A Randomized, Double Blind, Placebo-Controlled Study to Assess Efficacy, Safety and Tolerability of ISIS 304801 in Patients with Partial Lipodystrophy with an Open-Label Extension

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**Indicates those who are allowed to obtain informed consent*

Protocol Overview

Protocol Title:	A Randomized, Double Blind, Placebo-Controlled Study to Assess Efficacy, Safety and Tolerability of ISIS 304801 in Patients with Partial Lipodystrophy with an Open-Label Extension
Abbreviated Title:	ApoC-III ASO in lipodystrophy
IRB:	NIDDK/NIAMS
Research Type:	Phase II Clinical Trial
Multi-site Collaboration:	No
Intramural Collaboration:	No

Ionizing Radiation Use: (X-rays, e.g., CT; radioisotope, e.g. PET; etc.)	Research Indicated
Investigational New Drug/Device:	Yes
Patient Self-Referral Allowed:	Yes
List Protocol On Web:	Yes
Is tissue being collected for research purposes:	Yes

Conflict of Interest
The protocol involves no drugs/devices/products that may lead to payments and/or royalties to be paid to the investigators or the NIH.

The investigators have no equity, consultative, or other financial relationship with a non-NIH source related to this protocol which might be considered a conflict of interest**.**

Time Frame
Start Date: 12/1/2015 End Date: 4/30/2020

Table of Contents

Contents

Precis – Abstract

Background

Lipodystrophy is a rare disease of deficient adipose mass, characterized by severe hypertriglyceridemia as well as insulin resistance, diabetes mellitus, fatty liver disease, acute pancreatitis, and early cardiovascular events. Apolipoprotein C-III (apoC-III) regulates triglyceride metabolism, and apoC-III levels strongly correlate with serum triglycerides in a variety of patient populations. Patients with genetically low levels of apoC-III have lower triglycerides and reduced cardiovascular disease, while individuals with genetically elevated levels of apoC-III have higher triglycerides and increased non-alcoholic fatty liver disease and insulin resistance. Pharmacologic reduction of apoC-III using anti-sense oligonucleotides (ASOs) reduce triglycerides by ~60-70% in a tested patient populations.

Aim

The purpose of this study is to determine if apoC-III reduction using an ASO to apoC-III (ISIS 304801) will reduce triglycerides and improve insulin resistance, diabetes, and hepatic steatosis in patients with lipodystrophy. The primary hypothesis to be tested is:

1. ISIS 304801will reduce log10 fasting serum triglycerides.

Secondary and tertiary hypotheses to be tested are:

2. ISIS 304801will improve glucose metabolism by improving insulin resistance.
3. ISIS 304801 will reduce hepatic steatosis.
4. ISIS 304801 will improve cardiovascular risk markers.

We will also explore the mechanism of action of apoC-III ASO by studying lipoprotein lipase activity and lipoprotein particle distribution.

Methods

This study will enroll up to 20 patients with partial lipodystrophy with a goal of 10 study completers. The study will be conducted in two phases. The first is a 16-week, randomized, double-blind, placebo-controlled design. Subjects will be treated with ISIS 304801 at a target dose of 300 mg per week or placebo. Following this phase, all subjects will enter a 12 month open-label extension in which they will receive active drug. Patients who experience benefit (triglyceride lowering ≥50%) may receive an additional 12 months of open-label drug (up to 24 months, total). Measurement of the primary outcome (serum triglycerides) and key secondary outcomes will be performed at baseline prior to the intervention, after 16 weeks (primary and secondary outcomes) of blinded drug or placebo, and after an additional 4 months of active drug in subjects initially randomized to placebo.

STUDY GLOSSARY

Abbreviation	Definition
2'-MOE	2'-*O*-(2-methoxyethyl)
AE	adverse event
ALT	alanine aminotransferase (SGPT)
ANA	antinuclear antibody
apoA1	apolipoprotein A1
apoB	apolipoprotein B
apoC-III	apolipoprotein C-III
apoE	apolipoprotein E
aPTT	activated partial thromboplastin time
ASO	antisense oligonucleotide
AST	aspartate aminotransferase (SGOT)
BMI	body mass index
BP	blood pressure
DEXA	dual-energy X-ray absorptiometry
DSMB	Data and Safety Monitoring Board
EKG	electrocardiogram
eCRF	electronic Case Report Form
FPG	fasting plasma glucose
FPL	familial partial lipodystrophy
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HBV	Hepatitis B virus
HbA1C	Hemoglobin A1C
HCV	hepatitis C virus
HDL-C	high-density lipoprotein cholesterol
hERG	human ether-a-go-go
HIV	human immunodeficiency virus
IDL	intermediate-density lipoprotein
IRB	Institutional Review Board
ISIS 304801	antisense inhibitor of apolipoprotein C3
LDL-C	low-density lipoprotein cholesterol
LPL	lipoprotein lipase
MRI	magnetic resonance imaging
NCS	not clinically significant
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NOAEL	no observable adverse effect level
NYHA	New York Heart Association

OLE	open-label extension
on study	The patient is 'on study' from signing of the informed consent until their last study visit
PCOS	Polycystic ovary syndrome
PFS	pre-filled syringes
PK	pharmacokinetic(s)
PL	partial lipodystrophy
PT	prothrombin time
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAT	subcutaneous adipose tissue
SC	subcutaneous(ly)
Study Day 1	defined as the first day Study Drug product is administered to the patient
Study Drug	ISIS 304801 or placebo
SUSAR	suspected unexpected serious adverse reaction
TChol	total cholesterol
TG	triglyceride(s)
TRL	triglyceride-rich lipoprotein
ULN	upper limit of normal
VAT	visceral adipose tissue
VLDL-C	very low-density lipoprotein cholesterol
WBC	white blood cell

1. Study Objectives

1.1 Primary Objective

To evaluate the efficacy of 16 weeks of ISIS 304801 compared to placebo on the change in log10 fasting triglycerides (TG).

1.2 Secondary/Tertiary Objectives

To evaluate the efficacy of 16 weeks of ISIS 304801 as compared to placebo (between group comparison) on additional lipid, glycemic, hepatic, and cardiovascular parameters (see Outcome Measures), as well as safety and tolerability of ISIS 304801. In addition, the effects of ISIS 304801 (defined as tertiary outcomes) as compared to immediately prior to treatment (pre-post within subject comparision) for 16 weeks on these outcomes will be evaluated.

2. Introduction

2.1 Lipodystrophy

Lipodystrophy syndromes are a group of rare metabolic diseases characterized by selective loss of adipose tissue that leads to ectopic fat deposition in liver and muscle and the development of insulin resistance, diabetes, dyslipidemia and fatty liver disease [1-4]. These syndromes are categorized according to the distribution of fat loss into generalized or partial and according to the underlying etiology as inherited or acquired [2-4]. This classification scheme yields 4 major lipodystrophy subtypes: congenital generalized lipodystrophy, acquired generalized lipodystrophy, familial partial lipodystrophy, and acquired partial lipodystrophy. These syndromes constitute a significant medical unmet need as these patients are refractory to current therapies mainly used to treat diabetes and elevated TG levels in an attempt to reduce the risk of serious associated complications (coronary artery disease, diabetic nephropathy, cirrhosis and pancreatitis).

Deficiency of adipose tissue in patients with lipodystrophy leads to a deficiency of adipose-derived hormones, including leptin, which in turn contributes to the metabolic complications of lipodystrophy. Between 2000 and 2014, the NIDDK intramural research program conducted an open-label clinical trial the recombinant human leptin analog, metreleptin, in patients with generalized and partial forms of lipodystrophy. This study showed that metreleptin ameliorates metabolic and endocrine abnormalities in lipodystrophy, including reducing food intake, improving insulin resistance and diabetes, reducing ectopic lipid, and normalizing reproduction[5-14]. The best results were achieved in patients with generalized lipodystrophy who had low leptin levels [mean (SD): 1.3 (1.1) ng/mL], while patients with PL who had a wider range of baseline leptin values [mean (SD): 4.9 (3.1) ng/mL] had a more varied and attenuated response. Based on these data, metreleptin was approved by the FDA in February 2014, for patients with generalized, but not partial, lipodystrophy. Because of the risks associated with the development of neutralizing antibodies and lymphoma, Myalept is available only through a risk evaluation and mitigation strategy (REMS) program, which requires prescriber and pharmacy certification and special documentation[15].

The current study focuses on partial lipodystrophy for which there is no specific treatment and an unmet medical need. Partial Lipodystrophy (PL) has a higher prevalence (estimated ~2-3 in one million) than generalized lipodystrophy, but the true prevalence is unknown because these patients are greatly under-diagnosed[2,3]. Like generalized lipodystrophy, both genetic and acquired forms exist. Acquired lipodystrophies may be caused by medications, autoimmune mechanisms or other unknown mechanisms

(idiopathic). An acquired form seen in patients with the human immunodeficiency virus (HIV) on protease inhibitors has become the most prevalent form of PL, with an estimate of 100,000 patients in the United States alone, with many more patients in other countries. No specific pharmacologic treatment currently exists for the non-HIV-associated forms of PL.

The diagnosis of PL is mainly clinical and needs to be considered in patients presenting with the triad of insulin resistance (with or without overt diabetes), significant dyslipidemia in the form of hypertriglyceridemia, and fatty liver [16]. Patients often present with diabetes and severe insulin resistance requiring high doses of insulin. Other evidence of severe insulin resistance is provided by the presence of acanthosis nigricans and PCOS (with symptoms like hyperandrogenism and oligomenorrhea). Some patients develop severe hypertriglyceridemia resulting in episodes of pancreatitis. In many patients, the TG levels remain persistently elevated despite fully optimized therapy or diet modifications. Radiographic evidence of hepatic steatosis or steatohepatitis with hepatomegaly and/or elevated transaminases is common [17].

Careful clinical assessment of fat distribution through visual and physical examination can confirm the diagnosis. Patients with FPL have reduced subcutaneous fat in the limbs and truncal regions and may have excess subcutaneous fat deposition in neck, face and intra-abdominal regions. Patients with the Dunnigan variety have normal body fat distribution in childhood and gradually lose subcutaneous fat from the extremities and trunk around the time of puberty. In women, the loss of fat may be most striking in the buttocks and hips. At the same time these patients accumulate fat on the face ("double chin"), neck and upper back ("Cushingoid appearance with buffalo hump"). The extent of adipose tissue loss usually determines the severity of the metabolic abnormalities. Patients display prominent muscularity and phlebomegaly (enlarged veins) in the extremities and complain of disproportionate hyperphagia. The condition in females is more easily recognized than in men, and so is reported more often. Patients may also have a family history of similar physical appearance and/or fat loss. Genetic testing, when available, is confirmatory.[2,16,18]

Current treatment includes lifestyle modification such as reducing caloric intake and increasing energy expenditure via exercise. Conventional therapies used to treat severe insulin resistance (metformin, thiazolidinediones, GLP-1s, insulin), and/or high TGs (dietary fat restriction, fibrates, fish oils) are not very efficacious in these patients [3].

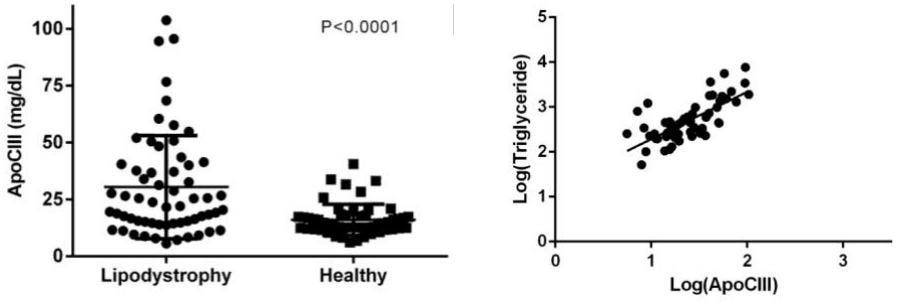
Partial Lipodystrophy is an orphan indication for which there is a significant unmet medical need. Diabetes and/or hypertriglyceridemia associated with this condition can lead to serious complications [17] such as:

- Acute pancreatitis, especially when triglyceride levels are >1,000 mg/dL
- Accelerated microvascular complications from uncontrolled diabetes
- Accelerated cardiovascular disease from lipid abnormalities and insulin resistance
- Steatohepatitis that can progress to cirrhosis
- Proteinuric nephropathies which can progress to end stage renal disease

In patients with generalized lipodystrophy the metabolic complications are partially related to leptin deficiency, and can be ameliorated in part by leptin replacement. However, leptin deficiency alone cannot explain the severity of metabolic disease in patients with PL who have variable leptin levels. Unpublished

data from our group showed that elevated apoC-III plays a role in the hypertriglyceridemia seen in both generalized lipodystrophy and PL and might therefore represent a therapeutic target in these patients.

These data show (left graph) that apoC-III is significantly higher in lipodystrophy patients compared to age, sex, and race matched obese controls (30.4 ± 22.7 mg/dL in lipodystrophy versus 16.1 ± 6.9 in controls, p<0.0001). In addition, apoC-III levels are very strongly correlated with triglyceride levels in patients with lipodystrophy (right graph, R2 = 0.52, p<0.0001), such that an elevated triglyceride level is highly predictive of an elevated apoC-III level.



By reducing apoC-III and TG levels, ISIS 304801 may improve the metabolic profile of patients with PL and reduce their risk of acute pancreatitis. In addition, apoC-III inhibition may also improve insulin sensitivity in these patients and potentially lead to a reduction in the complications associated with diabetes. It is unknown whether this mechanism could improve hepatic steatosis and reduce cirrhosis risk.

2.2 Therapeutic Rationale

ApoC-III is a 79 amino acid glycoprotein synthesized principally in the liver [19] (Figure 1). ApoC-III is a major regulator of lipoprotein metabolism and plays a pivotal role in regulating plasma TG levels [20]. It is a component of TG-rich lipoproteins (TRLs) and a potent inhibitor of lipoprotein lipase (LPL) [21]. At higher concentrations, apoC-III also inhibits the activity of hepatic lipase[22], an enzyme which plays an important role in the conversion of dense VLDL to intermediate-density lipoprotein (IDL) and to LDL [23], as well as in the remodeling of HDL [24]. In addition, increased apoC-III content adversely affects apoE-mediated hepatic uptake of TG-rich remnants [25]. Thus, elevated plasma apoC-III levels are associated with impaired hydrolysis and retarded clearance of TG-rich particles, resulting in the accumulation of VLDL-TG and chylomicrons in plasma and the development of hypertriglyceridemia [26].

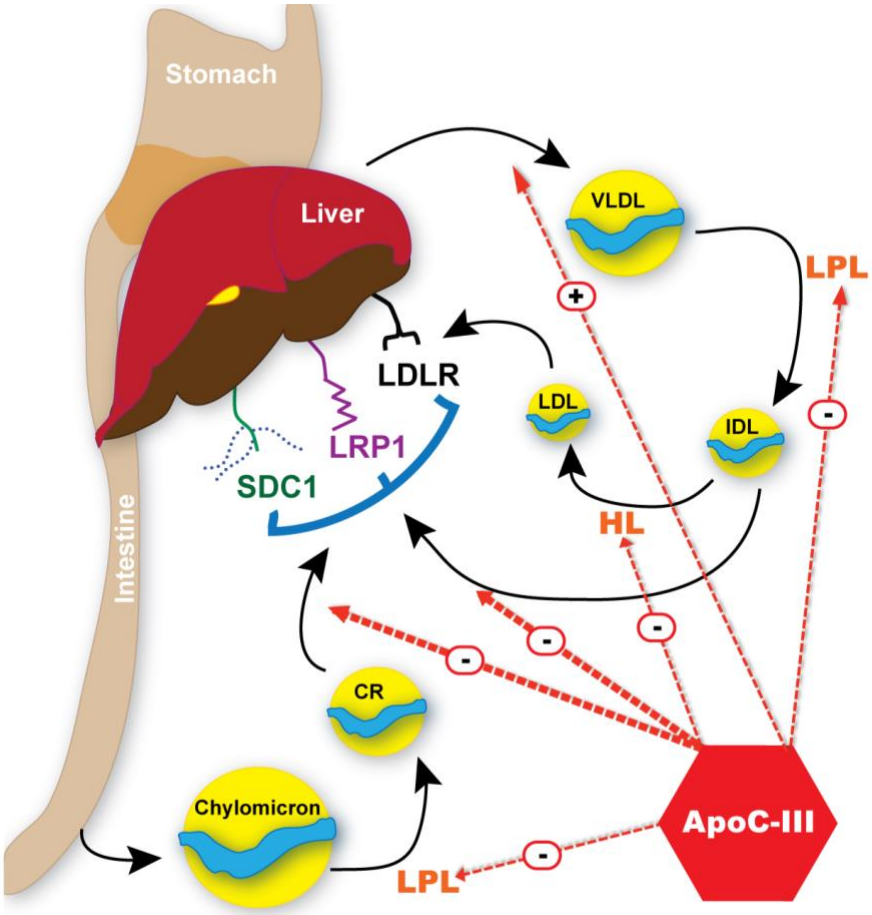


Figure 1 ApoC-III Regulates Lipoprotein Metabolism by Multiple Mechanisms

Courtesy of P. Gordts and J. Esko.

HL=hepatic lipase, IDL=intermediate-density lipoprotein, LDL=low-density lipoprotein, LDLR=LDL receptor, LRP1=Low density lipoprotein receptor-related protein 1 ; LPL=lipoprotein lipase, LSR=lipolysis-stimulated receptor, , SDC1=Syndecan-1TG=triglyceride, VLDL=very low-density lipoprotein.

In humans, decreased apoC-III has been associated with low triglycerides and protection from cardiovascular disease, while gene variants with elevated apoC-III have been associated with high triglycerides and fatty liver disease. A heterozygous null mutation in the apoC-III gene (R19X) in Old Order Amish individuals causing life-long apoC-III deficiency (~50% of normal) resulted in lower plasma TG levels (fasting and post-prandial), a cardioprotective lipid profile (i.e., higher HDL-C and lower LDL-C) and reduced atherosclerosis (as measured by coronary artery calcification) compared with noncarriers [27].

Jorgensen et al. had similar findings and showed that lifelong low levels of nonfasting triglycerides due to three loss-of-function mutations (R19X, IVS2+1G→A, A43T) in apoC-III were associated with reduced risk of ischemic heart disease in the general population [28]. Recent genetic studies involving mendelian randomization suggest that this association may be causal [29,30]. By contrast, gain of function polymorphisms in Apo-CIII that lead to lifelong increases in Apo-CIII have been associated with increased triglycerides and non-alcoholic fatty liver disease.

The clinical effects of apo-CIII inhibition in hypertriglyceridemia have been recently published. In one study, apoC-III inhibition with ISIS 304801 led to reduced apoC-III and triglyceride levels in various animal models and in humans in recent phase 1 clinical trials [31,32]. A recent phase 2 clinical trial in three patients with severe hypertriglyceridemia due to the familial chylomicronemia syndrome likewise showed robust reduction of apoC-III levels, with concomitant 56-86% reduction in fasting triglycerides [33].

In patients with lipodystrophy, preliminary data from our group has demonstrated that apo-CIII levels are elevated compared to controls, and correlate strongly with triglycerides [34]. Moreover, triglyceride reductions with leptin replacement correlated with reductions in apo-CIII. These data support the idea that apo-CIII excess plays a role in the hypertriglyceridemia of lipodystrophy, and that apo-CIII lowering could represent a promising therapeutic target in this condition.

2.3 ISIS 304801

2.3.1 Mechanism of Action

ISIS 304801 is a second-generation antisense oligonucleotide (ASO) drug targeted to human apoC-III. It is complementary to a region within the 3′ untranslated region of the apoC-III mRNA and binds to the mRNA by Watson and Crick base pairing. The hybridization (binding) of ISIS 304801 to the cognate mRNA results in the RNase H-mediated degradation of the apoC-III mRNA, thus preventing production of the apoC-III protein. Maximal antisense-mediated reduction of target mRNA levels is typically greater than 90% of control levels in sensitive tissues [35,36]. Furthermore, reduction in target mRNA levels using this approach correlates directly with a subsequent reduction in target protein levels.

