpha-1 antitrypsin deficiency, Wilson's disease, hemochromatosis, or autoimmune hepatitis
- Use of GH or GHRH within the past 1 year
- Change in lipid lowering or anti-hypertensive regimen within 3 months of screening

- HgB < 11.0 g/dL, CD4 < 100 th/mm^3, or HIV viral load > 400 copies/mL
- Active malignancy
- For men, history of prostate cancer or evidence of prostate malignancy by PSA > 5 ng/mL
- Severe chronic illness judged by the investigator to present a contraindication to participation
- History of hypopituitarism, head irradiation or any other condition known to affect the GH axis
- Use of physiologic testosterone (men) or estrogen or progesterone (women) *unless* stable use for a year or more prior to study entry
- Routine MRI exclusion criteria such as the presence of a pacemaker or cerebral aneurysm clip
- Weight loss surgery within 1 year before study entry.
- For women, positive pregnancy test performed in a CLIA certified laboratory using a test with a sensitivity of at least 25mIU/mL, or breastfeeding.
- Known hypersensitivity to tesamorelin or mannitol
- Unwillingness to abstain from the conception process during the study (i.e., must agree not to participate in an active attempt to become pregnant or impregnate, donate sperm, or participate in *in vitro* fertilization)
- Unwillingness to use one (for males) or two (for females) reliable methods of contraception while engaging in heterosexual intercourse during the study. Acceptable methods for women include hormonal contraception (estrogen/progesterone or progesterone-only formulations) if stable for a year or more prior to study entry, intrauterine device, or barrier methods (condom, or diaphragm with spermicide). Acceptable methods for males include condom use. This requirement does not apply to women who have been post-menopausal for at least 24 consecutive months or have undergone surgical sterilization, or to men who have undergone surgical sterilization or have documented azoospermia.
- Not willing or able to adhere to dose schedules and required procedures per protocol.
- Unwilling to agree to use of study samples for related research (criterion for NIH only)

10.1.3 Co-Enrollment Criteria:
Participants will be asked to inform the study team if they are enrolled in any other research studies to determine if it is safe and appropriate for them to enroll in this study.

10.2 Recruitment Process
We will recruit patients with HIV infection and known or suspected fatty liver disease.

At NIH, recruitment efforts will be based on existing NIH cohorts following patients with these conditions. In addition, NIH will utilize the OP8 Clinic recruitment strategies that are in place and include a full-time patient recruiter and community-based outreach within local area clinics specializing in HIV.

At MGH, subjects will be recruited from local primary care, infectious disease, and gastroenterology practices as well as community organizations and the MGH Clinical Research Program's Research Subject Volunteer database (RSVP). We anticipate that some subjects will be known to have NAFLD or NASH and may already be followed by (and referred from) gastroenterologists, whereas other patients may not have a known diagnosis and will be either self-referred or referred from their primary care or infectious disease providers due to high likelihood of NAFLD or NASH based on clinical factors such as abdominal adiposity and elevated transaminases. For self-referred patients, phone screening will be used to select volunteers for screening who have a high likelihood of qualifying for the study based on either history of elevated transaminases or significant abdominal adiposity and/or metabolic abnormalities.

Participants who are interested in the study will be asked to contact us. Flyers with our contact information will be provided to medical practices for distribution to appropriate patients, and flyers will also be sent to community organizations. In addition, if a physician obtains permission from his/her patient for us to contact the patient, then we may contact the patient.

Advertisement of the study in community organizations will increase participation of women and minorities.

10.3 Participant Retention

Every effort will be made to maintain subject participation by calling participants approximately once per month between visits to ensure that they are feeling comfortable with the study drug and that they do not have any unanswered questions. Subjects who wish to discontinue participation for any reason will be asked to discuss possible solutions that may allow them to continue participation, and subjects will also be reminded that their participation is voluntary and they are free to discontinue at any time.

11. INTERVENTIONS

11.1 Biomedical Interventions (Study Drug)

11.1.1 Regimen

- Double blind phase (first 12 months): subjects will receive tesamorelin vs. identical placebo, formulated as described below, according to their randomization.
- Open label phase (last 6 months): subjects will receive tesamorelin, formulated as described below
- Tesamorelin and identical placebo will be provided by Theratechnologies, Inc. (Montreal, Canada). The dose of tesamorelin will be 2 mg SC QD, with provision for a dose adjustment to 1mg SC QD if subject's IGF-1 is ≥3 (see below and Section 14.4.1). All doses will be self-administered by the subject, after s/he receives instruction in reconstitution and administration and demonstrates competence in self-injection. Dosing is based on prior studies of this compound in HIV-infected patients with fat accumulation in which $GHRH^{1-44}$ at 2mg significantly reduced visceral fat, improved lipid parameters, while achieving a generally physiologic increase in IGF-I of 80% vs. placebo over 6 months [54].
- Dose adjustment: If a subject who is receiving tesamorelin in either the double-blind phase or open-label phase has an IGF-1 z-score ≥3, a dose reduction to 1mg will be performed. If the subject is in the double-blind phase, this will be accompanied by a dummy dose reduction for a placebo subject in order to maintain blinding, as described in 14.4.1. If the subject's next IGF-1 level shows z-score ≥3, s/he will be discontinued from the study. If the next IGF-1 z-score is <3, the subject will continue in the study at

the 1mg dose. Dose reductions will remain in place for the remainder of the subject's participation in the study.

11.1.2 Study Product Formulation and Preparation

A three month supply of tesamorelin or identical-appearing placebo will be dispensed at each study visit. The dose of study drug is 2mg subcutaneous daily, which requires administration of two reconstituted vial of medication. Each vial of tesamorelin contains 1.1mg, which are reconstituted to provide 1mg dose. Each vial of placebo contains 55 mg of mannitol. Subjects will be instructed to refrigerate the vials between 36-46° F, and will be expected to dilute, mix, and administer daily injections of the study drug or placebo. Dr. Grinspoon holds an IND for the use of $GHRH^{1-44}$ in HIV lipodystrophy (IND 77,473, S. Grinspoon) that has been amended to include this protocol. $GHRH^{1-44}$ (Tesamorelin) has been approved by the FDA for use in HIV lipodystrophy. Medication adherence will be assessed by vial count and medication diary.

Patients will receive detailed instruction regarding proper storage and self-administration of the study agent in accordance with the manufacturer's product information.

11.1.3 Device Studies: N/A

11.1.4 Study Product Supply and Accountability

Tesamorelin and identical placebo will be procured from Theratechnologies, Inc. (Montreal, Canada). Medication will be dispensed by the MGH and NIH Research Pharmacies. A three month supply of medication will be dispensed to patients at the baseline, 3, 6, 9, 12, and 15 month visits. Medication and supplies dispensed will be as follows:

- 190 vials of study drug, comprising a 95 day supply
- 100 vials of sterile water, to be used to reconstitute study drug. A total of 2.2 cc of sterile water is used for the reconstitution of the medication in both vials, and 2cc solution is withdrawn and administered.
- 100 3cc syringes with 18 gauge, 1.5 inch needles.
- 100 additional 18 gauge, 1.5 inch needles
- 95 27 gauge, ½ inch needles
- Box of alcohol swabs
- Sharps container
- Cooler with 2 ice packs for transporting medication home
- Study drug diary

At each dispensation, the subject's identity will be confirmed using 2 identifiers that match the prescription labeling, and the randomization number will also be verified. The number of vials provided will be recorded, along with the Lot # for the medication and, if applicable, kit and/or box #s as well.

The MGH and NIH research pharmacies will receive vials of tesmorelin and placebo from Theratechnologies. The pharmacies will be able to identify which vials are active vs. placebo, but this will not be apparent to investigators or subjects by inspecting the vials.

Subjects will be asked to return used and unused medication at the 3, 6, 9, 12, 15, and 18 month visits. The number of used and unused vials will be recorded prior to destruction of used and unused drug.

Each site will keep track of study drug supply on hand, as well as study drug dispensed to each patient. Overall accountability for this will be shared by the investigator and the research pharmacy, with specific responsibilities delegated by the investigator at each site.

