16.1.9 Documentation of Statistical Methods

This appendix includes the following statistical analysis plans:

AXA1957-002 SAP Final, Version 1.0, 25 November 2020

AXA1957-002 SAP Final, Version 2.0, 04 February 2021

Statistical Analysis Plan

Axcella Health Inc.

AXA1957-002

A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver

Protocol Version: 13-Dec-2019

Sponsor: Axcella Health Inc.
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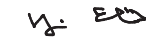
Version	Date
Draft 0.1	15 Jul 2019
Draft 0.2	26 Aug 2019
Draft 0.3	04 Sep 2019
Final 1.0	25 Nov 2020

Approval

Upon review of this document, including the table, listing, and figure shells, the undersigned approves the statistical analysis plan. The analysis methods and data presentation are acceptable.

Signature	Date
Signature:  Electronically signed by: Hongxia Yan Reason: I am the author of this document Date: Nov 25, 2020 16:21 EST Email: Hongxia.Yan@synteract.com Title: Biostatistician II Hongxia Yan Biostatistician II Synteract	
Signature:  Electronically signed by: Eunsil Yim Reason: I am the approver of this document Date: Nov 25, 2020 16:20 EST Email: euns.yim@gmail.com Title: Statistical Consultant Eunsil Yim Statistical Consultant Axcella Health Inc	

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LIST OF ABBREVIATIONS

Abbreviation	Full Notation
AE	Adverse Event
ATC	Anatomical/Therapeutic/Chemical
BMI	Body Mass Index
bpm	Beats Per Minute
CSR	Clinical Study Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ICH	International Council for Harmonisation
IWRS	Interactive web response system
MedDRA	Medical Dictionary For Regulatory Activities
MRI	Magnetic Resonance Imaging
PT	Preferred Term
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
VAS	Visual Analogue Scale
WHO DDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

This document outlines the statistical methods to be implemented during the analyses of data collected within the scope of Axcella Health Inc. protocol AXA1957-002 [A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver]. The purpose of this plan is to provide specific guidelines from which the statistical analyses will proceed. As the study was terminated early, no interium analyses and physiological effects analyses will be conducted, and a reducted scope of the analysis will be included in this plan. Any deviations from this plan will be documented in the clinical study report (CSR).

2 STUDY DOCUMENTS

The following study documents are used for the preparation of the statistical analysis plan (SAP):

- Protocol version 3, 13-Dec-2019
- Annotated electronic case report form (eCRF), version 3.0, 13-Feb-2020
- Data management plan v1.0, 09Aug2019

3 STUDY OBJECTIVES

Safety and tolerability will be assessed by:

- Reported clinical adverse events (AEs)
- Physical examinations, including changes in body weight and body composition such as lean mass and fat mass
- Vital sign assessments
- Electrocardiograms (ECGs)
- Clinical laboratory tests including changes in standard hematology, chemistry, and lipid panels

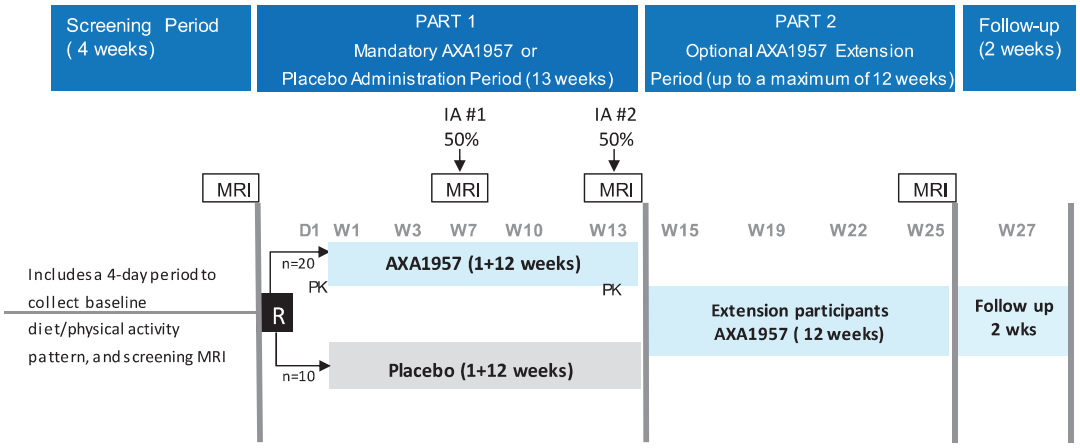
Physiological effects on liver structure and function will be assessed by:

- Multiparametric magnetic resonance imaging (MRI) assessments of liver structure (fat content and inflammation changes)
- Blood tests of liver function, including markers of inflammation and fibrosis

4 STUDY DESIGN AND PLAN

This is a randomized, placebo-controlled study conducted in 2 study parts: a mandatory 13-wk administration period (Study Part 1) and an optional extension period up to a maximum of 12 additional weeks (Study Part 2); see study schematic as Figure 1 below.

Figure 1: Study Schematic



Total duration of the study from Screening to the end of Part 2 follow-up is anticipated to be approximately 31 weeks. It is anticipated that there may be a total of up to 12 study visits during the entire study (Screening and Parts 1 and 2).

In Part 1, following up to a 4-week screening period, eligible subjects will be randomized in a 2:1 ratio to receive either AXA1957 or placebo for a 13-week administration period.

Subjects will be centrally randomized with gender (male/female) as the stratification factor to AXA1957 or placebo groups. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and prior to the Day 1 Visit. Study food product amounts will be gradually escalated through the first week of study participation to assess tolerability issues to the food product during the initial week, and to enable subjects and their caregivers to get accustomed to the twice daily regimen (Days 1 to 3: 1 stick pack twice daily [total of 2 stick packs daily]; Days 4 through the remainder of the study: 2 stick packs twice daily [total of 4 stick packs daily]). Safety and tolerability will be monitored throughout the study.