2.3.2 Chemistry

Chemically, ISIS 304801 is a synthetic chimeric oligomer of 20 nucleotides (i.e., a 20-mer) that are connected sequentially by phosphorothioate linkages. The nucleotide sequence of ISIS 304801 (Figure 2) is complementary to a 20-nucleotide stretch within the 3′ untranslated region of the apoC-III mRNA transcript at base position 489-508. Structurally, the oligonucleotide has 3 regions. Two of them, the 5 nucleotides at the 5′ end and the 5 nucleotides at the 3′ end, are composed of 2′-O-(2-methoxyethyl) (MOE)-modified ribonucleotides. These MOE-modified nucleotides confer (1) increased affinity to the target mRNA [37,38], (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue)[39], and (3) amelioration of some of the high dose toxicities thereby resulting in an improved safety profile compared to first generation antisense drugs containing phosphorothioate modified oligodeoxynucleotides (DNA)[40]. The third region, the central portion of the oligonucleotide, is composed of 10 oligodeoxynucleotides. This chimeric design is called a MOE-Gapmer, and ISIS 304801 employs this chimeric structure to enable use of the RNase H-mechanism for antisense activity. This is because while the 2′-MOE modifications confer increased stability and affinity, they do not support RNase H catalysis of RNA hybridized to 2′-MOE-modified nucleotides[38]. This is caused by conformational changes induced in the heteroduplex by 2′-alkoxy:RNA hybrids that are not recognized by RNase H enzymes[41,42]. By limiting the number of 2′-MOE modifications to nucleotides flanking the phosphorothioate oligodeoxynucleotide core, the beneficial attributes of the 2′-MOE chemistry are preserved while also retaining RNase H recognition.

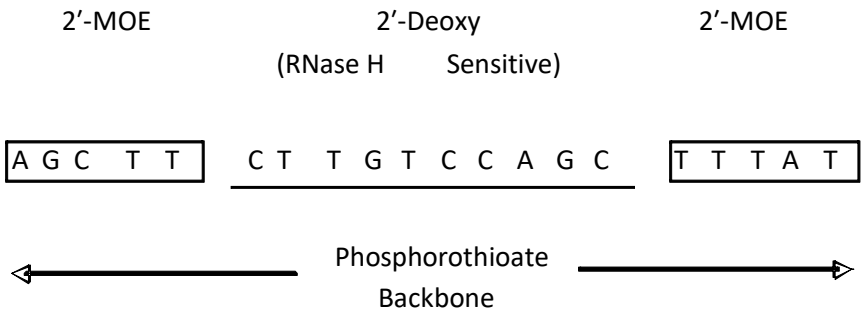


Figure 2 Design of Chimeric 2′-MOE Phosphorothioate Oligonucleotides (MOE-Gapmer). The sequence of ISIS 304801 is shown.

2.3.3 Preclinical Experience

Inhibition of apoC-III using ISIS 304801 has been shown to potently reduce hepatic apoC-III mRNA as well as plasma apoC-III protein and TG in a dose- and time-dependent fashion in several species, including human apoC-III transgenic mice, and other rodent and monkey models. Treatment with ISIS 304801 also resulted in reduced VLDL and chylomicron TG and reduced postprandial TG in hypertriglyceridemic monkeys[43].

The pharmacokinetics (PK) and toxicity of ISIS 304801 have been assessed in mice, rats, and cynomolgus monkeys. General toxicity studies with ISIS 304801 for up to 39 weeks of treatment followed by a 20-week recovery period are complete. The preclinical toxicology program for ISIS 304801 consists of single-dose safety pharmacology studies in the monkey (cardiovascular) and mouse (neurobehavioral and pulmonary assessment studies), *in vitro* and *in vivo* genetic toxicity assessment, repeat-dose fertility and/or developmental reproductive toxicology studies in the mouse and rabbit and evaluation in the *in vitro* human Ether-á-go-go -related gene (hERG) assay.

ISIS 304801 treatment-related findings in mice and monkeys were generally consistent with those expected for the 2′-MOE-class of ASOs. Treatment-related findings included effects consistent with drug accumulation in tissues in rodents and monkeys, species-dependent proinflammatory response in rodents and monkeys and complement activation and reductions in platelet counts in the monkey. In the mouse and monkey general toxicity studies, reduction of hepatic apoC-III mRNA (>75%) was not associated with any findings that could be considered related to the pharmacologic inhibition of apoC-III for up to 39 weeks of treatment.

The long-term, chronic toxicology studies in rodents with ISIS 304801 produced a significant proinflammatory response that led to subsequent effects on the heart at high doses. The rodent species are most sensitive to the proinflammatory effects of antisense molecules. Importantly, these effects on the heart were not observed in the monkey chronic toxicity studies even at the highest doses tested where drug exposure was higher than that in rodents. Because these changes were not observed in monkeys, the findings in the heart are considered to be rodent-specific and not relevant to humans. Nevertheless, such effects are monitorable by measurement of echocardiograms and cardiac troponin T that will be included in this clinical study.

ISIS 304801 caused no untoward effects in safety pharmacology studies (in vitro and in vivo) and was non-genotoxic (in vitro and in vivo). ISIS 304801 had no effects on fertility or embryo/fetal development in the mouse or rabbit reproductive toxicity studies. In these studies, ISIS 304801 was detected in placental tissue but not in fetal tissue indicating that little, if any, drug was able to cross the placenta to reach the fetus. Reduction of apoC-III mRNA (64% in males and 47% in females) also did not affect fertility or cause untoward effects on embryo/fetal development in mice.

Detailed information concerning the preclinical studies conducted with ISIS 304801 can be found in the ISIS 304801 Investigator's Brochure.

2.3.4 Clinical Experience

Detailed information concerning the clinical studies conducted with ISIS 304801 can be found in the ISIS 304801 Investigator's Brochure. A summary is included below.

ISIS 304801 has been evaluated in one Phase 1 study and two Phase 2 studies, all double blinded and placebo controlled. The total exposures comprise 99 patients and healthy volunteers administered ISIS 304801 from 50 to 400 mg subcutaneously up to 3 months (compared to 37 administered placebo).

All clinical trials of ISIS 304801 have shown very large and clinically meaningful reductions in fasting apoC-III and TG (~80% and 70%, respectively, mean reduction from baseline with 300 mg dose) with a very high degree of consistency of response between the different patient groups. This includes healthy volunteers, patients with moderate to severe hypertriglyceridemia not on background TG-lowering therapy, patients with moderate to severe hypertriglyceridemia on a background of stable fibrate therapy, patients with Familial Chylomicronemia Syndrome (FCS), and patients with hypertriglyceridemia and T2DM. Comparison of the effects of ISIS 304801 at the 300 mg/week dose across several patient populations is shown in Table 2.

Table 2 Mean Percent Change from Baseline of Lipid Parameters in Hypertriglyceridemia Patients Treated with ISIS 304801 (300 mg/wk for 12 weeks) in Phase 2 Studies

Mean % (SD) Change from Baseline	Monotherapy in Hypertriglyceridemia and T2DM (N = 7) Mean Baseline TG 259 mg/dL	Monotherapy in Hypertriglyceridemia (N = 11) Mean Baseline TG 559 mg/dL	Add-on to Fibrate in Hypertriglyceridemia (N = 10) Mean Baseline TG 394 mg/dL
ApoC-III	**-88% (6.0)***	**-80% (9.3)*****	**-71% (13.0)****
Triglycerides	**-72% (8.3)***	**-71% (14.1)*****	**-64% (8.9)****
HDL-C	**+40% (19.8)***	**+46% (24.0)*****	**+52% (23.7)****

* $p \leq 0.05$ vs. placebo
** $p \leq 0.01$ vs. placebo
*** $p \leq 0.001$ vs. placebo

In addition to the lipid analysis, the effect of lowering apoC-III on glycemic control and insulin sensitivity was evaluated in the subgroup of patients with hypertriglyceridemia and type 2 diabetes, by measuring

fructosamine, glycated albumin, and HbA1c and by performing a hyperinsulinemic euglycemic clamp at baseline and at the end of the treatment period.

Statistically significant decreases were observed in the mean change from Baseline in % glycated albumin and in fructosamine 1 week after the last dose and in HbA1c and fructosamine at the end of the Follow-up Period (3 months after the last dose). These significant effects later in the Follow-up Period were likely a result of the long duration of response of ISIS 304801. Trends were also observed towards improvement in all measures of peripheral insulin sensitivity, but the small sample size of patients with valid clamps performed within the protocol-specified time window likely precluded statistical significance.

In clinical studies conducted to date, ISIS 304801 has been well tolerated and has shown a favorable safety profile. There has been no clinical or laboratory evidence of drug-drug interactions.

To date, there have been no ISIS 304801 associated laboratory abnormalities suggestive of an effect on the renal (serum creatinine, proteinuria) or hepatic systems (alanine aminotransferase [ALT], aspartate aminotransferase [AST], bilirubin) despite many patients in the Phase 2 clinical trials receiving concomitant medications that are known to be associated with elevations in hepatic enzymes, such as fibrates and statins. In Phase 2 studies, there was a mild decrease of platelet count associated with ISIS 304801 administration that recovered in the post-treatment period and was not associated with platelet-related adverse events (AEs).

The most frequently observed AEs with ISIS 304801 were local reactions at the injection site. Local cutaneous reactions at the injection site, defined as those events presenting as either pain, tenderness, erythema, pruritus or swelling occurring on the day of injection and persisting for at least 2 days (~15% of injections), were almost always mild, resolved spontaneously, were non-progressive, and were not associated with systemic sequelae.

2.4 Rationale for Dose and Schedule of Administration

The dose and schedule selected for this study is 300 mg of ISIS 304801 or placebo per week for 16 weeks, followed by a 12 month open label extension of 300 mg of ISIS 304801 per week. Patients who experience benefit (triglyceride lowering ≥50 may receive an additional 12 months of open label drug (up to 24 months, total). The dose of 300 mg per week is supported by both the cumulative nonclinical data available to date and the Phase 2 clinical data. In nonclinical studies, ISIS 304801 treatment-related effects in rodents and monkeys were consistent with drug accumulation in tissues and also include species-specific proinflammatory responses. In the monkey, the no observable adverse effect level (NOAEL) was defined by the cumulative clinical pathology results and histopathology data provide an adequate clinical safety margin. Nonclinical findings were not considered to be related to the pharmacologic inhibition of apoC-III.

The safety data to date suggest that ISIS 304801 has been well tolerated at dose levels up to 300 mg in patients with high TG (including type 2 diabetes patients and administration in combination with fibrates and statins) with the most common AEs being local to the injection site and predominantly mild. A pooled analysis of safety for the Phase 2 studies did not demonstrate any clear difference in safety or tolerability between the different doses tested (100 mg, 200 mg, and 300 mg per week for 13 weeks). Analysis of the cohorts in which ISIS 304801 was studied at different doses demonstrates dose-dependent pharmacology with

respect to pharmacodynamic effect on the target, apoC-III, with a clear difference in the TG-lowering effect between the different doses in the 100 mg to 300 mg dose range as compared to placebo.

In addition to the nonclinical and clinical experience of ISIS 304801, the dose and schedule of administration is supported by the preclinical and clinical safety experience of several other 2′-MOE-modified ASOs that have been administered intravenously and SC in multiple clinical studies at doses up to 1200 mg and for treatment durations that exceed 24 months [44].

3. Subject Eligibility Assessment and Enrollment

3.1 Inclusion Criteria

1. Age ≥18 years at enrollment (the time of informed consent, week -1)
2. Fasting TG levels ≥500 mg/dL (≥5.7 mmol/L) at enrollment. If the fasting TG value is <500 mg/dL (<5.7 mmol/L) but ≥350 mg/dL (≥4.0 mmol/L) up to two additional tests may be performed in order to qualify, and a single level ≥500 mg/dL will permit enrollment.

   OR
3. Fasting TG levels ≥ 200 mg/dL (2.6mmol/L) with a hemoglobin A1C over 7%
4. Willing to maintain their customary physical activity level and to follow a diet moderate in carbohydrates and fats with a focus on complex carbohydrates and replacing saturated for unsaturated fats
5. Clinical diagnosis of lipodystrophy based on deficiency of subcutaneous body fat in a partial fashion assessed by physical examination, and low skinfold thickness in anterior thigh by caliper measurement: men (≤ 10mm) and women (≤ 22 mm), plus one of the following:

   a) Genetic diagnosis of familial PL (e.g., mutations in LMNA, PPAR-γ, AKT2, or PLIN1 genes)
   OR
   b) Family history of familial PL or abnormal and similar fat distribution PLUS 1 minor criterion (below), OR
   c) 2 minor criteria (below) in the absence of genetic diagnosis of family history

   MINOR Criteria

   a. Diabetes mellitus with requirement for high doses of insulin, eg, requiring ≥200 U/day, ≥2 U/kg/day, or currently taking U-500 insulin
   b. Presence of acanthosis nigricans on physical examination
   c. History of polycystic ovary syndrome (PCOS) or PCOS-like symptoms (hirsutism, oligomenorrhea, and/or polycystic ovaries)
   d. History of pancreatitis associated with hypertriglyceridemia
   e. Evidence of non-alcoholic fatty liver disease
      - Hepatomegaly and/or elevated transaminases in the absence of a known cause of liver disease or
      - Radiographic evidence of hepatic steatosis (e.g., on ultrasound or CT)

6. Satisfy one of the following:

a) Females: Non-pregnant and non-lactating; surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy), post-menopausal (defined as 12 months of spontaneous amenorrhea in females >55 years of age or, in females ≤55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and follicle-stimulating hormone (FSH) levels in the postmenopausal range for the laboratory involved), abstinent*, or if engaged in sexual relations of child-bearing potential, patient is using an acceptable contraceptive method (refer to Section 7.4.1) from time of signing the informed consent form until at least 13 weeks after the last dose of Study Drug administration.

b) Males: Surgically sterile, abstinent*, or if engaged in sexual relations with a female of child-bearing potential, patient is utilizing an acceptable contraceptive method (refer to Section 7.4.1) from the time of signing the informed consent form until at least 13 weeks after the last dose of study drug administration.

* *Abstinence is only an effective method of birth control when this is the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.*

3.2 Exclusion Criteria

1. A diagnosis of generalized lipodystrophy
2. Current or history of autoimmune diseases (even with a diagnosis of PL) unless approved by the Investigator and Sponsor Medical Monitor
3. Acute pancreatitis within 4 weeks of enrollment
4. History within 6 months of enrollment of acute or unstable cardiac ischemia (myocardial infarction, acute coronary syndrome, new onset angina), stroke, transient ischemic attack, or unstable congestive heart failure requiring a change in medication
5. Major surgery within 3 months of enrollment
6. History of heart failure with New York Heart Association functional classification (NYHA) greater than Class II
7. Uncontrolled hypertension (blood pressure [BP] >160/100 mm Hg)
8. Any of the following laboratory values at enrollment:
    a. Cardiac troponin T >ULN
    b. Measured or estimated (in case of triglycerides > 400 mg/dL) LDL-C >130 mg/dL on maximal tolerated statin therapy
    c. Hemoglobin HbA1c ≥9.5%
    d. Hepatic:
        i. Total bilirubin >ULN
        ii. ALT >3.0 x ULN. Higher levels will be permitted after a safety review by a hepatologist, that includes no evidence of cirrhosis.
        iii. AST >3.0 x ULN. Higher levels will be permitted after a safety review by a hepatologist, that includes no evidence of cirrhosis.

    e. Renal:

i. Persistently positive (2 out of 3 tests ≥ trace positive) for blood on urine dipstick. In the event of a positive test eligibility may be confirmed with urine microscopy showing ≤ 5 red blood cells per high power field (urine will be screened at admission and repeated if abnormal). If RBC are high every effort will be made to determine if the source is renal or benign, such as from menstrual bleeding. If the presence of RBC is determined to be from a non-renal source, the PI will make the decision to proceed with protocol testing and/or randomization.

ii. Two out of three consecutive tests ≥ 1+ for protein on urine dipstick. In the event of a positive test eligibility may be confirmed by either a spot urine albumin to creatinine ratio <1000mg/g or a quantitative total urine albumin measurement of < 1g/24 hrs (urine will be screened at admission and repeated if abnormal)

iii. Estimated creatinine clearance calculated according to the formula of Cockcroft and Gault <60 mL/min

f. Platelet count below 140,000 K/uL

g. Clinically significant (as determined by the Investigator or Sponsor) abnormalities on laboratory examination that will increase risk to the patient or interfere with data integrity

9. Uncontrolled hypothyroidism (abnormal thyroid function tests should be approved by the Investigator)
10. History within 6 months of enrollment of screening of drug or alcohol abuse
11. History of bleeding diathesis or coagulopathy or clinically significant abnormality in coagulation parameters at enrollment
12. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to enrollment
13. Known history of or positive test for human immunodeficiency virus (HIV), hepatitis C or chronic hepatitis B
14. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated
15. Treatment with another investigational drug, biological agent, or device within one month of enrollment, or 5 half-lives of investigational agent, whichever is longer
16. Unwilling to comply with contraceptive and lifestyle (diet/exercise) requirements
17. Use of any of the following:
    a. Use of metreleptin within the last 3 months prior to enrollment
    b. Antidiabetic, lipid lowering, or atypical antipsychotic medication, unless on a stable dose for at least 3 months prior to enrollment
    c. Insulin unless on a stable daily insulin dose regimen (± 20 %) for at least 4 weeks prior to enrollment
    d. Use of nicotinic acid or derivatives within the last 4 weeks prior to enrollment
    e. Systemic corticosteroids or anabolic steroids within 6 weeks prior to enrollment unless approved by the Investigator
    f. Antihypertensive medication unless on a stable dose for at least 4 weeks prior to enrollment
    g. Tamoxifen, estrogens or progestins unless on a stable dose for at least 4 months prior to enrollment and dose and regimen expected to remain constant throughout the study
    h. Oral anticoagulants unless on a stable dose for at least 4 weeks prior to enrollment and regular clinical monitoring is performed
    i. Prior exposure to ISIS 304801

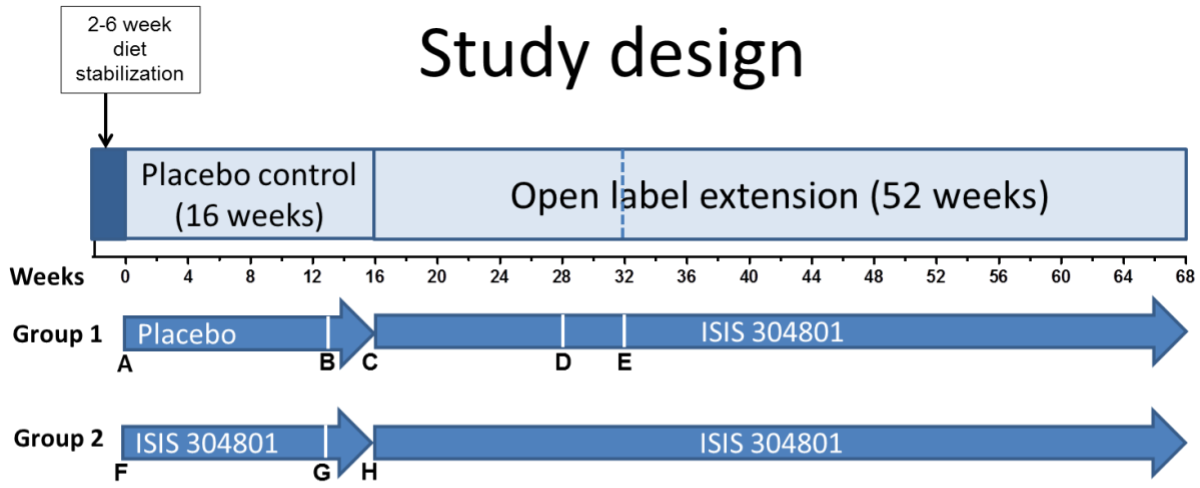
j. Anti-obesity drugs [e.g., the combination of phentermine and extended-release topiramate (Osymia, orlistat (Xenical), liraglutide [rDNA origin] injection (Saxenda) and lorcaserin (Belvig), phentermine, amphetamines, herbal preparations] within 12 weeks prior to screening
k. Any other medication unless stable at least 4 weeks prior to enrollment (occasional or intermittent use of over-the-counter medications will be allowed at Investigator's discretion)

18. Blood donation of 50 to 499 mL within 30 days or of >499 mL within 60 days
19. Have any other conditions, which, in the opinion of the Investigator or the Sponsor would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study

4. Study Design

4.1 Design/Randomization

This study has two components. Initially, subjects will receive ISIS 304801 or placebo, in a 1:1 randomized, double-blinded fashion for 16 weeks. Randomization will occur after verification that all eligibility criteria have been met. The randomization will be stratified by use of lipid-lowering medications. After completion of 16 weeks, all subjects will receive ISIS 304801 for 12 months in an open-label extension (OLE) phase. After discontinuation of ISIS 304801 (due to study end or any other reason) subjects will be followed for an additional 12 week withdrawal phase when possible. Patients who experience benefit (triglyceride lowering ≥50%) may receive an additional 12 months of open label drug (up to 24 months, total). This period may initiate immediately following the standard 12 month OLE, or following the withdrawal phase.



