Clinical Study Protocol

Title	**A 16-Week, Single-Blind Randomized, Placebo- Controlled Food Study of the Safety and Tolerability of AXA1125 and AXA1957 in Subjects with Non-Alcoholic Fatty Liver Disease (NAFLD)**
Protocol Number	AXA1125-003
Date (Version)	01April2019, Version 4.0
Sponsor Address	Axcella Health Inc. 840 memorial Drive, 3rd Floor Cambridge, MA 02139

SPONSOR PROTOCOL APPROVAL PAGE

Signature

Manu Chakravarthy, MD, PhD

Sponsor Responsible Person

Chief Medical Officer

Title

01-April-2019

Date

INVESTIGATOR'S SIGNATURE OF AGREEMENT PAGE

Protocol Title: A 16-Week, Single-Blind Randomized, Placebo- Controlled Food Study of the Safety and Tolerability of AXA1125 and AXA1957 in Subjects with Non-Alcoholic Fatty Liver Disease (NAFLD)

Protocol Number: AXA1125-003

I, the undersigned, have reviewed this protocol, including appendices and I will conduct the study as described in compliance with this protocol, Good Clinical Practice (GCP) and relevant International Council for Harmonization (ICH) guidelines.

Once the protocol has been approved by the Investigational Review Board (IRB)/Investigational Ethics Committee (IEC), I will not modify this protocol without obtaining prior approval of Axcella Health (Sponsor) and of the IRB/IEC. I will submit the protocol modifications and/or any informed consent form (ICF) modifications to Axcella Health and the IRB/IEC, and approval will be obtained before any modifications are implemented. except where necessary to eliminate an immediate hazard(s) to the study participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

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

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all Case Report Forms (CFRs), laboratory samples or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Axcella Health, to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

Principal Investigator/Title (printed name):
_____

Institution: _____

Address: _____

Telephone number:
_____

Signature: _____

Date: _____

SPONSOR CONTACT INFORMATION

Sponsor Responsible Person and Study Director:	Name: Manu Chakravarthy, MD, PHD Address: 840 Memorial Drive, 3rd floor, Cambridge, MA 02139 USA Telephone: +1 857 320 2218 E-mail: mchakravarthy@axcellahealth.com
SAE Reporting	SAE form in the EDC system **or** Medpace Clinical Safety Telephone: +1 (513) 579-9911, extension 12999 Toll Free: +1-(866)-336-0930 Fax: +1 (866) 336-5320 E-mail: Medpace-safetynotification@medpace.com *The preferred means of SAE reporting is via the eDC system, reference the Case Report Completion Guidelines for instructions*

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1 SYNOPSIS

Protocol Number	AXA1125-003
Protocol Title	A 16-Week, Single-Blind, Randomized, Placebo-Controlled Food Study of the Safety and Tolerability of AXA1125 and AXA1957 in Subjects with Non-Alcoholic Fatty Liver Disease (NAFLD)
Study Objectives	This study is intended to assess the safety and tolerability of proprietary amino acid food products, AXA1125 and AXA1957, in subjects with NAFLD.
Study Assessments	Safety and tolerability will be assessed by: • Reported clinical adverse events (AEs) • Physical examinations, including body weight • Vital sign assessments • Multiparametric magnetic resonance imaging (MRI) to characterize and quantify liver tissue (e.g. liver fat/inflammation) and body composition • Electrocardiograms (ECGs) • Clinical laboratory tests including changes in hematology, chemistry, lipid profiles, glucose homeostasis, and other blood markers of inflammation and fibrosis.
Number of Study Sites	Up to 20 sites in the United States
Study Population	Adult subjects with NAFLD
Study Food Products	AXA1125 and AXA1957 are composed of naturally occurring amino acids, which are dietary ingredients of normal food or those readily available as dietary supplements. Placebo product is calorie-, excipient-, and color-matched to AXA1125, but without any amino acids.
Number of Subjects	A sufficient number of subjects will be screened to have 105 subjects enrolled in the study, as described below. Subjects will be randomized in a 2:2:2:1 ratio to one of the following groups: • AXA1125 24g BID (n = 30) • AXA1957 20.3g BID (n = 30) • AXA1957 13.5g BID (n = 30) • Placebo 24g BID (n = 15). A randomization scheme using a stratified design in blocks of size 7 will be employed to allocate subjects evenly to the administration arms for both type 2 diabetes (T2D) subjects and non-T2D subjects.
Summary of Study Design	This is a 16-week, randomized, single-blind, placebo-controlled study to assess the food safety and tolerability of AXA1125 and AXA1957 in subjects with NAFLD.

	Subjects will sign an Informed Consent Form and be screened for eligibility per the inclusion and exclusion criteria below, up to 6 weeks before the start of the Administration Period. Note: The Screening Period can be for less than 6 weeks, i.e. subject can be randomized as soon as eligibility is confirmed. Eligible subjects will be randomized in a 2:2:2:1 ratio to receive either AXA1125 24g BID, AXA1957 20.3g BID, AXA1957 13.5g BID or placebo 24g BID. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and approximately 3-5 days prior to the Day 1 visit. Assigned study food product (AXA1125, AXA1957 or placebo) will be shipped to the clinical site upon randomization of each subject. Once randomization has occurred, subjects will present to the study site on Day 1 (Baseline/Visit 2) for their baseline assessments per the schedule of events in Table 1. Study Day 1 is the beginning of the 16-week Administration Period. Subjects will return to the study site at Week 1 (Visit 3), Week 2 (Visit 4), Week 4 (Visit 5), Week 8 (Visit 6), Week 12 (Visit 7) and Week 16 (Visit 8) to receive their study food product and/or return any unused study food product, to provide blood samples for biomarker and other laboratory testing, undergo liver imaging, and to complete other study safety assessments per the Schedule of Events in Table 1. There will be a safety Follow-up Visit, approximately 2 weeks after the last visit in the Administration Period (i.e., after the Week 16 visit or at the time of early termination) which will be the End of Study (EOS) Visit (Visit 9). There will be a total of 9 study visits, including the Screening and Follow up visits.
Study Dietary and Physical Activity Requirements	**Subjects are encouraged to maintain their usual dietary and physical activity patterns during the entire study (first visit to the last visit).** Maintenance of subjects' lifestyle regimen will be monitored via body weight assessments, recording changes in their diet, and recording changes in physical activity at every visit. The expectation is that subjects do NOT deviate from their usual baseline food intake and activity routines to maintain their body weight within 5% of their Day 1 (baseline) body weight. The primary purpose of monitoring dietary and physical activity during the study is to ensure that subjects are NOT deviating from their baseline food and physical activity patterns, such as by: initiating a new diet, introducing new meal items, taking away meal items that were previously part of their baseline diet, starting a new exercise regimen or stopping an exercise regimen that was part of their baseline activity routine. During each study visit, subjects will meet with a study dietician or other qualified study staff (e.g., Investigator, trained study nurse, etc.). The study dietician (or other qualified staff) will review any dietary or activity changes from baseline. Subjects will also be reminded to continue to adhere to their usual baseline dietary and activity patterns.

	Screening Period: During the last week of the Screening Period, and before a subject is scheduled for Day 1 procedures, subjects will be asked to keep a food and physical activity diary to record everything they typically eat and their usual physical activity patterns during that week. Subjects will be reminded to not alter their usual dietary or physical activity routines/patterns during this week. The purpose of the food and physical activity diary during the screening period is to establish a baseline for each subject's meal patterns, daily caloric intake, and physical activity regimen. The food and activity diary may be referenced during future study visits to remind/counsel subjects of their usual/baseline lifestyle routines/patterns. Subjects will also complete the hunger and satiety VAS to get acclimatized to completing the VAS worksheet. Day 1 Visit: At the Day 1 Visit, subjects will meet with a study dietician (or other qualified staff) to review the completed food and physical activity diary which will and confirm a baseline lifestyle pattern with each subject. Site staff will also ensure subjects are correctly filling out their VAS worksheets. Subjects will be reminded that they are expected to maintain their baseline lifestyle pattern for the duration of the entire study. Subjects will then be provided with a new food and physical activity diary to record their usual food and physical activity patterns between the Day 1 Visit until the Week 1 Visit. New VAS worksheets may also be provided. Week 1 Visit: At the Week 1 Visit, body weight, the food and physical activity diary, and the VAS worksheets will be reviewed with subjects in detail to ensure subjects are maintaining their baseline diet and activity patterns, and that subjects are able to complete their VAS assessments properly. If subjects experience a body weight change by more than 5% from their Day 1 baseline body weight, then the study dietician (or other qualified staff) should counsel subjects to make appropriate adjustments by reminding them of their usual (baseline) food and physical activity patterns that were established during the screening period, and the importance of adhering to the established baseline routine. Remaining Study Visits: Adherence to the maintenance of each subject's usual dietary and physical activity routines will be assessed by measuring subjects' body weight at every study visit. If subjects experience a body weight change by more than 5% from their Day 1 baseline body weight, then the study dietician (or other qualified staff) should counsel subjects to make appropriate adjustments by reminding them of their usual (baseline) food and physical activity patterns that were established during the

	screening period, and the importance of adhering to the established baseline routine.
Study Food Products	The study food products, AXA1125, AXA1957 and placebo, are provided as dry powder in stick packs. The specified number of stick packs (whether 2 or 3 or 4 stick packs, depending on the subject's group assignment) at each administration should be reconstituted in 8 oz (~240ml) of water and consumed orally immediately after mixing. Daily administration of the study food product must occur 30 ±5 minutes **before** meals twice daily (e.g., before breakfast and dinner or before lunch and dinner). The twice daily administrations should occur at least 4 hours apart. It is expected that subjects consume their total daily amount of the assigned study food product (AXA1125, AXA1957 or placebo). If subjects miss their study food product administration before a main meal, they may consume it after the meal, but must wait at least 30 minutes after the main meal to consume the study product. It is important to instruct subjects to not consume the study food product immediately before, with, or immediately after a main meal. If a subject experiences clinically significant gastrointestinal (GI) discomfort while taking their study food product prior to meals, administrations may occur approximately 30-60 minutes after the meal following consultation with the Medical Monitor and Sponsor. At any time during the study, if the Investigator determines any tolerability issues of clinical significance, the amount and/or frequency of AXA1125, AXA1957 or placebo may be reduced for that individual subject, but only after discussion with the Medical Monitor and Sponsor. Study food product compliance will be performed by study staff at each study visit.
Duration of Participation	The total duration of the study is 24 weeks (Screening through the Follow-up Visit).
Inclusion Criteria	Subjects must meet all the following criteria to be eligible to participate in the study: 1. Willing to participate in the study and provide written informed consent. 2. Male and female adults aged ≥18 years. 3. Female subjects of childbearing potential must have a negative serum beta-human chorionic gonadotropin (HCG) pregnancy test at Screening and must agree to and use an acceptable method of contraception during the entire study period and for 30 days following the last administration of study food product if they will be, or could possibly be, engaging in heterosexual intercourse. Childbearing potential refers to those female subjects who have not had a hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Female subjects are considered postmenopausal and not of child-bearing potential if they are either:

	a. >55 years of age, or b. >50 years of age with >12 months of amenorrhea, or c. have serum follicle stimulating hormone (FSH) values >40 mIU/ml with >12 months of amenorrhea 4. Subjects must <u>not</u> have participated in any diet/lifestyle intervention or observational studies, or engaged in any body weight altering regimens that resulted in body weight fluctuations (i.e. body weight loss or gain by ≥5%) in the preceding 3 months prior to Screening. 5. Historical liver biopsy that was obtained up to 6 months prior to Screening (local pathology interpretation will be used) with NAFLD Activity Score (NAS) ≥ 4 with a score of at least 1 in each of the NAS components: steatosis, ballooning degeneration, and lobular inflammation, and fibrosis stage of F2 or F3 only. **Note 1:** In order for the historical liver biopsy to qualify subjects into the study, the biopsy must have been obtained with no nonalcoholic steatohepatitis (NASH) treatment, medications that can cause NASH, or other investigational products for the treatment of NASH within 3 months prior to when the liver biopsy was obtained. **OR** <u>Each</u> of the following must be confirmed at the time of Screening: a. Fasting aspartate aminotransferase (AST) >20 IU/L, <u>and</u> b. Fibroscan conducted at Screening with Control Attenuation Parameter (CAP) ≥ 320 dB/m <u>and</u> kPa ≥ 8.0. **Note 2:** All subjects, regardless of qualifying historical liver biopsy in the last 6 months, or satisfying inclusion criteria #5a and 5b, must still meet the corrected T1 (cT1) and proton density fat fraction (PDFF) thresholds in inclusion criterion #8 below to qualify into the study. 6. Subjects may have a diagnosis of T2D, dyslipidemia, hypertension, hypothyroidism, and/or impaired glucose tolerance. If treated for these conditions, subjects must be well-controlled on a stable regimen (lifestyle or medications) for these conditions, i.e. for approximately 2 months prior to and during Screening, and anticipate no significant alterations to these regimens for the duration of the study. However, doses of certain medications [such as statins, anti-hypertensives, oral anti-diabetic drugs (OADs), etc.] to treat their stable chronic conditions may be modified during the study for tolerability or safety issues, if needed. See Section 8 below for a full list of excluded medications. **Note:** At the time of randomization, subjects will be stratified according to their T2D status to ensure subjects with T2D are evenly distributed across the four (4) administration arms.