All subjects will be provided diet and physical activity recommendations consistent with Guidance and Lifestyle Recommendations for Adolescents with Nonalcoholic Fatty Liver Disease; see protocol Appendix 1.

Following the mandatory Part 1 period, all subjects including those randomized to the placebo-arm in Part 1, will have an option to continue in the study in Part 2 for up to an additional maximum of 12 weeks on the AXA1957 food product. All subjects who opt to enter Part 2 will be administered AXA1957 at the same amount and regimen as in Part 1 (i.e. up to 2 stick packs twice daily starting from Week 14) and will continue to be provided the standard lifestyle guidance. Subjects who choose not to participate in Part 2, will undergo a safety Follow-Up Visit approximately 2-weeks after Visit 6 per the procedures in Schedule of Assessments (SOA) as Table 1. There will be a 2-week follow up period after subjects complete Part 2.

If subjects drop out at any time for any reason during either Part 1 or 2 of the study, including the follow up period, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1.

Table 1. Schedule of Assessments Table

Study Period	**Screening Period**	**Part 1** Mandatory AXA1957 or Placebo Administration Period (13 weeks)						**Part 2** Optional AXA1957 Extension Period (up to a maximum of 12 weeks)				**Follow-up Period** (2 weeks)	Early Term[1]
	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 W1 (+3 days)	Visit 3 W3 (±5 days)	Visit 4 **W 7** (±5 days)	Visit 5 W 10 (±5 days)	Visit 6 **W13** (±5 days)	Visit 7[14] W 15 (±5 days)	Visit 8 **W 19** (±5 days)	Visit 9 W 22 (±5 days)	Visit 10 **W 25** (±5 days)	Visit 11 W 27 (±3 days)	
Informed consent/Assent	X												
Confirm eligibility	X	X											
Randomization[16]		X											
Demographics and medical history	X												
Conconmitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[2]	X	X		X	X	X	X	X	X	X	X	X	X
Height, weight and waist circumference	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[4]	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X						X				X		X
MRI scan[5]	X				X		X				X		
Fibroscan or RUQ ultrasound	X												
Serum chemistry[11]	X	X			X		X		X		X	X	X
Hematology/coagulation	X	X			X		X		X		X	X	X
HbA1c[11]	X	X			X		X				X		X
Blood samples for metabolic, inflammation and fibrosis biomarkers [7,11]		X			X		X		X		X		X
Fasting lipid profile[11]	X	X					X				X		X
OWL lipidomic profile[11]		X					X				X		X
Blood sample for PBMC isolation[6,11]		X					X				X		

Study Period	**Screening Period**	**Part 1** Mandatory AXA1957 or Placebo Administration Period (13 weeks)						**Part 2**[17] Optional AXA1957 Extension Period (up to a maximum of 12 weeks)				**Follow-up Period** (2 weeks)	Early Term[1]
	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 W1 (±3 days)	Visit 3 W3 (±5 days)	Visit 4 **W 7** (±5 days)	Visit 5 W 10 (± 5 days)	Visit 6 **W13** (± 5 days)	Visit 7 W15 (±5 days)	Visit 8 **W19** (± 5 days)	Visit 9 W22 (±5 days)	Visit 10 **W25** (± 5 days)	Follow-Up[14] (±3 days)	
Blood sample for amino acid concentrations[12]		X					X				X		X
Urinalysis (quantitative)	X	X					X				X		X
Lifestyle guidance & monitoring		X	X	X	X	X	X	X	X	X	X	X	X
4-day baseline diet and exercise diary[15]	X												
Diet/Exercise lifestyle questionnaire[10]		X	X	X	X	X	X	X	X	X	X	X	X
Study food product administration diary[8]		X	X	X	X	X	X	X	X	X	X	X	
VAS[9]		X		X	X	X	X	X	X	X	X		
Dispense Study Food Product[13]		X		X	X	X	X[13]	X	X	X			
Study food product accountability		X	X	X	X	X	X	X	X	X	X		X
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess eligibility and willingness of subject to participate in Part 2							X						
Weekly Phone Calls[8]		X	X	X	X	X	X	X	X	X	X		

Abbreviations: ECG=electrocardiogram, MRI=magnetic resonance imaging, NFALD=nonalcoholic fatty liver disease OWL= one way liver, PBMC=peripheral blood mononuclear cells, RUQ=right upper quadrant, VAS=visual analog scale, W=week.
[1]Subjects who discontinue early in Part 1 or Part 2 will be asked to complete all assessments as indicated. Subjects who discontinue early and who have had the multiparametric MRI scan within 1 week of the date of discontinuation will not be required to repeat the early termination MRI.

[2] Physical examination will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose, throat, and neck, cardiovascular, musculoskeletal, and neurological systems.

[3]Vital signs include sitting systolic and diastolic blood pressure, heart rate, respirations and temperature. Blood pressure should be obtained after the subject has been sitting calmly for at least 5 minutes

[4]Serum pregnancy test at screening visit and urine pregnancy test at all other visits.

[5]The Screening MRI scan should occur approximately within 7 days prior to randomization and must NOT occur until each of the inclusion criteria #1 through #7 have been confirmed. Subjects are required to be fasting for at least 4 hours prior to all MRI scans. Scans should be scheduled based on scanner availability which may not correlate with the same day as the study clinic visit. MRIs at Visits 7 and 13 are not required to occur the same day as the study clinic visit, but must occur within the ± 5 day visit window. Every attempt should be made to schedule scans at roughly the same time of the day to reduce diurnal fluctuation in daily liver lipid levels.