11.1.5 Assessment of Participant Adherence
Participants will be asked to keep a study drug diary in which they record their daily injections and/or note if an injection was missed. The number of missed injections will be tallied as a measure of adherence. Independently, the count of used study drug vials, as compared to the expected number of used vials, will also be used as a measure of adherence.

11.1.6 Concomitant Medications and Procedures
All concomitant prescription medications taken during study participation will be documented in CRIMSON (NIH) or the written CRF (MGH). 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 CRIMSON or the CRF are concomitant prescription medications, over-the-counter medications, and non-prescription medications taken at the time of adverse events (all grades).

11.1.7 Permitted Medications and Procedures
Subjects will be asked to discuss all new medications or procedures with study investigators. Unless specified below, medications and medical procedures will generally be permitted as long as there is no concern for participant safety.

11.1.8 Precautionary Medications and Procedures
Tesamorelin has not been shown to have significant drug-drug interactions in clinical studies to date. Theoretical interactions may affect the metabolism of other drugs that are metabolized by CYP450. Concomitant medications will be recorded at each visit, and subjects will be carefully monitored.

11.1.9 Prohibited Medications and Procedures
Treatment with insulin or thiazoledinediones (TZDs); chronic corticosteroid use except intermittent use of topical steroid creams and/or prior short-term physiologic corticosteroid use in the ≤6 months prior to baseline visit; chronic use of methotrexate, amiodarone, or tamoxifen; use of GH or GHRH within the past 1 year; or use of physiologic testosterone (men) or estrogen or progesterone (women) unless stable for 1 year prior to study participation will not be permitted unless discussed with and approved by the study team.

11.1.10 Required Medications: N/A

11.1.11 Rescue Medications: N/A

11.2 Behavioral Intervention
All subjects will be counseled in standard lifestyle modification 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 [68, 69]. Please see Appendix A for the schedule of planned counseling sessions.

12. STUDY PROCEDURES/EVALUATIONS

12.1 Clinical Procedures and Evaluation (Please also see Appendix A, Schedule of Events)

All subject encounters will take place at the MGH GCRC or the NIH Clinical Center. All female subjects will have a pregnancy test at each visit. All subjects will receive standard nutrition counseling at baseline and every 6 months thereafter.

12.1.1 Evaluation of Preliminary Eligibility
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.

12.1.2 Informed Consent
Written informed consent will be signed prior to screening evaluation and testing by a licensed physician investigator. Subjects will be informed that they may withdraw from participation in the study at any point.

12.1.3 Screen Visit (to determine eligibility)

- Informed consent
- Detailed H&P and medications (past and current), Lifetime Drinking History assessment
- Blood sampling (AST, ALT, creatinine, total bilirubin, PT (and INR) and PTT, CBC, CD4, HbA1c, HIV viral load, Hepatitis B sAg, Hepatitis C antibody*, PSA (male subjects). *Positive Hep C antibody patients will be tested for HCV RNA.
- ^{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.)

12.1.4 Randomization and Subsequent Visits
Eligible subjects will return for 9 visits. After signing consent and, prior to the baseline visit, subjects will be randomized in a 1:1 ration to receive GHRH$^{1-44}$ 2mg SC QD versus identical-appearing placebo as above. Randomization will be stratified by study site and vitamin E use ≥400 IU daily of any formulation. Permuted block randomization will be performed by an MGH biostatistician, with randomization lists distributed to the MGH and NIH pharmacies. Study staff, investigators, and subjects will be blinded to assignment. During the first 12-month treatment phase, subjects will have visits at baseline, 2 weeks, and months 3, 6, 9 and 12. After month 12, all patients will receive open label GHRH regardless of their original randomization. Subsequent visits will take place at months 12.5, 15 and 18 months. Subjects seen at MGH will receive stable isotope study and euglycemic hyperinsulinemic clamp in addition to other study procedures.

12.1.5 Baseline assessment: The baseline assessment will be completed within 3 months of the screening visit. In order to complete all of the procedures in the baseline assessment, subjects may come for up to 3 different visits, all of which should take place within a 2 week period. Subjects will begin the study drug upon completion of the baseline assessment, and subsequent visits will be timed according to the day study drug is started.

- H&P
- Fasting labs: IGF-I, AST, ALT, GGT, alkaline phosphatase, lipid panel including direct LDL, frozen serum for adiponectin, IL-6, CRP, CK18, and other relevant inflammatory biomarkers, as guided by the histological results
- Height, metabolic weight, anthropometrics
- Whole body DEXA scan for regional fat
- 4-day food record and Modifiable Activity Questionnaire
- OGTT

- Transient elastography ("FibroScan")
- Liver biopsy. Biopsy will not be done if performed in the 6 months prior to baseline and, in the interval since biopsy, no new treatment initiated and no weight change ≥8% of baseline weight
- Peripheral blood mononuclear cells (PBMCs) will be processed and stored
- Subjects at MGH will receive stable isotopes and clamp for metabolic assessment (see below).

12.1.6 Safety Visits

At 0.5, 3, 6, 9, 12.5, and 15 months: **(1) i**nterval H&P; **(2)** fasting glucose, AST, ALT, IGF-1. *At 6 month only:* ^{1}H-MRS/MRI of abdomen for quantification of liver fat and VAT

12.1.7 12-month visit

Identical to baseline visit (including liver biopsy) but **excluding** (1) collection of PBMCs and **including (1)** HbA1c, CD4+, CBC, and HIV viral load; **(2)** ^{1}H-MRS for quantification of liver fat and MRI of abdomen for quantification of VAT that were obtained at the screening visit

12.1.8 18 month visit

- Interval history, H&P
- Fasting labs: IGF-I, AST, ALT, GGT, alkaline phosphatase, lipid panel including direct LDL, HbA1c
- Height, weight, anthropometrics
- Whole body DEXA scan for regional fat
- ^{1}H-MRS for quantification of liver fat, MRI of the abdomen for quantification of VAT
- Transient elastography
- 4-day food record and Modifiable Activity Questionnaire
- OGTT

12.2 Laboratory Evaluations

12.2.1 Specimen Preparation, Handling, and Shipping

Blood samples drawn during visits will have 1 of 3 possible dispositions: (1) assay through NIH/MGH lab services; (2) central assay, for IGF-1 levels only, requiring sample shipment; (3) processing and frozen storage for later analysis. Samples of liver tissue will be sent to Dr. David Kleiner at NIH; those samples gathered at MGH will require shipment. Samples will be handled using universal precautions and only by individuals who have had biohazard training. Samples that require shipment will be shipped only by individuals who have IATA training in biohazard shipment, and shipment will follow all IATA regulations. All protocol specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

12.2.2 Biohazard Containment

Transmission of HIV and other blood borne pathogens can occur through contact with contaminated needles, blood, and blood products. Respiratory pathogens such Mycobacterium tuberculosis (MTB) are transmitted by inhalation of droplet nuclei. Appropriate blood, secretion, and respiratory precautions will be employed by all personnel in the collection of clinical samples and the shipping and handling of all clinical samples and isolates for this study, as

currently recommended by the Centers for Disease Control and Prevention in the United States, the WHO internationally and the National Institutes of Health.

12.2.3 Total Blood Volume

The total blood drawn for subjects completing the study is equivalent to approximately 730 cc over a period of 18 months for those subjects in the subset receiving euglycemic hyperinsulinemic clamp and stable isotopes at MGH, and approximately 540cc over 18 months for those at NIH (where clamp/isotope will not be performed). The maximum amount of blood drawn at any given visit will be approximately 170cc, which will be drawn at the baseline and 12 month visits. A total of 730cc (less than 2 blood donations) over 18 months does not pose excessive risk to patients, nor does withdrawal of up to 170cc at a single timepoint.

Patients with a hemoglobin < 11 g/dL will be excluded from the study.

The amount of blood drawn for research purposes will be within the limits allowed for adult subjects at the NIH Clinical Center (Medical Administrative Series Policy M95-9 Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf). The amount is also within Partners Human Research Committee guidelines (MGH IRB).