	7. Subjects taking vitamins and/or other dietary/herbal supplements are permitted as long as ALL the following are met: a. Vitamin(s) or other supplement(s) are not on the list of prohibited medications (see Section 8), and b. Subject has been on stable doses and regimens for at least 2 months prior to and during Screening; however, vitamin E at doses ≥400 IU/day must be discontinued prior to Randomization, and c. There are no anticipated dose adjustments or changes (i.e. stopping or adding) of vitamins/supplements for the study duration. 8. Corrected T1 (cT1) ≥830 msec **and** PDFF ≥10%, using Liver *Multi*Scan MRI acquisition protocols. **Note:** The Screening MRI should NOT occur until each of the above entry criteria have been confirmed. If the Screening MRI is within approximately 14 days of Study Day 1, then the Screening MRI can serve as the Day 1/Baseline MRI. If the Screening MRI was more than approximately 14 days from Day 1, then a separate Day 1 (baseline) liver MRI scan is required.
Exclusion Criteria	Subjects are not eligible to participate in the study if any of the following criteria are met: 1. Current or history of significant alcohol consumption (>30 g/day for males & >20 g/day for females), and/or inability to reliably quantify alcohol consumption based upon judgement of the Investigator. 2. History or presence of liver disease (other than NAFLD/NASH), including but not limited to: alcoholic liver disease, Wilson's disease, hemochromatosis, primary biliary cholangitis, primary sclerosing cholangitis, history of any bile duct obstruction/biliary diversion, autoimmune hepatitis, alpha-1-anti-trypsin deficiency, known or suspected hepatocellular carcinoma, history of or planned liver transplant or current Model for End-Stage Liver Disease (MELD) score > 12. 3. Known history of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), including subjects previously or currently receiving treatment for HCV, or drug-induced liver injury/disease at the time of screening. 4. History or presence of cirrhosis on historical liver biopsy (i.e. F4 fibrosis stage) and/or history or presence of hepatic decompensation, including ascites, hepatic encephalopathy, esophageal varices, any other bleeding disorders/coagulopathies, and/or has one or more of the following laboratory abnormalities, suggestive of decompensated liver disease: a. Platelet count < 140,000/uL b. Serum albumin <3.5 g/dL c. International normalized ratio (INR) >1.3 d. Total bilirubin ≥2.0 md/dL. 5. History of Inborn Errors of Metabolism and/or genetic deficiencies that

	impact amino acid metabolism [e.g., urea cycle disorders, carnitine deficiency, carnitine palmitoyltransferase (CPT) I or II deficiency, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria, etc.] 6. Screening ALT or AST ≥ 4x upper limit of normal (ULN) or total bilirubin ≥ 2 mg/dL (unless history of Gilbert's Syndrome). However, subjects with Gilbert's syndrome with direct bilirubin above ULN in addition to total bilirubin ≥ 2 mg/dL, or with evidence of hemolysis contributing to elevated total bilirubin, should be excluded. 7. Any diabetes other than Type 2 [e.g. type 1 diabetes, Maturity onset diabetes of the young (MODY), latent autoimmune diabetes of adults (LADA), etc.]. 8. Uncontrolled type 2 diabetes is defined as either: a. HbA1c >9.5% on their current T2D regimen at Screening, or b. Requiring >10% insulin dose adjustments within approximately 2 months prior to Screening, or c. Requiring a complex OAD regimen needing 3 or more OADs to maintain glycemic control, or d. With a history of severe hypoglycemia on their anti-diabetic medical regimen(s). 9. T2D subjects maintained on thiazolidinediones (TZDs) and/or glucagon-like peptide-1 (GLP-1) analogues/GLP-1 receptor agonists, and/or prandial insulins (e.g. Lispro, Aspart, Glulisine, Regular, etc.). **Note:** Use of insulin mixtures (e.g. 70/30), NPH insulin, or other basal insulins (e.g. detemir, glargine, concentrated insulins such as U500 etc.) are permitted only if they are at stable doses and regimen for approximately 2 months prior to and during Screening, and no clinically significant dose adjustments in these insulin regimens are anticipated during the study. **Note:** See Section 8 for a complete list of contraindicated diabetes medications. 10. Uncontrolled hypertension defined as systolic blood pressure >160mmHg and/or diastolic blood pressure >100mmHg at Screening, including any history of malignant hypertension. 11. Uncontrolled lipids defined as triglycerides >500 mg/dL and/or low-density lipoprotein (LDL) >200 mg/dL at Screening. 12. Impaired renal function defined as glomerular filtration rate ≤ 60 mL/min/1.73 m^2. 13. History of clinically significant cardiovascular event within 6 months prior to Screening [includes acute cardiovascular episode, stroke, transient ischemic attack, coronary heart disease (unstable angina pectoris, myocardial infarction, revascularization procedures), clinically significant heart failure (e.g., New York Heart Association Classification II-IV or history of left

	ventricular ejection fraction <30%), and/or cardiac arrhythmias, including any clinically significant findings on the Screening ECG]. 14. History of clinically significant diseases of the GI tract, such as inflammatory bowel disease, malabsorption syndromes, chronic constipation, steatorrhea/loose stools, peptic ulcer disease. 15. Known sensitivity and/or history of clinically significant food intolerance/allergies to proteins (including whey, soy, casein, amino acids, etc.), gluten, fats, carbohydrates (e.g., lactose), nuts, or any ingredient in the study food product formulation. 16. History or presence of clinically significant pulmonary (e.g., exacerbations of asthma, emphysema, bronchitis, pneumonia, etc.), and rheumatological diseases (e.g., flares of rheumatoid or psoritric arthritis). However, subjects on stable medication/dose regimen (i.e., ≥2 months prior to and during Screening) and anticipate remaining on the same medication/dose regimen for the duration of the study (except the excluded medications listed in Section 8) can enroll into the study at the discretion of the Investigator. 17. Any active uncontrolled psychiatric disorder. Subjects who are on a stable dose(s) of anti-depressant medication(s) for at least 6 months prior to and during Screening and are considered to be psychiatrically stable in the judgement of the study investigator(s), may be enrolled. 18. Prior history of or planned (i.e., to occur during the study period) bariatric surgery; however, prior laproscopic-band, intra-gastric balloon, or other weight loss device which was removed >12 months prior to Screening, is not exclusionary. 19. Use within 2 months prior to and during Screening of systemic glucocorticoids, methotrexate, amiodarone, tamoxifen, tetracyclines, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, obeticholic acid, ursodeoxycholic acid, TZDs, GLP-1 analogues/receptor agonists, prandial insulins, anti-obesity compounds (e.g., orlistat, locaserin, Qsymia®, etc.), or any other known hepatotoxin. 20. Current use of any other excluded medications listed in Section 8 not otherwise specified in the entry criteria. 21. Current or planned use of any dietary supplement intended for general metabolic health, weight maintenance, weight loss, OR those containing proteins, amino acids, ketones, or fish oils, from the time the ICF is signed through the end of the study. **Note:** Common examples of excluded protein, amino acid, and other dietary supplements include, but are not limited to: ketones, protein powders, shakes, bars, gels containing whey, soy, casein, collagen, amino acids, and their derivatives/metabolites, N-acetylcysteine, L-carnitine, etc. A few examples of supplements used for weight loss include green tea, green coffee bean extracts, garcinia cambogia, any ketogenic products, etc.

	22. Currently on or planning to be on any extreme or unbalanced diet such as Ketogenic, Atkins, Paleo , Vegan, etc.) from the time the ICF is signed through the end of the study. 23. Female subjects who are pregnant, breast feeding, postpartum within 6 months prior to and during Screening, or planning to become pregnant during the study. 24. Male subjects with female partners of child-bearing potential who are unwilling or unable to adhere to contraception requirements. 25. Active substance use (oral, inhaled, injected, patches, etc.) within 3 months prior to and during Screening (including marijuana). 26. History of malignancy, other than non-melanoma skin cancer, within 3 years prior to Screening. 27. Any other serious medical condition with likely life expectancy < 2 years. 28. Received an investigational new drug or food/dietary supplement(s) within 1 month or 5 half-lives (whichever is longer), prior to Screening. 29. Any contraindications to a MRI scan, such as subjects with non-removable ferromagnetic implants, pacemakers, aneurysm clips or other foreign bodies, and/or inability to fit into a MRI scanner to adequately conduct the Liver *Multi*Scan assessments, and/or clinically significant claustrophobia. Subjects experiencing mild anxiety due to transient claustrophobic symptoms may be treated with anxiolytics for mitigation of those symptoms, if necessary. 30. Any other condition that, in the opinion of the Investigator, renders the subject at risk for compliance, compromises the well-being of the subject, or hinders study completion.
Statistical Methods	A stratified random sampling scheme will be used to allocate subjects in a ratio of 2:2:2:1 to AXA1125 24g BID, AXA1957 20.3g BID, AXA1957 13.5g BID or placebo 24g BID in blocks of size 7. Safety and tolerability will be evaluated using descriptive statistics and listings of AEs, physical examination, including liver imaging to assess changes in liver fat and inflammation, clinical laboratory test values, vital signs, body weight, body temperature, ECGs and other safety parameters. Analyses of AEs will be performed for those events that are considered study product-emergent, where study product-emergent is defined as any AE with onset (or worsening of a pre-existing condition) after the first administration of the study food product.

2 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BCAA	Branched-Chain Amino Acids
BMI	Body Mass Index
CRF	Case Report Form
cT1	Corrected T1
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
EOS	End of Study
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP-1	Glucagon Like Peptide 1
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
I	Isoleucine
ICH	International Council for Harmonization
INR	International Normalized Ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System
L	Leucine
MRI	Magnetic Resonance Imaging
NAC	N-acetylcysteine
NAFLD	Nonalcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic Steatohepatitis
NEFA	Non-Esterified Fatty Acids
OAD	Oral Anti-Diabetic Drug
OGTT	Oral Glucose Tolerance Test
PBMC	Peripheral Blood Mononuclear Cells
PDFF	Protein Density Fat Fraction

PE	Physical Examination
PK	Pharmacokinetic
Q	Glutamine
R	Arginine
S	Serine
SAE	Serious Adverse Event
T2D	Type 2 Diabetes
TZD	Thiazolidinediones
ULN	Upper Limit of Normal
V	Valine
VAS	Visual Analog Scale

Table 1. Schedule of Events										
Study Period	Screening	Administration Period							Follow-up	Early Term[1]
	Up to 6 weeks prior to Day 1	Day 1 (Baseline)	Week 1 +/- 2 days	Week 2 +/- 2 days	Week 4 +/- 2 days	Week 8 +/- 4 days	Week 12 +/- 3 days	Week 16 +/- 4 days	Week 18 +/- 3 days	
Informed consent	X									
Determination of study eligibility	X	X								
Demographics and Medical History	X									
Physical examination[2]	X	X				X		X	X	X
Height[3], weight, BMI, waist circumference	X	X	X	X	X	X	X	X	X	X
Pregnancy test[4]	X	X			X	X	X	X	X	X
Fasting multiparametric MRI Scan[5]	X[5]	X[5]				X[5]		X[5]		X
Fibroscan	X[14]									
HbA1c	X	X				X	X	X		X
Fasting blood samples for metabolic, inflammation and fibrosis biomarkers[6]		X			X	X	X	X		X
Fasting serum sample for OWL metabolomics		X				X		X		X
Fasting plasma for free amino acids		X				X		X		X
Fasting lipid profile[6]	X	X			X	X	X	X		X
Fasting lactate, pyruvate, glycerol, BHB, NEFA, insulin, glucose, HOMA-IR and adipo-IR		X		X	X	X	X	X		X
OGTT[7]		X				X		X		X
Vital signs[8]	X	X	X	X	X	X	X	X	X	X
Hematology, including PBMC isolation	X	X[9]		X	X	X[9]	X	X[9]	X	X
Blood chemistry panel (fasting)	X	X		X	X	X	X	X	X	X
Urinalysis		X				X		X		X

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Study Period	Screening	Administration Period							Follow-up	Early Term[1]
	Up to 6 weeks prior to Day 1	Day 1 (Baseline)	Week 1 +/- 2 days	Week 2 +/- 2 days	Week 4 +/- 2 days	Week 8 +/- 4 days	Week 12 +/- 3 days	Week 16 +/- 4 days	Week 18 +/- 3 days	
ECG	X					X		X	X	X
Randomization	X[10]									
Dispense AXA1125, AXA1957 or placebo		X	X	X	X	X	X			
Study food product accountability			X	X	X	X	X	X		X
Subject completes detailed food and physical activity diary	X[12,13]	X[12,13]								
Subject completes study food product administration diary		X	X	X	X	X	X	X		
Review of the weekly hunger and satiety VAS[11]	X[11]	X	X	X	X	X	X	X		X
Site reviews meals and physical activity pattern with subject [12]		X	X	X	X	X	X	X		X
Record adverse events	X	X	X	X	X	X	X	X	X	X
Record concomitant medications	X	X	X	X	X	X	X	X	X	X

[1]Subjects who discontinue prior to Week 16 will be asked to complete all assessments as indicated. Subjects who discontinue early and who have had a liver MRI within 1 week of the date of discontinuation will not be required to repeat the early termination MRI.