[6]PBMC isolation required on Day 1 and W13 and Week 25 (extension period)

[7]See Protocol Table 5– Clinical Laboratory Evaluations for a list of biomarkers to be collected at the indicated time points. A retained sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

[8]The study dietician (or other qualified staff) will confirm that subjects are administering their assigned study food product accurately, and answer any questions on usual Lifestyle Recommendations for Adolescents with NAFLD (See protocol Appendix 1)

[9]Subjects will complete a hunger and satiety VAS at the indicated time points biweekly and must return completed VAS worksheets for review by study site qualified staff at each study visit.

[10]Subjects are expected to follow the study Guidance and Lifestyle Recommendations for Adolescents with NAFLD (Protocol Appendix 1). The Diet and Exercise questionnaire will be a tool for the study dietician (or other qualified staff) to help monitor adherence to the guidance throughout the study. The questionnaire is easy to use and requires a simple "yes/no" response.

[11] Subjects are required to fast approximately 8 hours prior to their clinic visits on days when blood draws are obtained. On those days, the morning administration of their assigned study food product should be held until blood draws are completed. Subjects may consume their assigned study food product after the blood draw; site staff should inspect the venipuncture site(s) prior to subject's discharge.

[12] On Day 1, Week 13, and Week 25, subjects will arrive at the study site after having fasted approximately 8 hours. They will have a fasted blood draw (T=0), will be administered their assigned study food product at the study site, and then approximately T=1-2 hours later, another blood sample will be collected for plasma amino acid concentrations. The exact clock times of the T=0 (pre-administration) and T=1-2 hr (post-administration) samples should be recorded in the source documents.

[13] AXA1957 will be provided to all subjects entering Part 2, there is no placebo.

[14]The Follow-Up Visit will occur 2 weeks after Visit 6 for subjects participating in Part 1 only. The Follow-Up Visit will occur 2 weeks after Visit 10 for those participating in Part 2.

[15] Diary must be filled out over 4 days which should include 2 weekdays and 2 weekend days. Subjects will record to the best of their ability all the typical foods/beverages they eat/drink on those days, and their usual physical activity routines to establish a baseline lifestyle pattern

[16]Randomization will occur only after eligibility is confirmed (including MRI criteria).

[17] A gap between Part 1 and Part 2 of up to 1 week is permitted.

5 DETERMINATION OF SAMPLE SIZE

As this is food product study, no formal sample size calculations will be conducted. A sufficient number of subjects will be screened to have approximately total 30 subjects complete the study (20 for AXA 1957, 10 for Palcebo). 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.

6 GENERAL ANALYSIS CONSIDERATIONS

The statistical analyses will be reported using summary tables and listings. The International Council for Harmonisation (ICH) numbering convention will be used for all tables and listings. No statistical tests will be conducted on the data. Efficacy data will not be analyzed.

Continuous variables will be summarized with means, SD, medians, minimums, maximums, and number of non-missing cases.

Categorical variables will be summarized by counts and by percentage of subjects in corresponding categories. Percentages are based on the total category count excluding the missing category if not otherwise mentioned. Percentages showing a rate relative to the total number of subjects in this group are given in certain tables (eg, AE tables). Footnotes will specify the percent basis in those cases.

All summary tables will be presented by study group.

Individual subject data obtained from the eCRFs, external vendors, central clinical laboratory and any derived data will be presented by subject in data listings. Listings will be sorted by study group assigned in Part 1, subject, date, visit, and time.

All analyses and tabulations will be performed using SAS® software Version 9.4 or higher. Tables, listings, and figures will be presented in RTF format.

The process for SAS program validation and quality control (QC) for programs and outputs is documented in the Synteract working instruction "SAS Programming Quality Control."
Study-specific QC requirements can be found in Appendix D: SAS Programming QC Requirements.

Conventions for handling partial dates and missing dates for AEs and prior and concomitant medications are given in Appendix B: Missing or incomplete dates of AEs. Listings will present the dates in their original format (without any imputation).

For all analyses, unscheduled visits will not be included for summarization and will only be included in the listings. Unscheduled visits will be used in the determination of baseline values, when applicable.

7 NOTATION OF TREATMENT GROUPS, VISITS AND BASELINE

Notation of treatment groups

The following notation of study groups will be used throughout the report, the overall group will only be on specified tables, e.g. Baseline tables.

Full notation (as used in the study protocol)	*Notation as used throughout all tables, listings and figures*
AXA1957 Placebo	AXA1957 Placebo

Visit terminology

Visit	*Notation as used throughout all tables, listings and figures*
SCREENING PERIOD	Screening
RANDOMIZATION	Randomization
VISIT 1 DAY 1	Day 1
VISIT 2 WK 1	Week 1
VISIT 3 WK 3	Week 3
VISIT 4 WK 7	Week 7
VISIT 5 WK 10	Week 10
VISIT 6 WK 13	Week 13
VISIT 7 WK 15	Week 15
VISIT 8 WK 19	Week 19
VISIT 9 WK 22	Week 22
VISIT 10 WK 25	Week 25
EARLY TERM	Early Termination
FOLLOW-UP	Follow-up

Days are measured from date of study food product administration.

Study days corresponding to measurements are calculated as:

- Assessment date – date of study food product administration + 1, if assessment date is on or after the date of study food product administration.
- Assessment date – date of study food product administration, if measurement date is before the date of study food product administration.

Baseline

Unless specified otherwise, baseline will be defined as the last non-missing value prior to the start of study food product or placebo administration.