- 1° outcome (Δ in log10 triglycerides):
  - C vs D
  - F vs G
- 2°/3° outcomes – within subject comparisons:
  - C vs E
  - F vs H
- Placebo vs drug comparisons
  - C vs H

1

4.2 Outcome Measures

4.2.1 Primary outcome:

Efficacy of 16 weeks of ISIS 304801 as compared to placebo (between group comparison) on the change in log10 fasting triglycerides (TG).

4.2.2 Secondary outcomes:

Efficacy of 16 weeks of ISIS 304801 as compared to placebo (between group comparison) on:

Lipids

- Percent change in fasting TG
- Absolute change in fasting TG
- Change in lipolysis rate measured using stable isotope tracers
- Change from baseline in liver volume and hepatic steatosis (as assessed by magnetic resonance imaging [MRI] and magnetic resonance spectroscopy [MRS])
- Lipoprotein lipase activity

Glycemic

- Change in total body insulin sensitivity using the hyperinsulinemic euglycemic clamp
- Change in hepatic glucose production and hepatic insulin sensitivity using stable isotope tracers in combination with the hyperinsulinemic euglycemic clamp
- Change in hemoglobin A1c (HbA1c)
- Change in fasting plasma glucose

Safety

- Safety and tolerability of ISIS 304801
- Prospectively adjudicated acute pancreatitis events (Atlanta classification) and Major Adverse Cardiovascular Events (MACE)

Pharmacokinetic

- Plasma ISIS 304801 level

4.2.3 Tertiary/Exploratory outcomes:

Efficacy of 16 weeks and 16 months of ISIS 304801 as compared to immediately prior to treatment (pre-post within subject comparison) on:

Lipids

- Change in other fasting lipid measurements: high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TChol), very low-density lipoprotein cholesterol VLDL-C, non-HDL-C, apolipoprotein B (apoB), apolipoprotein B-48 (apoB48), apolipoprotein A1 (apoA1), apolipoprotein CIII (apoC-III; total, chylomicron, VLDL, LDL and HDL), and free fatty acids
- Change in lipoprotein particle size/number by nuclear magnetic resonance (NMR)
- Triglyceride (and other lipid particle) clearance following mixed meal testing

Glycemic

- Change in 24-h glucose (using 7-point plasma blood glucose)
- Change in fasting and area under the curve for insulin and C-peptide during mixed meal testing
- Reduction in insulin use

Adipose tissue

- Change in skinfold thickness and dual-energy X-ray absorptiometry (DEXA)
- Change in abdominal VAT and SAT volumes
- Change in adiponectin and leptin
- Change in body weight and waist circumference

Cardiovascular

- Carotid intima-media thickness (by ultrasound)
- Coronary artery wall thickness and diameter change after hand-grip exercise (by MRI)

Patient Reported Outcomes

- Change in Quality of Life (EQ-5D, SF36)
- Change in hunger visual analog scale
- Change in pain

Other

- Change in testosterone
- Metabolomics
- Liver histopathology

Efficacy of 16 weeks of ISIS 304801 as compared to placebo (between subject comparison) on the primary and secondary outcomes, above.

5. Statistical Considerations

5.1 Sample Size Justification

The primary outcome variable is the change in log10 fasting triglycerides from before treatment to16weeks of active drug treatment compared to placebo groups.

Preliminary data are available for 28 patients treated with ISIS 304801 and 24 treated with placebo. For the ISIS 304801 group, these data show a difference from baseline to follow-up of -423 (SD 400, 95% CI -578 to -268) mg/dL and -0.485 (SD 0.221, 95% CI -0.57 to -0.40) log10 mg/dL. Converting the difference in log10 mg/dL to a percent change [100*(10^-0.485-1)] results in a percent change of -67 % (95% CI -73% to -60%). For the placebo group, the absolute change is -12 (SD 406, 95% CI -12 to 184) mg/dL and -0.011 (SD 0.224, 95% CI -0.105 to 0.084) log10 mg/dL. The percent change for the placebo group is -3% (95% CI -21% to 21%.

For comparison of placebo and ISIS 304801 we have chosen a SD of 0.25 log10 mg/dL and a difference of 0.50 log10 mg/dL. A trial with 5 patients in each group has 79% power to detect a difference of 0.50 log10 mg/dL triglycerides in the change from baseline to follow-up between the ISIS and placebo groups, assuming a SD of 0.25 log10 mg/dL and a two-sided significance level of 0.05.

Where preliminary data were available, sample size calculations were performed for secondary and tertiary study outcomes in addition to the primary outcome. The preliminary data and power estimates for a sample size of 10 subjects (using one-sample t-test for off versus on drug comparisons) are summarized in the table below. Based on these calculations, the study should be adequately powered to detect differences in total body insulin sensitivity, hepatic insulin sensitivity, fasting glucose, apoC-III serum level, HDL-C, VLDL-C, and apolipoprotein B48.

Based on these calculations, we plan to enroll a maximum of 20 patients, with a goal of 10 patients completing the study (defined as completing 16 weeks of active drug treatment). The accrual ceiling of 20 is planned because subjects will consent to the study prior to screening, thus we are accounting for up to 10 screen failures in the accrual ceiling.

	Outcome	Model	Treatment	Treat-ment Duration	Mean of Δ on-off drug (SD)	Power
Primary outcome	Absolute change log[serum triglycerides] (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-0.48 (0.22)	>0.95
	% change serum triglycerides (%)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-63 (20)	>0.95
	Absolute change serum triglycerides (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-423 (400)	0.85
Secondary outcomes	Hemoglobin A1C (%)	Type 2 diabetes	ApoCIII ASO 300 mg/wk	16 wk	-0.2 (0.36)	0.35
	Total body insulin sensitivity (glucose infusion rate during clamp, $mg*kg^{-1}*min^{-1}$)	Type 2 diabetes	ApoCIII ASO 300 mg/wk	16 wk	1.6 (1.2)	>0.95
	Hepatic insulin sensitivity (% suppression of endogenous glucose production during clamp)	Type 2 diabetes	ApoCIII ASO 300 mg/wk	16 wk	11.0 (5.6)	>0.95
	Fasting plasma glucose (mg/dL)	Type 2 diabetes	ApoCIII ASO 300 mg/wk	16 wk	-35 (36)	0.78

	ApoCIII serum level (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-15.3 (7.5)	>0.95
	HDL-C (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	13.3 (10.1)	>0.95
Tertiary Outcomes	LDL-C (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	31.3 (47.3)	0.46
	Total Cholesterol (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-32 (72)	0.24
	VLDL-C (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-77 (60)	0.95
	non-HDL-C (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-46 (74)	0.42
	Apolipoprotein B (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-5.8 (30)	0.09
	Apolipoprotein B-48 (mg/dL)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-0.44 (0.43)	0.82
	Free Fatty Acids (uEq/L)	Familial chylomicron-emia	ApoCIII ASO 300 mg/wk	16 wk	-0.40 (0.44)	0.73
	Body Weight (kg)	High TG +/- diabetes and/or fibrate	ApoCIII ASO 300 mg/wk	16 wk	-0.87 (1.69)	0.30

5.2 Statistical Methods for Data Analysis

Descriptive statistics such as mean and standard deviation (or median and interquartile range) will be used to summarize data in raw scale or change from baseline per visit.

The primary outcome, change in log10 TG at Week 16 from baseline, and continuous secondary outcomes will be compared between ISIS 304801 and placebo groups using either two-sample t-test or Wilcoxon rank sum test as appropriate based on change from Baseline at Week 16 . Changes from baseline based on tertiary/exploratory outcomes as well as change on log10 TG at Week 16 will be examined using either one-sample t-test or Wilcoxon signed rank test as appropriate.

Repeated measures analysis of covariance using linear mixed models will be conducted in order to investigate trends on selected response variables and to compare active and placebo group while possible covariates are taken into account. There are three possible covariates: (1) Baseline level of the outcome of interest, (2) Baseline level of ApoCIII, and (3) Change in ApoCIII before and after the 16 week active drug intervention.

Inclusion of covariates will be checked using linear mixed models by checking Akaike Information Criteria (AIC) with inclusion of one covariate at a time

Analyses will include all available data even if patients do not adhere to their assigned treatments. Additional analyses will examine the effect of adherence to study results. We will also investigate the effect of missing data on the results using standard methods for missing data.

A p-value of 0.05 or less is considered significant.

6. Experimental Plan

6. 1 Diet

2-8 weeks prior to the first study visit, all patients will be counseled by an NIH dietician (in person or via long-distance contact) to follow a moderate diet composed of ~50% kcal from carbohydrates (focusing on complex carbohydrates and avoidance of concentrated sugars like juice and candy) and 30% or less kcal from fat (focusing on decreasing intake of trans and saturated fats and increasing intake of mono-unsaturated fats), with an emphasis on mixed meals (combination of protein, carbohydrate and fat). Patients should maintain their customary physical activity level throughout the study. Patients will be advised to limit alcohol consumption to moderate use (not more than 3 drinks/week and not more than 1 drink/day for females and not more than 5 drinks/week and not more than 2 drinks/day for males). One drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of hard liquor.

6.2 Medications

6.2.1 Pre-study Medications

With the exception of insulin and sulfonylureas subjects will continue their pre-admission medications at stable doses throughout the study. This includes oral hypoglycemic agents, and other medications either related or unrelated to lipodystrophy or its complications. Insulin and sulfonylurea doses may be reduced per investigator judgment in case of hypoglycemia.

6.2.2 ISIS 304801

6.2.2.1 Formulation, storage conditions/stability:

Study Drug (ISIS 304801 or placebo) characteristics are listed below.

The Study Drug is contained in glass prefilled syringes (PFS). The Study Drug must be stored securely at 2° to 8° Celsius and be protected from light. The investigative team will provide patients with instructions in Study Drug storage and administration.

Study Drug Characteristics

Study Drug	ISIS 304801	Placebo
Strength	**200 mg/ mL**	**Not Applicable**
Volume/Formulation	**1.5 mL solution per PFS**	**1.5 mL solution per PFS**

Dose	**300 mg once weekly**	**Not applicable**
Route of Administration	**SC**	**SC**

6.2.2.2 Availability

ISIS 304801 and placebo are supplied by Ionis Pharmaceuticals.

6.2.2.3 Initial Drug Shipment and Re-Supply

A completed Drug Request Form must be emailed, at least 5 days prior to the expected delivery date, to Ionis Clinical Supplies.

6.2.2.4 Study Drug Accountability

The study staff is required to document the receipt, dispensing, and return/destruction of Study Drug supplies provided by the Sponsor. At the end of the study, and after full accountability is performed on study drug or site closure, study drug destruction will occur at NIH.

6.2.2.5 Study Drug Administration

For each individual patient, Study Drug will be administered SC as a single 1.5 mL injection once weekly for Weeks 1-68. Self-administration will be allowed after appropriate training of the patient and/or caregiver. Patients should receive 1 dose per week, with weeks always defined relative to Study Day 1. For example, if a patient receives the first dose on a Monday, subsequent doses should be given on Mondays, if possible. If a patient misses an injection, or if dosing on the usual day is not possible, the patient can reschedule the injection provided that 2 doses are administered at least 2 days apart.

Every effort should be made to ensure the previous week's dose is given 7 days prior to a scheduled clinic visit. Subjects will not be removed from the study because of missed dosing; however, subjects missing more than 2 doses before Week 13 may not be evaluable for the primary endpoint.

6.3 Study Procedures

6.3.1 Study Schedule

A detailed schedule of study visits and testing to be performed at each visit is given in Appendix A.

6.3.2 Prescreening

Prior to the first study visit, patients will undergo prescreening via an in-person NIH visit under another protocol, or via long-distance review of medical history and records, to maximize the likelihood that subjects will meet all inclusion/exclusion criteria at the time of the initial study visit. Dietary counseling will be provided in-person or via long-distance communication 2-8 weeks prior the initial study visit as per section 6.1, and prior to the written informed consent process. If an outside LDL-C lab result is available and is within 3 months of a subject's initial NIH visit, the outside value will be considered as part of their enrollment criteria. The test will be remeasured once at NIH but will ensure there are no delays in study procedures as a result of this test not being available until ~7 days after it is drawn.

6.3.3 Initial Visit – Includes Screening (Week -1) and the first week of dosing (Week 1)
Subjects will be admitted to the Metabolic Research Unit in the NIH Clinical Center. Upon admission, they will undergo a history and physical examination, and baseline labs will be obtained. Height, weight, blood pressure, resting pulse, and temperature will be obtained. Subjects will undergo the informed consent process prior to any study-related testing.

Testing used to assess subject eligibility includes:

- Physical examination (including skin-fold measurements, blood pressure)
- Urinalysis and/or 24 hour urine for protein and blood
- Lipid panel
- Fasting insulin
- Pregnancy test (females)
- Cardiac troponin T
- Hemoglobin A1c
- Hepatic panel
- Serum creatinine
- Thyroid function tests
- Coagulation panel (PT, aPTT)
- Viral hepatitis studies
- 12-Lead EKG
- Echocardiogram

If a subject meets all eligibility criteria, he/she will undergo randomization and will proceed with the Week 1 schedule per Appendix A.

6.3.4 In-person follow-up visits
Patients will come to NIH for follow-up visits every 4 months. Testing will be performed as per the Study Schedule (Appendix A)

6.3.5 Home nurse visits
Home nurse visits for safety and/or efficacy labs and vital signs will be conducted every week (unless the patient is coming for an in-person visit) for the duration of the study, as detailed in Appendix A. Home (or visits at a location convenient for the subject) visits will be completed by trained service providers, sourced through a home health care agency, GlobalCare Clinical Trials. Service providers will receive detailed training on all necessary study procedures and are also required to comply with patient privacy and data protection laws as well as regulatory guidelines for Good Clinical Practices and ICH guidelines. Home health care is provided to ease patient burden while still allowing for adequate safety monitoring. If feasible, patients can also complete these visits as outpatient visits at the NIH clinical center. Visits will be approximately 1 hour in length. In the unlikely event a service provider is unavailable to collect blood from a subject, the NIH study team will send a prescription to the subject so that they can have their labs drawn by a local lab facility. Prior approval to cover the cost of the lab tests is required by NIH. Patients will be made aware there is a

possibility they may need to cover the costs through either their health insurance provider or pay out of pocket.

All visits (home and in-person) will have a visit window of at least +/- 7 days. All reasonable attempts should be made to ensure compliance with the visit schedule as outlined in Appendix A. However, in the event that a visit does not occur or is delayed, all subsequent visits should be calculated based on the time elapsed since Day 1 rather than from the date of the previous visit.

6.3.6 Telephone follow-up

Telephone contact between the study team and each subject for review of adverse events, concomitant medications, and general study requirements will be conducted every month for the placebo controlled period and the first 6 months of the open label extension, and every 2 months thereafter (unless the patient is coming for an in-person visit), as detailed in Appendix A.

6.3.7 Post-Treatment

After completion of the open label extension (week 68), patients will enter the 12-week post-treatment evaluation period. This period consists of home platelet monitoring every week for six weeks and then again at a single NIH visit on week 80 as outlined in Appendix A.

Immediately after week 68, or after the post-treatment evaluation period, patients may choose to receive an additional 12 months of open label drug (up to 24 months, total). They will be offered this option if their triglycerides improved by ≥ 50%. Justification for this additional extension are:

a. The patients received ≥50% improvement in triglycerides and they have no other effective treatment options.
b. To collect additional safety data over a longer term
c. To collect additional efficacy data over a longer term

Home health care visits will not be available during the extended open-label period. Patients must obtain platelet and other safety monitoring locally through their health insurance provider or pay out of pocket. This monitoring will include weekly CBC for platelet count, and monthly CBC with differential, lipid panel, coagulation studies, urine protein:creatinine ratio, liver function tests, and chemistry panel. Hemoglobin A1c will be measured every 3 months. Study staff will remain in weekly contact with patients to review safety labs and any adverse events. Patients will return to NIH at the end of the 12 month extended open-label use period, or earlier if there are any safety concerns that, in the judgment of the PI, require in-person evaluation. During the NIH visit at the end of the 12 month period, patients may undergo optional evaluation including any test listed in section 6.4 except liver biopsy. All concomitant medications, including those for diabetes or hyperlipidemia, may be adjusted as clinically appropriate per the judgment of the investigator during the extended open-label period.

6.3.8 Follow-up Visits for Early Termination from Treatment Period

Any patient who discontinues early from the placebo-controlled or OLE periods will be encouraged to attend a final in-person follow-up visit (Week 80 visit assessments per Appendix A) approximately 12 weeks after their last dose of Study Drug. If the patient declines or is unable to participate in the above, a home nurse

visit for safety/efficacy labs (equivalent to week 74 visit in Appendix A) and telephone follow-up will be attempted, ideally ~12 weeks from the last dose of Study Drug.

6.3.9 Follow-up Visits for Early Termination from Post-Treatment Follow-up Period
The patient who requests to withdraw from the study during the Post-Treatment Follow-up Period should be encouraged to have a home nurse visit for safety/efficacy labs (equivalent to week 74 visit in Appendix A) and telephone follow-up, ideally ~12 weeks from the last dose of Study Drug.

If a patient withdraws due to an AE at any time during the study, the Investigator will arrange for the patient to have appropriate follow-up until the AE has resolved or stabilized. Appropriate follow up may include home nursing visits or visits to NIH if necessary.

6.3.10 End of Study
After completion of this study, subjects may continue to receive care through NIH via protocol 76-DK-0006 (Studies of Molecular Genetics of Insulin Secretion, Insulin Action and Diabetes Mellitus), but cannot continue to take the study drug through this study.

6.3.11 Post-Study Obligations
There are no anticipated post-study obligations.

6.4 Study Assessments

6.4.1 Patient Reported Outcomes

1. Hunger and Widespread Pain Diaries: All patients will complete hunger and pain questionnaires at each in-person visit. (Appendix H).

2. Quality of Life Assessments: All patients will complete Quality of Life Questionnaires (EQ-5D and SF-36) at each in-person visit. (Appendix F & G).

6.4.2 Detailed Metabolic Phenotyping

1. Anthropometric measurements: Physical exams and vital signs will be performed as indicated in the Schedule of Procedures (Appendix A). Height will be measured in the early morning on the first full day of the first inpatient visit. Vital signs will include weight, blood pressure, pulse rate, respiratory rate and body temperature. Blood pressure and pulse rate will be recorded after the patient has been in a sitting position for at least 5 minutes. Systolic and diastolic blood pressure should always be measured on the same arm (preferentially on the left arm). Waist circumference will be measured at the iliac crest in triplicate to the nearest tenth of a cm using a stretch-less, tension-sensitive tape measure at the times indicated in the Schedule of Procedures (Appendix A). Skinfold measurements will be obtained in triplicate to the nearest mm using regularly calibrated Lange skinfold calipers at the times indicated in the Schedule of Procedures (Appendix A).

2. Fasting labs: Blood samples will be obtained following a minimum 8 hour fast for analytes described in Appendix A. Ideally the morning blood draw will be coordinated so that it will be collected 10 hours after the evening meal. Additional serum and plasma samples will be stored for future analysis.

3. DEXA scan for total body composition: DEXA scans will be conducted prior to administration of the first dose of study drug and repeated after the 16 week placebo controlled period, and again after 16 weeks of open-label drug in those previously assigned to placebo. A DEXA scan (iDXA, GE Healthcare, Madison WI) will be performed to determine total and regional body fat and lean soft tissue masses, bone mineral content and density. DEXA produces photons at two different energy levels, 40 and 70 KeV. The photons pass through tissues and attenuate at rates related to elemental composition. Bone mineral, with highly attenuating calcium and phosphorous, is readily distinguished from soft tissues. The different elemental profiles of fat and bone-mineral free lean components allows for the analysis of soft tissue fat content, so that bone mineral, fat, and bone mineral fat-free lean components may be resolved.