[2]Screening and Baseline (Day 1) PE will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose throat, neck, cardiovascular, musculoskeletal, and neurological systems. Subsequent PE examinations will be abbreviated and will consist of general appearance, skin, thorax/lungs, cardiovascular system, and abdomen.

[3]Height is collected only at Screening.

[4]Serum pregnancy test at Screening and urine pregnancy test on all other days.

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Study Period	Screening	Administration Period							Follow-up	Early Term[1]
	Up to 6 weeks prior to Day 1	Day 1 (Baseline)	Week 1 +/- 2 days	Week 2 +/- 2 days	Week 4 +/- 2 days	Week 8 +/- 4 days	Week 12 +/- 3 days	Week 16 +/- 4 days	Week 18 +/- 3 days	

[5]Subjects are required to be fasting for at least 4 hours prior to all MRI assessments. Multiparametric MR imaging will be conducted at the indicated time points to characterize and quantify liver tissue (e.g. liver fat/inflammation) and body composition using Liver *Multiscan.* The Screening MRI scan data will be used as the Baseline (Day 1) MRI assessment provided that the scan occurs within approximately 14 days prior to Day 1. If the Screening MRI occurs more than approximately14 days prior to Day 1, another separate MRI will be performed as part of the Day 1 procedures. However, the Day 1 (if applicable), Week 8 and Week 16 MRI must occur **before** the OGTT assessment (preferably the day prior to the OGTT).

[6]See Table 7– Clinical Laboratory Evaluations for a list of biomarkers to be collected at the indicated time points. A retain sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

[7]OGTT requires subjects to present to the clinic/study center after an overnight fast of ~10 hours (i.e. no food or drinks except for water during fast). Once at the clinic, blood samples are collected (fasted) for paired plasma glucose and insulin levels. After fasting samples are collected, 75 g of an oral glucose solution is administered at time 0, following which blood is collected for paired plasma glucose and insulin levels at time 30 (±5), 60 (±5), and 120 (±5) minutes.

[8]Vital signs include sitting systolic and diastolic blood pressure, heart rate and temperature. Respiration rate will be measured at Day 1, Week 8, Week 16 and Week 18 visits.

[9]PBMC isolation required on Day 1, Week 8 and Week 16 only.

[10]Randomization will occur after eligibility is confirmed and approximately 3-5 days prior to the Day 1 visit as assigned study food product will be shipped upon randomization of each subject.

[11]Subjects will complete a hunger and satiety VAS once during the final week of screening and then one day each week of the Administration Period (subjects may choose the day of the week the VAS is completed and it is recommended they adhere to the same day of the week for all their subsequent VAS assessments). On the days the VAS is completed subjects will fill-out the VAS worksheets in conjunction with each study food product administration that day at two timepoints: 1) immediately prior to the study food product administration; and 2) immediately prior to eating their meal where the study food product is administered. Subjects must return completed VAS worksheets for review at each study visit.

[12]During the week prior to the Day 1/Baseline Visit and for the first week of the Administration Period (i.e., from Day 1 to the Week 1 visit), subjects will keep a detailed diary of all food and beverage consumed as well as a record of their physical activity. Subjects should NOT change their usual dietary/physical activity patterns as the intent of the diary is to understand the subject's usual patterns and to establish a baseline. **If subjects experience a body weight change by more than 5% from their Day 1 baseline body weight, then the study dietician (or qualified staff) should counsel subjects to make appropriate adjustments to ensure they maintain their pre-study caloric intake and/or physical activity regimen.**

[13]The study dietician (or other qualified staff) will confirm that subjects are administering their assigned study product accurately per the group they are assigned to. Tolerability to assigned study food product should also be discussed with subjects at the Week 1 visit. Adjustments to the administration regimen can be made (e.g. for clinically significant tolerability issues) with permission from the Sponsor (See Section 7.7).

[14] A Fibroscan is required at Screening **only** for subjects who do not have qualifying historical liver biopsy results (See Inclusion criterion #5)

Abbreviations: Adiopo-IR = adipose tissue insulin resistance; BHB = beta-hydroxybutarate; BMI = body mass index; BW = body weight; cT1 = corrected T1; ECG = electrocardiogram; HbA1c = hemoglobin A1c; HOMA-IR = homeostatic model assessment of insulin resistance; MRI = magnetic resonance imaging; OGTT = oral glucose tolerance test; PBMC = peripheral blood mononuclear cell; PDFF = proton density fat fraction; VAS = visual analogue scale.

3 INTRODUCTION

3.1 Background and Rationale for the Proposed Study

Nonalcoholic fatty liver disease (NAFLD) encompasses the spectrum of steatosis, steatohepatitis, fibrosis, and cirrhosis complicated by portal hypertension and hepatocellular carcinoma (Do & Lim, 2016). NAFLD, considered the liver manifestation of the metabolic syndrome, is a major cause of liver disease worldwide. While the overall global prevalence of NAFLD is over 25% (Younossi, et al., 2016), NAFLD prevalence is 80-90% in obese adults, 30-50% in subjects with type 2 diabetes, and up to 90% in subjects with dyslipidemia (Bellentani, Scaglioni, Marino, & Bedogni, 2010). Lifestyle modification, including dietary changes and increase in physical activity to induce weight loss is recommended for these subjects as these steps may reduce liver inflammation and improve hepatic steatosis (Chalasani, Younossi, & Lavine, 2012). Currently, there is no approved pharmacological treatment for subjects with NAFLD.

Extensive prior literature suggests amino acids can have important physiological and health-related consequences. There is evidence that liver health as well as metabolism may be enhanced by specific combinations of amino acids. For example, branched-chain amino acids (BCAAs), cysteine, and arginine have been implicated in enhancing liver health [(Tajiri & Shimizu, 2013), (Khoshbaten, et al., 2010), (Kakumitsu, Shijo, & Yokoyama, 1998)]. Investigations in subjects with type 2 diabetes (T2D) showed that the usually reduced insulin response after carbohydrate ingestion can almost be tripled by co-ingestion of a free amino acid/protein mixture that contains whey protein hydrolysate, free leucine, and phenylalanine (van Loon, et al., 2003). Thus, selective supplementation of certain amino acids may have the potential to restore optimal physiological processes in the liver.

Axcella's amino acid food products, AXA1125 and AXA1957, consist of specific combinations of natural amino acids and substances that are naturally produced by the body, all of which are normal food ingredients and/or readily available as dietary supplements. The intention and objective of this study is to primarily understand the safety and tolerability profile of AXA1125 and AXA1957 when administered to subjects with NAFLD.

3.2 Rationale for the Study Design and Amounts of Study Food Product Selected

This study is primarily intended to assess the safety and tolerability profile of AXA1125 and AXA1957 in subjects with NAFLD. The study would also add to the body of knowledge regarding the role(s) of amino acids in hepatic physiology in subjects with the metabolic syndrome/NAFLD. While studies in the literature have established the safety of amino acid administration in nonhumans and humans over extended periods of time over years [(Scolapio, McGreevy, Tennyson, & Burnett, 2001), (Huynh & Tayek, 2002), (Scarna, et al., 2003), (Williams, Olivi, & Li, 2004), (Dreyer, Drummond, & Pennings, 2008), (Borsheim, Bui, & Tissier, 2009), (Jablecka, et al., 2012) (Hurt, Ebbert, & Schroeder, 2014), (Coker, Deutz, & Schutzler, 2015), (McClure, Baker, & Gipson, 2015)], the effect of these amino acids especially as it pertains to hepatic structure and function remains poorly understood. Hence, a 16-week administration period is proposed, a duration that should not only provide sufficient exposure to robustly assess the safety and tolerability profile of the AXA1125 and AXA1957 food products, but also to study the physiological changes in liver structure and function over this duration.

The amount of amino acids to be administered in this study are within the known, well-tolerated ranges for individual or mixtures of amino acids administered to humans in several published studies, including repeated administrations in people with T2D and/or metabolic syndrome (see Table 2 below). These amounts are also similar to readily available over-the-counter amino acid/dietary supplement formulations.

Table 2. Summary of Studies Supporting the Administration of the Components within the AXA1125 and AXA1957 Food Products

Components within AXA1125 and/or AXA1957	Daily amounts within AXA1125	Daily amounts within AXA1957	Amounts /duration used in relevant prior clinical studies	Summary of safety/tolerability
Leucine (L), Isoleucine (I), Valine (V)	8g/day of L, 4g/day each of I & V; 12 g of BCAA total	9g/day of L & I at a 2:1 ratio (i.e. 6g L & 3g I per day total); 0g V	- 12g/day of L/I/V at 2:1:1 ratio → 6/3/3 g/day each of LIV in patients with liver cirrhosis over a 2-year period (Muto, et al., 2005) - 30g/day of oral BCAA (13.5g L, 9g I) in patients with cirrhosis and prior hepatic encephalopathy over 56 weeks (NCT00955500)	- Adverse events reported in 12% of BCAA cohort in Muto study primarily gastrointestinal problems such as abdominal distension, diarrhea and constipation (Muto, et al., 2005) - No safety/tolerability reported in NCT009555000
Arginine (R)	12 g/day (given as 14.6 g R-HCl)	8 g/day (given as 9.67g R-HCl)	- 9g/day R given to patients with T2D for 3 months to study microcirculation (NCT00902616) - 9g/day R given to T2D patients for 1 month to improve peripheral and hepatic insulin sensitivity (Piatti, et al., 2001)	- No safety/tolerability reported in NCT00902616 or Piatti et al, 2001
Glutamine (Q)	16g/day	4 g/day	- 15g/day for 8 weeks used to treat patients with Irritable Bowel Syndrome (TID as 5g/dose) (NCT01414244)	- No AEs reported in the study
N-acetylcysteine (NAC)	1.2 g/day	2.6 g/day	- NAC improves liver function in patients with non-alcoholic steatohepatitis (NASH) at 1.2 g/day for 3 months (Khoshbaten, Aliasgarzadeh, & Masnadi, 2010) - 3-4g/day for 14 days in mild traumatic brain injury (Hoffer, Balaban, Slade, Tsao, & Hoffer, 2013)	- No AEs in patients with NASH - NAC produced no side effects in subjects with blast-induced traumatic brain injury

Table 2. Summary of Studies Supporting the Administration of the Components within the AXA1125 and AXA1957 Food Products

Components within AXA1125 and/or AXA1957	Daily amounts within AXA1125	Daily amounts within AXA1957	Amounts /duration used in relevant prior clinical studies	Summary of safety/tolerability
L-carnitine	--	2 g/day	– 2g/day supplement added to diet for 24 weeks improved liver function and histological manifestations of NASH (Malaguarnera, et al., 2010)	– Carnitine was well tolerated in all patients, with mild AEs reported in 5/36 subjects: 1 nausea, 2 headaches, 2 abdominal pain (Malaguarnera, et al., 2010)
Serine (S)	--	15 g/day	– 20g/day S was administered for 14 days in NAFLD patients (Mardinoglu, et al., 2017)	– No AEs reported, S was well tolerated

In addition, the amounts selected in this study are also directly informed by completed/ongoing clinical studies and preclinical work conducted by the Sponsor with these food products.