For Study Part 1, the baseline is defined as the last nonmissing value prior to the first dose of study food product administration. For the cases when no time was collected, the value collected on Visit 1 will be taken as the baseline since the first administration will be supervised at site and the assessments will be taken before the study food administration. For the subjects who enroll in Part 2, the Part 2 baseline is defined as the last non-missing value by the Visit 6. Data collected during Part 2 will only be included in the listings.

If there are multiple assessments that qualify as baseline and are collected on the same scheduled date, the average of these values will be used for baseline.

8 ANALYSIS SETS

- Safety Analysis Set: will include all subjects who receive at least 1 administration of either placebo or AXA1957. Any subject who is screened but who does not receive study food product will not be included in study reporting.

  The group assignment in the Safety Analysis Set will use actual study food product administration, which is defined as the sample dispensed to the subject on the first day. Since the primary objective for this study is safety and tolerability, the primary analysis population will be the safety analysis set.

9 STUDY POPULATION

9.1 Subject Disposition

Subject disposition information will be summarized for all enrolled subjects by randomized study group and overall. Summaries will include: the number of screened, enrolled subjects, the number of subjects in the Safety Analysis Set, and the number and percentages of subjects that

- completed the mandatory Part 1
- discontinued Part 1 and the primary reason for discontinuation
- enrolled in Part 2
- completed Part 2
- discontinued Part 2 and the primary reason for discontinuation

Reasons for early termination will also be summarized and percentages are calculated using the total number of early terminated subjects as denominators.

9.2 Protocol Deviations

Major protocol deviations that could potentially affect the physiological effect or safety conclusions of the study will be identified prior to database lock. Major protocol deviations may include, but are not limited to:

- Randomly assigned subjects who did not satisfy selected inclusion and exclusion criteria;
- Randomly assigned subjects who developed withdrawal criteria during the study but were not withdrawn;
- Subjects who received the wrong study group or incorrectamount;
- Subjects who received an excluded concomitant treatment;
- Failure to comply with good clinical practice guidelines;

- Subject who did not have at least one postbaseline Magnetic Resonance Imaging measurement and 80% -125% compliance rate of the assigned study food product administration in any 2 close visits where MRI procedure is performed

A listing of all protocol deviations including the deviation designation (major or minor), category, if deviation significant, deviation details and the action taken will be presented in a data listing. Any COVID-19-related protocol deviations will be flagged in the listing.

9.3 Subject Characteristics

9.3.1 Demographic and Baseline Characteristics

The demographic and baseline characteristics will be summarized in the Safety Analysis Set.

The demographic characteristics include age collected as years on consent date, sex, race and ethnicity. The baseline characteristics include baseline height (cm), baseline weight (kg), body mass index (BMI; kg/m^2), baseline waist circumference (cm) and childbearing potential.

Descriptive statistics will be presented for age, height, weight, BMI, and other continuous variables. Frequency counts and percentages will be presented for sex, ethnicity, race and other categorical variables based on the total number of subjects in the Safety Analysis Set.

All demographic and baseline information will be listed according to product administration group by subject.

Baseline Fibroscan or right upper quadrant ultrasound data will only be listed.

9.3.2 Medical History

Medical history will be coded using the MedDRA version 22.0. The number and percentage of subjects with any medical history will be summarized overall and for each system organ class (SOC) and preferred term (PT). Percentages will be calculated based on number of subjects in the Safety Analysis Set.

By subject medical history data including specific details will be presented in a listing.

9.3.3 Prior and Concomitant Medications

Prior and concomitant medication verbatim terms in the eCRFs will be mapped to Anatomical/Therapeutic/Chemical (ATC) class and Preferred Names using the World Health Organization Drug Dictionary Enhanced (WHO DDE) Drug Reference List (Version Mar.1.2019) B3 format.

- Prior medications are medicationsthat started and ended before the study food product administration.

- Concomitant medications are defined as any medication used prior to Screening with stop date at/or after date of first study medication, that are ongoing from Screening/baseline, or that are taken at/after date of first study medication intake.
- If the start or stop date is incomplete and the allocation to previous or concomitant is not clear, the medication will be considered to be concomitant.

All medications will be listed along with a flag to indicate whether the medication was a prior medication or a concomitant medication.

10 PHYSIOLOGICAL EFFECT ANALYSES

No physiological effects data will be listed or summarized.

11 PHARMACOKINETIC ANALYSES

Pharmacokinetic analyses will be described in a separate plan.

12 SAFETY ANALYSES

12.1 Product Administration Compliance and Exposure

The study product administration data will be listed only.

12.2 Adverse Events

All AE summaries will be restricted to study food product-emergent AEs, where study food product-emergent AE is defined as any AE with onset (or worsening of a pre-existing condition) after the first administration of the study food product. The algorithms in Appendix B will be applied to missing and incomplete start and stop dates when identifying the study food product-emergent AEs. If it cannot be determined whether the AE is study food product-emergent due to a partial onset date, then it will be counted as such. The Part 2 study food product-emergent AEs for subjects from Part 1 placebo group is defined as any AE with onset (or worsening of a pre-existing condition) after the Visit 6. The study food product-emergent AEs will be summarized for Part 1. The AE occurs in Part 2 will only be included in listing with a column indicating which Part the AE occurred in. Verbatim terms in the eCRFs will be mapped to PT and SOC using MedDRAversion 22.0.

Each AE summary list as below will be displayed by Part 1 study group and overall. Adverse events summaries will be constructed displaying AEs in decreasing order of frequency under the AXA1957 group according to the numbers of subjects reporting the AE (not the number of events) in SOC and PT within each SOC.