12.3 Study Methods

12.3.1 ^{1}H Magnetic Resonance Spectroscopy(MRS) & MRI

After a 12-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. A voxel measuring 20 x 20 x 20 mm (8 ml) will be placed within the right lobe of the liver, avoiding vessels or artifact, and with position within the liver confirmed by 3 planes of view. ^{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. Image series will include Axial T1-weighted localizers. No intravenous contrast will be used. The total amount of time in the magnet will be approximately 1 hour. A subset of patients at the NIH (no more than 5) may be offered an opportunity for a repeat validation scan to establish test-retest reliability and will be compensated an additional $50 for this procedure. Proton density fat fraction will be calculated from integral lipid and water peak areas as previously described [70, 71]. The imaging procedure will be identical at both the MGH and NIH sites. Dr. Torriani will read all the MRS scans centrally at MGH, including data sent from MRS scans obtained at NIH, to ensure uniformity in the assessment hepatic fat by MRS across both sites.

12.3.2 Transient Elastography

Liver stiffness will be determined using transient elastography.[72]. At least ten measurements will be made using the M-probe according to the manufacturer's recommendations and the median will be expressed in kilopascal (kPa) units. If measurements from the M-probe are unsuccessful at the baseline assessment, the XL probe may be used at the operator's discretion. Use of the XL probe at baseline will be noted, and the XL probe will also be used for subsequent assessment of that subject.

12.3.3 Liver Biopsy

Following MRS/MRI, a percutaneous liver biopsy will be performed under ultrasound guidance using an 18-gauge needle by experienced interventional radiologists. Participants with any contraindication to liver biopsy, including platelets < 75,000, elevated PT or PTT, or chronic use of aspirin or other anti-platelet agents will not undergo biopsy. Use of other anti-coagulants will

be considered and managed on a case-by-case basis by the site PI in conjunction with the physician performing the biopsy and the subject's primary care physician. Subjects will not be asked to discontinue necessary therapeutic medications such as warfarin in order to undergo biopsy. Individuals on aspirin may undergo biopsy following discontinuation of aspirin for ≥7 days. Resumption of anti-coagulants will be under the direction of the interventional radiologist performing the procedure. A maximum of 3 passes will be performed during the biopsy. A biopsy fragment will be fixed in 10% formalin. These will be sent to the NIH for central review by Dr. David Kleiner using standard histopathologic techniques. Semi-quantitative scoring will use validated scoring systems, including the modified Histologic Activity Index [73] and NASH Clinical Research Network scoring system [74]. If sufficient tissue exists, a second fragment will be placed in an RNA stabilization reagent (RNAlater, Qiagen) for gene expression analysis. Microarray of liver tissue will be used to study the expression of inflammation, fibrosis and metabolism related genes in liver tissue.

12.3.4 Genetic Analysis
PBMC's will be stored and analyzed for single nucleotide polymorphisms that may affect liver fat content. This analysis will be performed at the NIH.

12.3.5 Oral Glucose Tolerance Test
75 g Oral Glucose Tolerance Test will be performed with sampling for insulin and glucose at 0, 30, 60, 90, and 120 min.

12.3.6 Whole Body DEXA will be performed to determine total body and regional percent fat and lean body mass. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass[75]. Trunk, extremity and trunk to extremity ratio will also be assessed [76, 77].

12.3.7 Nutritional Analysis
Four day food record at baseline, 12 months, and 18 months will be analyzed for protein, carbohydrate, fat, micronutrient, dietary supplements and alcohol intake (Nutrition Data Systems).

12.3.8 Anthropometric Measurements
Measurements of waist to hip ratio, leg circumference, arm circumference, shoulders, back of neck, and neck circumference will be performed using a standardized technique [78].

12.3.9 Activity
Modifiable Activity Questionnaire (MAQ) will be used to assess physical activity [79].

12.3.10 History and Physical Examination
The **initial history**, performed at the screening visit, will consist of a comprehensive review of the subject's medical history, including any previous surgical procedures and any previous significant medical conditions. A comprehensive review of systems will also be performed. A detailed medication history will be taken. Subjects will specifically be asked about any previous use of growth hormone or growth hormone releasing hormone, as well as antiretroviral medication history. Allergies and all current medications will be recorded. **Interval history**, performed at all subsequent visits, will consist of questioning subjects about any new medical issues or any noted changes in health, as well as any symptoms of developing or worsening diabetic retinopathy. The most recent medication list will be reviewed with subjects to assess for any changes in dosing, new medications, or medication discontinuation. **Physical examination** will consist of a review of vital signs obtained by nursing staff as well as examination of general appearance (visualization), heart (auscultation), lungs (auscultation),

abdomen (palpation and auscultation), and extremities (visualization and palpation). Additional elements of the physical examination may be performed at the investigator's discretion.

12.3.11 Stable Isotopes and Clamp Subjects who participate at the MGH will undergo stable isotope and euglycemic hyperinsulinemic clamp studies, in order to assess the following: de novo lipogenesis (stable isotopes), hepatic insulin sensitivity (stable isotopes and low dose clamp to measure insulin suppression of gluconeogenesis), and peripheral insulin sensitivity ("full" dose clamp). Assessment of hepatic gluconeogenesis and de novo lipogenesis will utilize 6,6-2H-glucose and deuterium oxide stable isotopes, respectively. Two intravenous (IV) catheters will be inserted: (1) one placed in the hand when access is available, otherwise in the forearm or antecubital space, to be used for venous sampling; (2) one placed in the antecubital space or proximal forearm and used for administration of stable isotopes, insulin, and 20% dextrose as below. Starting at 7am, 1g/kg of deuterated water will be consumed PO and a priming dose (3.0 mg/kg) of 6,6-2H-glucose will be administered intravenously, followed by a continuous infusion (0.03 mg/kg/min) of 6,6-2H-glucose until 11am. Baseline fasting samples for isotopic analysis will be collected at -20, -10, and 0 minutes. Samples will be collected at 60, 100, 110, and 120 minutes for 6,6-2H-glucose enrichment for assessment of fasting gluconeogenesis. At 120 minutes, a "low dose" euglycemic hyperinsulinemic clamp will be performed with an insulin dose of 20 mU/m^2/min to detect differential suppression of hepatic gluconeogenesis. Blood will be sampled for isotopic enrichment of 6,6-2H-glucose at 180, 220, 230, and 240 minutes. At 240 minutes, a "full dose" insulin clamp will commence with an insulin infusion of 80mU/m^2/min. Blood will be sampled for deuterium incorporation into palmitate at 340, 350, and 360 minutes. Samples for isotopic enrichment will be assayed by Metabolic Solutions. Although DNL occurs in both liver and adipocytes, measured DNL will reflect largely hepatic DNL because DNL plays only a minor role in adipose tissue metabolism [17] and does not significantly contribute to circulating FFA. Peripheral insulin sensitivity will be determined during the last 20 minutes of the full dose clamp using the DeFronzo method [80].
Clamp procedure will be as follows.

- Insulin infusate will be prepared by the MGH pharmacy.
- The hand/forearm with the IV that will be used for venous sampling will be placed in a warming box for the duration of the clamp procedure, although it may be withdrawn if the subject finds the warmth uncomfortable.
- At time "120" (approximately 10am), a priming dose of 100mU/m^2/min of insulin will be given for 2 minutes followed by a continuous infusion of 20mU/m^2/min for the next 118 minutes, comprising the "low dose" clamp.
- At 240 minutes (approximately 12pm), the dose of insulin will be increased to a priming dose of 400mU/m^2/min for 2 minutes followed by a continuous infusion of 80mU/m^2/min from 240-360 minutes, comprising the "full dose" clamp.
- From 0-240 minutes, a sample (≤ 0.5cc) of venous blood will be drawn every 5 minutes for assessment of glucose using a Hemocue glucose analyzer. Each sample is run twice in the Hemocue analyzer, with an additional third run if the difference between the first two runs is ≥4mg/dL. Samples for serum insulin concentration will be drawn at times 120, 200, 220, 240, 320, 340, 360.
- Starting at 125 minutes (5 minutes after commencing insulin infusion), a variable 20% dextrose infusion will be administered to achieve a target glucose of 90mg/dL (range 85-95mg/dL). The rate will be adjusted by the investigator, an MD or NP who is trained in clamp procedure. The initial infusion rate of 20% dextrose will be 0-30cc/hr, with the exact rate determined by the investigator based on the subject's fasting glucose, available clinical information, and, if available, data from the subject's previous clamp procedure for the study. (For example, a subject who is known clinically to be insulin

resistant and has a fasting glucose of 110mg/dL would be started at a rate of 0cc/hr, whereas a lean, muscular subject who exercises regularly and has a fasting glucose of 70mg/dL would be started at 30cc/hr.)