4. Glucose and Lipid Turnover: Stable isotope tracers will be used to measure glucose and lipid turnover. Endogenous glucose production will be measured using steady-state infusion of [6,6-$^{2}H_2$]glucose tracer. The fractional rate of gluconeogenesis will be measured using steady-state oral dosing of deuterated water ($^{2}H_2O$) [45]. The rate of lipolysis will be measured using steady-state infusion of deuterium-labeled $^{2}H_5$-glycerol, and the fatty acid turnover rate will be measured using steady-state infusion of [U-$^{13}C_{16}$] palmitate. Subjects will fast after 8 pm. A total of 3 grams per kg lean body mass of $^{2}H_2O$ will be given in four divided doses every two hours at 9 pm, 11 pm, 1 am, and 3 am in order to enrich the subject's body water pool to approximately 0.5% $^{2}H_2O$. Beginning at ~5 am, a primed [6,6-$^{2}H_2$]glucose infusion will be given for 3 hours, after which blood samples to measure isotope enrichment will be measured over a period of 30 minutes at steady state. Two hours after the [6,6-$^{2}H_2$]glucose infusion begins, a primed $^{2}H_5$-glycerol infusion, and unprimed [U-$^{13}C_{16}$] palmitate infusion will be given for one hour, and isotope enrichment will be measured over a period of 30 minutes at steady state. To assess the effects of hyperinsulinemia on glucose turnover, the glucose tracer infusion will be continued during the euglycemic, hyperinsulinemic clamp study, below, with blood samples for tracer enrichment obtained during the steady-state period of the clamp.

5. Euglycemic Hyperinsulinemic Clamp: This is the gold-standard test to measure insulin sensitivity. For insulin treated subjects, an overnight insulin drip may be given beginning at 6 pm the night prior to the clamp to maintain euglycemia. After an 8-hour fast, insulin will be infused at a dose of 120 mcU/m^2*minute. This dose is designed to suppress endogenous glucose production and near-maximally stimulate glucose uptake in this group of very insulin resistant subjects. 20% dextrose enriched with 2.5% [6,6-$^{2}H_2$]glucose tracer will be infused at a variable rate to maintain blood glucose at approximately 100 mg/dL. If subjects remain hyperglycemic without dextrose infusion after 1 hour of insulin infusion (by which time maximum insulin effect is expected), a higher steady state blood glucose concentration for the clamp may be used. During the insulin infusion, blood samples (0.5 mL) will be obtained every 5 minutes to measure blood glucose at the bedside. At steady state, the rate of dextrose infusion provides an estimate of insulin-stimulated glucose disposal. Additional samples will be drawn to measure the glucose and lipid tracers (described above), insulin,

and other hormone levels. If technical problems occur with a clamp study (e.g. IV failure or failure to attain steady-state glucose and insulin levels), the clamp may be repeated if blood volume limits permit.

6. Mixed meal test: The effect of a standardized liquid meal (Boost-plus) with fixed, balanced macronutrient content will be assessed. Blood samples will be obtained for glucose, insulin, C-peptide, lipid analysis, and research storage at -10, 0, 30, 60, 120, 180, 240, 300 and 360 minutes after consuming the liquid meal. Visual analog scales for hunger and satiety will be obtained at the 0 and 60 minute time points.

7. Lipoprotein lipase activity: Measurement of lipoprotein lipase and hepatic lipase activities in plasma will be performed using blood samples obtained 10 minutes after intravenous infusion of 60 units/kg of unfractionated heparin using the methods of Iverius and Nilsson-Ehle[46,47]. Lipoprotein lipase and hepatic lipase are bound to vessel walls and only released into the blood with an infusion of heparin.

8. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS): Hepatic, muscle and central body fat will be measured by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). Studies are performed on a 3T MRI scanner using phased-array coils. Hepatic and muscle fat fraction will be determined[48]. Visceral and subcutaneous fat measurements will be obtained using standard T1 Spin Echo technique which presents fat as a bright signal that can be segmented for area and volume measurements. In addition, MRI scanning will be performed for angiography (without gadolinium), vessel wall, and endothelial function[49]. Each subject will be asked to perform a hand-grip exercise to obtain arterial dimensions. Before entering the magnet, we shall measure the maximal force production while hand squeezing a nonmagnetic hand-grip device. Later in the magnet at roughly one quarter of his/her maximal force, we will obtain MR data before, during, and after this exercise. The use of the device will be continuously monitored and the subject will be able to switch hands if one hand becomes fatigued.

9. Carotid intima-media thickness (cIMT): cIMT is an established marker of coronary artery disease and cardiovascular risk. This is a non-invasive test using ultrasonography of the carotid arteries in the neck.

10. 7-point plasma glucose: Plasma glucose will be measured prior to each meal, 90 minutes after each meal, and at bedtime. Area under the curve for glucose will be calculated using the trapezoidal method.

11. Liver biopsy: An optional liver biopsy will be performed in patients at the initial visit and at the end of the open label extension period (after 12-16 months of exposure to ISIS 304801). Biopsies will be performed transcutaneously unless there is a clinical reason for another approach (e.g. transjugular). These biopsies are performed primarily for research purposes, although clinical data about the severity of non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) will be obtained as well. Patients will sign a research consent for each liver biopsy prior to the procedure. Patients will be allowed to refuse the biopsies and continue to participate in the rest of the study. Platelet count will be obtained prior to percutaneous liver biopsy, and the biopsy will not be

performed if patients have evidence of coagulopathy unresponsive to vitamin K (INR >1.5) or low platelets (Platelets < 75,000 k/uL) which may place them at a potentially higher risk for bleeding. About half of the liver biopsy sample will be fresh frozen in liquid nitrogen and will be stored for molecular analyses and Metabolomics studies in collaboration with Dr. Elif Oral at the University of Michigan.

Histologic analysis: Specimens will be graded for the severity of NAFLD/NASH pathology. Histological features of NAFLD/NASH will be studied using the validated NASH-CRN (NASH Clinical Research Network) scoring system (3). This scoring system comprises histological features, 4 of which [steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2) and fibrosis (0-3 with grade 1 divided in three categories as well)] are evaluated semi-quantitatively. NAFLD activity score (NAS) is the unweighted sum of steatosis, lobular inflammation and hepatocellular ballooning scores. Total of this together with the fibrosis score constitutes the total NASH score.

12. Metabolomics: Studies will be done in the Molecular Phenotyping Core of the Michigan Metabolomics and Obesity Center at the University of Michigan in collaboration with Dr. Elif Oral. Plasma samples collected in the fasting state and liver biopsy samples will be extracted according to the method of Bligh and Dyer [50] for analysis. Lipomic studies will include measurement of subclasses of lipids and fatty acid composition. Unbiased metabolomics studies of plasma will be conducted using LC/MS and GC/MS.

6.4.3 Pharmacokinetic Analysis

Each subject will have a single (not serial) plasma PK completed at the end of their week 1 visit. The plasma PK of ISIS 304801 following extensive sampling will be assessed at Week 16 (Week 1 OLE) for all subjects, and Weeks 32 (Month 4 OLE). Additionally, plasma trough levels throughout the 16-week and 12-month OLE treatment period and post-treatment levels during the 12-week follow up period will be measured. Details are given in Appendices A and C.

For all patients who receive ISIS 304801 treatment, non-compartmental PK analysis of ISIS 304801 will be carried out on each individual subject data set. The maximum observed drug concentration (C_{max}) and the time taken to reach C_{max} (T_{max}) will be obtained directly from the concentration-time data. Partial areas under the plasma concentration-time curve from zero time (predose) to selected times (t) after the SC administration (AUC_t) will be calculated using the linear trapezoidal rule. The plasma disposition half-life associated ($t_{1/2\lambda z}$) with the apparent terminal elimination phase will be calculated using a non-compartment method, if appropriate, using available data (Week 68 and later), from the equation $t_{1/2\lambda z}=0.693/\lambda_z$, where λ_z is the rate constant associated with the apparent terminal elimination phase. A minimum of three data points will be used to define λ_z and the correlation of determination values (r^2) had to be at or greater than 0.8 for the estimate to be accepted. The samples at Week 68 (assumed pre-dose value for Week 69), Week 74, and Week 80 will be used to calculate the half-life. Other pharmacokinetic parameters, as appropriate, may be determined or calculated at the discretion of the pharmacokinetic scientist.

Plasma concentrations and PK parameters will be summarized with and without stratification by immunogenicity status using descriptive statistics. Additional details regarding the PK analysis will be described in the Statistical Analysis Plan.

Potential relationships between selected PD and PK measures may also be explored, where deemed appropriate.

6.4. 4 Immunogenicity Analysis

Immunogenicity (IM) results (screen positive/negative, confirmed positive/negative or unevaluable, and when applicable, titer of anti-ISIS 304801 antibodies) before, during, and after treatment with study drug (ISIS 304801) (i.e., sample ADA status) will be listed by treatment and study day. Subject ADA status (positive/negative or unevaluable) for all evaluable patients, along with the study day associated with the first positive IM status emerged (T_{first}, i.e., onset of ADA development), the last positive IM status observed (T_{last}), the duration of ADA response (number of days between T_{first} and T_{last}) if appropriate, the last ADA sample collection day, and subject maximum titer if applicable, will be listed by treatment and study day.

Additionally, the sample and subject IM incidence (number) and incidence rate (percent) will be summarized as the total number and percent of evaluated subjects with antibody negative, positive, and unknown status by treatment. Furthermore, onset, duration, and titer of the ADA response, if applicable, will be summarized as median and range.

Additional details regarding the immunogenicity data analysis will be described in the SAP.

6.4.5 Safety Assessments

1. Liver function, Kidney function, Platelets, LDL, and coagulation tests (detailed in Appendix B): Monitoring of the above (except for platelets) will be performed every 4 weeks for the first 6 months of treatment, and every 8 weeks thereafter, per the Study Schedule (Appendix A). Platelets will be monitored every week for the duration of the study.

2. Self-monitoring of blood glucose: In subjects with diabetes, a home glucometer will be distributed to patients who do not have one already at the first inpatient visit for home-based glucose monitoring ("Self-Monitored Plasma Glucose" or SMPG). Training on the use of the glucometer will be provided as needed. Fasting SMPG will be self-measured in the fasting state: daily if the patient is on insulin therapy, and weekly if on oral antidiabetic therapy.

3. Urinalysis: Urinalysis and urine protein to creatinine ratio will be conducted as per the Study Schedule (Appendix A).

4. Echocardiogram: Echocardiography for assessment of left and right ventricular function and mitral and aortic valve function will be conducted per the Study Schedule (Appendix A). Cardiologists reviewing echocardiograms at NIH will not be aware of treatment assignment during the randomized period. Results will be provided to the DSMB for their review.

5. EKG: Standard 12-lead EKGs will be performed after the patient has been resting in a supine position for at least 5 minutes per the Study Schedule (Appendix A).

6. Troponin T: Cardiac troponin T will be measured per the Study Schedule (Appendix A).

7. Pregnancy test: Pregnancy tests will be performed on female patients with reproductive potential at each inpatient visit.

8. Pancreatitis: All patients will be given an instruction card that must be presented to the treating physician if a suspected case of pancreatitis occurs. The card will explain that the patient is in a clinical trial and that serum lipase and/or amylase measures should be done in order to adjudicate the event, based on Atlanta Classification[51]. If serum or amylase activity is less than 3 x ULN, imaging, preferably contrast-enhanced computed tomography (CT), should be considered to confirm the diagnosis of acute pancreatitis per the Atlanta Criteria.

7. Human Subject Protection

7.1 Data and Safety Monitoring Plan

Safety data will be monitored on ***an ongoing basis*** by the Principal Investigator and captured by the Sponsor on the CRF. Appropriately trained personnel will be granted access to Express 5.4, the Electronic data capture system for this trial.

At least monthly, the Principal Investigator or designee will review vital signs (including at a minimum heart rate, systolic and diastolic blood pressure), adverse events (including all pre and post dosing adverse events), serum chemistry (including at a minimum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, blood urea nitrogen, and creatinine), coagulation (including at a minimum aPTT, PT, and INR), and urinalysis (including at a minimum urine protein (by dipstick), urine blood, and urine white blood cells). At least weekly, the Principal Investigator or designee will review hematology (at a minimum hemoglobin, absolute lymphocytes, absolute neutrophils, platelet count, WBCs) to advise on possible medication dose changes for safety reasons.

The Ionis Medical Advisor will answer queries and provide safety management recommendations to the Principal Investigator as needed. Anticipated AE's are fully described in the Investigator Brochure. Any safety issues identified in other apoC-III clinical trials will be promptly communicated by the Ionis to the Principal Investigator.

A monthly cumulative summary safety report for all apo-CIII studies, including the current study, will be issued by Ionis Pharmaceuticals, including an assessment of fatal cases, SAEs, treatment discontinuations, treatment-related AEs with severity and relatedness, laboratory analysis, ECG and vital signs. Particular attention will be paid to protocol defined monitoring/stopping rules. This report will be sent monthly to the Principal Investigator and will be used by the Data and Safety Monitoring Board (DSMB) for their ongoing data safety review.

7.1.1 Investigational New Drug application

The investigators will be conducting this study with the oversight of the Food and Drug Administration (FDA) under an Investigational New Drug (IND) application. NIDDK will serve as IND sponsor. Ionis Pharmaceuticals will provide NIDDK with a letter of cross reference authorization to their existing IND for ISIS 304801.

7.1.2 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be assembled by Ionis Pharmaceuticals and include an NIDDK statistician to review safety, tolerability and efficacy (as needed) data collected on ISIS 304801 during this study. The membership of the DSMB will be determined by the PI in collaboration with the Company. Based on its ongoing assessment of the safety and tolerability of ISIS 304801, the DSMB will provide recommendations to the Principle Investigator for modifying, stopping or continuing the study as planned. The DSMB will meet approximately 3 times per year (exact schedule to be determined by DSMB Chair), and additional ad hoc meetings may be called by the Chair if an imminent significant safety concern arises (e.g. unexpected death of study participant, new serious safety issue identified in the drug development program). Study data (blinded during the initial 4 month placebo controlled period) will be provided to the DSMB via from the study database maintained by Ionis Pharmaceuticals. The NIDDK statistician will have access to the unblinding scheme, and can provide this to the DSMB on request. The Investigator will review and respond to the DSMB recommendations in writing. Any recommendations of the DSMB will not be binding but require due consideration by the Investigator in consultation with the NIDDK/NIAMS IRB. If the DSMB recommends continuation of the study without modification, no formal response will be required. However, if the recommendations request action, such as a recommendation for termination of the study or modification of the protocol, the Investigator will provide a formal written response to the DSMB stating whether the recommendations will be followed and the plan for addressing the issues.

7.1.3 Study monitoring

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

As required by FDA 21 CFR 312.50 (and NIH OHSRP's SOP 23), trial procedures will be subject to review and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements with the NIDDK quality assurance program plan. Audit and/or monitoring visits results will be reported to the Principal Investigator/Sponsor for further reporting to the FDA consistent with applicable regulations. The specific monitoring plan will be developed with the Principal Investigator and frequency of monitoring visits determined by such factors as study enrollment, data collection status and regulatory obligations. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

7.1.4 Adjudication Committees

All SAEs that occur during the study that are consistent with a major acute cardiovascular event (MACE) will be adjudicated by a blinded, independent committee as outlined in the MACE Adjudication Charter.

All AEs and SAEs that occur during the study that are consistent with an event of acute pancreatitis will be adjudicated by a blinded, independent committee according to the Atlanta classification of acute pancreatitis[51] and as outlined in the Pancreatitis Adjudication Charter. In addition, data for prior episodes of acute pancreatitis or suspected pancreatitis will be collected by review of each patient's medical chart and these events will also be adjudicated.

7.2 Adverse Events, Protocol Deviations, and Unanticipated Problems

The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team.

7.2.1 Definitions

Adverse events, protocol deviations, unanticipated problems (UP), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.") with further details and modifications given below. All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded, and will be summarized for the NIDDK/NIAMS IRB by the investigative team and to the FDA by NIDDK at the time of annual review.

Adverse Event (AE)

An adverse event is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the Study or use of investigational drug product, whether or not the AE is considered related to the investigational drug product.

Adverse Reaction and Suspected Adverse Reaction

An adverse reaction is any AE caused by the Study Drug. A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Serious Adverse Event (SAE)

A SAE is any AE that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event
  An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization
  Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE.
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- Results in a congenital anomaly or birth defect in the offspring of the patient (whether the patient is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

Protocol Deviation:
Any change, divergence, or departure from the NIDDK/NIAMS IRB-approved research protocol. The impact of a PD is characterized by designation as serious or not serious. A protocol deviation is serious if it meets the definition of a Serious Adverse Event (see above) or if it compromises the safety, welfare or rights of subjects or others**.**

7.2.2 Monitoring and Recording Adverse Events
Any pre-existing conditions or signs and/or symptoms present in a patient prior to the start of the Study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a single unifying diagnosis if possible.

Serious Adverse Events
The collection of SAEs will begin after the patient signs the informed consent form and stop at the end of the patient's follow-up period. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. An Initial Serious Adverse Event Form should be completed and a copy should be faxed to the Sponsor and to Ionis or designee.

Detailed information should be actively sought and included on Follow-Up Serious Adverse Event Forms as soon as additional information becomes available. All SAEs will be followed until resolution. SAEs that remain ongoing past the patient's last protocol-specified follow-up visit will be evaluated by the Investigator and Ionis. If the Investigator and Ionis agree the patient's condition is unlikely to resolve, the Investigator will determine the follow-up requirement.

Non-Serious Adverse Events
The recording of non-serious AEs will begin after the patient signs the informed consent form and will stop at the end of the patient's follow-up period. The Investigator will monitor each patient closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

7.2.3 Evaluation of Adverse Events (Serious and Non-Serious)
The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form:

Relatedness

The event's relationship to the Study Drug (ISIS 304801 or placebo) is characterized by one (1) of the following:

- Related: There is clear evidence that the event is related to the use of Study Drug, e.g., confirmation by positive re-challenge test
- Possible: The event cannot be explained by the patient's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug (ISIS 304801 or placebo) administration
- Unlikely/Remote: An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug (ISIS 304801 or placebo) administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- Not Related: The event can be readily explained by the patient's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

Severity

The event's severity is characterized by one of the following:

- Mild: The event is easily tolerated by the patient and does not affect the patient's usual daily activities
- Moderate: The event causes the patient more discomfort and interrupts the patient's usual daily activities
- Severe: The event is incapacitating and causes considerable interference with the patient's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated.

Action Taken with Study Drug

Action taken with Study Drug (ISIS 304801 or placebo) due to the event is characterized by one of the following.

- None: No changes were made to Study Drug (ISIS 304801 or placebo) administration and dose
- Permanently Discontinued: Study drug was discontinued and not restarted
- Temporarily Interrupted, Restarted – Same Dose: Dosing was temporarily interrupted or delayed due to the AE and restarted at the same dose
- Reduced Dose: Dosing frequency was reduced

Treatment Given for Adverse Event

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form. Treatment should also be recorded on the concomitant treatment or ancillary procedures CRF, as appropriate.

Outcome of the Adverse Event

If the event is a non-serious AE, then the event's outcome is characterized by one of the following:

- AE Persists: Patient terminates from the trial and the AE continues
- Recovered: Patient recovered completely from the AE

- Became Serious: The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- Change in Severity (if applicable): AE severity changed

If the event is an SAE, then the event's outcome is characterized by one of the following:

- Ongoing: SAE continuing
- Persists (as non-serious AE): Patient has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE eCRF (the SAE resolution date should be entered as the date of onset of that AE)
- Recovered: Patient recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- Fatal: Patient died (the date of death should be entered as the SAE resolution date)

7.3 Reporting

Serious unanticipated problems and serious protocol deviations will be reported to the NIDDK/NIAMS IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the NIDDK/NIAMS IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Non-serious protocol deviations will be reported to the NIDDK/NIAMS IRB (as soon as possible but not more than 14 days after the PI first learns of the event) only if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

For studies conducted under an IND, any event that is a serious, unexpected, and suspected adverse reaction (SUSAR) (related to study drug) will be reported by the Investigator to the NIDDK Clinical Director, NIDDK/NIAMS IRB, Food and Drug Administration (FDA), and Ionis Pharmaceuticals as soon as possible **and no later than 7 days** (for a death or life-threatening event) **or 15 days** (for all other SAEs) **after the investigator's or institution's initial receipt of the information.** All serious, unexpected, and suspected adverse reactions (SUSARS) will be reported in aggregate at the time of continuing review.

IND Safety Reports will be submitted to the FDA by NIDDK.

If only limited information is initially available, follow-up reports are required. If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent to the FDA. All SAEs should be followed to resolution or stabilization.

Deaths will be reported by the investigative team to the NIDDK Clinical Director and NIDDK/NIAMS IRB within 7 days after the PI first learns of the event. All deaths that have occurred among study participants since the previous review will be summarized at the time of continuing review.