- Overall summary (number of AEs, study food product-emergent AEs, related study food product-emergent AEs, serious AEs (SAEs), severe study food product-emergent AEs, study food product-emergent AEs leading to study product discontinuations, interruption or product administration amount reduction and study food product-emergent AEs leading to death).

- Summary table of study food product-emergent AEs by MedDRA SOC and PT.
- Summary table of study food product-emergent AEs by MedDRA SOC, PT and highest severity. At each level of subject summarization, a subject is classified according to the highest severity if the subject reported 1 or more events.
- Summary table of study food product-emergent AEs by MedDRA SOC, PT, and closest relationship to study food product (Related/Not Related). Related AEs are those reported as "Definitely Related"or "Possibly Related," and unrelated AEs are those reported as "Unlikely Related." At each level of subject summarization, a subject is classified according to the closest relationship if the subject reported 1 or more events.

AEs with missing severity and relationship will be kept as missing and will be summarized as a separate group in corresponding tables.

All AEs, SAEs, all deaths regardless of causality and all AEs leading to study discontinuation, interruption or reduction of study product will be presented in separate listings.

12.3 Clinical Laboratory Evaluation

International System (SI) units and normal ranges are provided by the Central Laboratory for the examined parameters (serum chemistry, hematology, lipid panels). Laboratory shift tables will be provided for all laboratory parameters where low/normal/high can be ascertained to assess changes in laboratory values from baseline to post baseline. Normal ranges provided by the laboratory should be used in the analysis. Laboratory shift tables will only be provided for Study Part 1.

Detailed subject listings of all safety laboratory data collected during the study will be provided. Laboratory values outside normal limits will be identified in the subject listing with flags for low (L) and high (H).

Coagulation and urinalysis results will not be summarized but will be provided in a data listing.

12.4 Vital Signs

All vital sign results will be listed for the Safety Analysis Set.

12.5 Physical Examination

Since abnormal physical exams will be summarized as adverse events, no separate summary tables will be presented. All physical exam results will be listed for the Safety Analysis Set.

12.6 Electrocardiogram

All 12-lead ECG data by subject will be presented in a listing.

12.7 Other Safety Evaluations

Assessment results of hunger and satiety visual analog scale (VAS) self-assessment, diet/exercise lifestyle questionnaire, fibroscan or right upper quadrant (RUQ) ultrasound, Biosmarkers, fasting lipid profile, Metabolic Panel I, pyruvic acid, plasma glucose/insulin, peripheral blood mononuclear cell (PBMC), lifestyle counseling, telephone call, and pregnancy test results will be provided in listings.

13 CHANGES TO PROTOCOL-SPECIFIED ANALYSES

Note the following modifications and/or clarifications to the methodology specified in the protocol.

Due to the early closure of the study, the statistical analysis will only be conducted on the baseline and safety data. No table or listings will be created for the physiological effects data.

REFERENCES

US Department of Health and Human Services (DHHS), Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry ICH E9 Statistical principles for clinical trials. September 1998 [cited 2018 Aug 03]. Available from: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073137.pdf

14 APPENDICES

Appendix A: Presentation of Data and Programming Specifications

General

- Specialized text styles, such as bold, italics, borders, shading, superscripted, and subscripted text will not be used in tables, figures, and data listings unless they add significant value to the table, figure, or data listing.
- Only standard keyboard characters are to be used in tables and data listings.
- Special characters, such as nonprintable control characters, printer-specific, or font-specific characters, will not be used on a table, figure, or data listing.
- Hexadecimal character representations are allowed (eg, μ, α, ß).
- All footnotes will be left justified and at the bottom of a page. Footnotes must be used sparingly and must add value to the table, figure, or data listing.

Tables

- Formal organization of tabulations may be changed during programming, if appropriate, eg, tables for the different variables may be combined into a single table, or tables with more than 1 variable may be split into several tables.
- Means and medians will be presented to 1 more decimal place than the raw data. Standard deviations will be presented to 2 more decimal places than the raw data. Minimums and maximums will be reported with the same number of decimal places as the raw data.
- Percentages will be presented to the tenths place.
- For frequency counts of categorical variables, categories whose counts are zero will be displayed for the sake of completeness. For example, if none of the Subjects discontinue due to "lost to follow-up," this reason will be included in the table with a count of 0. Categories with zero counts will not have zero percentages displayed.
- Lower and upper CI values must be presented to 1 decimal place more than the raw/derived data (ie, to the same number of decimal places as the mean).
- Percentiles (eg, 25%, 75%) must be presented to 1 decimal place more than the raw/derived data.
- For all inferential analyses, *P* values will be rounded to 4 decimal places (or at the highest level of precision) with a leading zero (0.0001). *P* values less than 0.0001 will be presented as "<0.0001."
- The last footnotes will be

  "Source: xxx", where xxx indicates the source table number(s) if applicable (in case aggregated results like mean or median are plotted) or the source listing(s) (in case individual responses are plotted) and/or source dataset(s) (eg, ADaM) and "PROGRAM SOURCE: ...\\xx.sas, DATA CUT OFF DATE: DDMMMYYYY, RUN DATE: DDMMYY hh:mm", where extract date is the datestamp of the data snapshot used.

Listings

- Formal organization of the listing may be changed during programming, if appropriate, eg, additional variables may be included, change in the column order, or the listing may be split into multiple parts due to space constraints.
- If not otherwise specified, all data listings will be sorted by sequence/treatment, center, Subject number, visit, and date/time, as appropriate.
- All date values will be presented in a SAS date (eg, 29AUG2001) format.