- The rate of 20% dextrose infusion will be adjusted up or down for the duration of the clamp procedure by the investigator. Rate changes of 0-40cc/hr will be made by the investigator every 5 minutes, based on the subject's venous glucose and rate of change in glucose, to achieve target glucose of 90mg/dL.
- At 360 minutes, the insulin infusion will be discontinued, and the glucose infusion will continue for 30 more minutes. During this time, subjects will be given a meal. At 390 minutes, venous glucose will be checked, and the glucose infusion will be discontinued if venous glucose ≥80mg/dL. If glucose is <80mg/dL, the glucose infusion will be continued and will be weaned by the investigator at the bedside, with repeat venous glucose sampling every 10 minutes until the glucose is ≥80mg/dL and the infusion is stopped.
- After the clamp, all subjects are counseled concerning the symptoms of hypoglycemia and are asked to report immediately any symptoms. Subjects are observed on the CRC for at least 20 minutes following discontinuation of glucose infusion.

12.4 Schedule of Procedures/Evaluations: Timing and Definitions

12.4.1 Screening: The period that commences at the signing of informed consent and ends when subjects are either (1) informed that they are not eligible for participation; (2) informed that they are eligible but decline participation; or (3) informed that they are eligible, choose to participate, and are scheduled for a baseline assessment and randomized.

12.4.2 Enrollment: For IRB purposes, participants who sign a consent form are considered to be enrolled. However, the sample size of 60 refers to the number of individuals who screen, are eligible, choose to participate, and are randomized to treatment.

12.4.3 HIV Counseling and Testing: Individuals will not be invited to screen for the study unless they are known to have HIV infection. Thus HIV counseling and testing will not take place under this protocol.

12.4.4 Follow-Up: Please see description of follow-up visits in section 12.1

12.4.5 Early Termination Visit: Subjects who end the study early will be asked come for a study termination visit (please see Appendix A).

12.4.6 Pregnancy Visit: Every effort will be made, both through absolute eligibility criteria and ongoing counseling about contraception and risk for participating women of child-bearing age, to prevent pregnancy during the study. Should pregnancy occur, a participant would be asked to discontinue the medication immediately and to come for a visit in order to discuss the situation with the Site PI. Such participants would also be asked to remain in contact with the PI and study team through the completion of the pregnancy in order to document the outcome.

12.4.7 Other Visits: Aside from the visits described in this section and in 12.1, no other visits are anticipated.

12.4.8 Final Study Visit: Please see description of 18 month visit above. Subjects who leave the study early will be asked to come for an early termination visit in lieu of their next scheduled study visit as discussed above.

13. ASSESSMENT OF SAFETY

13.1 Safety Assessment Overview

Assessment of safety will be performed by a study investigator at each visit, with subjects asked to describe any changes or adverse effects that they have noted since the previous study visit. In addition, subjects will be repeatedly asked to call the study team if they experience any adverse effects, have any new medical conditions, start any new medications, have any procedures, or require hospitalization, such that safety can be monitored between study visits. All adverse events will be collected and reported as described below. The Co-PI's (Hadigan and Grinspoon) will periodically review adverse events, and the study will also be monitored by the NIAID and the FDA, as the protocol is conducted under an IND for tesamorelin (77,473 to S.Grinspoon).

13.2 Adverse Event Procedures and Reporting Requirements

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded on the Adverse Event Case Report Form (AE CRF) or electronic database, and
- reported as outlined below (e.g., IND Sponsor, IRB).

13.2.1 Definitions

Adverse Event (AE)

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), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)

An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)

An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)

A Serious Adverse Event is an AE that results in one or more of the following outcomes:

- 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.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is
1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated Problem that is not an Adverse Event (UPnonAE)
Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as
1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation: A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as
1. Serious: Non-compliance that
   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH-HRPP
   d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

*13.2.2 **Investigator Reporting Responsibilities to the IND Sponsor***

Adverse Events
Line listings, frequency tables, and other summary AE data will be submitted to the IND Sponsor when needed for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

Serious Adverse Events
All SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the IND Sponsor within 1 business day of site awareness. Any SAE submitted to the FDA will also be submitted to the DAIDS Clinical Representative within 24 hours of FDA notification. SAEs that are unexpected and deemed to be possibly related to the study drug will also be reported to both IRBs, regardless of the site at which they occur.

Unanticipated Problems
Unanticipated Problems that are also adverse events must be reported to the IND Sponsor and the DAIDS Clinical Representative no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the IND Sponsor.

Pregnancy
Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the IND Sponsor via fax or email within 3 business days from site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the IND Sponsor within 3 business days of the site's awareness.

In the event of a pregnancy, the DSMB and the IRB will be notified. The participant will
- Discontinue the study agent
- Withdraw from the study but continue in follow-up for safety
- Be advised to notify her obstetrician of study agent exposure

*13.2.3 **Reporting Procedures to the NIAID IRB***

Expedited Reporting to the NIAID IRB
Serious and non-serious Unanticipated Problems, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. Serious Adverse Events that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

Annual Reporting to the NIAID IRB
The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:
- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs and deaths granted a waiver of reporting.
- Serious and Non-Serious Protocol deviations

- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

*13.2.4 **Reporting Procedures to the MGH IRB***

Partners Human Research Committee policy dictates the following reporting procedures.

Expedited Reporting to the MGH IRB

The following will be reported within 7 calendar days of investigator awareness:

- Internal adverse events that are unexpected, and related or possibly related to the research and that indicate there are new or increased risks to subjects
- External adverse events that are serious, unexpected, and related or possibly related to the research and that indicate there are new or increased risks to subjects that require some action (e.g., modification of the protocol, consent process, or informing subjects)
- Deviation from the approved research protocol or plan without IRB approval in order to eliminate apparent immediate hazard to subjects or harm to others
- Deviation from the approved research protocol or plan that placed subjects or others at an increased risk of harm regardless of whether there was actual harm to subjects or others
- Breach of confidentiality or violation of HIPAA (e.g., lost or stolen laptop)
- Medication, procedural or laboratory error (e.g., errors in drug administration or dosing, surgical or other procedure, or testing of samples or test results) regardless of whether subjects experienced any harm
- Interim analysis, safety monitoring report, publication in a peer-reviewed journal, or other finding that indicates that there are new or increased risks to subjects or others or that subjects are less likely to receive any direct benefits from the research
- Change in FDA labeling (e.g., black box warning), withdrawal from market, manufacturer alert from the sponsor, or recall of an FDA-approved drug, device, or biologic used in the research
- Complaint by/on behalf of a research subject that indicates that the rights, welfare, or
- safety of the subject have been adversely affected or that cannot be resolved by the
- investigator
- Incarceration of a research subject during participation in research that is not approved for involvement of prisoners as subjects
- Noncompliance with applicable regulations or requirements or determinations of the IRB identified by the research team or others (e.g., FDA Form 483 or Warning Letter) that indicates that the rights, welfare, or safety of subjects have been adversely affected
- Suspension or termination of the research, in whole or in part, based on information that indicates that the research places subjects at an increased risk of harm than previously known or recognized (e.g., FDA clinical hold)
- Suspension or disqualification of an investigator by FDA, sponsor, or others
- Scientific misconduct
- Any other problem that indicates that the research places subjects or others at an increased risk of harm or otherwise adversely affect the rights, welfare or safety of subjects or others.

Annual Reporting to the MGH IRB

The following items will be reported to the MGH IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems

- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

*13.2.5 **Follow-up of Adverse Events and Serious Adverse Events***

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open).

SAEs that occur after the study follow-up period that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related must be reported to the IND Sponsor, as described above.