The sponsor has conducted both preclinical and clinical pharmacokinetic (PK) studies in healthy humans with AXA1125. These studies confirm a well-behaved PK profile for each of the amino acid components within AXA1125, with a Tmax of approximately 1-1.5 hrs and t1/2 of approximately 3-4 hrs with dose-proportionality in both AUC and Cmax. A 6-week open-label safety and tolerability study of AXA1125 24 g TID (i.e. 72 g/day) in healthy human volunteers was recently completed and demonstrated that AXA1125 was generally well tolerated with no clinically significant safety signals. Three (3) of the 10 subjects enrolled reported a total of 4 mild adverse events (AEs): diarrhea in 1 subject, abdominal cramping in 1 subject and abdominal cramping and abdominal bloating in another subject. All AEs were "possibly related" to study product by the Investigator, and were mild, transient, self-resolving, and all subjects remained in the study to completion.

Additionally, a 12-week study of AXA1125 24g TID (i.e. 72 g/day) in subjects with T2D and NAFLD is currently ongoing with 32 subjects enrolled. Of the 32 enrolled subjects, 9 subjects terminated the study early, with 6 withdrawals due to AEs, 2 due to withdrawal of consent and 1 lost to follow-up. The AEs leading to discontinuation were: stomach flu, diarrhea (2 subjects), right toe infection, cholecystitis and uncontrolled diabetes. Twenty-one (21) of the enrolled subjects reported at least one AE. Most of the reported AEs were mild to moderate intensity and "Unlikely" or "Not Related" to AXA1125. Two of the reported AEs-- right toe infection and cholecystitis--were considered serious adverse events (SAEs) due to hospitalization for these events. The AEs that were considered by the study Investigators to be "Possibly" or "Definitely Related" to AXA1125 are summarized in Table 3 below.

Table 3. Summary of Related/Possibly Related Adverse Events from Study AXA1125-002			
Adverse event	**Number of AEs Reported**	**Number of Subjects Reporting the AE**	**Number of Subjects Withdrawing Due to AE**
Cholecystitis*	2	1	1
Diarrhea	7	6	2
Frequent urination	1	1	0
Headache	3	3	0
Heartburn	1	1	0
Toe infection*	1	1	1
*These AEs were considered "related" or "possibly related" by the study Investigator. After evaluation of the individual clinical cases for these events in consultation with the Investigator and the study Medical Monitor, the Sponsor considers these events to be "not reasonably associated" with AXA1125 administration because these are relatively common complications of T2D/obesity/poor glycemic control, and therefore, not an unexpected finding given these subjects' medical history of long standing diabetes.			

With regards to other safety assessments in the AXA1125-002 study, no clinically meaningful changes in electrocardiograms (ECGs), vital signs or safety laboratory assessments were observed. There were no clinically significant ECG results. Of the 104 ECG recordings to date, 39 recordings were considered abnormal but not clinically significant. No AEs were reported for ECG findings. Two AEs of isolated elevated blood pressure were reported by 2 subjects. Both events were considered unrelated to AXA1125 administration and resolved without additional changes to their medical regimen. With regards to clinical laboratory assessments, one subject experience elevated creatine kinase (CK) that was considered to be clinically significant and was reported as an AE unrelated to AXA1125 administration. The CK returned to normal within a few days and was thought to be a result of excess physical activity.

In summary, AXA1125 administered up to 72g/day has been generally well-tolerated in both healthy subjects and in subjects with T2D and NAFLD for up to 12 weeks.

While AXA1957 has not been previously studied in humans, the composition and ratios of the constituents of AXA1957 are either identical to or highly similar to that of AXA1125. Leucine, isoleucine, arginine, glutamine and N-acetylcysteine (NAC) are present in both AXA1125 and AXA1957. All of these constituents within AXA1957 (except NAC) are either in similar or **lower** amounts than administered previously with AXA1125 in prior studies. NAC has been administered to subjects with NAFLD at amounts of 1.2g/day for 12 weeks in the AXA1125-002 study, to NASH patients at 1.2g/day for 3 months ((Khoshbaten, et al., 2010) and to individuals with mild traumatic brain injury at 4 g/day (Hoffer, Balaban, Slade, Tsao, & Hoffer, 2013). No AEs were reported in the Khoshbaten and Hoffer studies using NAC at these levels. Thus, the amount of NAC administered within the food products in this study (AXA1957 and AXA1125) is anticipated to be safe and well-tolerated based on prior Sponsor and published experience with NAC.

For those constituents that are specific to AXA1957 (i.e. L-carnitine and serine), these supplements have also been studied previously in similar populations (NAFLD and NASH patients; Malaguarnera, et al., 2010 and Mardinoglu, et al., 2017). Proposed amounts of these consituents within AXA1957 are either similar or lower than those used safely in prior studies. L-carnitine 2 g/day (similar amount to that present in AXA1957) has been administered for 24 weeks in biopsy-proven NASH subjects and shown to be generally safe and well-tolerated (Malaguarnera, et al., 2010). Serine administered at 20 g/day (higher amount than present in AXA1957) was provided to NAFLD subjects for 2 weeks with no AEs (Mardinoglu, et al., 2017).

Separately, the Sponsor has also completed studies with other similar amino acid compositions with much higher levels of BCAAs (i.e. 20 g/day vs. 9 or 12g/day in AXA1957 and AXA1125, respectively) in more compromised subjects with liver disease, such as those with mild to moderate hepatic insufficiency (Childs-Pugh A & B) and demonstrated a well-tolerated and safe profile.

Based on the cumulative data accumulated to date on these food products by the Sponsor and the extensive published literature [(Scolapio, McGreevy, Tennyson, & Burnett, 2001), (Huynh & Tayek, 2002), (Scarna, et al., 2003), (Williams, Olivi, & Li, 2004), (Dreyer, Drummond, & Pennings, 2008), (Borsheim, Bui, & Tissier, 2009), (Jablecka, et al., 2012) (Hurt, Ebbert, & Schroeder, 2014), (Coker, Deutz, & Schutzler, 2015), (McClure, Baker, & Gipson, 2015); see Table 2 above], on both specific amino acids as well as mixtures of amino acids across a variety of subject populations and disease conditions, it is anticipated that the safety and tolerability profile of AXA1957 would be similar to, or better, than that observed in prior studies. Importantly, the total daily amounts of AXA1957 proposed in this study (i.e. 13.5 g or 20.3 g BID) are lower than that of AXA1125 (24 g BID), further bolstering the expectation of a favourable safety and tolerability profile with AXA1957.

4 OBJECTIVES

4.1 Study Objective

This study is intended to assess the safety and tolerability of proprietary amino acid food products, AXA1125 and AXA1957, in subjects with NAFLD.

4.2 Study Assessments

Safety and tolerability will be assessed by:

- Reported clinical AEs
- Physical examinations, including body weight
- Vital sign assessments
- Multiparametric magnetic resonance imaging (MRI) to characterize and quantify liver tissue (e.g. liver fat/inflammation) and body composition
- ECGs
- Clinical laboratory tests including changes in hematology, chemistry, lipid profiles, glucose homeostasis, and other blood markers of inflammation and fibrosis.

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design and Plan

This is a 16-week, single-blind, randomized, placebo-controlled food study of the safety and tolerability of AXA1125 and AXA1957 in subjects with NAFLD.

Subjects will sign an Informed Consent Form and be screened for eligibility per the inclusion and exclusion criteria below, up to 6 weeks before the start of the Administration Period. Note: Screening Period can be for less than 6 weeks, i.e. subject can be randomized as soon as eligibility is confirmed.

Eligible subjects will be randomized in a 2:2:2:1 ratio to receive either AXA1125 24g BID, AXA1957 20.3g BID, AXA1957 13.5g BID or placebo 24g BID. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and approximately 3-5 days prior to the Day 1 visit. Assigned study food product (AXA1125, AXA1957 or placebo) will be shipped to the clinical site upon randomization of each subject.

Once randomization has occurred, subjects will present to the study site on Day 1 (Baseline/Visit 2) for their baseline assessments per the schedule of events in Table 1. Study Day 1 is the beginning of the 16-week Administration Period.

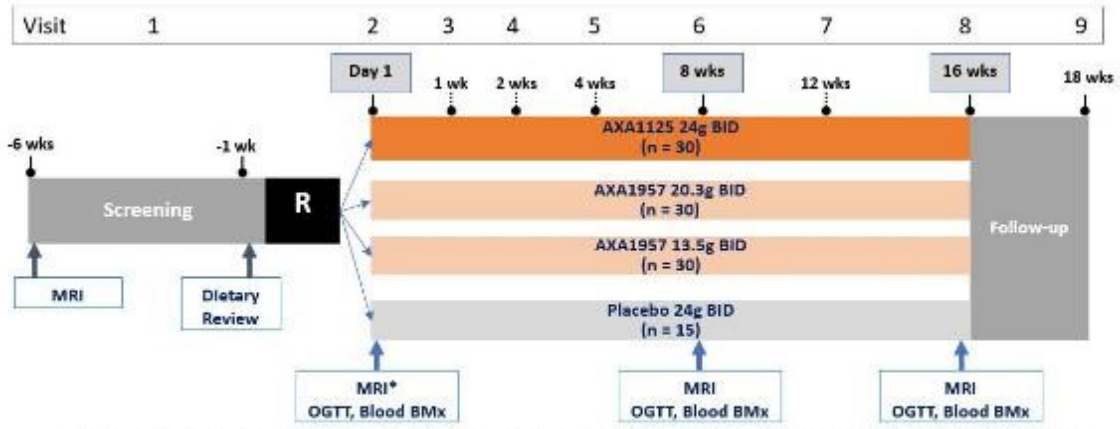
Subjects will return to the study site at Week 1 (Visit 3), Week 2 (Visit 4), Week 4 (Visit 5), Week 8 (Visit 6), Week 12 (Visit 7) and Week 16 (Visit 8) to receive their study food product and/or return any unused study food product, to provide blood samples for biomarker and other laboratory testing, undergo liver imaging, and to complete other study safety assessments per the Schedule of Events in Table 1.

There will be a safety Follow-up Visit, approximately 2 weeks after the last visit in the Administration Period (i.e., after the Week 16 visit or at the time of early termination) which will be the End of Study (EOS) Visit (Visit 9).

There will be a total of 9 study visits, including the Screening and Follow up visits.

A diagram of the study design is provided in Figure 1 below and schedule of assessments is presented in Table 1.

Figure 1. Study Design



*Screening MRI will be used as the Baseline MRI assessment if the Screening MRI occurs within approximately 14 days prior to Day 1.
MRI = magnetic resonance imaging; OGTT = oral glucose tolerance test; BMx = biomarkers

5.1.1 Screening

Following completion of informed consent procedures, subjects will enter the Screening Period. All screening assessments should be conducted, and eligibility confirmed, within 6 weeks prior to Day 1.

During the last week of the Screening Period, and before the subject is scheduled for Day 1 procedures, subjects will be asked to keep a food and physical activity diary to record everything they eat and their usual physical activity patterns during that week. Subjects should not alter their usual dietary or physical activity pattern during that week, nor during the course of the entire study. The purpose of the 1-week diary is to get a baseline assessment of the subject's meal patterns, daily caloric intake and physical activity regimen.

5.1.2 Randomization

Following completion of all Screening procedures, eligible subjects will be randomized in a 2:2:2:1 ratio to receive either AXA1125 24g BID, AXA1957 20.3g, BID AXA1957 13.5g BID or placebo 24g BID using a stratified randomization in blocks of size 7, with type 2 diabetes as the stratification factor. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and approximately 3-5 days prior to the Day 1 visit. Assigned study food product (AXA1125, AXA1957 or placebo) will be shipped to the clinical site upon randomization of each subject.

5.1.3 Administration Period

Once randomization has occurred, subjects will present to the study site on Day 1 (Baseline/Visit 2) for their baseline assessments per the schedule of events in Table 1. Study Day 1 is the beginning of the 16-week Administration Period.

Subjects will return to the study site at Week 1 (visit 3), Week 2 (visit 4), Week 4 (visit 5), Week 8 (visit 6), Week 12 (visit 7) and Week 16 (visit 8) to receive their study food product and/or return any unused product, to provide blood samples for biomarker and other laboratory testing, undergo liver imaging, and to complete other study safety assessments, including the submission/review of their daily/weekly study product/diet/activity compliance diaries and VAS, as per the Schedule of Events in Table 1.