- All observed time values will be presented using a 24-hour clock in the HH:MM:SS format (eg, 01:35:45 or 11:26). Seconds will only be reported if they were measured as part of the study.
- The last footnote will be
  "PROGRAM SOURCE: ...\\xx.sas, DATA CUT OFF DATE: DDMMMYYYY, RUN DATE: DDMMYY hh:mm", where extract date is the date tamp of the data snapshot used.

Appendix B: Missing or incomplete dates of AEs

The most conservative approach will be systematically considered. If the AE onset date is missing/incomplete, it is assumed to have occurred during the study food product administration phase (ie, considered a study product-emergent AE) except if the partial onset date or other data, such as the stop date, indicates differently. Similarly, a medication with partial start and stop dates could be considered as both a prior and concomitant treatment.

The following algorithms will be applied to missing and incomplete start and stop dates:

Start dates

- If the day portion of the start date is missing, then the start date will be estimated to be equal to the date of first dose of study drug, provided the start month and year are the same as the first dose of study drug and the stop date is either after the first dose of study drug or completely missing. Otherwise, the missing day portion will be estimated as "01."
- If both the day and month portions of the start date are missing, then the start date will be estimated to be equal to the date of first dose of study drug, provided the start year is the same as the first dose of study drug and the stop date is either after the first dose of study drug or completely missing. Otherwise, the event will be assumed to start on the first day of the given year (eg, ??-???-2013 is estimated as 01-JAN-2013).
- If the start date is completely missing and the stop date is either after the dose of study drug or completely missing, the start date will be estimated to be the day of study drug dosing. Otherwise, the start date will be estimated to be the first day of the same year as the stop date. All other non-AE and nonconcomitant medication day calculations where only partial dates are available will be handled as follows: the first day of the month will be used in the calculations if the day part of a start date is missing while January 1 will be employed if both the month and day parts of a start date are missing.

Stop dates

- If only the day of resolution is unknown, the day will be assumed to be the last of the month (eg, ??-JAN-2013 will be treated as 31-JAN-2013).
- If both the day and month of resolution are unknown, the event will be assumed to have ceased on the last day of the year (eg, ??-???-2013 will be treated as 31-DEC-2013).
- If the stop date is completely missing or the event is continuing, the event will be assumed to be after first dose of study drug and will be imputed using the last known date on the study.

Appendix C: Standard calculations

Variables requiring calculation will be derived using the following formulas:

- **Days** – A duration expressed in days between 1 date (date1) and another later date (date2) is calculated using the formulas noted below:

  duration in days = date2 – date1 + 1
- **Months –** A duration expressed in months is calculated using the INTCK function of SAS as follows: months=intck('month','date1'd,date2'd, 'continuous').
- **Years –** A duration expressed in years between 1 date (date1) and another later date (date2) is calculated as follows:
  duration in years = intck('year,'date1'd,date2'd, 'continuous').
- **Height** – Height entries made in inches (in) are converted to centimeters (cm) using the following formula: height (cm) = height (in) × 2.54.
- **Weight –** Weight entries made in pounds (lb) are converted to kilograms (kg) using the following formula: weight (kg) = weight (lb)/2.2046.
- **Temperature** – Temperature entries in degrees Fahrenheit are converted to degrees centigrade using the following formula:
  temp (degrees centigrade) = 5/9 × [temp (degrees Fahrenheit) – 32].
- **Change from baseline –** Change from baseline will be calculated as:
  Change = postbaseline value – baseline value.
- **Percent change from baseline** – Change from baseline will be calculated as:
  Percent change from baseline = (postbaseline value – baseline value)/baseline value × 100.

Appendix D: SAS Programming QC Requirements

Derived datasets are independently programmed by two programmers. The separate datasets produced by the 2 programmers must match 100%. Detailed specifications for the derived datasets are documented in the study analysis dataset specifications provided to the client at study conclusion.

Tables and listings are independently reprogrammed by a second programmer.
Listings are checked for consistency against corresponding tables, figures, and derived datasets.

The entire set of TLs is checked for completeness and consistency prior to its delivery to the client by the lead biostatistician and a senior level, or above, reviewer.

Appendix E: List of Tables, Figures, and Listings

The following proposal for Section 14 and 16.2 of the CSR is completed according to ICH E3 guidelines. The ICH heading numbers and description are in **bold**. Minor changes from this planned index do not need to be amended in the SAP.

Formal organization of tabulations may be changed during programming, if appropriate, (eg, tables for the different variables may be combined into a single table, or tables with more than 1 variable may be split into several tables).

TABLES, FIGURES AND GRAPHS

Table Number	Table Title	Comment
14	**TABLES, FIGURES, AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT**	
14.1	**DEMOGRAPHIC DATA**	
14.1.1	Subject Disposition	All Subjects
14.1.2	Demographic and Baseline Characteristics	Safety Analysis Set
14.1.3	Medical History	Safety Analysis Set
14.3	**Safety data**	
14.3.1	**Displays of Adverse Events**	
14.3.1.1	Overall Summary of Study Food Product-Emergent Adverse Events: Study Part 1	Safety Analysis Set
14.3.1.2	Study Food Product-Emergent Adverse Events by System Organ Class and Preferred Term: Study Part 1	Safety Analysis Set
14.3.1.3	Study Food Product -Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity: Study Part 1	Safety Analysis Set
14.3.1.4	Study Food Product -Emergent Adverse Events by System Organ Class, Preferred Term, and Closest Relationship to Study Food Product: Study Part 1	Safety Analysis Set
14.3.4	**Laboratory**	
14.3.4.1	Serum Chemistry – Shift from Baseline: Study Part 1	Safety Analysis Set
14.3.4.2	Haematology – Shift from Baseline: Study Part 1	Safety Analysis Set
14.3.4.3	Lipids – Shift from Baseline: Study Part 1	Safety Analysis Set