14. CLINICAL MANAGEMENT

Clinical Management of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All AEs occurring from the time the informed consent is signed through the study follow-up period will be documented, recorded, and reported.

The Investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

Severity

The Investigator will grade the severity of each AE according to the “Division of AIDS Table for Grading fhe Severity of Adult and Pediatric Adverse Events” Version 2.0, November, 2014, which can be found at:

https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

Some grade 1 lab parameters on the DAIDS Toxicity Table (Fibrinogen, Potassium (low), Uric Acid (males only, elevated)) fall within the NIH lab reference range for normal values. These normal values will not be reported as grade 1 adverse events. The grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100-176 mg/dL
- Potassium (low): 3.0-3.3 mmol/L
- Uric Acid (males): 8.7-10.0 mg/dL

Causality
Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

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.

14.1 Other Disease Events
Occurrence of other disease events will be immediately brought to the attention of the Site PI. The participant's clinical care providers will be contacted to discuss his/her participation in the study and to discuss whether or not the study drug should be discontinued, either temporarily or permanently, and whether or not the participant can safely continue in the protocol.

14.2 Pregnancy
Women able to bear children will be repeatedly counseled about the risks of pregnancy during the study and the importance of contraception, and women will be instructed to immediately discontinue the study drug if they suspect pregnancy. If a participant becomes pregnant during the study, she will
- Discontinue the study agent
- Withdraw from the study but continue in follow-up for safety
- Be advised to notify her obstetrician of study agent exposure

14.3 Criteria for Discontinuation

14.3.1 Criteria for Permanent Intervention Discontinuation for an Individual Participant
The criteria for permanent discontinuation of further study intervention for an individual participant are

- Fasting glucose >150mg/dL, or >180 mg/dL for individuals with known type 2 diabetes at study entry, at any visit, confirmed by re-draw
- Erythema and/or pruritis extending ≥3cm beyond the immediate injection site, indicating allergic reaction
- Pregnancy
- Active malignancy
- Evolving medical condition (e.g., progressive liver disease, acute critical illness) such that continued participation in the study may incur risk will also be discontinued.
- Significant symptoms of GH excess felt to be related to the study drug
- IGF-1 level that remains ≥ 3 standard deviations above the mean following a dose adjustment
- Development or worsening of retinopathy
- 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)

14.3.2 Criteria for Premature Study Discontinuation for an Individual Participant
The criteria for premature discontinuation from the study for an individual participant are:

- Lost to follow up as evidenced by failure by the participant to attend a safety visit after multiple attempts at rescheduling, at the discretion of the site investigator
- Request by participant to withdraw
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of study results

14.4 ***Toxicity Management***
In the event of noted side effects, treating physicians will provide whatever available treatment is considered best to protect patient safety and well-being; compliance with study requirements will not compromise such treatment. Guidelines for the management of known side effects of tesamorelin that do not require immediate discontinuation of study drug are as follows.

14.4.1 Elevated IGF-1: IGF-1 will be checked at each visit, and results will be reviewed by an independent endocrinologist at MGH who has the randomization code for both study sites. If a subject receiving tesamorelin has an IGF-1 z-score of ≥ 3, the endocrinologist will contact the site investigator to recommend a dose-decrease of study drug to 1mg daily. If the subject is in the double-blind phase of treatment, the independent endocrinologist will simultaneously recommend a dose decrease for a placebo subject from the same site whose visit was temporally closest to the visit of the tesamorelin subject requiring dose reduction. Dose reductions should be enacted within 1 month of the IGF-1 level being drawn. Any dose reductions taking longer than this will be reported as protocol deviations. If a tesamorelin subject continues to have IGF-1 z-score ≥ 3 following dose reduction, the subject will be discontinued from the study.

14.4.2 Injection site reaction: A mild degree of non-pruritic swelling and mild erythema at the site of the injection that resolves within a few hours is relatively common with

tesamorelin and does not necessarily represent a hypersensitivity reaction. Subjects who present with this reaction will be asked to monitor for resolution and to let the study investigator know of any spread beyond the injection site or any pruritis, either of which may represent hypersensitivity. Erythema and pruritis extending >3cm beyond the injection site will be treated as a likely hypersensitivity reaction. If this is accompanied by any systemic symptoms such as shortness of breath or swelling suggestive of anaphylaxis, subjects will be discontinued from the study immediately and will be directed to immediate care. If the erythema and pruritis are not accompanied by any systemic symptoms, subjects will be asked to hold the study drug until resolution of the symptoms. At the study investigator's discretion, the subject may restart study drug once to determine if the reaction recurs. With recurrence, subjects will be discontinued from the study.

14.4.3 Joint pain: Joint pain of varying severity is a possible side effect of tesamorelin. For **mild joint pain**, subjects will be offered the choice of continuing the study drug or discontinuing the medication to see if the joint pain resolves. If joint pain resolves with discontinuation and thus is presumably related to tesamorelin, the subject may elect to discontinue or to continue in the study with careful monitoring as long as the joint pain is mild. Lack of resolution will prompt referral to the subject's usual care providers for further evaluation. For **moderate to severe** joint pain, i.e., pain that interferes with sleep or affects physical function, subjects will be asked to discontinue the study drug to determine if the joint pain resolves. If the joint pain resolves and thus is presumably related to tesamorelin, subjects will be discontinued. If joint pain does not resolve, subjects will continue to hold the study drug and will be referred to their usual care providers for evaluation. If an alternate etiology is found, subjects may elect to resume study drug.

14.4.4 Peripheral edema: Peripheral edema will be carefully assessed at baseline, as many subjects may have some degree of edema. If peripheral edema worsens during the study, subjects will be asked to have evaluation with their regular care provider for possible etiologies (cardiac, renal), with the understanding that an increase in edema may also be due to study drug. If no other etiology is found and if the edema does not interfere with physical function, the subject may continue in the study with careful monitoring if s/he wishes.

14.4.5 Hyperglycemia/glucose intolerance: Fasting glucose will be checked at every visit. For any fasting glucose of >150mg/dL, or >180 mg/dL for individuals with known type 2 diabetes at study entry, subjects will be contacted to arrange a re-draw. If fasting glucose persists >150mg/dL, or >180 mg/dL for individuals with known type 2 diabetes at study entry, subjects will be discontinued. Subjects without diabetes at study entry who develop fasting glucose >126mg/dL but <150mg/dL during the study, or any subjects who are found to have increases in HbA1c or adverse changes in glucose tolerance, will be reminded that tesamorelin may contribute to increased blood sugar and will receive additional counseling regarding limiting caloric intake. They will also be asked to discuss their glucose control with their regular care provider. Such subjects may continue in the study if they wish, with continued monitoring of glucose per protocol.

14.4.6 Malignancy: There is no evidence linking tesamorelin to the development of or worsening of malignancy. Nonetheless, there is theoretical concern that increasing growth hormone levels may promote tumor growth. Consequently, subjects with active malignancy will not be allowed in the study, and subjects with any new malignancy discovered during the study will be discontinued from the study.

15. STATISTICAL CONSIDERATIONS

15.1 Overview and General Design Issues

Examination of data from the double-blind study phase, comparing changes between tesamorelin and placebo groups after 12 months, will comprise the primary statistical analysis. The final 6 month open-label phase of the study is designed to improve subject retention and to gather longer-term safety data.

15.2 Study Endpoints

15.2.1 Primary Endpoint: change in hepatic fat fraction as measured by ^{1}H-MRS after 1 year of randomized treatment.

15.2.2 Secondary endpoints will include changes in the following: overall NAS score; fibrosis stage and NAS scores for ballooning degeneration, inflammation, and steatosis; transient elastography measurement; AST and ALT; fasting lipids; fasting insulin, glucose, HOMA-IR; adiponectin and markers of inflammation and cellular injury including circulating CK-18 and hepatic mRNA expression of IL-6 and TNFα; and, in a subset, hepatic DNL and gluconeogenesis.