5.1.4 Follow Up

There will be a safety Follow-up Visit, approximately 2 weeks after the last visit in the Administration Period (i.e., after the Week 16 visit or at the time of early termination) which will be the End of Study (EOS) Visit (Visit 9).

5.1.5 Early Discontinuation

Subjects who prematurely discontinue for any reason will be asked to return to the clinical site for a Follow-up Visit following the last active study day. Week 16 study procedures will be conducted at the Follow-up Visit for Early Discontinuations.

5.1.6 Study Dietary and Physical Activity Requirements

Subjects are encouraged to maintain their usual dietary and physical activity patterns during the entire study (first visit to the last visit).

Maintenance of subjects' lifestyle regimen will be monitored via body weight assessments, recording changes in their diet, and recording changes in physical activity at every visit. The expectation is that subjects do NOT deviate from their usual baseline food intake and activity routines to maintain their body weight within 5% of their Day 1 (baseline) body weight. The primary purpose of monitoring dietary and

physical activity during the study is to ensure that subjects are NOT deviating from their baseline food and physical activity patterns, such as by: initiating a new diet, introducing new meal items, taking away meal items that were previously part of their baseline diet, starting a new exercise regimen or stopping an exercise regimen that was part of their baseline activity routine.

During each study visit, subjects will meet with a study dietician or other qualified study staff (e.g., Investigator, trained study nurse, etc.). The study dietician (or other qualified staff) will review any dietary or activity changes from baseline. Subjects will also be reminded to continue to adhere to their usual baseline dietary and activity patterns.

6 SELECTION OF STUDY POPULATION

Subjects who meet inclusion criteria and do not meet any of the exclusion criteria will be eligible for enrollment into the study.

6.1 Inclusion Criteria

Subjects must meet all the following criteria to be eligible to participate in the study:

1. Willing to participate in the study and provide written informed consent.
2. Male and female adults aged ≥18 years.
3. Female subjects of childbearing potential must have a negative serum beta-human chorionic gonadotropin (HCG) pregnancy test at Screening and must agree to and use an acceptable method of contraception during the entire study period and for 30 days following the last administration of study food product if they will be, or could possibly be, engaging in heterosexual intercourse. Childbearing potential refers to those female subjects who have not had a hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Female subjects are considered postmenopausal and not of child-bearing potential if they are either:
    a. >55 years of age, or
    b. >50 years of age with >12 months of amenorrhea, or
    c. have serum follicle stimulating hormone (FSH) values >40 mIU/ml with >12 months of amenorrhea
4. Subjects must <u>not</u> have participated in any diet/lifestyle intervention or observational studies, or engaged in any body weight altering regimens that resulted in body weight fluctuations (i.e. body weight loss or gain by ≥5%) in the preceding 3 months prior to Screening.
5. Historical liver biopsy that was obtained up to 6 months prior to Screening (local pathology interpretation will be used) with NAFLD Activity Score (NAS) ≥ 4 with a score of at least 1 in each of the NAS components: steatosis, ballooning degeneration, and lobular inflammation, and fibrosis stage of F2 or F3 only.

    Note 1: In order for the historical liver biopsy to qualify subjects into the study, the biopsy must have been obtained with no nonalcoholic steatohepatitis (NASH) treatment, medications that can cause NASH, or other investigational products for the treatment of NASH within 3 months prior to when the liver biopsy was obtained.

    OR

Each of the following must be confirmed at the time of Screening:

a. Fasting aspartate aminotransferase (AST) >20 IU/L, and

b. Fibroscan conducted at Screening with Control Attenuation Parameter (CAP) ≥ 320 dB/m and kPa ≥ 8.0, and

Note 2: All subjects, regardless of qualifying historical liver biopsy in the last 6 months, or satisfying inclusion criteria #5a, and 5b must still meet the corrected T1 (cT1) and proton density fat fraction (PDFF) thresholds in inclusion criteria #8 below to qualify into the study.

6. Subjects may have a diagnosis of T2D, dyslipidemia, hypertension, hypothyroidism, and/or impaired glucose tolerance. If treated for these conditions, subjects must be well-controlled on a stable regimen (lifestyle or medications) for these conditions, i.e. for approximately 2 months prior to and during Screening, and anticipate no significant alterations to these regimens for the duration of the study. However, doses of certain medications [such as statins, anti-hypertensives, oral anti-diabetic drugs (OADs), etc.] to treat their stable chronic conditions may be modified during the study for tolerability or safety issues, if needed.

   See Section 8 below for a full list of excluded medications.

   Note: At the time of randomization, subjects will be stratified according to their T2D status to ensure subjects with T2D are evenly distributed across the four (4) administration arms.

7. Subjects taking vitamins and/or other dietary/herbal supplements are permitted as long as ALL the following are met:
   a. Vitamin(s) or other supplement(s) are not on the list of prohibited medications (see Section 8), and
   b. Subject has been on stable doses and regimens for at least 2 months prior to and during Screening; however, vitamin E at doses ≥400 IU/day must be discontinued prior to Randomization, and
   c. There are no anticipated dose adjustments or changes (i.e. stopping or adding) of vitamins/supplements for the study duration.
8. Corrected T1 (cT1) ≥830 msec **and** PDFF ≥10%, using Liver *Multi*Scan MRI acquisition protocols.

   Note: The Screening MRI should NOT occur until each of the above entry criteria have been confirmed. If the Screening MRI is within approximately 14 days of Study Day 1, then the Screening MRI can serve as the Day 1/Baseline MRI. If the Screening MRI was more than approximately 14 days from Day 1, then a separate Day 1 (baseline) liver MRI scan is required.

6.2 Exclusion Criteria

Subjects are not eligible to participate in the study if any of the following criteria are met:

1. Current or history of significant alcohol consumption (>30 g/day for males & >20 g/day for females), and/or inability to reliably quantify alcohol consumption based upon judgement of the Investigator.
2. History or presence of liver disease (other than NAFLD/NASH), including but not limited to: alcoholic liver disease, Wilson's disease, hemochromatosis, primary biliarycholangitis, primary sclerosing cholangitis, history of any bile duct obstruction/biliary diversion, autoimmune hepatitis, alpha-1-anti-trypsin deficiency, known or suspected hepatocellular carcinoma, history of or planned liver transplant or current Model for End-Stage Liver Disease (MELD) score > 12.
3. Known history of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), including subjects previously or currently receiving treatment for HCV, or drug-induced liver

injury/disease at the time of screening.

4. History or presence of cirrhosis on historical liver biopsy (i.e. F4 fibrosis stage) and/or history or presence of hepatic decompensation, including ascites, hepatic encephalopathy, esophageal varices, any other bleeding disorders/coagulopathies, and/or has one or more of the following laboratory abnormalities, suggestive of decompensated liver disease:
    a. Platelet count < 140,000/uL
    b. Serum albumin <3.5 g/dL
    c. International normalized ratio (INR) >1.3
    d. Total bilirubin ≥2.0 md/dL.
5. History of Inborn Errors of Metabolism and/or genetic deficiencies that impact amino acid metabolism [e.g., urea cycle disorders, carnitine deficiency, carnitine palmitoyltransferase (CPT) I or II deficiency, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria, etc.]
6. Screening ALT or AST ≥ 4x upper limit of normal (ULN) or total bilirubin ≥ 2 mg/dL (unless history of Gilbert's Syndrome). However, subjects with Gilbert's syndrome with direct bilirubin above ULN in addition to total bilirubin ≥ 2 mg/dL, or with evidence of hemolysis contributing to elevated total bilirubin, should be excluded.
7. Any diabetes other than Type 2 [e.g. type 1 diabetes, Maturity onset diabetes of the young (MODY), latent autoimmune diabetes of adults (LADA), etc.].
8. Uncontrolled type 2 diabetes is defined as either:
    a. HbA1c >9.5% on their current T2D regimen at Screening, or
    b. Requiring >10% insulin dose adjustments within approximately 2 months prior to Screening, or
    c. Requiring a complex OAD regimen needing 3 or more OADs to maintain glycemic control, or
    d. With a history of severe hypoglycemia on their anti-diabetic medical regimen(s).
9. T2D subjects maintained on thiazolidinediones (TZDs) and/or glucagon-like peptide-1 (GLP-1) analogues/GLP-1 receptor agonists, and/or prandial insulins (e.g. Lispro, Aspart, Glulisine, Regular, etc.).

    Note: Use of insulin mixtures (e.g. 70/30), NPH insulin, or other basal insulins (e.g. detemir, glargine, concentrated insulins such as U500 etc.) are permitted only if they are at stable doses and regimen for approximately 2 months prior to and during Screening, and no clinically significant dose adjustments in these insulin regimens are anticipated during the study.

    Note: See Section 8 for a complete list of contraindicated diabetes medications.
10. Uncontrolled hypertension defined as systolic blood pressure >160mmHg and/or diastolic blood pressure >100mmHg at Screening, including any history of malignant hypertension.
11. Uncontrolled lipids defined as triglycerides >500 mg/dL and/or low-density lipoprotein (LDL) >200 mg/dL at Screening.
12. Impaired renal function defined as glomerular filtration rate ≤ 60 mL/min/1.73 m^2.
13. History of clinically significant cardiovascular event within 6 months prior to Screening [includes acute cardiovascular episode, stroke, transient ischemic attack, coronary heart disease (unstable angina pectoris, myocardial infarction, revascularization procedures), clinically significant heart failure,

history of left ventricular ejection fraction of <30%, and/or cardiac arrhythmias, including any clinically significant findings on the Screening ECG].

14. History of clinically significant diseases of the GI tract, such as inflammatory bowel disease, malabsorption syndromes, chronic constipation, steatorrhea/loose stools, peptic ulcer disease.
15. Known sensitivity and/or history of clinically significant food intolerance/allergies to proteins (including whey soy, casein, amino acids, etc.), gluten, fats, carbohydrates (e.g., lactose), nuts or any ingredient in the study food product formulation.
16. History or presence of clinically significant pulmonary (e.g., exacerbations of asthma, emphysema, bronchitis, pneumonia, etc.), and rheumatological diseases (e.g., flares of rheumatoid or psoritric arthritis). However, subjects on stable medication/dose regimen (i.e. ≥2 months prior to and during Screening) and anticipate remaining on the same medication/dose regimen for the duration of the study (except the excluded medications listed in Section 8) can enroll into the study at the discretion of the Investigator.
17. Any active uncontrolled psychiatric disorder. Subjects who are on a stable dose(s) of anti-depressant medication(s) for at least 6 months prior to and during Screening and are considered to be psychiatrically stable in the judgement of the study investigator(s), may be enrolled.
18. Prior history of or planned (i.e., to occur during the study period) bariatric surgery; however, prior laproscopic-band, intra-gastric balloon, or other weight loss device which was removed >12 months prior to Screening, is not exclusionary.
19. Use within 2 months prior to and during Screening of systemic glucocorticoids, methotrexate, amiodarone, tamoxifen, tetracyclines, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, obeticholic acid, ursodeoxycholic acid, TZDs, GLP-1 analogues/receptor agonists, prandial insulins, anti-obesity compounds (e.g., orlistat, locaserin, Qsymia®, etc.), or any other known hepatotoxin.
20. Current use of any other excluded medications listed in Section 8 not otherwise specified in the entry criteria.
21. Current or planned use of any dietary supplement intended for general metabolic health, weight maintenance, weight loss, OR those containing proteins, amino acids, ketones, or fish oils, from the time the ICF is signed through the end of the study.

    Note: Common examples of excluded protein, amino acid, and other dietary supplements include, but are not limited to: ketones, protein powders, shakes, bars, gels containing whey, soy, casein, collagen, amino acids, and their derivatives/metabolites, N-acetylcysteine, L-carnitine, etc. A few examples of supplements used for weight loss include green tea, green coffee bean extracts, garcinia cambogia, any ketogenic products, etc.
22. Currently on or planning to be on any extreme or unbalanced diet such as Ketogenic, Atkins, Paleo , Vegan, etc.) from the time the ICF is signed through the end of the study.
23. Female subjects who are pregnant, breast feeding, postpartum within 6 months prior to and during Screening, or planning to become pregnant during the study.
24. Male subjects with female partners of child-bearing potential who are unwilling or unable to adhere to contraception requirements.
25. Active substance use (oral, inhaled, injected, patches, etc.) within 3 months prior to and during Screening (including marijuana).
26. History of malignancy, other than non-melanoma skin cancer, within 3 years prior to Screening.