Section 16.2: **List of Data Listings**

ICH Listing Number	Listing Title	Comment
16.2	**SUBJECT DATA LISTINGS**	
16.2.1	**Discontinued Subjects**	
16.2.1.1.1	Subject Disposition: Study Part 1	All Subjects
16.2.1.1.2	Subject Disposition: Study Part 2	All Subjects
16.2.2	**Protocol deviations**	
16.2.2	Protocol Deviations	Safety Analysis Set
16.2.3	**Subjects excluded from the efficacy analysis**	
16.2.3	Inclusion/Exclusion Criteria	Safety Analysis Set
16.2.4	**Demographic data**	
16.2.4.1	Demographics	All Subjects
16.2.4.2	Medical history	Safety Analysis Set
16.2.4.3	Prior and Concomitant Medications	Safety Analysis Set
16.2.5	**Compliance and/or drug concentration data**	
16.2.5.1	Study Food Product Administration	Safety Analysis Set
16.2.7	**Adverse events listings**	
16.2.7.1	Adverse Events	Safety Analysis Set
16.2.7.2	Serious Adverse Events	Safety Analysis Set
16.2.7.3	Adverse Events Leading to Study Discontinuation	Safety Analysis Set
16.2.7.4	Deaths	Safety Analysis Set
16.2.8	**Listing of individual laboratory measurements by subject, when required by regulatory authorities**	
16.2.8.1	Hematology	Safety Analysis Set
16.2.8.2	Chemistry	Safety Analysis Set
16.2.8.3	Coagulation	Safety Analysis Set
16.2.8.4	Urinalysis	Safety Analysis Set
16.2.8.5	Vital Signs	Safety Analysis Set
16.2.8.6	Physical Examination	Safety Analysis Set
16.2.8.7	Pregnancy Test	Safety Analysis Set
16.2.8.8	Electrocardiogram	Safety Analysis Set
16.2.8.9	Fibroscan or Right Upper Quadrant (RUQ) Ultrasound	Safety Analysis Set
16.2.8.10.1	Biomarkers - Metabolic, Inflammation and Fibrosis	Safety Analysis Set
16.2.8.10.2	Biomarkers - Enhanced Liver Fibrosis (ELF)	Safety Analysis Set
16.2.8.10.3	Biomarkers - Apolipoproteins	Safety Analysis Set
16.2.8.11	Fasting Lipid Profile	Safety Analysis Set
16.2.8.12	Metabolic Panel	Safety Analysis Set
16.2.8.13	Pyruvic Acid, Plasma Glucose/Insulin, and Lactatel	Safety Analysis Set
16.2.8.14	Peripheral Blood Mononuclear Cells	Safety Analysis Set
16.2.8.15	Diet/Exercise Lifestyle Questionnaire	Safety Analysis Set
16.2.8.16	Hunger and Satiety Visual Analog Scale (VAS)	Safety Analysis Set

ICH Listing Number	Listing Title	Comment
16.2.8.17	Lifestyle Counseling	Safety Analysis Set
16.2.8.18	Telephone Call	Safety Analysis Set

Statistical Analysis Plan

Axcella Health Inc.

AXA1957-002

A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver

Protocol Version: 13-Dec-2019

Sponsor: Axcella Health Inc.
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Version	Date
Draft 0.1	15 Jul 2019
Draft 0.2	26 Aug 2019
Draft 0.3	04 Sep 2019
Final 1.0	25 Nov 2020
Final 2.0	04 Feb 2021

Approval

Upon review of this document, including the table, listing, and figure shells, the undersigned approves the statistical analysis plan. The analysis methods and data presentation are acceptable.

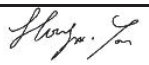
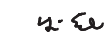
Signature	Date
Signature:  *Electronically signed by: Hongxia Yan Reason: I am the author of this document Date: Feb 4, 2021 15:04 EST* **Email:** Hongxia.Yan@synteract.com **Title:** Biostatistician II Hongxia Yan Biostatistician II Synteract	
Signature:  *Electronically signed by: Eunsil Yim Reason: I am the approver of this document Date: Feb 4, 2021 14:40 EST* **Email:** euns.yim@gmail.com **Title:** Principal Statistician Eunsil Yim Statistical Consultant Axcella Health Inc	

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Full Notation
AE	Adverse Event
ATC	Anatomical/Therapeutic/Chemical
BMI	Body Mass Index
bpm	Beats Per Minute
CSR	Clinical Study Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ICH	International Council for Harmonisation
IWRS	Interactive web response system
MedDRA	Medical Dictionary For Regulatory Activities
MRI	Magnetic Resonance Imaging
PT	Preferred Term
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
VAS	Visual Analogue Scale
WHO DDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

This document outlines the statistical methods to be implemented during the analyses of data collected within the scope of Axcella Health Inc. protocol AXA1957-002 [A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver]. The purpose of this plan is to provide specific guidelines from which the statistical analyses will proceed. As the study was terminated early, no interium analyses and physiological effects analyses will be conducted, and a reducted scope of the analysis will be included in this plan. Any deviations from this plan will be documented in the clinical study report (CSR).