15.2.3 Exploratory endpoints: none

15.3 Study Aims and Hypotheses

Specific Aim 1: Tesamorelin will significantly decrease liver fat in HIV-infected patients with NAFLD. We hypothesize that, after 12 months, compared to placebo
a) tesamorelin will significantly decrease hepatic fat fraction as measured by ^{1}H-MRS (primary endpoint).
b) improvements in hepatic fat fraction will be reflected in reductions in steatosis as assessed by histological examination of liver biopsy.

Specific Aim 2: Tesamorelin will reduce inflammation and improve histological findings. We hypothesize that a significant proportion of our patients will demonstrate histologically confirmed NASH on biopsy and that after 12 months, compared to placebo,
a) tesamorelin will improve hepatocellular ballooning and reduce inflammation on liver biopsy.
b) tesamorelin will reduce fibrosis on liver biopsy, and as measured by transient elastography (e.g. FibroScan®).
c) expression of pro-inflammatory genes in the liver will be reduced, and adiponectin expression will increase.
d) tesamorelin will significantly decrease AST and ALT.

Specific Aim 3: Reduction in liver fat with tesamorelin will be associated with beneficial changes in hepatic glucose and lipid metabolism.
a) tesamorelin will decrease hepatic de novo lipogenesis as measured by stable isotopes.
b) reduction in liver fat will be associated with improved hepatic insulin sensitivity, as measured by hepatic gluconeogenesis in the fasting and hyperinsulinemic states, and peripheral insulin sensitivity.
c) hepatic expression of lipogenic genes will decrease.

15.4 Sample Size Considerations

In our previous study of GHRH in HIV, 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 11%. Using this data we estimate that an initial sample size of 60 will provide 82% power to detect a clinically relevant treatment difference of 9.4% or larger 1 year mean changes in liver fat (lipid to water %) at $\alpha = 0.05$ if the

discontinuation rate is 20% (48 evaluable patients), or 80% power if the discontinuation rate is 25%. For secondary variables collected at both sites, the sample size will provide 85% power to determine a 0.89 SD difference in 1 year mean changes between treatment groups at $\alpha = 0.05$ with 48 evaluable patients, which is considered a clinically meaningful effect size. For example, we will have 85% power to detect a change of 7 U/L in AST and 8 U/L in ALT. Power will be lower for secondary assessment of insulin sensitivity assessed by euglycemic clamp and stable isotopes, performed only at MGH.

15.5 Stratification/Randomization/Blinding Procedures/Unblinding Procedures

- Randomization will be stratified by site (NIH or MGH) and vitamin E use (≥400 IU daily or not)
- Randomization lists will be prepared by an MGH biostatistician (Hang Lee, PhD) using 1:1 treatment allocation, using a permuted block algorithm with randomly varying block sizes
- Dr. Lee will send the site specific randomization lists to the NIH and MGH research pharmacies.
- Randomization procedure:
    - Eligible participants who have a baseline visit scheduled will be randomized in the 1-2 weeks prior to the baseline visit, so that the research pharmacy can prepare their study drug.
    - At the time of randomization, study staff will assign the next randomization number in sequence and write this in the randomization form as well as on the prescription sent to pharmacy. (e.g., numbers 101, 102, then 103 will be assigned sequentially for subjects at MGH who are not using Vitamin E.)
    - Upon receiving a prescription for a new participant, the pharmacy will record the participant's name next to the appropriate randomization number on their list.
    - Randomization numbers will not be re-used, even if a participant is randomized and then fails to come for baseline assessment. The next randomization number in sequence will always be assigned.
- Blinding will be maintained, as the only parties who will know the randomization assignment of subjects will be the research pharmacists and an independent endocrinologist at MGH who reviews IGF-1 levels. Reports of IGF-1 levels will not be made available to study staff, as this would unblind the study.
- Should a situation arise in which a subject or subject's medical provider needed to know their treatment assignment for medical reasons, the Site PI would contact the appropriate research pharmacy to obtain the randomization assignment. The PI at the other site, as well as the DSMB, will be made aware of the unblinding.

15.6 Maintenance of Trial Treatment Randomization Codes

As above, randomization codes will be held by each site's research pharmacy, and pharmacy staff will record participant assignments. These lists will be distributed to the PIs and other study staff only after every participant has completed the double-blind phase of the study, and all data have been entered, verified, and locked.

15.7 Participant Enrollment and Follow-up

Please see Section 10 for details regarding recruitment and enrollment of participants, and please see section 12 for details regarding study visits, including all follow-up visits.

15.8 Data and Safety Monitoring

15.8.1 Planned Interim Analyses and Stopping Guidelines: N/A

15.8.2 Data and Safety Monitoring Board:

Because this study will generate randomized, blinded data, NIAID intramural policy mandates that it be reviewed by the NIAID intramural DSMB. The DSMB has been constituted to review the data and analysis plans of all intramural NIAID clinical studies that require DSMB oversight and consists of experts in infectious diseases, biostatistics, and clinical trials. The DSMB is responsible for reviewing the IRB approved protocol, informed consent documents, the data and safety monitoring plan, and the stopping guidelines prior to initiation of the study, unless otherwise waived by the NIAID Clinical Director.

During the course of the study, the DSMB will review cumulative study data twice per year to evaluate safety, efficacy, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study subjects. The DSMB will also assess the performance of overall study operations and any other relevant issues, as necessary. The DSMB may also convene outside of the biannual meeting if other safety issues arise that the Principal Investigator would like the DSMB to address.

Study Pause Criteria: There will be no predetermined stopping criteria based on efficacy or lack of efficacy, however if 3 or more similar grade 4 events that are possible, probably or definitely related to the study drug, or a single SAE that is possibly, probably or definitely related to study drug are reported, the DSMB will be asked to convene to determine if early stopping is warranted.

Items reviewed by the DSMB include:

- Interim/cumulative data for evidence of study-related adverse events;
- Data quality, completeness, and timeliness;
- Demographic information on study subjects;
- Site monitoring reports related to adherence to the protocol and applicable regulations;
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations, unblinding, etc.); and,
- Factors external to the study such as scientific or therapeutic developments that may impact subject safety or the ethics of the study.

Prior to opening the study to enrollment, the statistician will provide the DSMB Executive Secretary with blinding codes in case the DSMB requires this information to make its recommendations. If the DSMB decides to unblind the safety data, the DSMB Executive Secretary will inform the Principal Investigator of the decision in writing or via e-mail as soon as possible.

The trial will be conducted in compliance with Title 21 Code of Federal Regulations, the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) and any applicable regulatory requirements.

The Investigator is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected and must be signed and dated by the person recording and/or

reviewing the data. Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the subject's medical records, laboratory reports, radiologist's reports, subject's diaries, biopsy reports, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the study.

15.8.3 Progress and Safety Monitoring: Reports on subject accrual and toxicity will be provided to the Division of AIDS Clinical Representative every month (or more frequently if necessary). In addition, periodic reports of data pooled over arm will be provided to the team that details accrual, participant status, delinquency of forms and specimens, and toxicity.

15.8.4 Analysis Plan

The analysis will be based on intention to treat analysis population using all available data, including interim data on subjects not completing the study. Wilk-Shapiro test will be used to assess for normality of distribution of all variables. 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 gluconeogenesis, and liver histology, 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. Following primary analyses to assess treatment effect, we will perform adjusted analyses to assess whether covariates may have influenced apparent treatment effects. We anticipate that some patients will have simple steatosis and some will have steatohepatitis on biopsy, and secondary stratified analyses will be performed for these subgroups. We will also perform secondary stratified analyses by those with vs. without elevation in transaminases. We also anticipate using gender, age, change in caloric intake, and change in BMI as covariates, as these may be potential confounders of hepatic steatosis. We will also carefully assess changes in physical activity, Vitamin E use, and macronutrient composition between the groups. If any subject demonstrates dramatic changes in physical activity or diet during the course of the study, we will consider secondary sensitivity analyses excluding such subjects.

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.

For the 6-month open-label extension, hepatic fat on MRS and relevant secondary endpoints including HOMA-IR and OGTT, fibrosis as assessed by transient elastography, and AST and ALT will be determined. 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, glucose homeostasis and

liver fibrosis as measured by transient elastography. We will use paired t-test to compare data for subjects initially randomized to tesamorelin at 12 and 18 months, to assess whether there were additional changes beyond 12 months of continued treatment and, if so, if these changes represented continued benefit or reversal of benefit. The extension will also provide longer-term safety data in this group.