7.4 Procedures for Handling Special Situations

7.4.1 Contraception and Pregnancy

All patients of childbearing potential must refrain from sperm/egg donation and practice effective contraception from the time of signing the informed consent form until at least 3 months after their last dose of study treatment.

For the purposes of this study, women of childbearing potential are defined as any female who has experienced menarche, and who does not meet one of the following conditions:

- Postmenopausal: 12 months of spontaneous amenorrhea in females >55 years of age or, in females ≤55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved

- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Post hysterectomy

For the purposes of the study, effective contraception is defined as follows:

For male patients:

- Effective male contraception includes a vasectomy with negative semen analysis at follow-up, or the use of condoms together with spermicidal foam/gel/film/ cream/suppository. Male patients must also encourage their female partner to use effective contraception from the time of signing the informed consent until 3 months after the patient's last dose of study treatment. Effective contraceptive for the female partner includes: surgical sterilization (e.g., bilateral tubal ligation), hormonal contraception, intrauterine contraception/device, or barrier methods (female condom*, diaphragm, sponge, cervical cap) together with spermicidal foam/gel/film/cream/suppository. Male patients with partners that are pregnant must use condoms to ensure that the fetus is not exposed to the study drug.

For female patients:

- Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), hormonal contraception, intrauterine contraception/device, or any 2 barrier methods (a combination of male or female condom* with diaphragm, sponge, or cervical cap) together with spermicidal foam/gel/film/cream/suppository.

*Note: A female condom and a male condom should not be used together as friction between the two can result in either product failing.

If a patient becomes pregnant or a pregnancy is suspected, or if a male patient makes or believes that he has made someone pregnant during the study, then the Investigator must be informed immediately. An Initial Pregnancy Form should be submitted to Ionis or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported on Follow-up Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the patient's responsibility.

Female patients: If a suspected pregnancy occurs while on the study (including follow-up), a pregnancy test will be performed. The patient with a confirmed pregnancy will be immediately withdrawn from treatment with Study Drug. However, the patient will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy. Regardless of continued study participation, the study physician will assist the patient in getting obstetrical care and the progress of the pregnancy will be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, the Investigator and Ionis or designee may require access to the mother and infant's medical records for an additional 8 weeks after birth.

Male patients: The progress of the pregnancy in a male patient's partner should be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). Male patients are not required to stop the study drug if their partner becomes pregnant. If the pregnancy results in the birth of a child, additional follow-up information may be requested for the mother and infant. Follow-up will be performed to the extent permitted by applicable regulations and privacy considerations.

7.4.2 Abnormalities of Laboratory Tests

Clinically significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value. The Investigator will consult with the Ionis Medical Advisor as needed, and for any exceptions to the above policy. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents.

7.4.3 Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the patient is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the patient's consent to participate in the Study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the patient's consent to participate in the Study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

7.4.4 Dosing Errors

Study Drug (ISIS 304801 or placebo) errors should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the patient was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional. Dosing details should be captured on the Dosing Case Report Form. If the patient takes a dose of Study Drug (ISIS 304801 or placebo) that exceeds protocol specifications and the patient is symptomatic, then the symptom(s) should be documented as an AE and be reported per Section 7.2.

Should an overdose occur, the Investigator or designee should refer to the Guidance to Investigator's section of the Investigator's Brochure and contact Ionis within 24 hours.

7.5 Safety Monitoring Rules

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations. For the purposes of safety monitoring baseline is defined as the average of Day 1 pre-dose assessment and the last measurement prior to Day 1.

Confirmation Guidance: At any time during the Study (Treatment or Post-Treatment Periods), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible (ideally within 3 days of the initial collection). For stopping rules, if the initial laboratory result is observed during the Treatment Period, the results from the retest **must be available** prior to administering the next dose of Study Drug (ISIS 304801 or placebo).

Re-dosing Guidance: Patients with initial laboratory test values that reach a stopping rule must not be re-dosed until the re-test results are available. In general, patients who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator will decide whether additional close monitoring of the patient is appropriate. If any of the stopping criteria described below are met below and are confirmed, the patient will be permanently discontinued from further treatment with Study Drug (ISIS 304801 or placebo), evaluated fully as outlined below and will be entered into the post-treatment evaluation portion of the study. Consultation will be conducted between the Investigator and the Ionis Medical Advisor as needed.

7.5.1 Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009. For a definition of baseline please refer to guidance above.

For patients with Baseline ALT or AST below 2 x ULN

In the event of an ALT or AST measurement that is >3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was >ULN) at any time during the Study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above. Additional, confirmatory measurements should also be performed if ALT or AST levels increase to 5 x ULN.

For patients with baseline ALT or AST between ≥2 and <3 x ULN
In the event of an ALT or AST measurement that is 2 x the baseline level any time during the study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above. Similarly, confirmatory measurements should also be performed if ALT or AST levels increase to 5 x ULN.

Frequency of Repeat Measurements: Patients with confirmed ALT or AST levels that are continuing to rise should have their liver chemistry tests (ALT, AST, ALP, INR and total bilirubin) retested at least bi-monthly until levels stabilize and begin to recover (ALT and AST levels become ≤1.2 x ULN or 1.2 x baseline value).

Further Investigation into Liver Chemistry Elevations: For patients with confirmed ALT or AST levels >3 x ULN [or the greater of 2 x baseline value or 3 x ULN if the baseline value was >ULN], the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases
2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
3. Obtain a history for exposure to environmental chemical agents and travel
4. Serology for viral hepatitis (HAV IgM, HBsAg, HCV antibody, CMV IgM, and EBV antibody panel)
5. Serology for autoimmune hepatitis (e.g., antinuclear antibody (ANA))

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic computed tomography (CT) or MRI scans, may be performed at the discretion of the Investigator, with consultation with the Sponsor Medical Monitor as needed. Repetition of the above evaluations should be considered if a patient's ALT and/or AST levels reach 5 x ULN.

7.5.2 Safety Monitoring Rules for Renal Function
Patients with confirmed persistent changes that are observed over 2 consecutive visits, at least 7 days apart, for the criterion below should be retested every two weeks with serum creatinine and urine chemistries until creatinine and P/C ratio stabilize:

- P/C ratio change from baseline > 50% and ≥ 1.5 ULN

7.5.3 Safety Monitoring Rules for Platelet Count Results
Actions to be taken in the event of reduced platelet count are shown in Table 3 in Section 7.6.3.

7.5.4 Safety Monitoring for Minor Bleeding Events
Minor bleeding events are those that do not fulfill the criteria for major bleeding or clinically relevant, non-major bleeding events (which are defined in Section 7.6.3), for example excess bruising, petechiae, gingival bleeding on brushing teeth. If a minor bleeding event occurs, additional testing of coagulation parameters (aPTT, PT, INR) and platelet count should be performed, and the Investigator will consult with the Sponsor Medical Monitor as needed.

7.5.5 Safety Monitoring for Constitutional Symptoms
Patients will be instructed to promptly report any signs of symptoms of fever or constitutional symptoms that may arise during the study and the Investigator should closely evaluate all potential causes, including

concomitant illness. If patients experience persistent constitutional symptoms, the Investigator will determine whether additional monitoring or laboratory tests are required, with consultation with the Sponsor Medical Monitor as needed.

7.5.6 Safety Monitoring for LDL-C Elevations

Beginning at Week 16 (after which treatment is unblinded), laboratory alerts will be in place to notify the Investigator if a patient has an LDL-C > 160 mg/dL on two consecutive visits. If this occurs, the Investigator will consider initiation or adjustment of treatment to lower LDL-C according to published guidelines (e.g., initiate statin therapy, increase the statin dose for patients who are already on treatment, add ezetimibe if on maximal statin therapy)[52].

7.5.7 Safety Monitoring Rule for Documented Severe Hypoglycemia

Classification of Hypoglycemia

1. Alert value for hypoglycemia
   ≤70 mg/dL (≤3.9 mmol/L) plasma concentration

2. Severe hypoglycemia
   Requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event. Neurological recovery following plasma glucose levels returning to normal is considered sufficient evidence that an event was induced by low plasma glucose concentration.

3. Documented symptomatic hypoglycemia
   Typical hypoglycemia symptoms accompanied by measured plasma glucose ≤70 mg/dL (≤3.9 mmol/L).

4. Asymptomatic hypoglycemia
   Not accompanied by typical hypoglycemia symptoms but with measured plasma glucose ≤70 mg/dL (≤3.9 mmol/L).

5. Probable symptomatic hypoglycemia
   Typical hypoglycemia symptoms not accompanied by plasma glucose determination but likely caused by plasma glucose ≤70 mg/dL (≤3.9 mmol/L).

A **documented severe hypoglycemic event** is defined as one in which the patient requires assistance of another person to obtain treatment for the event and has a plasma glucose level ≤70 mg/dL (≤3.9 mmol/L). The rescue treatment of hypoglycemia may include IV glucose or buccal or intramuscular glucagon. The definition of severe symptomatic hypoglycemia includes all episodes in which neurological impairment was severe enough to prevent self-treatment and which were thus thought to place patients at risk for injury to themselves or others. Note that "requires assistance" means that the patient could not help himself or herself. Someone being kind that assists spontaneously the patient when not necessary does not qualify as "requires assistance." Severe hypoglycemia will be qualified as a SAE only if it fulfills SAE criteria.

If a patient presents with symptoms of hypoglycemia, the Investigator will need to take immediate action to confirm the patient's glucose level and treat the patient accordingly. Patients must be instructed on the monitoring and management of hypoglycemic episodes at the Baseline Visit. Instructions should be provided to all patients on the appropriate use of a glucose meter. The experience of hypoglycemia may vary greatly from patient to patient. However, there are certain classical signs and symptoms, of which patients should be aware as a clue that their blood glucose may be low. Common symptoms include headache, heart pounding, confusion, disorientation, numbness or tingling, pale skin, shakiness or tremulousness, increased appetite, anxiousness or nervousness, lightheadedness or dizziness, sweating, and weakness. Many of these symptoms are listed in the diary provided to patients. These diaries will be used only to assist the investigator in assessing the event. Appropriate source documentation should capture the necessary information on the event with the aid of the diaries. Patients must be given adequate instructions on the use of the diaries.

If patients suspect they might be having a hypoglycemia reaction, they should check their blood glucose using their meters as soon as possible, before treatment if possible, provided they feel it is safe to do so. If there is doubt about safety they should treat the event first, using some sugar, milk, or juice for example, then obtain and record a blood glucose value as soon as possible thereafter. The time and nature of treatment should be noted, and especially if any blood glucose result was before or after treatment. It would be helpful for the patient to note if a contributory factor (eg, missed or reduced meals, unaccustomed physical activity) occurred earlier in the day of the event.

Doses of insulin and sulfonylureas may be reduced at the Investigator's discretion for hypoglycemia. Doses of other diabetes medications (e.g. metformin) should not be changed.

7.5.8 Monitoring Rule for Hyperglycemia

Assessment for rescue will be performed at each "visit" (including both telephone contacts and in-person visits). Routine fasting SMPG and central lab alerts on FPG (and HbA1c after week 12) are set up to ensure that glycemic parameters remain under predefined thresholds values. If one fasting SMPG value exceeds the specific glycemic limit on one day, the patient checks it again during the two following days. If all the values in three consecutive days exceed the specific limit, the patient should contact the investigator and a central laboratory FPG measurement (and HbA1c after week 12) is performed.

The threshold values are defined as follows, depending on study period:

- From baseline visit to week 12 *(including value at week 12)*: FPG >270 mg/dL (15.0 mmol/L)
- From week 12 to week 24 *(including value at week 24)*: FPG >240 mg/dL (13.3 mmol/L) or HbA1c>9% (for patients with baseline HbA1c <8%) and HbA1c increase of more than 1% from baseline (for patients with baseline HbA1c ≥8%).
- From week 24 up to week 68 : HbA1c >9% (for patients with baseline HbA1c <8%) and HbA1c increase of more than 1% from baseline (for patients with baseline HbA1c ≥8%).

In case of FPG/HbA1c above the threshold values, the investigator should ensure that no reasonable explanation exists for insufficient glucose control and in particular that:

- Plasma glucose was actually measured in the fasting condition (i.e. after at least 10 hours fast)

- Absence of intercurrent disease which may jeopardize glycemic control. In case of an emergency (e.g., surgery, infection), the investigator can take appropriate measures for glycemic control. If the measure does not exceed 7 days, then it will not be considered a rescue. If the measure lasts beyond 7 days then it will be treated as a rescue.
- Compliance to treatment is appropriate
- Compliance to diet and lifestyle is appropriate

If any of the above can reasonably explain the insufficient glycemic control, the investigator should undertake appropriate action, i.e.

- Investigation and treatment of intercurrent disease (to be reported in AE/concomitant medication parts of the e-CRF),
- Stress on the absolute need to be compliant to treatment,
- Organize a specific interview with a Registered Dietician or other qualified nutrition professional and stress on the absolute need to be compliant to diet and lifestyle recommendations,
- Schedule a FPG/HbA1c assessment at the next visit.

If none from the above-mentioned reason can be found, or if appropriate action fails to decrease FPG/HbA1c under the threshold values, rescue medication may be introduced at the investigator discretion and according to local guidelines.

Any patient who discontinues early from the placebo-controlled or OLE periods will be encouraged to attend a final in-person follow-up visit (Week 80 visit assessments per Appendix A) approximately 12 weeks after their last dose of Study Drug. If the patient declines or is unable to participate in the above, a home nurse visit for safety/efficacy labs.

If possible, an in-person visit for all assessments for primary and secondary efficacy and safety parameters planned in final primary endpoint assessment visit will be performed before adding the rescue medication. If an in-person visit cannot be conducted in a timely fashion, a home nurse visit for safety/efficacy labs will be attempted. If rescue medication is required, patients will continue the study treatment and remain in the study, although planned tests that will be affected by the use of rescue medication may be cancelled.

7.5.9 Acute Pancreatitis

If a patient has an episode of acute pancreatitis, dosing with study drug should be suspended temporarily until the patient is clinically stable. The suitability of the patient for continued dosing and the need for any modification to the treatment schedule will be determined by the Investigator, with consultation with the Sponsor Medical Monitor as needed.

7.6 Stopping Rules

For the purposes of stopping rules, baseline is defined as the average of Day 1 pre-dose assessment and the last measurement prior to Day 1. Prior to discontinuing study drug in any patient, the Investigator will consult with the Sponsor Medical Monitor when possible.

7.6.1 Stopping Rules for Liver Chemistry Elevations

In the event of confirmed laboratory results meeting the following criteria, **and the event is without an alternative explanation** as determined by the Investigator, dosing of a patient with Study Drug (ISIS 304801) will be stopped permanently:

1. ALT or AST > 8 x ULN, which is confirmed
2. ALT or AST > 5 x ULN, which is confirmed and persists for ≥ 2 weeks
3. ALT or AST > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN), which is confirmed **and** total bilirubin > 2 x ULN or INR > 1.5
4. ALT or AST > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN), which is confirmed, and the new appearance (i.e., onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia (> ULN) felt by the Investigator to be potentially related to hepatic inflammation

7.6.2 Stopping Rules for Renal Function Test Results

In the event of persistent changes that are observed over 2 consecutive weeks, for any of the criteria below, dosing of a patient with Study Drug (ISIS 304801 or placebo) may be stopped temporarily:

1. Quantitative total urine protein measurement of > 3.5 g/24 hours
2. Estimated creatinine clearance calculated according to the formula of Cockcroft and Gault ≤ 40 mL/min that is confirmed by a 24 hour urine collection

The possible dosing re-initiation or follow-up schedule for any events meeting either of these criteria will be determined by the Investigator, in consultation with the Sponsor Medical Monitor as needed.

7.6.3 Stopping Rule for Platelet Count Results

In the event of a confirmed platelet count less than 75,000/mm^3 that is associated with major bleeding or clinically relevant non-major bleeding (defined below[53]), dosing of a patient with study drug (ISIS 304801 or placebo) will be stopped permanently. The follow-up schedule for any events meeting this stopping criterion will be determined by the Investigator in consultation with the Sponsor Medical Monitor as needed.

In the event of any platelet count less than 25,000/mm^3, or a platelet count less than 50,000/mm^3 that occurs while the patient is on dosing at 300 mg every two weeks or 150 mg every week then dosing of a patient with Study Drug (ISIS 304801 or placebo) will be stopped permanently. Platelet count will be monitored daily until 2 successive values show improvement then monitored every 2-3 days until 2 successive values are > 75kmm^3, then monitored every 1 week.

Administration of steroids is recommended for patients whose platelet count is less than ≤ 50K/mm^3. Recovery in platelet count may be accelerated by administration of high dose steroids. Treatment guidelines for immune thrombocytopenia (Provan et al.; 2010) recommend Dexamethasone 40 mg daily for 4 days every 2-4 wk for 1-4 cycles; Prednis(ol)one 0.5-2 mg/kg/d for 2-4 weeks then taper; or Methylprednisolone 30 mg/kg/day for 7 days (**note:** may require continuation with oral steroids after methyl prednisolone).

In the event of a confirmed platelet count less than 75,000/mm^3, and in the absence of major bleeding or clinically relevant non-major bleeding (defined below[53]), dosing of a patient with study drug (ISIS 304801 or placebo) will be suspended temporarily until the platelet count has recovered to ≥100,000/mm^3. If dosing is continued it should be at a reduced dose frequency of 300 mg every two weeks or a reduced dose of 150 mg per week. The suitability of the patient for continued dosing and the need for any modification to treatment

schedule (refer to Section 7.7) will be determined by the Investigator (in consultation with the Ionis Medical Advisor as needed) and will be based on factors such as the original rate of decline in the patient's platelet count, whether any bleeding events were experienced by the patient, and the speed of recovery of platelet count upon holding of dosing.

If after the first dosing re-challenge the platelet count again falls below 50,000/mm^3, then dosing of the patient must be held until the platelet count again returns to at least 100,000/mm^3. The suitability of the patient for continued dosing and the need for any further modification to treatment schedule or dose (refer to Section 7.7) will be re-examined by the Investigator (in consultation with the Sponsor Medical Monitor as needed) based on (at least) the factors mentioned above.

If after the second re-challenge the platelet count falls below 50,000/mm^3 and is subsequently confirmed (see Section 7.5), dosing with Study Drug will be stopped permanently. The follow-up schedule for any events meeting this stopping criterion will be determined by the Investigator (in consultation with the Sponsor Medical Monitor as needed).

Following rechallenge platelet count should be tested every week until count is stable.

Any unreportable platelet count result must be rechecked and determined not to have met a stopping rule before dosing can continue.

If there is no reportable platelet count within 14 days of the last platelet count, the investigator will contact the patient to hold dosing until a new platelet count is obtained and reviewed.