27. Any other serious medical condition with likely life expectancy <2 years.
28. Received an investigational new drug or food/dietary supplement(s) within 1 month or 5 half-lives (whichever is longer), prior to Screening.
29. Any contraindications to a MRI scan, such as subjects with non-removable ferromagnetic implants, pacemakers, aneurysm clips or other foreign bodies, and/or inability to fit into a MRI scanner to adequately conduct the Liver *Multi*Scan assessments, and/or clinically significant claustrophobia. Subjects experiencing mild anxiety due to transient claustrophobic symptoms may be treated with anxiolytics for mitigation of those symptoms, if necessary.
30. Any other condition that, in the opinion of the Investigator, renders the subject at risk for compliance, compromises the well-being of the subject, or hinders study completion.

6.3 Number of Subjects Planned

A sufficient number of subjects will be screened to have 105 subjects enrolled in the study, as described below. Subjects will be randomized using a stratified design in blocks of size 7 in a 2:2:2:1 ratio to one of the following groups:

- AXA1125 24g BID (n = 30)
- AXA1957 20.3g BID (n = 30)
- AXA1957 13.5g BID (n = 30)
- Placebo 24g BID (n = 15).

This stratified design will allow for the even allocation of T2D and non-T2D subject to the four administration arms.

6.4 Removal of Subjects from the Study

A subject may be discontinued from the study at any time if the subject, the Investigator, or the Sponsor believes that it is not in the subject's best interest to continue. The following is a list of potential reasons for discontinuation from the study:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Subject experiences an AE that, in the opinion of the Investigator, would be in the best interest of the subject to discontinue
- Pregnancy
- Protocol violation
- Lost to follow-up
- Sponsor request for early termination of the study.

If a subject is withdrawn due to an AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue early from the study should come in for an early discontinuation visit (i.e. same assessments as the last study visit, as well as any others that are clinically indicated) as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents for early termination procedures.

Subjects who withdraw from the study may be replaced.

7 STUDY FOOD PRODUCT INFORMATION

7.1 Study Food Product Composition and Formulation Overview

Axcella will supply AXA1125, AXA1957 and placebo packaged in individual dry powder stick packs. AXA1125 and AXA1957 are mixtures of selected amino acids that are normally present in regular food and/or naturally produced by the body and/or readily available as dietary supplements and formulated as dry powder that will be reconstituted with water to form an orange flavored drink, which must be consumed orally immediately after mixing the product. The composition of AXA1125 with the total amount of amino acids contained in each stick pack is presented in Table 4 and the composition of AXA1957 is presented in Table 5.

Table 4. Amino Acid Composition of AXA1125	
Leucine	1.00g
Isoleucine	0.50g
Valine	0.50g
Arginine	1.81g
Glutamine	2.00g
n-acetyl cysteine	0.15g

Table 5. Amino Acid Composition of AXA1957	
Leucine	1.00g
Isoleucine	0.50g
Arginine HCl	1.61g
Glutamine	0.67g
Serine	2.50g
Carnitine	0.33g
n-acetyl cysteine	0.43g

7.2 Study Food Product Administration

During the 16-week Administration Period, subjects will consume their assigned study food product as instructed. The specified number of stick packs (e.g., 2 or 3 or 4 stick packs, depending on the subject's group assignment) are administered orally by reconstituting the study food product in 8 oz (~240 ml) of water and consuming immediately after mixing. Daily administration of the study food products must occur 30 ±5 minutes **before** meals twice daily (e.g., before breakfast and dinner or before lunch and dinner).

The twice daily administrations should occur at least 4 hours apart.

It is expected that subjects consume their total daily amount of the assigned study food product (AXA1125, AXA1957 or placebo). If subjects miss their assigned study food product (AXA1125, AXA1957 or placebo) administration before a main meal, they may consume the study food product after the meal, but must wait at least 30 minutes AFTER the main meal to consume the study product. It is important to instruct subjects to not consume the study food product immediately before, with, or immediately after a main meal.

If subjects experience clinically significant gastrointestinal (GI) discomfort while taking the study food product prior to meals, administrations may occur approximately 30-60 minutes after the meal following consultation with the Medical Monitor and Sponsor. At any time during the study, if the Investigator determines any tolerability issues of clinical significance, the amount and/or frequency of AXA1125, AXA1957 or placebo may be reduced, but only after discussion with the Medical Monitor and Sponsor.

Study food product preparation and administration instructions, as well as a daily schedule for study product administration, are provided in Table 6 below.

Table 6. Study Food Product Preparation and Administration Instructions				
Administration Group	**AXA1125 24g BID**	**AXA1957 20.3g BID**	**AXA1957 13.5g BID**	**Placebo 24g BID**
Formulation	Powder reconstituted in water to form an orange-flavored drink.			
Total daily Amount	48g of amino acids	40.6g of amino acids	27g of amino acids	48g of placebo
Preparation Instructions	Reconstitute **4 stick packs** in 8oz of water by mixing or shaking for at least 30 sec before consumption.	Reconstitute **3 stick packs** in 8oz of water by mixing or shaking for at least 30 sec before consumption.	Reconstitute **2 stick packs** in 8oz of water by mixing or shaking for at least 30 sec before consumption.	Reconstitute **4 stick packs** in 8oz of water by mixing or shaking for at least 30 sec before consumption.
Administration Instructions	Subjects will be provided with a 250mL Nalgene™ wide-mouth HDPE bottles with a screw-top lid (Thermo Scientific™ catalog #332189-0008), or similar container, that may be used for preparing and administering their assigned study food product. Subjects must drink all of their assigned study food product immediately after mixing with 8 oz of water. Administer reconstituted stick packs two times a day 30 ±5 minutes **before** meals twice daily (e.g., before breakfast and dinner or before lunch and dinner); The twice daily administrations should occur at least 4 hours apart. If the subject experiences GI discomfort taking their assigned product prior to meals, administrations may occur 30-60 minutes after the meal following consultation with the Medical Monitor and Sponsor. If the subject skips breakfast, the study food product must be consumed 30 ±5 minutes before lunch. The assigned study food product must not be consumed immediately before, with or immediately after a main meal.			
Missed Administrations	If subjects miss their administration **before** their main meal, subjects must consume the study food product at least 30-60 minutes **after** their main meal.			

7.3 Study Food Product Packaging, Labeling and Storage

AXA1125, AXA1957 and placebo will be supplied as pre-weighed powder in stick packs that will be reconstituted with water.

The individual labelled stick pack units are packaged into a secondary carton, containing a total of forty-two (42) stick packs per carton. The stick packs and exterior of the carton are labelled in accordance with local and national requirements, if applicable. The carton is sealed with tamper resistant tape.

The AXA1125, AXA1957 and placebo study products should be stored at 15-25°C (ambient temperature). The products are shipped at ambient temperature.

7.4 Method of Assigning Study Product to Subjects

Subjects will be screened sequentially and assigned a 5-digit subject number (3-digit site #- 2-digit subject #), as noted in the example below:

Site #001: 001-01, 001-02, 001-03 …

Site #002: 002-01, 002-02, 002-03 …

.
.
.
Site #017: 017-01, 017-02, 017-03 …

Randomization to one of 4 study food product groups will occur via an IWRS.

Randomization must occur approximately 3-5 days prior to the Day 1 visit; the assigned study food product will be shipped to the study site after randomization occurs.

7.5 Accountability, Disposal, Return, or Retention of Unused Study Product

The site personnel will document receipt of study product from the sponsor or its designee and preparation of the product for administration to subjects. Product administration and returns will be documented on product accountability log(s).

The site should maintain all unused product containers (cartons and stick packs) until final review of accountability is conducted by the study monitor, and instructions regarding return or disposal, as applicable, are provided.

7.6 Study Blinding

This is a single-blind, placebo-controlled study. Subjects will be blinded as to subject assignment to receive AXA1125, AXA1957 or placebo. Site personnel dispensing the product to subjects for administration and sponsor will be unblinded to subject assignment.

7.7 Modification of Study Food Product Administration Amount for Tolerability

At any time during the study, if the Investigator determines any tolerability issues of clinical significance, the amount and/or frequency of AXA1125, AXA1957 or placebo may be reduced after discussion with the Sponsor.

7.8 Compliance with Study Food Product Administration

Subjects will be asked to return the cartons and any remaining stick packs at each visit. Compliance will be assessed by reviewing the number of returned stick packs, if any, at each visit. Any apparent discrepancies between quantity of stick packs returned and the number anticipated based on the administration schedule will be discussed with the subject to ensure an understanding of the administration instructions. Subjects will also record the amount of the product consumed in a daily administration diary. Repeated non-compliance with administration instructions may necessitate discontinuation from the study, based on the Investigator's and/or Sponsor's judgment.

8 CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

8.1 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

If changes in antidiabetic medications are required during the study, the Investigator should discuss with study Medical Monitor and Sponsor whether the subjects should continue in the study or be withdrawn.

8.2 Prohibited Medication and Therapy

Concomitant use of the following is <u>prohibited</u> during the study:

- All prandial insulins (e.g. Lispro, Aspart, Glulisine, Regular, etc.)

  Note: Use of insulin mixtures (e.g. 70/30), NPH insulin, or other basal insulins (e.g. detemir, glargine, concentrated insulins such as U500 etc.) are permitted only if they are at stable doses and regimen for approximately 2 months prior to Screening, and no clinically significant dose adjustments in these insulin regimens are anticipated during the study duration.
- Systemic glucocorticoids
- Amiodarone (even if it was used ≥2 months prior to Screening)
- Methotrexate
- Tetracyclines
- Tamoxifen
- Estrogens at doses greater than those used for hormone replacement or contraceptives
- Anabolic steroids, including testosterone (patches, gels, injections)

- Valproic acid
- Obeticholic acid (OCA)
- Ursodeoxycholic acid (Ursodiol® and Urso®)
- Anti-obesity medications and dietary supplements for weight loss (e.g., orlistat, locaserin, Qsymia, green tea extract, green coffee bean extract, garcinia cambogia, any ketogenic products, etc.)
- Dietary supplements containing ketones, fish oils, protein, and amino acids including, but not limited to: protein powders, shakes, bars, and gels containing whey, soy, casein, collagen, amino acids or their derivatives/metabolites such as N-acetylcysteine, L-carnitine, and any ketogenic products.
- Vitamin E ≥400 IU/day
- GLP-1 analogs (e.g. exenatide, Byetta) or GLP-1 receptor agonists (e.g. liraglutide, dulaglutide, semaglutide)
- TZDs (e.g. pioglitazone, rosiglitazone)
- Other known hepatotoxins (acetaminophen is permitted as long as the dose is <1000mg/day)
- Any other investigational product.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy. The Medical Monitor, in consultation with the Study Sponsor, will make the final determination of which subjects are enrolled in the study.

9 STUDY ASSESSMENTS AND PROCEDURES

The schedule of assessments is summarized in Table 1. A description of study procedures and assessments is provided below.

9.1 Baseline and Disease Characteristics

Details regarding medical history will be collected during Screening. Prior and concomitant medications will also be recorded during this time.

9.2 Physical Examination, Vital Signs and Body Weight

Screening and Day 1/Baseline physical examination (PE) will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose throat, neck, and cardiovascular, musculoskeletal, and neurological systems. Subsequent PE examinations will be abbreviated and will consist of general appearance, skin, thorax/lungs, cardiovascular system, and abdomen. Physical examination findings will be documented in the patient's source documents. Any new PE finding that represents a new abnormal finding or a worsening from Baseline condition will be recorded as an AE.

Vital sign measurements include blood pressure, pulse, body temperature, and body weight and will be measured at every study visit. Vital signs should be conducted after subjects have been sitting quietly in a resting position for at least 5 minutes. If needed, blood pressure at screening may be re-tested two additional times at ≥15 minute increments to confirm eligibility.

Height will only be collected at Screening. Waist circumference will be measured at every study visit. Respiration rate will be measured at the Baseline/Day 1, Week 8, Week 16 and Week 18 visits.

9.3 12-Lead Electrocardiogram

An ECG will be performed using validated machinery available locally to each clinical site. The ECG reports will be reviewed by the Investigator or qualified sub-Investigator and assessed as (i) normal, (ii) abnormal – not clinically significant, or (iii) abnormal clinically significant. ECGs will be performed at Screening and at the Week 8, Week 16 and Week 18 visits.