16. DATA HANDLING AND RECORDKEEPING

16.1 Data Management Responsibilities

At NIH, Dr. Hadigan is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Data entry into CRIMSON will be performed by authorized individuals.

At MGH, Dr. Grinspoon is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data will be maintained in a written CRF and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. MGH will also maintain a deidentified study database into which data from both MGH and NIH subjects will be entered. Data entry will be performed by authorized individuals. The NIH site will enter data into REDCAP and periodically send de-identified data to MGH for maintenance of this database.

16.2 Essential/Source Documents and Access to Source Data/Documents

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. These will be kept at each site in subjects' study binders, which will stored in a locked and secured location, or electronically in the form of scanned documents. If stored electronically, access to the storage location will be protected by password. For regulatory purposes, individuals from the FDA, NIH, and each site's IRB may access these documents for inspection. The regulatory monitors will also access these documents for inspection (see Section 17).

16.3 Quality Control and Quality Assurance

The Site PIs (Hadigan and Grinspoon) will audit study charts and review data collection periodically to check for completeness. At monthly meetings with the PI's, Dr. Stanley (Co-I, MGH) will report on the completeness of the de-identified database to ensure timely and complete entry and troubleshoot any issues.

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Study records will be maintained by the Investigator for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law. Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator must provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written NIAID/OCRPRO permission must be received by the site prior to destruction or relocation of research records.

17. CLINICAL SITE MONITORING

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO and NIAID/DAIDS will visit the NIAID and MGH clinical research sites to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare CRIMSON data abstracts (NIH) or written CRF's (MGH) with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators' are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP, FDA) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts or CRFs) and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff, for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator(s) and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

18. HUMAN SUBJECTS PROTECTIONS

18.1 Institutional Review Board/Ethics Committee

The protocol has been approved by the Partners Human Research Committee (MGH IRB) and the NIH NIAID IRB, and research will be conducted with oversight from these IRBs. Each site PI will be responsible for reporting of study progress, AEs, protocol deviations, and other necessary reporting per the requirements of each site's IRB.

18.2 Vulnerable Participants

18.2.1 Pregnant women and fetuses are not included in this research. Pregnant women are excluded from this study because the effects of tesamorelin on the developing human fetus are unknown with the potential for teratogenic effects.

18.2.2 Prisoners are not included in this research. Should a study participant become newly incarcerated during participation in the study, the PIs will consult their IRBs for guidance.

18.2.3 Children are not included in this research. Because there are insufficient data regarding dosing or adverse events available in adults to judge the potential risk in children, children are excluded from participation in this study.

18.3 Informed Consent

18.3.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research subject and the researchers which begins before consent is given

and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

Written informed consent will be obtained by a physician investigator. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. The rights and welfare of the subjects 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.

18.3.2 Assent Process: N/A

18.3.3 Documentation of Informed Consent
The physician-investigator who obtains written informed consent will document this in the subject's chart. In addition, the consent form will be signed and dated by both the subject and the investigator, and the original signed/dated consent form will be kept in the subject's study record. A copy of the informed consent document will be given to the subject, and a copy may also be stored in the subject's medical record, according to site-specific guidelines.

18.3.4 Waiver of Informed Consent: N/A

18.3.5 Waiver of Documentation of Informed Consent: N/A

18.3.6 Stored Samples and Associated Data Considerations

- Subjects at both sites will be informed that de-identified tissue samples from liver biopsy will be sent to the NIH, where they will be analyzed and potentially banked.
- At MGH, subjects will be asked permission for researchers to also utilize their *blood* samples for future related research. Consent or non-consent is noted by a check-box and initials section on the consent form. Subjects who agree to have their blood samples used for related studies will have their samples used as described below, and will be informed that they can withdraw this consent in writing at any time for any remaining identifiable samples. Subjects at MGH who do not agree will have any remaining blood samples discarded after their study participation and protocol-specified analyses of banked samples are complete.
- At NIH, subjects must agree to use of samples for related research as an inclusion criterion for study participation.
- Use of samples for related research is described as below:
    - **Intended Use:** Samples and data collected under this protocol may be used to study the effects of tesamorelin on liver fat and liver histology and related health conditions in individuals with HIV. Genetic testing will be performed. Any other research or experimental treatments will be done under this or other protocols for which separate signed informed consent documents will be obtained.
    - **Storage:** Access to stored samples will be limited using either a locked freezer or (at NIH) a secure repository facility in Frederick. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

- **Tracking:** Samples and data acquired as part of this protocol will be tracked at NIH using the CRIMSON database system. Samples acquired and stored at MGH will be tracked by Dr. Grinspoon.
- **Disposition at the Completion of the Protocol:**
    - In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval.
    - At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.
- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**
    - Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of Protocol Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the IRB.
    - Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the IRB. Subjects at NIH will not be able to participate if they refuse to have samples stored but this decision will not affect the subject's participation in other protocols at NIH.

18.4 Risks

18.4.1 Radiation

The total amount of radiation exposure from 3 DEXA scans is 0.00009 – 0.00015 rem (0.9 to 1.5 microSeiverts). The radiation exposure in this study is below the limit of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee. There is no direct evidence that radiation exposure at this level is harmful, but, as with all radiation exposure, there may be a very slight increase in the risk of cancer.

The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. A copy of the pamphlet, "An Introduction to Radiation for NIH Research Subjects", will be provided to subjects by request.

18.4.2 Venipuncture/IV Catheter Placement

There will be minimal risk and discomfort associated with blood drawing and IV placement. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter.

18.4.3 Liver Biopsy

Ultrasound-guided percutaneous liver biopsy will be performed by trained interventional radiologists who specialize in gastrointestinal procedures at both the NIH and 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 known contraindication to liver biopsy, including platelets < 75,000, elevated PT or PTT, or chronic aspirin use will not undergo biopsy. Individuals on aspirin for primary prevention of MI may undergo biopsy following discontinuation of aspirin for ≥7 days. Post-procedure monitoring and

discharge will follow the site-specific policy for liver biopsy with and without sedation as appropriate. 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.

18.4.4 Study Drug

Administration of all medications will be overseen by the appropriate health care professional. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns. Participants will self-administer the study drug at home, with the exception of observed administration at the clinical research center during baseline and 12 month visits. Please see the Intervention section above for more information regarding study drug risks and benefits.

$GHRH^{1-44}$ analogue (tesamorelin, Theratechnologies, Inc.) for treatment purposes has been used in a number of studies, including the protocol in a prior completed R-01 grant described in preliminary data, with minimal side effects. In two large, Phase III studies conducted in individuals with HIV-infection and abdominal adiposity, 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). Clinically significant changes were not observed for liver function (alanine transaminase), kidney function (creatinine), or blood pressure (diastolic and systolic). The majority of the reported side effects were mild in severity. Tesamorelin is FDA approved for HIV-lipodystrophy and has now been prescribed to approximately 4000 patients with no additional signals of safety concern.

With regard to injection site reactions, subjects will be informed to notify a study investigator of any sign of erythema, swelling, or pruritis. Subjects will also be informed of theoretical risks associated with excess GH, including arthralgia, paresthesia, and hyperglycemia, although these were not seen frequently in previous studies of $GHRH^{1-44}$. In previous studies, there was no net effect of tesamorelin on glucose tolerance after 1 year of administration. 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 $GHRH^{1-44}$. 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. For potential participants with previous malignancy that has been treated and cured, their health care providers will be contacted to discuss history and risk of recurrence. The study investigators, potential participant, and participants' providers will work together to weigh the potential benefits of the study drug against the risk of recurrence; only if an agreement is reached that participation poses no increased risk of recurrence will subjects with previous history of cured malignancy be enrolled.