Definition of Major Bleeding Events[53]:

1. Fatal bleeding, and/or
2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarterial or pericardial, or intramuscular with compartment syndrome, and/or
3. Bleeding causing a fall in hemoglobin level of 20.0 g/L (1.24 mmol/L) or more within 24 hours, or leading to transfusion of two or more units of whole or red cells

Definition of Clinically Relevant, Non-Major Bleeding Events[53]:

1. Multiple-source bleeding
2. Spontaneous hematoma >25 cm^2
3. Excessive wound hematoma (not injection site related)
4. Macroscopic hematuria (spontaneous or lasting >24 hours if associated with an intervention)
5. Spontaneous rectal bleeding; epistaxis, gingival bleeding, hemoptysis, hematemesis
6. Bleeding after venipuncture for >5 minutes

Actions in Patients With Low Platelet Count

Platelet count	Dosing Rules	Monitoring Rules
Normal range, >140K/mm^3	No action	Monitor every week unless otherwise specified

100K/mm³ - 140K/mm³	Weekly 300 mg study drug administration	Monitor every 1 week unless otherwise specified Obtain additional lab tests if 2 occurrences of platelet count 100 K/mm³ -- 140K/mm³ or 1 occurrence of platelet count ≤ 100K/mm³**
75K/mm³ - 100K/ mm³	Permanently reduce dose frequency to 300 mg every 2 weeks	Monitor every 1week unless otherwise specified Obtain additional lab tests if 1 occurrence of platelet count ≤ 100K/mm³**
50K/mm³ - 75K/mm³	If occurs while on dose of 300 mg every 2 weeks or 150 mg every week then permanently discontinue Study Drug, otherwise dose pause. When platelet count returns to >100K/mm3 restart dosing at dose frequency of 300 mg every 2 weeks or 150 mg weekly[1] (in consultation with the Ionis Medical Advisor as needed).	Monitor every 2-3 days until two successive values are > 75K/mm³ then monitor every 1 week Consider discontinuation of antiplatelet/agents/NSAIDS/anticoagulant medication Obtain additional lab tests if 1 occurrence of platelet count ≤ 100K/mm³**
≤ 50 K/mm³	Permanently discontinue Study Drug	Monitor daily until 2 successive values show improvement, then monitor every 2-3 days until 2 successive values are > 75K/ mm³. Patient should be evaluated by a hematologist to provide diagnostic and therapeutic management Steroids recommended*. **It is <u>strongly</u> recommended that, unless the patient has a medical contraindication to receiving glucocorticoids, the patient receives glucocorticoid therapy to reverse the platelet decline.** Monitor triglyceride levels weekly and continue AE monitoring during steroid therapy. Discontinue antiplatelet agents/NSAIDS/anticoagulant medication while platelet count <50K/mm³ if

		possible Obtain additional lab tests if 1 occurrence of platelet count ≤ 100K/mm³**

*Recovery in platelet count may be accelerated by administration of high dose steroids. Treatment guidelines for immune thrombocytopenia (Provan et al. 2010) recommend Dexamethasone 40 mg daily for 4 days every 2-4 weeks for 1-4 cycles; Prednis(ol)one 0.5-2 mg/kg/d for 2-4 weeks then taper; or Methylprednisolone 30 mg/kg/day for 7 days (**note**: may require continuation with oral steroids after methyl prednisolone)

**See Appendix J

7.6.4 Stopping Rule for Documented Severe Hypoglycemia

In the event of a first instance of documented severe hypoglycemia, dosing of a patient with study drug (ISIS 304801) will be suspended temporarily. The need to adjust the background medication and the suitability of the patient for continued dosing will be determined by the Investigator in (in consultation with the Sponsor Medical Monitor as needed). In the event of a second instance of documented severe hypoglycemia, after a re-challenge, dosing of a patient with study drug (ISIS 304801) will be stopped permanently.

7.7 Adjustment of Dose and/or Treatment Schedule

Dose adjustments for platelet count reduction must be made in accordance with Section 7.6.3 and Table (above).

Other dose adjustments, including dose interruptions, and/or decreasing the dose frequency will be allowed for safety or tolerability, after consultation with the Ionis Medical Advisor if possible. Dose adjustments should not occur unless absolutely necessary prior to the primary analysis time point (Week 12).

7.8 Withdrawal Criteria

1. **Withdrawal of consent**. A subject wishes to withdraw from the study as stated in the informed consent (all subjects reserve the right to withdraw from the study without prejudice).
2. **Adverse event (or SAE)**. The patient develops laboratory test abnormalities that meet any of the stopping rules listed above, or any other adverse event that in the investigator's opinion necessitates withdrawal from the study.
3. **Investigator judgment**. An investigator feels it is in the subject's best interest to terminate participation. The detailed reasoning behind this decision will be documented.
4. **Pregnancy**.
5. **Ineligibility**. Includes study entry criteria violation.
6. **Significant Protocol Deviation**. Includes subject noncompliance or start of an unacceptable concomitant medication.

The reason for discontinuation of study treatment must be recorded in the electronic case report form (eCRF) and source documentation. All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from study, must be recorded in the eCRF.

Any patient who withdraws consent to participate in the study will be removed from further treatment and study observation immediately upon the date of request. These patients should be encouraged to complete the early termination study procedures and observations at the time of withdrawal. For patients withdrawn for reasons other than withdrawal of consent, every effort should be made to complete the early termination study procedures and observations at the time of withdrawal.

7.9 Rationale for Subject Selection

The primary anticipated benefit of ISIS 304801 is TG reduction. The severity of hypertriglyceridemia in patients with lipodystrophy is quite variable. In this study, we are targeting the patient population to those with severe hypertriglyceridemia (>500 mg/dL) who are not only at increased risk for cardiovascular disease, but also at risk for acute pancreatitis. Patients with generalized lipodystrophy already have available a highly effective, approved therapy: leptin replacement. However, there are currently no approved therapies for patients with partial lipodystrophy. Hence, the population of partial lipodystrophy patients with severe hypertriglyceridemia has substantial unmet medical need, and has the greatest benefit to risk ratio from participation in this study. Based on our experience, we expect that the majority of subjects who enroll in this study will be female as females typically present with greater metabolic derangement. In addition, we anticipate that the majority of subjects will be Caucasian based on the demographics of partial lipodystrophy. Patients under 18 years of age are excluded as severe metabolic complications of partial lipodystrophy typically develop in adulthood, and there currently is no safety data for ISIS 304801 in children. Because there is a prospect for direct benefit, non-institutionalized adults who are unable to give informed consent but are considered able to comply with the study demands, will be eligible. If we enroll patients who are unable to provide their own consent, we will follow the procedures specified in NIH SOP 14E (*"Research Involving Adults Who Are or May Be Unable to Consent"*). If the study team has a concern that an adult patient may be unable to consent, the NIH Ability to Consent Assessment Team will be contacted to independently assess the subject's ability to provide informed consent. If the NIH Ability to Consent Assessment Team (ACAT) determines that the subject is not able to consent, an appropriate surrogate (assessed by the NIH ACAT) will be assigned. Consent for research participation will then be obtained from either the surrogate or a legally authorized representative. Individuals designated as durable power of attorney (DPA) for health care or other valid advanced directive, or court-appointed guardians are accepted as legally authorized representatives. If after study enrollment, the patient is unable to comply with the protocol demands, they will be withdrawn from the protocol.

7.10 Risks/Benefits Analysis including Considerations of Alternatives to Participation

7.10.1 Benefits

This study is characterized as greater than minimal risk with a prospect of direct benefit. The primary anticipated benefit to patients is reduction in TG level, which may reduce the risk of cardiovascular disease and pancreatitis. All clinical trials of ISIS 304801 have shown very large and clinically meaningful reductions in fasting apoC-III and TG (~80% and 70%, respectively, mean reduction from baseline with 300 mg dose) with a very high degree of consistency of response between the different patient groups. This includes healthy volunteers, patients with moderate to severe hypertriglyceridemia not on background TG-lowering therapy, patients with moderate to severe hypertriglyceridemia on a background of stable fibrate therapy, patients with Familial Chylomicronemia Syndrome (FCS), and patients with hypertriglyceridemia and T2DM. In 2 subjects, triglycerides decreased from ~1000 mg/dL at the end of the placebo controlled phase, to <150 mg/dL after 4 months of open-label drug, and were maintained in that range thereafter. In 1 subject, triglycerides decreased from >500 at study entry to <150 at the end of the placebo controlled phase, and were maintained in that range thereafter. In the 3 subjects who have completed the placebo-controlled period, insulin sensitivity increased on open label drug, and hemoglobin A1c decreased in parallel (by ~1%). Two subjects have not yet completed the placebo controlled phase. Thus, based on current data, the drug appears highly effective in controlling triglycerides, improving insulin sensitivity, and lowering A1c.

Patients may also experience benefits in diabetes control. Small studies of ISIS 304801 have shown significant decreases in glycated albumin, fructosamine, and/or HbA1c, and trends towards improvement in insulin sensitivity.

The additional 12 months of open label extension is justified in subjects who have manifested ≥ 50% improvement in triglycerides on study drug because of the prospect of direct clinical benefit for individual subjects as well as the prospect of acquiring additional generalizable knowledge about the potential risks and benefits of this treatment in the patient population.

7.10.2 Risks/Discomforts

1. General: Some patients may find the time needed to complete the research studies an inconvenience in their routine lives.

2. ISIS 304801: A detailed discussion of side effects of ISIS 304801 is given in the Background section (2.3.3 and 2.3.4) of the protocol. Briefly, pre-clinical studies of ISIS 304801 showed a proinflammatory response in rodents and monkeys and complement activation and reductions in platelet counts in the monkey. In clinical studies conducted to date, ISIS 304801 has been well tolerated and has shown a favorable safety profile, with no effects on renal or hepatic function or markers. There has been no clinical or laboratory evidence of drug-drug interactions. Phase 2 studies of ISIS 304801 have shown mild decreases of platelet count that recovered in the post-treatment period and was not associated with platelet-related adverse events. The most frequently observed AEs with ISIS 304801 were local reactions (pain, tenderness, erythema, pruritus or swelling) at the injection site. Injection site reactions persisting for at least 2 days were infrequent (~15% of injections), were almost always mild, resolved spontaneously, were non-progressive, and were not associated with systemic sequelae.

   Thrombocytopenia: Phase 2 studies of ISIS 304801 have shown mild decreases of platelet count that recovered in the post-treatment period and was not associated with platelet-related adverse events. In Phase 3 studies of ISIS 304801 in patients with familial chylomicronemia, two of 56 subjects experienced SUSARs of severe low platelet count, with nadirs of 17,000 and 9,000/mm^3. Neither patient experienced bleeding during these events. No patient has developed severe thrombocytopenia in this study or in the multicenter industry sponsored study in partial lipodystrophy. The one patient in the NIH study who developed platelets <100K did so in the context of pancytopenia due to viral bone marrow suppression, which promptly rebounded to the normal range without intervention. Adverse events possibly associated with the study drug have included systemic inflammatory reactions, the current case of heart failure, and the case of pancreatitis reported separately to the IRB in this study. Due to N of 1 cases and lack of biological plausibility, it remains uncertain whether these events are causally related to study drug.

   Anaphylaxis: One patient with partial lipodystrophy enrolled in a drug-company sponsored study of ISIS 304801 experienced a SUSAR of anaphylactic reaction during the open label extension phase of the study, and recovered after standard therapy (fluids, antihistamines, steroids). The study drug was permanently discontinued in this patient after this event.

Heart failure: One patient with partial lipodystrophy enrolled in the NIH study of ISIS 304801 experienced a SUSAR of heart failure complicated by ascites during the 2nd open label extension phase of the study. The subject also experienced pulmonary hypertension. She was discharged home after an approximately two-week hospitalization and prescribed oral Lasix 40 mg twice a day (for continued diuresis) and close follow-up with her local cardiologist. Heart failure has not been observed in other patients (or animals) who have taken this drug, but a causal relationship with the study drug could not be ruled out so the study drug was permanently discontinued in this patient after this event.

3. Physical examination/Anthropometric measurements: These tests are similar to a typical examination in a doctor's office and do not present any special risk.

4. Self-monitoring of blood glucose: Home blood glucose monitoring will be performed at a frequency approximating standard of care for patients with type 2 diabetes, and thus should present no added inconvenience or risk to patients participating in this study.

5. Blood Sampling: Peripheral blood draws (venipuncture) performed during this study for research will not exceed 10.5 mL/kg, or 550 mL (whichever is smaller) per 8-week period for adults. Patients may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

6. DEXA: The effective radiation dose with total body DEXA scanning is 0.00003 rem. This is much less than the average daily background radiation from natural sources, and is not thought to increase lifetime cancer risk. Subjects in this study will have up to four DEXA scans (total radiation dose 0.00012 rem) per year.

7. Glucose and Lipid Turnover: The stable isotopes ^{13}C is not associated with any toxicity at the doses used in these studies [54]. Deuterium at the doses given may cause temporary dizziness. This risk will be minimized by dividing the $^{2}H_2O$ dose into four aliquots, and by giving the doses at night while subjects lie in bed. If subjects need to get out of bed (e.g. to use the bathroom) the patient will first sit upright in bed for a few minutes prior to standing, and a nurse will be available to supervise and assist. Palmitate must be complexed to albumin prior to delivery, and thus carries the risks associated with use of human blood products. To minimize exposure to human albumin, uniformly labeled $^{13}C_{16}$ palmitate will be used, permitting use of the minimum possible dose of this tracer.

8. Euglycemic Hyperinsulinemic Clamp Study: In order to assess insulin sensitivity, patients will receive an intravenous infusion of insulin and – via a second intravenous access – increasing amounts of glucose to maintain euglycemia. Insertion of the necessary intravenous lines may cause the above-mentioned discomfort and a possible skin infection. Insulin infusion can induce hypoglycemia and hypokalemia. Either event is very unlikely, since patients undergo frequent blood glucose measurements and are constantly observed for the presence of hypoglycemic symptoms by an experienced nurse and/or physician. However, in order to prevent complications, two patent, well-functioning intravenous lines are required during this procedure. A stat potassium will be measured at the end of the insulin infusion, and oral KCL 40 mEq will be given if potassium is less than 3.5.

9. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS): While MR scanning is thought to be safe, the procedure may cause anxiety in some patients since current equipment used at the Clinical Center uses a closed tube. Adult patients will be offered sedatives such as Valium if they express worry about being in a closed space. No intravenous contrast will be used in these studies. The hand-grip exercise may produce mild fatigue.

10. Lipoprotein lipase activity: The primary risk of lipase assays involving heparin infusion of 60 units/kg is bleeding (in <0.1% of subjects). Testing will not be performed in subjects at increased bleeding risk, including those with platelet counts, prothrombin time, and partial thromboplastin time outside the normal range within 48 hours of testing, those with history of recent peptic ulcer disease or gastrointestinal bleeding, or other bleeding diathesis. This test also involves minor risks associated with intravenous line placement.

11. Mixed meal: This test requires one IV, but no adverse effects are anticipated.

12. Carotid intima-media thickness: This is a non-invasive ultrasonographic test. No adverse effects are anticipated.

13. Hunger and pain questionnaires: Patients may find it a minor inconvenience to fill out the questionnaires, but no adverse effects are anticipated.

14. Liver biopsy: Risks and discomforts of liver biopsy are as follows:
    a. Pain. About 20% of persons who undergo a liver biopsy experience pain in the side of body over the liver after the biopsy. This pain usually lasts from a few minutes to several hours and may require pain medication. In rare cases, the pain lasts a day or two.
    b. Fainting. About 2% of persons pass out due to vasovagal reaction after a liver biopsy. This is unlikely to cause harm, as patients will be lying in bed during the biopsy. Atropine will be given to reverse the vasovagal reaction if it occurs, and may be given prior to the biopsy in individuals prone to these reactions.
    c. Infection. A rare complication of liver biopsy is infection that spreads to the rest of the body because the needle goes through skin that is not completely clean or sterile or goes through infected bile (as occurs in patients with gallstones sometimes). Antibiotics can treat such infections.
    d. Biopsy of another organ. In rare instances, the biopsy needle misses the liver and hits another internal organ, such as the lung, gallbladder, kidney, or intestine. This complication can be avoided by carefully selecting the site for the liver biopsy and using ultrasound to identify exactly where the liver is and the best position for the biopsy. Usually, the other organ is not damaged by the needle puncture; however, it is possible that surgery might be needed to repair the hole. The most important complication of liver biopsy is bleeding. A small amount of bleeding probably occurs in many patients after liver biopsy, but the amount of bleeding is usually too small to be detected or felt. Severe bleeding occurs in about one of every 1000 liver biopsies. Severe bleeding is most likely to occur in someone with cancer of the liver or with abnormal clotting studies, but bleeding can also occur in patients with mild forms of

liver disease. Most cases of severe bleeding stop on their own with bed rest and observation for several days in the hospital. In rare instances, a blood transfusion or even surgery is needed to sew up the tiny hole in the liver made by the biopsy. Very rarely, in less than one in 10,000 cases, death has occurred from bleeding after a liver biopsy. At the NIH Clinical Center, the Liver Diseases Branch has performed approximately 150 liver biopsies each year for the last 40 years. During this time, three patients developed serious, life-threatening complications of biopsy (two in the early1990s and one in 2014). The first patient, who had cirrhosis due to hepatitis C and advanced hepatocellular carcinoma, bled intraperitoneally from the liver biopsy and died after surgical attempts to stop the bleeding were unsuccessful. The second patient had Gaucher's disease and advanced cirrhosis with severe coagulopathy and was treated with platelet transfusions and fresh frozen plasma to correct the bleeding tendency; he tolerated liver biopsy well and was discharged a day later, but then returned in shock with a severe intra-abdominal bleed, and died despite surgical attempts at stopping the bleeding. The third patient had a history of Pre-B cell ALL status-post bone marrow transplantation and chronic coagulopathy. The patient was seen on consultation for concern of liver graft-versus-host disease and developed a severe intraperitoenal bleed after liver biopsy. Despite multiple interventions, including transfusions, arterial embolization and exploratory laparotomy, bleeding could not be stopped and the patient died. Subjects will be kept overnight in the Clinical Center after the liver biopsy with careful monitoring for any evidence of bleeding. The percutaneous liver biopsy will not be performed if the platelet level is below 75 K/ul.

e. Risks and discomforts of <u>medications</u> used during liver biopsy are as follows: Three medications are often used during a liver biopsy: Xylocaine to numb the skin, a sedative such as Versed (midazolam) to make subjects drowsy during the biopsy, and atropine if subjects have a tendency to faint. All three medications are safe when given in the usual doses. In rare instances, they can induce allergic reactions which can be a skin rash, itching, wheezing, an asthma attack, or even anaphylaxis - or allergic shock. All of the allergic complications can and will be treated if they arise. Xylocaine, when given in high doses can also cause heart blockage, seizures, coma, or even death; but, in the doses given in this study it has no obvious effect on the heartbeat. Atropine can cause dryness of the eyes and mouth, difficulty focusing the eyesight, constipation and difficulty passing urine. All these side effects will disappear within a few hours. Versed or Midazolam for sedation causes sleepiness and forgetfulness. In high doses, it can cause over-sedation with coma and depression of breathing and even death. This medication is given in small amounts with careful monitoring of sedation. Standard medications and equipment used for conscious sedation will be available during the procedure.

7.10.3 Alternatives to participation

Participation in clinical trials is completely voluntary. Refusal to participate will not affect a subject's ability to participate in other studies at NIH or elsewhere.

7.11 Financial Compensation

All subjects will receive financial compensation for their time per Clinical Center guidelines for on-site visits, and additional compensation will be provided for specific procedures based on inconvenience units. See Appendix E. There will be no compensation for the additional 12 month extended open-label period.

7.12 Consent Procedures

Written consent will be obtained at the first inpatient visit from each subject after detailed explanations of the planned procedures by the principal or an associated investigator (as listed on face sheet). The consent process will take place prior to any study procedures, however, the research team will talk with the subjects prior to coming to NIH to determine eligibility and to provide dietary recommendations. The current NIDDK/NIAMS IRB-approved informed consent document will be signed by the subject and the Investigator. A witness will additionally sign the document to attest only to the validity of the signature of the subject, not the validity or quality of the consent. A copy of the consent will be given to the subject for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS).

We do not plan or anticipate the enrollment of non-English speaking subjects. However, they are not excluded from participation either. If there is unexpected enrollment of a research participant for which there is no translated extant IRB-approved consent document, the Principal Investigator and/or those authorized to obtain informed consent will use the short form consent process as described in MAS Policy M77-2, NIH SOP 12, 45 CFR 46.117 (b), and 21 CFR 50.27 (b). The summary that will be used is the English version of the extant IRB-approved consent document. We request prospective IRB approval of the use of the short form consent process for up to a maximum of 5 requests (either for individual participants or families of participants) in a given language, and will notify the IRB at the time of continuing review of the frequency of the use of the short form. Should we reach the threshold of 5 subjects and/or families speaking a single language, we will request an additional use of the short form from the IRB and will notify the Board that we plan to have any consent documents frequently used with that population translated into the language(s) they speak.

Subjects have the right to withdraw participation from this protocol at any time.

7.13 Research Use, Storage and Disposition of Human Subjects' Samples and Data

For future reference and potential use, we will store all samples (blood or fluids) in our locked freezers for an unlimited period of time. Samples will be labeled with coded identifiers linked to patient identity only via a secured database. Research records and data with personal identifiers will be stored in our locked offices, the medical record department, and the electronic study database. The electronic study database will be maintained by Ionis Pharmaceuticals. Representatives of Ionis Pharmaceuticals will have access to research records and data with personal identifiers only when the representative is physically present in the NIH Medical Records department. The electronic study database will not contain any personal identifiers, although it will contain dates on which labs or tests are performed. This material will additionally be protected by medical record and computer access procedures. Access to records and data associated with personal information will be restricted to the Principal Investigator, Co-Investigators, study support staff, and NIH database support staff.

Stored samples and/or data may be sent to outside collaborating laboratories, or shared with other NIH collaborating investigators, to study questions related to lipodystrophy or its complications (including, for example: glucose metabolism, diabetes, obesity, weight, appetite, steatohepatitis, and lipid metabolism). Samples may be sent to outside commercial laboratories for analysis. Samples and data sent to outside laboratories and collaborators for analysis and/or testing will contain only coded numbers, without personal identifiers. Tech Transfer agreements will be completed before the exchange of samples and/or data with outside collaborators.