9.4 Hepatic Magnetic Resonance Imaging

Subjects will undergo multiparametric MRI examination up to 4 times during the study to characterize and quantify liver tissue (e.g. liver fat/inflammation) and body composition, as detailed in Table 1. Subjects are required to be fasting for at least 4 hours prior to all MRI scans. Scans should be scheduled whenever possible at roughly the same time of the day to reduce diurnal fluctuation in daily liver lipid levels.

Subjects will have a Screening MRI scan in order to qualify for the study (see Inclusion Criteria #8). The Screening MRI should occur after all other entry criteria have been confirmed. The Screening MRI may be used as the Baseline/Day 1 MRI scan provided that the Screening MRI scan occurs within approximately 14 days prior to the Baseline/Day 1 Visit. If the Screening MRI occurs more than approximately 14 days prior to Day 1, another MRI will be performed as part of the Day 1 procedures to serve as the Baseline MRI assessment; however, subjects will qualify for the study based on the Screening MRI results. For subjects who have a Screening MRI scan that occurs more than approximately 14 days prior to Baseline, the Baseline scan must occur up to approximately 14 days before the first administration of study food product commences.

All MRI scans (Day 1, Week 8 and Week 16) must occur prior to the oral glucose tolerance test (OGTT), preferably the day **before** the OGTT assessment.

Details of the imaging procedure will be described in the Imaging Review Charter and Imaging Guidelines.

9.5 Fibroscan Assessment

A Fibroscan assessment measuring hepatic steatosis (via controlled attenuation parameter, CAP) and stiffness (kPa, via transient elastography) is required at Screening only for those subjects who do not have a qualifying historical liver biopsy.

9.6 Oral Glucose Tolerance Test

Subjects will undergo a 2-hour OGTT at Baseline (Day 1), Week 8 and Week 16 to assess changes in glucose homeostasis.

The OGTT will be performed in the morning in the fasted state, following an overnight fast of at least 10 hours by administering a 75g glucose solution that will be consumed within 5 minutes. A blood sample for measurement of plasma glucose and insulin measurements will be collected immediately prior to administration of the glucose solution (i.e., at time 0), and then at 30min (+/- 5 min), 60 min (+/- 5 min), and 120 min (+/- 5 min) following glucose administration.

On study days when the OGTT is performed, the morning administration of the subject's assigned study product and any medications taken for T2D (if applicable) should not be administered until the OGTT has been completed, i.e. the first administration of study food product should occur after the OGTT (e.g., at lunch) and diabetes medications taken according to doctor's orders for a missed or late dose.

9.7 Clinical Laboratory Assessments

Blood samples for clinical laboratory evaluations include hematology, fasting blood chemistry, fasting lipid panel, markers of liver inflammation, apoptosis, and fibrosis, insulin, free fatty acids/non-esterified fatty acids (NEFA), beta-hydroxybutyrate, free amino acids, glucose, etc. The complete list of laboratory analytes is available in Table 7 below. A retain sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

Samples will be analyzed at a central laboratory. Laboratory reports will be reviewed by the Investigator or designee and filed in the source document. Clinical laboratory findings that represent a worsening from a baseline value and are considered by the Investigator to be clinically significant will be recorded as an AE.

Table 7. Clinical Laboratory Evaluations

Chemistry (fasted)	Hematology (CBC)	Lipid panel (fasted)	Metabolites (fasted)	Metabolic Panel (fasted)	Biomarkers (fasted)	Biomarkers (fasted)	Other
• Albumin • Alkaline phosphatase (ALP) • ALT • AST • Blood urea nitrogen (BUN) • Calcium • Chloride • Creatinine • Gamma-glutamyl transferase (GGT) • Glucose (serum) • Insulin (serum) • Phosphorus • Potassium • Sodium • Bilirubin (total) • Bilirubin (direct) • Total CO2 (measured as bicarbonate) • Total protein • Uric acid • eGFR	• Hematocrit • Hemoglobin • Mean corpuscular hemoglobin (MCH) • Mean corpuscular hemoglobin concentration (MCHC) • Mean corpuscular volume (MCV) • Platelet count • Red blood cell count • White blood cell (WBC) count • WBC differential (% & absolute) • Basophils • Eosinophils • Lymphocytes • Monocytes • Neutrophils • PBMC isolation • Prothrombin time/INR	• Total cholesterol • High-density lipoprotein cholesterol (HDL-C) • Low-density lipoprotein (LDL-C) • Triglycerides • Non-HDL-C • Apolipoprotein B (ApoB) • Apolipoprotein CIII (Apo-CIII) • Lipoprotein a [Lp(a)]	• Lactate • Glycerol • Pyruvate	• Insulin (plasma) • Glucose (plasma) • Beta-hydroxybutyrate • Non-esterified fatty acids (NEFA) • Free amino acids • Hemoglobin A1c (HbA1c) • Homeostasis model assessment of insulin resistance (HOMA-IR; calculated) • Adipose tissue insulin resistance (Adipo-IR; calculated) • OWL NASH Index	• Adiponectin • FGF-21 • IL-1beta • High sensitivity C-reactive protein (hsCRP) • MCP-1 • CK-18 (M30 and M65) • YKL-40 • Alpha-2 macroglobulin, • Enhanced liver fibrosis (ELF) Score (TIMP-1, PIIINP & hyaluronic acid)	• N-terminal fragment of type III collagen (ProC3) • Internal epitope in the 7S domain of type IV collagen (ProC4) • Released N-terminal pro-peptide of type VI collagen (ProC6) • Internal epitope in the N-terminal pro-peptide of type 1 collagen (P1NP)	• Serum pregnancy test for Women of Childbearing Potential at Screening; urine pregnancy test for all other days. • FSH*

Table 7. Clinical Laboratory Evaluations	
Urinalysis	• pH • Specific gravity • Protein • Glucose • Ketones • Bilirubin • Blood • Nitrate • Urobilinogen • Leukocyte esterase

*FSH per Inclusion #3c will be tested at Screening in women considered postmenopausal (regardless of age) with amenorrhea >12 months.

9.8 Contraception and Pregnancy

No human studies of the effects of AXA1125 and AXA1957 on conception, pregnancy, or lactation have been performed. Therefore, females should not be exposed to the product if pregnant, breastfeeding, or attempting to conceive.

To prevent pregnancy subjects must follow the guidelines for acceptable contraception (See Table 8) from the time the ICF is signed until 30 days after the last study food product administration **unless** they meet any of the following criteria:

- Practice true abstinence for at least 90 days prior to screening and throughout the study (including 30 days after the study). *Periodic abstinence (e.g., calendar, ovulation, symptothermal, etc. methods) are NOT acceptable*.
- Men with documented confirmed infertility
- Women are considered postmenopausal and not of child-bearing potential if they are >55 years of age, or >50 years of age with >12 months of amenorrhea, or have serum follicle stimulating hormone (FSH) values >40 mIU/ml with >12 months of amenorrhea
- Women with documented confirmed hysterectomy or bilateral oophorectomy/salpingo-oophorectomy

Table 8. Acceptable Methods of Contraception

	Male Subject with Female (non-study) of Child Bearing Potential	**Female Subject with Male (non-study) Partners of Child Bearing Potential**
Vasectomy at performed >6 months from screening	Yes	Yes
Bilateral tubal occlusion/ligation performed >6 months prior to screening	Yes	Yes
Male or Female condom with spermicide	Yes	Yes
Female barrier contraception (e.g., diaphragm, cervical cap or sponge) with spermicide	Yes	Yes
Hormonal contraceptive use for at least 60 days prior to screening	Yes	Yes
Intrauterine device > 90 days prior to screening	Yes	Yes

*Male and female condom use is prohibited due to potential for breakage.

Pregnancy of a female partner of a male subject must be reported to the Investigator, and, in turn, the pregnancy must be reported to the Sponsor or its representative within 24 hours of the Investigator's awareness of the pregnancy.

In the case of pregnancy of a female subject in the study, AXA1125, AXA1957 or placebo should be discontinued and the Sponsor informed immediately. Follow-up of the pregnancy will occur until the outcome is available, including premature termination. The follow-up period will be deemed to have ended when the health status of the child has been determined upon birth.

9.9 Food and Activity Diaries & Dietary Recommendations

Subjects are encouraged to maintain their usual dietary and physical activity patterns during the entire study (first visit to their last visit). Subjects' diets and physical activity will be monitored during the study visits with an expectation that subjects do not change their body weight by more than 5%.

9.9.1 Study Dietary and Physical Activity Requirements: Screening Period

During the last week of the Screening Period, and before a subject is scheduled for Day 1 procedures, subjects will be asked to keep a food and physical activity diary to record everything they typically eat and their usual physical activity patterns during that week. Subjects will be reminded that they are not to alter their usual dietary or physical activity routines/patterns during that week. The purpose of the food and physical activity diary during the screening period is to establish a baseline for each subject's meal patterns, daily caloric intake, and physical activity regimen, such that any deviations noted later in the study could be referenced back to this baseline pattern to remind/counsel subjects of their usual lifestyle routines/patterns. Subjects will also complete the hunger and satiety VAS to get acclimatized to completing the VAS.

9.9.2 Study Dietary and Physical Activity Requirements: Day 1 Visit

At the Day 1 Visit, subjects will meet with a study dietician (or other qualified staff) to review the completed food and physical activity diary which will and confirm a baseline lifestyle pattern with each subject. Site staff will also ensure subjects are correctly filling out their VAS worksheets. Subjects will be reminded that they are expected to maintain their baseline lifestyle pattern for the duration of the entire study. Subjects will then be provided with a new food and physical activity diary to record their usual food and physical activity patterns between the Day 1 Visit until the Week 1 Visit. New VAS worksheets may also be provided.

9.9.3 Study Dietary and Physical Activity Requirements: Week 1 Visit

At the Week 1 Visit, body weight, food and physical activity diary, including the VAS worksheets, will be reviewed with the subject in detail to ensure compliance with the preceding counseling, and ensure subjects are able to complete their VAS assessments properly. If subjects experience a body weight change by more than 5% from their Day 1 baseline body weight, then the study dietician (or other qualified staff) should counsel subjects to make appropriate adjustments by reminding subjects of their usual (pre-study) food and physical activity patterns that were established during the screening period, and the importance of adhering to the established baseline routine.

9.9.4 Study Dietary and Physical Activity Requirements: Remaining Study Visits:

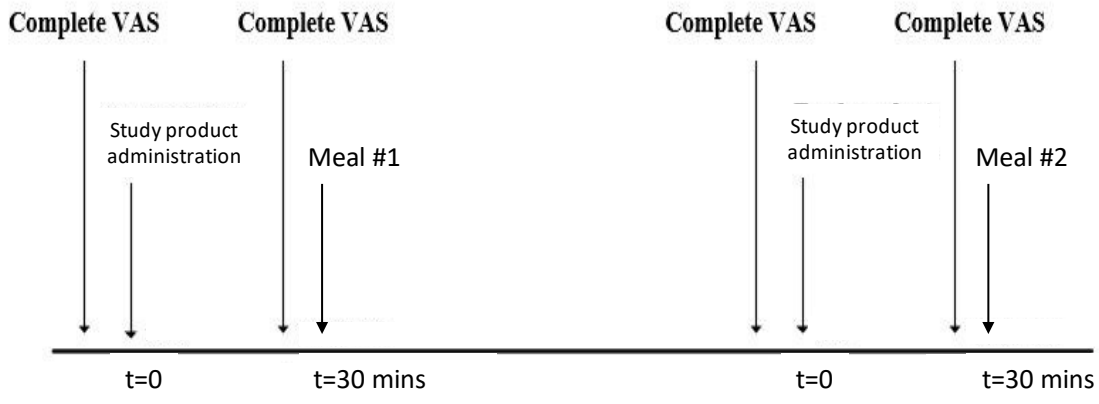
Adherence to the maintenance of each subject's usual lifestyle routines will be assessed by weighing subjects at every study visit. If subjects experience a body weight change by more than 5% from their Day 1 baseline body weight, then the study dietician (or other qualified staff) should counsel subjects to make appropriate adjustments by reminding subjects of their usual (pre-study) food and physical activity patterns there were established during the screening period, and the importance of adhering to the established baseline routine.

9.10 Hunger and Satiety Visual Analog Scale

In the week prior to the Day 1 visit, subjects will be asked to complete the hunger and satiety VAS to get them familiarized with the VAS self-assessment. Subjects will be asked several questions about hunger and fullness (satiety) and activity by marking on a scale associated with each question. Each of the questions in the VAS includes a scale that is 100 mm long. The study staff will measure the distance (in mm) from the left to right side of the scale, to the line that the subject marked as their answer. This length (in mm) is the score for that question.