IGF-1 Z-score ≥3 for subjects on tesamorelin will prompt a dose-decrease. IGF-1 levels will be monitored by an endocrinologist not otherwise involved in the study in order to maintain blinding of study physicians and study staff. This endocrinologist will know the study drug assignment. If a subject receiving tesamorelin has an IGF-1 Z-score ≥3 (i.e., an IGF-1 level of 3 SD or more above the mean for age and gender), then the endocrinologist will contact the site investigator to request a dose decrease to 1mg. If the subject is in the double-blind portion of the study (first 12 months), a dummy dose decrease will also be requested for a placebo patient seen at the same site during the same time-frame in order to maintain blinding. If the IGF-1 Z-score for the

tesamorelin subject remains ≥3 at the next visit, tesamorelin administration will be discontinued but the subject will continue to be followed for safety.

18.4.5 Euglycemic Hyperinsulinemic Clamp/Insulin (MGH Only)

Administration of insulin infusion can cause hypoglycemia. Blood sugar will be monitored every 5 minutes during the insulin clamp, and 20% dextrose is infused per protocol to achieve target blood glucose of 90mg/dL. In the event of hypoglycemia, 50% dextrose is available at the bedside, and a physician or nurse practitioner is present throughout the insulin clamp procedure.

18.4.6 Other Risks

It is possible that incidental abnormal findings may be found during this study. In this case, the subject and his/her primary care physician will be notified.

18.5 Social Impact Events

Individuals enrolled in this study may experience personal problems resulting from the study participation. Such problems are termed social impact events. Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that participants may experience stigmatization or discrimination as a result of being perceived as being HIV-infected or at risk for HIV infection. For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities. Problems may also occur in circumstances in which study participation is not disclosed, such as impact on employment related to time taken for study visits. In the event that a participant reports a social impact event, every effort will be made by study staff to provide appropriate assistance, and/or referrals to appropriate resources.

Social impact events are documented and reviewed on a scheduled basis by the protocol team leadership with the goal of reducing their incidence and enhancing the ability of study staff to mitigate them when possible.

Social impact events that are judged by study investigators to be serious, unexpected, or more severe or frequent than anticipated, will be reported to the responsible site's IRB promptly, or otherwise in accordance with the IRB's requirements.

18.6 Benefits

There may be no benefit to participants from participation in this study.

Participants will receive information about their nutritional and metabolic health. In addition, subjects may experience benefits from the study drug, which half of subjects will receive from study onset and which all subjects will receive for at least 6 months during the final open-label extension. Tesamorelin is established to reduce visceral fat in a majority of patients and to lower triglyceride, on average. In addition, based on our preliminary data, subjects may experience reductions in liver fat, and potentially related improvements in metabolism and reductions in inflammation. Further, the information obtained from this study will inform the use of GHRH analogue and possibly other strategies to treat NAFLD in HIV. Therefore, the benefits are felt to outweigh the risks described above.

18.7 Compensation

18.7.1 MGH Compensation Plan

Subjects will receive reimbursement for parking and for transportation to study visits from within the greater Boston area. In addition, subjects will receive monetary compensation as below:

Patient Stipend	Amount
Screen	$50
Baseline	$100
Biopsy Stipend	$100
Clamp Stipend	$50
Safety visits (0.5, 3, 6, 9, 12.5, 15 months)	$25
12 mo visit	$150
18mo visit	$100

Given these amounts, the maximum compensation amounts for each visit (including clamp stipend and/or biopsy stipend when applicable) are as follows:

- Screen visit -- $50
- Baseline visit -- $150, plus $100 if liver biopsy
- Safety visits -- $25 for each visit
- 12 month visit -- $200, plus $100 if liver biopsy
- 18 month visit -- $100

18.7.2 NIH Compensation Plan

Subjects will be reimbursed for their time and inconvenience according to the following and in keeping with NIH NIAID OP8 Clinic reimbursement practices in similar protocols.

- Screen visit -- $50
- Baseline visit -- $100, plus $300 if liver biopsy
- Safety visits -- $25 for each visit
- 12 month visit -- $100, plus $300 if liver biopsy
- 18 month visit -- $100

18.8 Participant Privacy and Confidentiality

Data at each site 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. For regulatory purposes, individuals from the FDA, NIH, and each site's IRB may access these documents for inspection. The regulatory monitors will also access these documents for inspection (see 17 below). 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.

Any data or samples shared between sites will be de-identified such that no protected health information is shared between sites.

18.9 Certificates of Confidentiality: N/A

18.10 Critical Event Reporting: N/A

18.11 Communicable Disease Reporting

Subjects will be tested for Hepatitis B and Hepatitis C at the screen visit. Positive results will be communicated to the subject and, with the subject's permission, his/her provider, and, if required by state law, will be reported to the Department of Public Health per their reporting requirements. There are no plans to report Hepatitis B or C status except as required by the state law governing each site. HIV testing will not be performed, and there are no plans to report HIV status.

18.12 Incidental Findings

Incidental findings discovered on imaging will be discussed with the radiologist and subsequently be reported to the subject and, with the subject's permission, his/her provider. The possibility of incidental findings is discussed with subjects as part of the informed consent process.

18.13 New Findings

Any new scientific findings related to the research that affect the risk/benefit assessment of study participation, or that substantially affect the importance of the research question, will be reported to the IRBs overseeing the research and will be shared will any active and future research subjects.

18.14 Study Discontinuation

The study may be discontinued at any time by the IRB, NIAID, the FDA, or other government entities as part of their duties to ensure that research participants are protected.

18.15 Ancillary Benefits: N/A

18.16 Community Advisory Board and Other Relevant Stakeholders: N/A

19. ADMINISTRATIVE PROCEDURES

19.1 Regulatory Oversight

As above, the protocol will be monitored by the NIAID as well as the site IRB's. In addition, the protocol is being conducted under an IND (77,473 to Dr. Steven Grinspoon) and thus falls under FDA regulatory oversight.

19.2 ClinicalTrials.gov

This protocol will be registered in ClinicalTrials.gov.

20. PUBLICATION POLICY

Drs. Grinspoon and Hadigan will share responsibility and decision making regarding publication of study results.

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22. APPENDICES

Appendix A: Schedule of procedures/evaluations

Test/Procedure	screen	Base-line	0.5 mo	3 mo	6 mo	9 mo	12 mo	12.5 mo	15 mo	18 mo	ETV*
Informed consent	x										
Assessment of eligibility criteria	x										
Pregnancy test**	x	x	x	x	x	x	x	x	x	x	x
Review of medical history	x										
Interval history		x	x	x	x	x	x	x	x	x	x
Review of concomitant medications	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x	x	x	x	x	x	x	x	x	x	x
Nutrition Counseling		x			x		x			x	
Vital Signs	x	x	x	x	x	x	x	x	x	x	x
Body weight	x	x	x	x	x	x	x	x	x	x	x
Anthropometrics		x					x			x	x
Assessment of adverse events		x	x	x	x	x	x	x	x	x	x
AST	x	x	x	x	x	x	x	x	x	x	x
ALT	x	x	x	x	x	x	x	x	x	x	x
Creatinine	x										
total Bilirubin	x										
PT and INR	x										
PTT	x										
CBC	x						x				
CD4, HIV Viral Load	x						x				
HbA1c	x						x			x	
Hepatitis B sAg	x										

Test/Procedure	screen	Base-line	0.5 mo	3 mo	6 mo	9 mo	12 mo	12.5 mo	15 mo	18 mo	ETV*
Hepatitis C antibody	x										
PSA	x										
HCV RNA	x										
IGF-1		x	x	x	x	x	x	x	x	x	x
GGT		x					x			x	x
Alkaline Phosphatase		x					x			x	x
Lipid Panel		x					x			x	x
Fasting Glucose			x	x	x	x		x	x		x
Glucose (OGTT)		x					x			x	
Frozen serum: CK18, adiponectin, CRP, IL-6, other		x					x				
1H-MRS/MRI	x				x		x			x	x
Liver Biopsy		x					x				
DEXA		x					x			x	x
FibroScan		x					x			x	x
4-day food record		x					x			x	
Modifiable Activity Questionnaire		x					x			x	x
Collection of Peripheral Blood Mononuclear Cells		x									
Stable Isotopes and Clamp (MGH only)		x					x				

*ETV: Early termination visit.
**A urine or serum pregnancy test with sensitivity of at least 25 mIU/ml will be used.