Subjects may request that unused samples be removed from our freezers and returned to the subject, or be destroyed. If no such request is made, we will keep samples until they are completely used or no longer of scientific value, at which time they will be destroyed. We do not plan to destroy personal medical information or stored data. The Principal Investigator will report loss or destruction of data or samples to the NIDDK/NIAMS IRB.

7.14 Collaborations

Metabolomic studies of plasma and liver biopsy specimens will be conducted in collaboration with Dr. Elif Oral at University of Michigan. Deidentified samples will be shipped to the University of Michigan under a Material Transfer Agreement. Raw data from Dr. Oral will not be sent to us, but overall results will be shared.

Collaboration has been established with Professor Stephen O'Rahilly in Cambridge, England. Our direct physician contacts are Dr. Robert Semple and Dr. David Savage. The purpose of this collaboration is to share coded de-identified clinical data and results collected from subjects enrolled under this protocol at NIH. The Laboratory at Cambridge University will use the coded data and results, including laboratory, anthropometric, imaging and demographic data, to characterize the phenotype of patients affected by lipodystrophy and to study the association between phenotypes and causative genetic mutations, in order to better understand the pathogenesis and natural history of lipodystrophy, improve the diagnosis, and predict and assess response to treatment.

Collaboration has been established with Abhimanyu Garg, MD at the University of Texas Southwestern. This collaborator is a former associate principal investigator of our original leptin protocol, and is a world's expert on syndromes of lipodystrophy. The purpose of this agreement is to transfer coded de-identified metabolic and clinical data, including laboratory, anthropometric, imaging and demographic data, to characterize the phenotype of patients affected by lipodystrophy and to study the association between phenotypes and causative genetic mutations, in order to better understand the pathogenesis and natural history of this disease, improve the diagnosis, and predict and assess response to treatment.

Collaboration has been established with Dr. Morey W. Haymond, a board-certified pediatric endocrinologist and Professor of Pediatrics and Medicine at Baylor College of Medicine. Our direct site contact will be Shaji Chacko, Ph.D. The purpose of the collaboration is to assist in the conduct of the clinical studies determining the rates of glucose production and gluconeogenesis in subjects with a variety of forms and severity of lipodystrophy. This will include the analysis of coded human subject samples and interpretation of the results.

Collaboration has been established with Robert E. Eckel, M.D. and Kimberley D. Bruce, Ph.D. are professors at the University of Colorado Anschutz Medical Campus. The secondary study objective of this protocol was to explore the mechanism of action of apoC-III ASO by measuring the lipoprotein lipase (LPL) activity and lipoprotein particle distribution to evaluate if we can strengthen the connection between LPL activity and the metabolic disturbances which is an area that has not been researched. Therefore, the pre- and 10 minutes post-heparin infusion samples collected on the 5 enrolled partial lipodystrophy patients who were treated with ApoC3 antisense oligonucleotide will be shared with the collaborators who will run LPL assays. Samples will be coded and the collaborators will share the results of their analysis.

Collaboration has been established with Katsuyuki Nakajima, Ph.D. and Dr. Masami Murakami, professor of Gunma University Graduate School of Medicine (Department of Clinical Laboratory Medicine) located in Maebashi, Gunma, Japan. The collaborators are interested in determining 4 types of markers found in the plasma of patients with lipodystrophy. These markers (or "assay systems") were developed in Dr. Murakami's laboratory and include: (1) LPL activity (post-heparin plasma) and mass, (2) HTGL activity (post-heparin plasma) and mass, (3) GPIHBP1 mass, and (4) GPIHBP1 autoantibody. They will run the above stated assays using specimens collected before and after leptin treatment. Research has shown that Leptin lowers triglycerides in these patients, and hence we would hypothesize that LPL and HTGL activity might be increased after leptin treatment. Therefore, we will send to the collaborators deidentified plasma collected from the lipodystrophy patients to test this hypothesis.

Appendix A: Schedule of Study Procedures

Study Period	Pre-Screen	Screen	Double-Blind						Open Label Extension										Post Treatment		
Study Week (from enrollment)	**-8 to -2**	**-1**	**1**	**4**	**8**	**12**	**13**	**16 Primary Endpoints**	**20**	**24**	**28**	**32 Primary Endpoints**	**36**	**40**	**48**	**56**	**64**	**68**	**74**	**80**
Study week (from start of open label extension)									**4**	**8**	**12**	**16**	**20**	**24**	**32**	**40**	**48**	**52**		
Inpatient Visits		x						x				x						x		x
Home Nurse visit**				x	x	x	x		x	x	x		x	x	x	x	x		x	
Telephone contact				x	x	x	x		x	x	x		x	x	x	x	x		x	
Inclusion/Exclusion Criteria	x	x																		
Medical History Review	x	x																		
Vital Signs/Weight/Height		x						x				x						x		x
Physical Exam		x						x				x						x		x
Waist Circumference		x						x				x						x		x
Skinfold measurements		x																		
DEXA Scan			x					x				x						x		
Glucose and Lipid Turnover			x					x				x						x		x
Euglycemic Hyperinsulinemic Clamp			x					x				x						x		x
Mixed Meal Test			x					x				x						x		x
Urinalysis & protein:creatinine ratio		x		x	x		x	x	x	x	x	x		x		x		x		x
Liver Biopsy			x															x		
MRI/MRS liver/heart			x					x				x						x		
Carotid intima-media thickness			x					x				x						x		
12-Lead EKG		x						x				x[1]						x		

Echocardiogram			x						x				x[1]						x		
Fasting Blood Draws	Chemistry Panel		x		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	CBC w/diff*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Serum lipid panel		xx[2]		x	x	x	x	xx[2]	x	x	x	xx[2]	x	x	x	x	x	xx[2]	x	xx[2]
	Apolipoprotein panel		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Coag (PTT, PT, INR)		x		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Hepatitis B, C, HIV		x																		
	Troponin T		x						x				x						x		
	Thyroid function (TSH, free T4)		x																		
	HbA1c		x						x				x				x	x	x		x
	Plasma glucose		x		x	x			x	x	x		x	x	x	x	x	x	x	x	x
	Insulin, c-peptide		x						x				x						x		x
	hsCRP		x						x				x						x		x
	Lipoprotein particle size/# (NMR)		x						x				x						x		x
	Sedimentation rate		x						x				x						x		x
	Complement (C5a, Bb)		x						x				x						x		x
	Plasma PK ISIS 304801 serial sampling (peak and trough)				x	x		x	X[3]				X[3]						x	x	x
	Single plasma PK			x																	
	Anti ISIS 304801 Antibodies			x	x	x			x				x						x		x
	FSH (women only, if applicable)		x																		
	Testosterone (women only)		x						x				x						x		x
	Serum pregnancy		x						x				x						x		x
	Free Fatty Acids		x						x				x						x		x
	Leptin		x						x				x						x		x
	Lipoprotein lipase activity			x					x				x						x		x
	Adiponectin		x						x				x						x		x
	Research serum/plasma		x						x				x						x		x

7-point plasma glucose			x					x				x						x		x
Weekly Study Drug SQ Injection			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Glucometer Dispensation/Training			x																	
Hunger Diary			x					x				x						x		x
Widespread Pain Diary			x					x				x						x		x
Quality of Life Assessments			x					x				x						x		x
Review SMPG and Insulin Dose			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Diet/Alcohol Counseling	---x---		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medication Review	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

[1] Patients previously treated with placebo, only

[2] One sample obtained on each of two consecutive days

[3] Sampling schedule can be found in Appendix C.
Unless specified, otherwise, PK and Antibodies will be collected predose.
PK and immunogenicity testing does not need to be collected fasting.

* Platelets will be assessed every week

** Home Health Nurse Visits (or lab draw by local lab facility when needed) will take place every week unless patient will be at NIH for in-person visit.

Appendix B: List of Laboratory Analytes

Based on emerging data from this or future studies, additional tests not listed below may be performed on stored samples to better characterize the profile of ISIS 304801.

Clinical Chemistry Panel

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Total protein
- Albumin
- Calcium
- Magnesium
- Phosphorus
- BUN
- Creatinine
- Uric Acid
- Total bilirubin
- Direct (conjugated) bilirubin
- Indirect (unconjugated) bilirubin
- ALT
- AST
- Alkaline phosphatase

Screening Tests

- Hepatitis B surface antigen
- Hepatitis C antibody
- HIV antibody
- FSH (women only)
- Serum βhCG (women only)
- TSH, T3, T4

Coagulation

- aPTT (sec)
- PT (sec)
- INR

Lipid Panel

- Total Cholesterol
- LDL-C
- HDL-C
- Triglycerides
- VLDL-C
- Non-HDL-C
- apoC-III (total, chylomicron, VLDL, LDL, HDL)

Hematology

- Red blood cells
- Hemoglobin
- Hematocrit
- MCV, MCH, MCHC
- Platelets
- White blood cells
- WBC Differential (% and absolute)
- Neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- Monocytes

Pharmacokinetics[1] & Immunogenicity

- ISIS 304801 levels in plasma
- Anti-ISIS 304801 antibodies in plasma

Urinalysis

- Color
- Appearance
- Specific gravity
- pH
- Protein
- Blood
- Ketones
- Urobilinogen
- Glucose
- Bilirubin
- Leukocyte esterase
- Nitrate
- Microscopic examination[2]

Other assessments

- hsCRP
- Sedimentation Rate
- C5a, Bb
- HbA1c
- Glucose
- Insulin
- C-peptide

	• apoA-1 • apoB • apoB-48 • Lipoprotein particle size/number • Free fatty acids	• Troponin T • Adiponectin • Leptin • Testosterone (women only)

1 Plasma PK samples may also be used for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity testing (or possibly for purposes of immunogenicity assay development and/or validation), or to assess other actions of ISIS 304801 with plasma constituents

2 Will be performed on abnormal findings unless otherwise specified

Appendix C: PK Sampling Schedules

Blood PK Sampling Schedule: Week 16 (Week 1 OLE)

Week 16			
D1	D2	D3	D4
Predose1, 2, 3, 4, 6, 8,12 hours post SC injection	24 hours	48 hours	72 hours

Blood PK Sampling Schedule: Week 32 (Month 4 OLE)

Week 32			
D1	D2	D3	D4
Predose, 1, 2, 3, 4, 6, 8,12 hours post SC injection	24 hours	48 hours	72 hours

Appendix D: Investigator Qualifications and Roles

Rebecca J. Brown, M.D., M.H.Sc. is a board certified pediatric endocrinologist, and an Assistant Clinical Investigator of the Diabetes Endocrinology, and Obesity Branch. Dr. Brown has six years of experience conducting clinical studies in children adults with diabetes, and has a Master's degree in Clinical Research. She is the Principal Investigator of this study, and is responsible for the study design, implementation, and interpretation.

Elaine K. Cochran, M.S.N., CRNP, is a nurse practitioner in the Diabetes, Endocrinology and Obesity Branch, and is lead associate investigator of the ongoing study of leptin treatment in lipodystrophy patients. She is an expert in the administration of leptin to patients with lipodystrophy. Her role in this project will be to assist in clinical management and education of patients.

Ahmed Gharib, M.D. is board certified in radiology and nuclear medicine. He has more than 10 years of experience in cardiac imaging including MR and CT in addition to MR spectroscopy. He is a tenure track investigator in the Biomedical and Metabolic a Imaging Branch (BMIB) in NIDDK with focus on imaging metabolic disorders and their effects on various organs. His role in this project is to plan, perform, and interpret MR and CT imaging studies.

Phillip Gorden, M.D. is a board certified endocrinologist, Senior Investigator in the Diabetes, Endocrinology and Obesity Branch, and Director Emeritus of the NIDDK. He has over four decades of experience conducting clinical studies in patients with rare disorders of extreme insulin resistance, and is Principal Investigator of the ongoing study of leptin treatment in lipodystrophy patients. His role in this project will be to work with Dr. Brown on study strategy, design, and interpretation.

Theo Heller, M.D. is a board certified hepatologist and gastroenterologist and Chief of the Translational Hepatology Unit in the NIDDK Liver Diseases Branch. He has 16 years of experience studying the connections between the innate immune system and liver-related damage and repair. His role in this study will be to perform liver biopsies and provide interpretation of the results.

Christopher Koh, M.D., M.H.Sc. is a board certified hepatologist and gastroenterologist and staff clinician in the Liver Diseases Virology Section of the NIDDK Liver Diseases Branch. His role in this project will be to perform liver biopsies and interpret their results.

Megan Startzell, RN, MPH is a research nurse in the Diabetes, Endocrinology and Obesity Branch. Ms. Mattingly's role in this project will be to assist Dr. Brown with the coordination of patient visits, patient education and communication.

Elif Oral, MD, is an associate professor of internal medicine in Metabolism, Endocrinology, and Diabetes at the University of Michigan. She played a critical role in the identification of three disease genes for lipodystrophy and pioneered the development of leptin therapy in lipodystrophy. Her role in this project is to aid in study design and interpretation, and to perform metabolomic studies.

Robert Shamburek, M.D. is a lipidologist and board certified internist and gastroenterologist. He is a Staff Clinician in the Lipid Metabolism Section, Cardiovascular and Pulmonary Branch of the National Heart, Lung, and Blood institute. His role in this project is on the study design an interpretation.

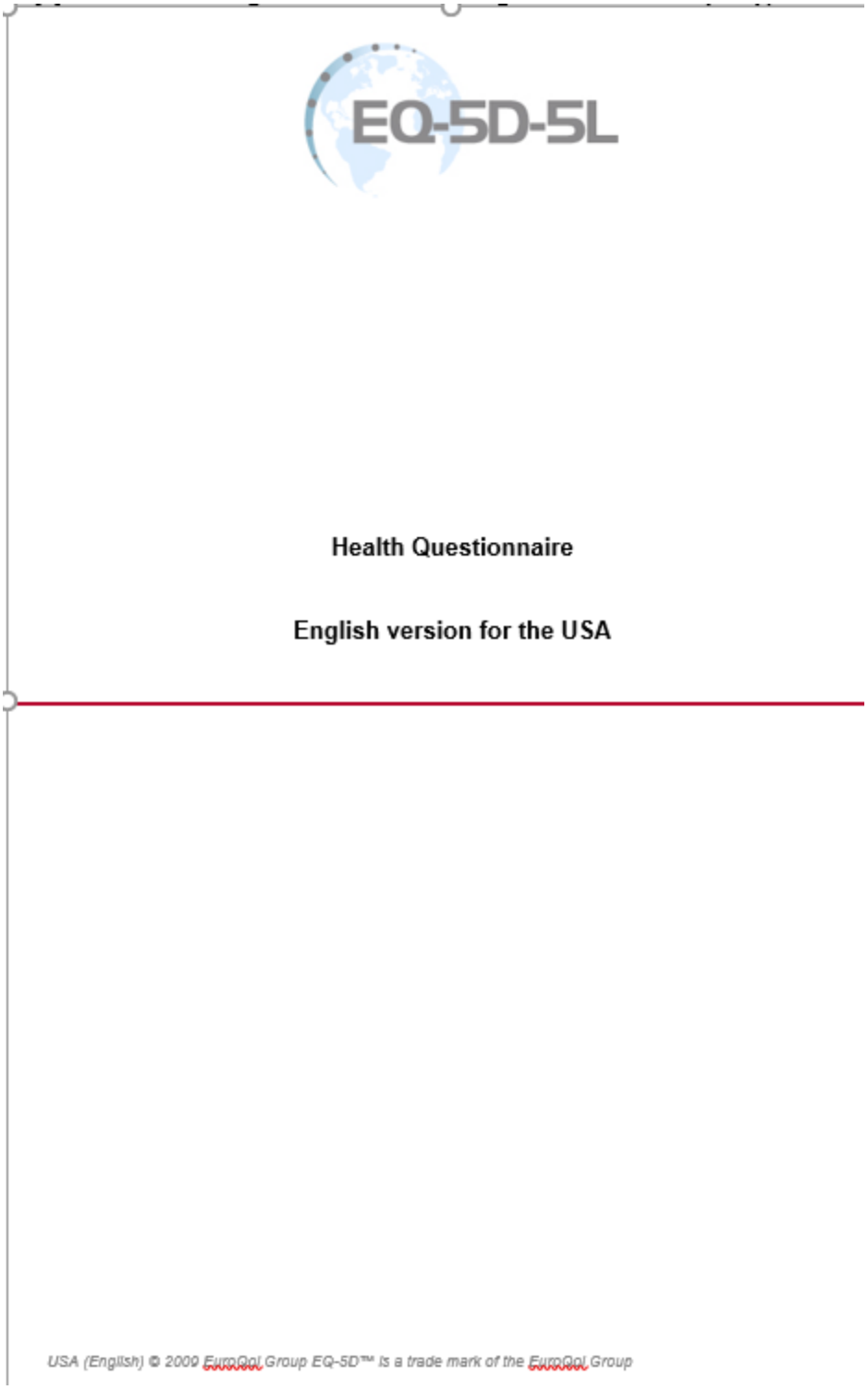
Monica Skarulis, M.D. is a board-certified endocrinologist, and Special Volunteer with the Diabetes, Endocrinology and Obesity Branch. Her role in this project is to work with Dr. Brown on study design and interpretation.

Marissa Lightbourne, M.D. is a board-certified pediatrician, and clinical fellow in the National Institutes of Child Health and Development. Her role in this project is to work with Dr. Brown on study design, interpretation, and publications.

Appendix E: Financial Compensation

			Baseline admission		Week 16		Week 32		Week 68		Post-treatment	
Procedure	**Inconvenience Units**	**Compensation**	**Count**	**Total**	**Count**	**Total**	**Count**	**Total**	**Count**	**Total**	**Count**	**Total**
Inpatient stay	n/a	40	9	360	9	360	9	360	8	320	4	160
Home labs	1	10	0	0	15	150	15	150	34	340	4	40
7 point plasma glucose	3	30	1	30	1	30	1	30	1	30	1	30
DEXA	1	10	1	10	1	10	1	10	1	10	0	0
MRI	5	50	1	50	1	50	1	50	1	50		0
Mixed meal test	2	20	1	20	1	20	1	20	1	20	1	20
Euglycemic Clamp	5	50	1	50	1	50	1	50	1	50	1	50
Liver Biopsy	5	50	1	50	1	50	0	0	0	0	0	0
Study diaries	1	10	1	10	1	10	1	10	1	10	1	10
Visit completion bonus						250		250		250		250
Total				580		980		930		1080		560
											Grand total	**4130**

Appendix F: EQ-5D-5L Health Questionnaire (English and Spanish versions)



Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY

- I have no problems walking ❑
- I have slight problems walking ❑
- I have moderate problems walking ❑
- I have severe problems walking ❑
- I am unable to walk ❑

SELF-CARE

- I have no problems washing or dressing myself ❑
- I have slight problems washing or dressing myself ❑
- I have moderate problems washing or dressing myself ❑
- I have severe problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

- I have no problems doing my usual activities ❑
- I have slight problems doing my usual activities ❑
- I have moderate problems doing my usual activities ❑
- I have severe problems doing my usual activities ❑
- I am unable to do my usual activities ❑

PAIN / DISCOMFORT

- I have no pain or discomfort ❑
- I have slight pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have severe pain or discomfort ❑
- I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

- I am not anxious or depressed ❑
- I am slightly anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am severely anxious or depressed ❑
- I am extremely anxious or depressed ❑

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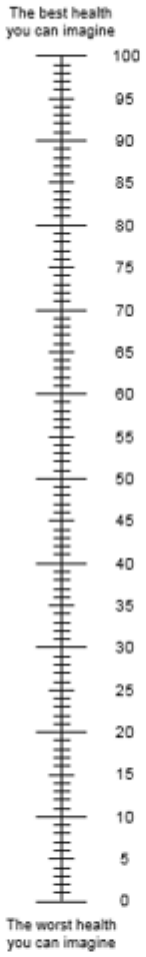
- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



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Cuestionario de Salud

Versión en español para los EE. UU.

(Spanish version for the USA)

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Debajo de cada encabezamiento, marque UNA casilla, la que mejor describe su salud HOY.