Starting on Day 1, and then one day each week of the Administration Period subjects will complete the VAS; subjects may choose the day of the week the VAS is completed. On the days the VAS is completed, subjects will fill-out the VAS worksheets in conjunction with each study food product administration for that day (e.g., morning and evening) at two timepoints: 1) immediately prior to the study food product administration; and 2) immediately prior to eating the meal where the study food product is administered (see Figure 2). For consistency, subjects should be instructed to try to complete the VAS on the same day of the week throughout the study. Subjects must return completed VAS worksheets for review at each study visit.

Figure 2: Timing of Visual Analog Scale Completion



9.11 Study Food Product Administration Diary

Subjects will be provided a diary to self-record study food product administration. Subjects will be required to complete the diary daily and return the diary to the site during each study visit for the site staff to review.

10 ADVERSE EVENTS AND SAFETY REPORTING

The Investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol.

10.1 Definitions

10.1.1 Adverse Events

An AE is any untoward medical occurrence associated with the use of a study product or with study participation, regardless of the relationship of the occurrence to study product or protocol. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the product, whether or not considered related to the product. An AE can arise from any use of the product, and from any route of administration, formulation or dose, including an overdose.

From the time of informed consent up to first administration of study product on Day 1, any untoward medical occurrence considered related to study procedures will be recorded as an AE. Adverse events that occur from the first administration of study product on Day 1 through the Follow-up Visit will be considered treatment-emergent AEs.

For each AE, start date, stop date, causality (relationship to study product), action taken, outcome, and severity will be recorded in the source document and on the case report form (CRF).

10.1.2 Serious Adverse Event Definition

An SAE is any untoward medical occurrence at any dose that results in one or more of the following:

- Results in death
- Is life-threatening (at risk of death at the time of the event)
- Requires subject hospitalization or prolongation of existing hospitalization
- NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered to be an AE.
- Results in disability/incapacity
- NOTE: The term disability is defined as a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All SAEs must be reported to the Sponsor or designee within 24 hours of the site's awareness of the event. All available information should be included in the initial report. Additional follow-up information should also be reported within 24 hours.

10.1.3 Adverse Reaction and Suspected Adverse Reaction

All noxious and unintended responses to the study product related to any amount administered should be considered adverse reactions. Suspected adverse reactions are any AEs for which there is a reasonable possibility that the product caused the AE.

AEs associated with the use of study product outside what is described in the protocol, including misuse and abuse of the product, are considered adverse reactions.

10.1.4 Causal Relationship Assessment

The Investigator is required to provide an assessment of relationship of AEs and SAEs to study product or protocol procedures, if applicable. To promote consistency, the following guidelines should be taken into consideration, along with good clinical and scientific judgement, when determining the relationship of AEs to study products:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the study product administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study product should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the study product administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the withdrawal of the study product may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to the study product administration, and which other drugs or chemicals or underlying disease provide plausible explanations.

10.2 Categorization of Adverse Events

The intensity of an AE will be categorized as follows:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with daily activity.
Severe:	Severe events are those which incapacitate and prevent usual activity.

10.3 Recording and Reporting of All Non-Serious and Serious Adverse Events

Subjects will be required to report any AE that occur after informed consent is signed.

All AEs will be recorded from the time of informed consent until the last follow-up visit/call. Subjects will be instructed to report all AEs and will be asked a general health status question at each study visit.

An AE should be followed until it is either resolved, has returned to baseline, or is determined to be a stable or chronic condition.

If a non-serious AE is ongoing at the follow-up visit/call, the AE will be recorded as ongoing. AEs that are ongoing at the follow-up visit will be followed to resolution at the discretion of the Investigator.

At each study visit, all AEs that have occurred since the previous visit must be recorded. The Investigator or appropriate designee must determine the intensity of the AE.

Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually.

All study product-related AEs/SAEs will be followed to resolution (the subject's health has returned to his/her baseline status or all variables have returned to normal), or until an outcome is reached, stabilization occurs (the Investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained, regardless of whether the subject is still participating in the study. Where appropriate, medical tests and examinations will be performed to document resolution of the event(s).

10.4 Serious Adverse Event Reporting Procedures

All SAEs, from the signing of informed consent through the last follow-up visit, will be reported to the Sponsor or its representative, within 24 hours of the Investigator's first knowledge of the event, even if the event does not appear to be related to the study product or the protocol.

The initial SAE report must be as complete as possible, including details of the current illness and (serious) AE, and an assessment of the causal relationship between the event and the food product. Information not available at the time of the initial report (e.g., an end date for the event, laboratory values received after the initial report, or hospital discharge summary) must be documented on a follow-up report. All follow-up information must be reported in the same timelines as the initial report.

At any time after completion of the AE reporting period, if an Investigator becomes aware of an SAE that is suspected by the Investigator to be related to the study product, the event must be reported to the Sponsor or its representative.

The following information is a minimum set of information required for all initial SAE reports:

- Investigator name
- Subject identifiers
- AE term(s)
- Suspect study product
- Study product event relationship
- Reason why the event is serious.

SAEs will be entered in the electronic data capture (EDC) system; the project contact for SAE receipt will be notified via the EDC system upon entry of an SAE in the system. In circumstances where the EDC system is not available, notification by telephone is acceptable (See Section 10.4.1). For telephone reports, the SAE must be entered into the EDC system as soon as the system is available for data entry.

The site will notify the sponsor or its representative of additional information or follow-up to an initial SAE Report as soon as relevant information is available and within 24 hours of awareness. Follow-up information is reported in the EDC system.

10.4.1 Safety/SAE Reporting Contact Information

The preferred means of SAE reporting is via the eDC system, reference the Case Report Completion Guidelines for instructions. Initial SAE reporting may also be communicated to Medpace Clinical Safety utilizing the contact information below.

Medpace Clinical Safety
Telephone: +1 (513) 579-9911, extension 12999
Toll Free: +1-(866)-336-0930
Fax: +1 (866) 336-5320
E-mail: Medpace-safetynotification@medpace.com

10.5 Data and Safety Monitoring Committee

There will not be an independent Data and Safety Monitoring Committee established for this study. Investigators will monitor all data for safety and protection of subjects.

10.6 Reporting to IRBs and Ethics Committees

The Sponsor or its designee is responsible for notifying the investigational sites of all expedited SAEs.

The Investigator will notify institutional review boards (IRBs) or Ethics Committees (ECs) of serious, related AE(s) or significant risks to subjects, per local country requirements. The Investigator must keep copies of all AE information, including correspondence with the Sponsor, IRBs and ECs on file. It is the responsibility of the Principal Investigator to notify the IRB/EC of all SAEs that occur at his or her site.

11 STATISTICAL ANALYSIS

A statistical analysis plan describing the specific analyses to be performed for this study will be completed prior to database lock.

11.1 Sample Size

It is anticipated to enroll and administer study product to approximately 105 subjects in a 2:2:2:1 stratified randomization schema (AXA1125 24g BID, AXA1957 20.3g BID, AXA1957 13.5g BID and placebo 24g BID, respectively) using blocks of size 7. Approximately 90 subjects will receive active study product and approximately 15 subjects will receive placebo. This study is exploratory in nature, and the sample size is based on clinical judgement that this number of subjects will be sufficient to provide a characterization of the product safety. As this is food product study, no formal sample size calculations will be conducted.

11.2 Analysis Sets

The analysis set for the primary outcome variable will include any subject who receives at least 1 administration of the assigned study product. This will be noted as the Safety analysis set. Any subject who is screened but who does not receive study product will not be included in study reporting.

11.2.1 Safety Analyses

Safety and tolerability will be evaluated using descriptive statistics and listings of AEs, clinical safety laboratory test values, information from liver MRI exams, vital signs, body weight, ECG, and other safety parameters.

Analyses of AEs will be performed for those events that are considered product-emergent, where product emergent is defined as any AE with onset (or any changes in a pre-existing condition) after the first administration of the test product. All efforts will be made to record start and stop dates for AEs. Adverse events with partial dates will be assessed using the available date information to determine product-emergent status. Adverse events with completely missing dates will be assumed to be product-emergent.

Adverse events will be summarized by administration group using subject incidence rates. Therefore, in any tabulation, a subject contributes only once to the count for a given AE (preferred term). Separate tabulations will be produced for all product-emergent AEs, product-related AEs (those considered by the Investigator as possibly study product related), SAEs, and discontinuations due to AEs. By subject listings will be provided for any deaths, SAEs, and AEs leading to discontinuation of administration. If the number of AEs seen on the study is very small, listings only will be produced.

11.3 Interim Analysis

No interim analysis is planned.

12 ETHICS AND ADMINISTRATIVE DETAILS

12.1 Ethics

This study is to be performed in accordance with the protocol, the Declaration of Helsinki, the International Council for Harmonization (ICH) Harmonized Tripartite Guideline for Good Clinical Practice (GCP), and all applicable local regulations.

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a subject's name to a subject identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by health authorities. The Investigator must also comply with all applicable privacy regulations.

The protocol and consent form will be reviewed and approved by the IRB/EC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/EC in accordance with the standard operating procedures and policies of the IRB/EC, and the Investigator will keep the IRB/EC informed as to the progress of the study. The Investigator will obtain assurance of IRB/EC compliance with regulations.

Any documents that the IRB/EC may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning subject recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/EC. The IRB/EC's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/EC's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/EC approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/EC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/EC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB/EC; new information that may affect adversely the safety of the subjects of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

All study findings and documents will be regarded as confidential. Subject confidentiality will be strictly maintained to the extent possible under law. Subjects will be identified in the research records, case report forms and other documents submitted to the Sponsor or its designated representative, by their assigned subject number. Documents that identify the subject (e.g. the signed informed consent form) should not be submitted to the Sponsor or its designated representative, and must be maintained in confidence by the Investigator.

12.2 Informed Consent

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, the EU Data Protection Directive 95/46/EC and local regulations.

The Investigator will prepare the informed consent form and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/EC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/EC. The written consent document will embody the elements of informed consent as described in the ICH GCP E6 guideline and will also comply with local regulations. The Investigator will send an IRB/EC-approved copy of the informed consent form to the Sponsor or designee for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the informed consent form, a legal representative may sign for the subject. A copy of the signed consent form will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

12.3 Administrative Details

12.3.1 Monitoring

The Sponsor or its representative (e.g., clinical research associate) may conduct a study center visit to verify the qualifications of each Investigator, inspect study center facilities, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study conduct and documentation.

Throughout the study, the monitor will make study center visits to discuss the progress of the study with the Investigator or his/her representatives, review the informed consent forms, review the CRFs for completeness and accuracy, review protocol compliance, compare CRFs and individual subject's medical records and source documentation, assess product accountability, and ensure that the study is being conducted according to pertinent regulations and GCP. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

Monitoring visits will be conducted by the Sponsor or its representatives according to ICH GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor or designee, and appropriate authorities, to conduct on-site monitoring and/or auditing of all appropriate study documentation.

12.3.2 Recording, Access and Retention of Source Data

The Investigator must make study data and documentation accessible to the monitor, and other authorized representatives of the Sponsor (or designee) and IRB/ECs upon request.

A file for each subject must be maintained that includes the signed informed consent form and copies of all source documentation related to that subject. The Investigator must ensure the reliability, integrity and availability of source documents from which the information on the CRF was derived.

All study documents (subject files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of 7 years following the completion of the study unless there is prior written agreement with the Sponsor. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

12.3.3 Study termination

If the Sponsor or Investigator discovers conditions arising during the study that suggest the study should be halted, then this can happen only after appropriate consultation between the Sponsor and Investigator. Conditions that may warrant study termination include, but are not limited to:

- The discovery of any unexpected, significant, or unacceptable risk to the subjects enrolled in the study.
- Failure of the Investigator to enter subjects at an acceptable rate.
- Unsatisfactory subject enrollment with respect to quality and/or quantity or data recording is inaccurate and/or incomplete on a chronic basis.
- Insufficient adherence to the protocol requirements.

- A decision on the part of the Sponsor to suspend or discontinue development of study product.

12.3.4 Protocol Violations

A protocol violation occurs when the subject, Investigator or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with GCP guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be recorded and reviewed with the Investigator.

12.3.5 Data Quality Assurance

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study product.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific CRFs when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee) but will be identified by subject number and initials.

If a correction is required for a CRF, the time and date will be recorded by the person updating CRF data to create an audit trail. The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

The data will be entered into a validated database. A Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

12.3.6 Case Report Form

An electronic data capture (EDC) system will be used in the study.

12.3.7 Publication

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws.

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