MOVILIDAD

- No tengo problemas para caminar ❑
- Tengo problemas leves para caminar ❑
- Tengo problemas moderados para caminar ❑
- Tengo problemas graves para caminar ❑
- No puedo caminar ❑

CUIDADO PERSONAL

- No tengo problemas para lavarme o vestirme solo/a ❑
- Tengo problemas leves para lavarme o vestirme solo/a ❑
- Tengo problemas moderados para lavarme o vestirme solo/a ❑
- Tengo problemas graves para lavarme o vestirme solo/a ❑
- No puedo lavarme o vestirme solo/a ❑

ACTIVIDADES DE TODOS LOS DÍAS *(Ej.: trabajar, estudiar, hacer las tareas domésticas, actividades familiares o actividades de ocio)*

- No tengo problemas para realizar mis actividades de todos los días ❑
- Tengo problemas leves para realizar mis actividades de todos los días ❑
- Tengo problemas moderados para realizar mis actividades de todos los días ❑
- Tengo problemas graves para realizar mis actividades de todos los días ❑
- No puedo realizar mis actividades de todos los días ❑

DOLOR / MALESTAR

- No tengo dolor ni malestar ❑
- Tengo dolor o malestar leve ❑
- Tengo dolor o malestar moderado ❑
- Tengo dolor o malestar intenso ❑
- Tengo dolor o malestar extremo ❑

ANSIEDAD / DEPRESIÓN

- No estoy ansioso/a ni deprimido/a ❑
- Estoy levemente ansioso/a o deprimido/a ❑
- Estoy moderadamente ansioso/a o deprimido/a ❑
- Estoy muy ansioso/a o deprimido/a ❑
- Estoy extremadamente ansioso/a o deprimido/a ❑

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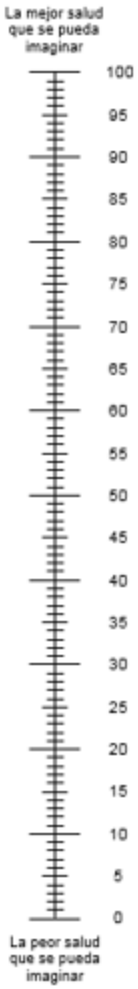
- Nos gustaría saber lo buena o mala que es su salud HOY.
- La escala está numerada de 0 a 100.
- 100 representa la mejor salud que se pueda imaginar.
  0 representa la peor salud que se pueda imaginar.
- Por favor haga una X en la escala para indicar cuál es su estado de salud HOY.
- Ahora, por favor escriba en la casilla que encontrará a continuación el número que ha marcado en la escala.

SU SALUD HOY = ☐



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Appendix G: SF-36v2 Health Survey (English and Spanish versions)

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. ***Thank you for completing this survey!***

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c. Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d. Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e. Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f. Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g. Walking more than a mile	☐ 1	☐ 2	☐ 3
h. Walking several hundred yards	☐ 1	☐ 2	☐ 3
i. Walking one hundred yards	☐ 1	☐ 2	☐ 3
j. Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

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(SF-36v2® Health Survey Standard, United States (English))

4. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**

None	Very mild	Mild	Moderate	Severe	Very severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?**

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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Su Salud y Bienestar

Esta encuesta le pide sus opiniones acerca de su salud. Esta información permitirá saber cómo se siente y qué tan bien puede hacer usted sus actividades normales. ***¡Gracias por contestar estas preguntas!***

Para cada una de las siguientes preguntas, por favor marque con una ☒ la casilla que mejor describa su respuesta.

1. **En general, diría que su salud es:**

Excelente	Muy buena	Buena	Pasable	Mala
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **<u>Comparando su salud con la de hace un año</u>, ¿cómo la calificaría en general <u>ahora</u>?**

Mucho mejor ahora que hace un año	Algo mejor ahora que hace un año	Más o menos igual ahora que hace un año	Algo peor ahora que hace un año	Mucho peor ahora que hace un año
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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3. Las siguientes preguntas se refieren a actividades que usted podría hacer durante un día típico. ¿Su estado de salud actual lo/la limita para hacer estas actividades? Si es así, ¿cuánto?

	Sí, me limita mucho	Sí, me limita un poco	No, no me limita en absoluto
a. Actividades vigorosas, tales como correr, levantar objetos pesados, participar en deportes intensos	☐ 1	☐ 2	☐ 3
b. Actividades moderadas, tales como mover una mesa, empujar una aspiradora, jugar al bowling o al golf o trabajar en el jardín	☐ 1	☐ 2	☐ 3
c. Levantar o cargar las compras del mercado	☐ 1	☐ 2	☐ 3
d. Subir varios pisos por la escalera	☐ 1	☐ 2	☐ 3
e. Subir un piso por la escalera	☐ 1	☐ 2	☐ 3
f. Doblarse, arrodillarse o agacharse	☐ 1	☐ 2	☐ 3
g. Caminar más de una milla	☐ 1	☐ 2	☐ 3
h. Caminar varias cuadras (varios cientos de metros)	☐ 1	☐ 2	☐ 3
i. Caminar una cuadra (unos cien metros)	☐ 1	☐ 2	☐ 3
j. Bañarse o vestirse	☐ 1	☐ 2	☐ 3

4. **Durante las últimas 4 semanas, ¿cuánto tiempo ha tenido usted alguno de los siguientes problemas con el trabajo u otras actividades diarias regulares a causa de su salud física?**

	Siempre	Casi siempre	Algunas veces	Casi nunca	Nunca
a. Ha reducido el tiempo que dedicaba al trabajo u otras actividades	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Ha logrado hacer menos de lo que le hubiera gustado	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Ha tenido limitaciones en cuanto al tipo de trabajo u otras actividades	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Ha tenido dificultades en realizar el trabajo u otras actividades (por ejemplo, le ha costado más esfuerzo)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **Durante las últimas 4 semanas, ¿cuánto tiempo ha tenido usted alguno de los siguientes problemas con el trabajo u otras actividades diarias regulares a causa de algún problema emocional (como sentirse deprimido/a o ansioso/a)?**

	Siempre	Casi siempre	Algunas veces	Casi nunca	Nunca
a. Ha reducido el tiempo que dedicaba al trabajo u otras actividades	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Ha logrado hacer menos de lo que le hubiera gustado	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Ha hecho el trabajo u otras actividades con menos cuidado de lo usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Appendix H: Brief Pain Inventory (Short Form) (English and Spanish versions)

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Brief Pain Inventory (Short Form)

Date: ____/____/____ Time: _____

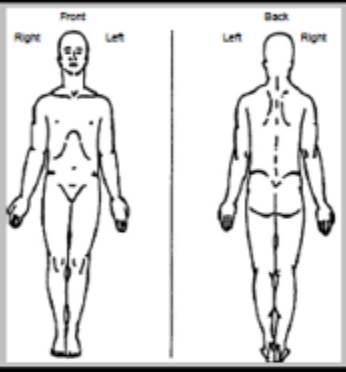
Name: _____ _____ _____

Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

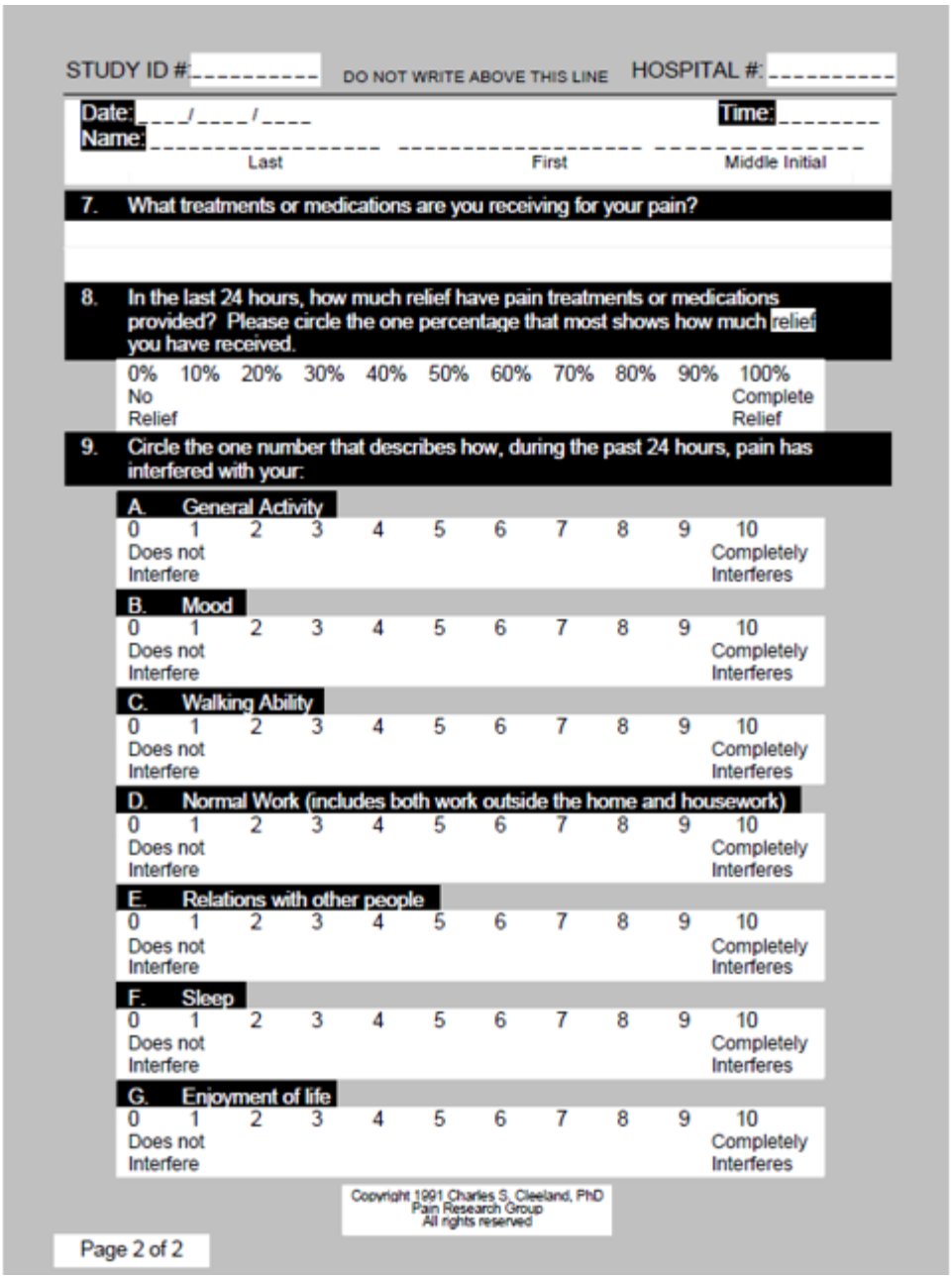
5. Please rate your pain by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

Page 1 of 2



STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Date: ____/____/____ Time: _____

Name: _____ _____ _____

Last First Middle Initial

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
No Relief										Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

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Page 2 of 2

ESTUDIO N°: _____ NO ESCRIBA SOBRE ESTA LINEA HOSPITAL N°: _____

Cuestionario Breve del Dolor (Forma Corta)

Fecha: ____/____/____ **Hora:** _____

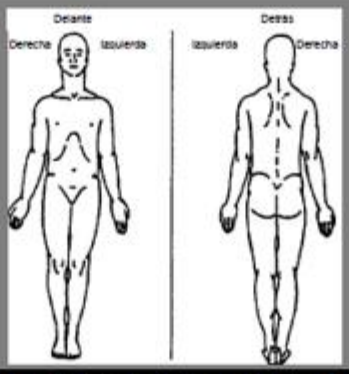
Nombre: _____ _____ _____

Primer nombre Apellido Inicial del segundo nombre

1. Todos hemos tenido dolor alguna vez en nuestra vida (por ejemplo, dolor de cabeza, contusiones, dolores de dientes). ¿En la actualidad, ha sentido un dolor distinto a estos dolores comunes?

1. Sí 2. No

2. Indique en el diagrama las zonas dónde siente dolor sombreando la parte afectada. Marque con una cruz la zona que más le duele.



3. Por favor, evalúe su dolor rodeando con un círculo el número que mejor describa la intensidad máxima de su dolor en las últimas 24 horas.

0 1 2 3 4 5 6 7 8 9 10
Ningún Dolor — El Peor Dolor imaginable

4. Por favor, evalúe su dolor rodeando con un círculo el número que mejor describa la intensidad mínima de su dolor en las últimas 24 horas.

0 1 2 3 4 5 6 7 8 9 10
Ningún Dolor — El Peor Dolor imaginable

5. Por favor, evalúe su dolor rodeando con un círculo el número que mejor describa la intensidad media de su dolor.

0 1 2 3 4 5 6 7 8 9 10
Ningún Dolor — El Peor Dolor imaginable

6. Por favor, evalúe su dolor rodeando con un círculo el número que mejor describa la intensidad de su dolor ahora mismo.

0 1 2 3 4 5 6 7 8 9 10
Ningún Dolor — El Peor Dolor imaginable

Página 1 de 2

ESTUDIO N°: _____ NO ESCRIBA SOBRE ESTA LINEA HOSPITAL N°: _____

Fecha: ____/____/____ Hora: _____

Nombre: _____ _____ _____

Primer nombre Apellido Inicial del segundo nombre

7. ¿Qué tratamiento o medicación está recibiendo para el dolor?

8. En las últimas 24 horas, ¿hasta qué punto le han aliviado los tratamientos o la medicación para el dolor? Por favor, rodee con un círculo el porcentaje que corresponda al grado de alivio que ha sentido.

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
Ningún alivio Alivio total

9. Rodee con un círculo el número que mejor describa hasta qué punto el dolor lo ha afectado en los siguientes aspectos de la vida, durante las últimas 24 horas:

A. Actividad en general

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

B. Estado de ánimo

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

C. Capacidad de caminar

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

D. Trabajo habitual (incluye tanto el trabajo fuera de casa como las tareas domésticas)

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

E. Relaciones con otras personas

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

F. Sueño

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

G. Disfrutar de la vida

0 1 2 3 4 5 6 7 8 9 10
No me ha afectado Me ha afectado por completo

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Appendix I: Ionis Pharmaceuticals, Inc. "Dosing and Potential Injection Site Responses" Patient Handout

Ionis Pharmaceuticals, Inc.

Volanesorsen (ISIS 304801) Dosing and Potential Injection Site Responses

Study Drug Dosing

1

Remove a pre-packaged syringe from the refrigerator and allow it to stand at room temperature for about 30 minutes but not more than 2 hours prior to injection.

Peel back the cover from the syringe packaging and remove the syringe from the tray. You will see an air bubble in the syringe. This is normal. Twist off the cap from the end of the syringe.

With the needle cap on, carefully twist the needle onto the syringe.

2

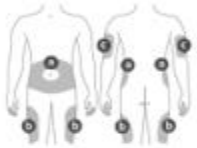
Visually inspect the liquid to verify it is clear and free of foreign particulate matter.

If the liquid is cloudy or foreign particulate matter is found, discard needle and syringe in sharps disposal container. Start again from Step 1 with a new syringe and needle.

3

Locate and cleanse chosen injection area with a fresh alcohol swab:

a) abdomen
b) thigh
c) outer area of upper arm



4

Remove needle cap just prior to dosing. Do not remove the air bubble. Gently pinch skin together at the center of the cleansed injection site. Insert needle at a 90° angle and let go of skin.

- Depress plunger slowly taking at least 15 seconds to complete the injection.
- Hold for at least 5 seconds before withdrawing the needle from the skin

5

Withdraw needle from skin. Briefly hold a cotton ball at injection site. Do not recap needle. Discard used syringe and needle into sharps disposal container.

Possible Injection Site Reactions

General Characteristics

- ☑ Pain
- ☑ Redness
- ☑ Brief muscle twitching or tightness
- ☑ Hard area
- ☑ Itchiness
- ☑ Swelling

ISRs are rarely severe and typically resolve on their own with no long-lasting effects. Improvements in ISRs may be seen with repeat injections.

Continued on reverse

Self Administration

You may be able to reduce the occurrence of ISRs by following the study drug administration instructions above and by doing the following:

- Rotate your injection sites to keep the skin healthy
- Give injections at least 1 inch (2.5 cm) apart (from the outer borders of previous injections) if administered in the same location
- Do not give injections in areas that are burned, reddened, inflamed, swollen, or have existing side effects from a prior injection in that area
- For injections in the abdomen, do not use the area within 1 inch
- (2.5 cm) of the outer border of the navel
- Avoid injecting at the waist line where pressure or rubbing may occur from your clothing

In addition, the following should be done starting with the first dose:

1. **Ice and do not disturb** the area of injection for **10 to 15 minutes before** dosing while you are in a **resting position**
2. **Pinch** the injection site area **just enough** to ensure the dose is given subcutaneously, and not as an intramuscular injection
   - Pinching as gently as possible avoids disturbances to the surrounding tissue
3. Give the injection while you are **in a resting position** that you can **remain in** for **10 to 15 minutes after the injection**
4. Ice the area of injection for **10 to 15 minutes after dosing** while you are in a **resting position**

Contact study staff if you have any questions or concerns

Site staff are trained to help answer your questions and advise on how to alleviate any discomfort, if needed.

IONIS PHARMACEUTICALS

Version 1.0: 01FEB2016

Appendix J: Labs which may be performed in the Event a Platelet Count is Less Than the Lower Limit of Normal (x 2) or < 100,000/mm3 (x 1)

ln patients who have any 2 occurrences of platelet count less than the lower limit of normal or who have any 1 occurrence of platelets <100,000/mm3

Note: The actual labs that may be performed are subject to the PI's decision and may change as we learn what the cause of the low platelets are.

To Be Performed at Local Lab
Peripheral smear (should be performed locally, fixed and sent to central lab for review) Fibrinogen split products or D-dimer on fresh blood
To Be Performed at Central Lab
Citrated sample for platelets Coagulation panel (PTI/INR, aPTT) CBC with reticulocytes
Folate (folic acid)
Vitamin B12
Fibrinogen
von Willebrand factor
Total globulins, total lgA, lgG and lgM
Complement: total C3, total C4, 8b, C5a
hsCRP
Helicobacter pylori (breath test)
Serology for:
H8V, HCV, HIV (if not done recently for screening)
Rubella
CMV
EBV
Parvo 819
Auto-antibody screen:
Antiphospholipid
Rheumatoid factor
Anti-dsDNA
Anti-thyroid
Auto-antibody screen:
Antiphospholipid
To Be Performed at Specialty Lab(s)
Antiplatelet antibodies and Anti-PF4 assay
Anti-ASO antibody

Appendix K: Single Patient Allowed to Stay on Study

This amendment (for Appendix K) is in response to the Problem Report dated 6-2-17. Briefly, a patient with partial lipodystrophy and known steatohepatitis was enrolled in the protocol with an ALT of 140. This represents a protocol deviation as the exclusion criteria state that ALT needs to be <3xULN (NIH ULN is 33U/L). The study team inadvertently missed this when enrolling the patient and were alerted to problem when reviewing her week 4 laboratory results and noted an ALT of 167.

After identifying this protocol deviation, the subject's study medication was held pending discussions with the IRB and the drug manufacturer, Ionis Pharmaceuticals. The PI spoke with the Ionis medical advisor, Andres Digenio, on 5-31-17. He affirmed that, as NIDDK is the sponsor of the study, we have the authority to allow this patient to continue participating in the study. He also affirmed that, as a representative of Ionis, he was fine with allowing the patient to continue study drug as a protocol violation. The company would not wish us to change the exclusion criteria for the study, however, as the drug is currently in development and the company wishes to avoid any ambiguity about potential liver safety signals. He affirmed that, although the drug is metabolized by the liver, there is no known safety signal for hepatoxicity with this drug. However, the drug has never been tested in patients with liver enzymes as high as this patient's.

The PI obtained expert consultation from two NIDDK hepatologists, Chris Koh (an AI on the study) and Yaron Rotman (not associated with this study). Both documented (attached to problem report) that they did not feel that this patient's liver disease, and specifically, having an ALT >3x ULN, would increase risk to this subject. The risk of idiosyncratic drug-induced liver injury (DILI) is not increased in patients with underlying liver disease, such as this patient. Patients with advanced liver disease (e.g. cirrhosis) may be at increased risk for hepatic decompensation due to DILI if it occurs. However, this patient does not have evidence of advanced liver disease (she has stable or improving ALT/AST over several years, no cirrhosis on biopsy, and normal liver synthetic function).

Therefore, given this patient's unchanged prospect of direct benefit from study participation, without any increase in risk, we plan to continue her participation in the study and resume dosing of the study drug. Because we respect the company's request to not change the overall study exclusion criteria, we will not change the ALT/AST exclusion criteria, and will not enroll additional subjects who violate these exclusion criteria.

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