2 STUDY DOCUMENTS

The following study documents are used for the preparation of the statistical analysis plan (SAP):

- Protocol version 3, 13-Dec-2019
- Annotated electronic case report form (eCRF), version 3.0, 13-Feb-2020
- Data management plan v1.0, 09Aug2019

3 STUDY OBJECTIVES

Safety and tolerability will be assessed by:

- Reported clinical adverse events (AEs)
- Physical examinations, including changes in body weight and body composition such as lean mass and fat mass
- Vital sign assessments
- Electrocardiograms (ECGs)
- Clinical laboratory tests including changes in standard hematology, chemistry, and lipid panels

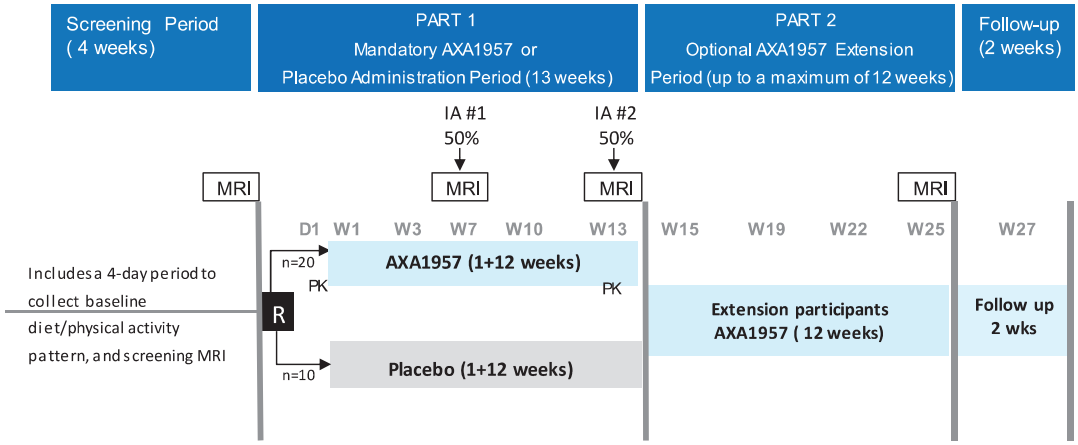
Physiological effects on liver structure and function will be assessed by:

- Multiparametric magnetic resonance imaging (MRI) assessments of liver structure (fat content and inflammation changes)
- Blood tests of liver function, including markers of inflammation and fibrosis

4 STUDY DESIGN AND PLAN

This is a randomized, placebo-controlled study conducted in 2 study parts: a mandatory 13-wk administration period (Study Part 1) and an optional extension period up to a maximum of 12 additional weeks (Study Part 2); see study schematic as Figure 1 below.

Figure 1: Study Schematic



Total duration of the study from Screening to the end of Part 2 follow-up is anticipated to be approximately 31 weeks. It is anticipated that there may be a total of up to 12 study visits during the entire study (Screening and Parts 1 and 2).

In Part 1, following up to a 4-week screening period, eligible subjects will be randomized in a 2:1 ratio to receive either AXA1957 or placebo for a 13-week administration period.

Subjects will be centrally randomized with gender (male/female) as the stratification factor to AXA1957 or placebo groups. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and prior to the Day 1 Visit. Study food product amounts will be gradually escalated through the first week of study participation to assess tolerability issues to the food product during the initial week, and to enable subjects and their caregivers to get accustomed to the twice daily regimen (Days 1 to 3: 1 stick pack twice daily [total of 2 stick packs daily]; Days 4 through the remainder of the study: 2 stick packs twice daily [total of 4 stick packs daily]). Safety and tolerability will be monitored throughout the study.

All subjects will be provided diet and physical activity recommendations consistent with Guidance and Lifestyle Recommendations for Adolescents with Nonalcoholic Fatty Liver Disease; see protocol Appendix 1.

Following the mandatory Part 1 period, all subjects including those randomized to the placebo-arm in Part 1, will have an option to continue in the study in Part 2 for up to an additional maximum of 12 weeks on the AXA1957 food product. All subjects who opt to enter Part 2 will be administered AXA1957 at the same amount and regimen as in Part 1 (i.e. up to 2 stick packs twice daily starting from Week 14) and will continue to be provided the standard lifestyle guidance. Subjects who choose not to participate in Part 2, will undergo a safety Follow-Up Visit approximately 2-weeks after Visit 6 per the procedures in Schedule of Assessments (SOA) as Table 1. There will be a 2-week follow up period after subjects complete Part 2.

If subjects drop out at any time for any reason during either Part 1 or 2 of the study, including the follow up period, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1.

Table 1. Schedule of Assessments Table

Study Period	**Screening Period**	**Part 1** Mandatory AXA1957 or Placebo Administration Period (13 weeks)						**Part 2** Optional AXA1957 Extension Period (up to a maximum of 12 weeks)				**Follow-up Period** (2 weeks)	Early Term[1]
	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 W1 (+3 days)	Visit 3 W3 (±5 days)	Visit 4 **W 7** (±5 days)	Visit 5 W 10 (±5 days)	Visit 6 **W13** (±5 days)	Visit 7[14] W 15 (±5 days)	Visit 8 **W 19** (±5 days)	Visit 9 W 22 (±5 days)	Visit 10 **W 25** (±5 days)	Visit 11 W 27 (±3 days)	
Informed consent/Assent	X												
Confirm eligibility	X	X											
Randomization[16]		X											
Demographics and medical history	X												
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[2]	X	X		X	X	X	X	X	X	X	X	X	X
Height, weight and waist circumference	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[4]	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X						X				X		X
MRI scan[5]	X				X		X				X		
Fibroscan or RUQ ultrasound	X												
Serum chemistry[11]	X	X			X		X		X		X	X	X
Hematology/coagulation	X	X			X		X		X		X	X	X
HbA1c[11]	X	X			X		X				X		X
Blood samples for metabolic, inflammation and fibrosis biomarkers [7,11]		X			X		X		X		X		X
Fasting lipid profile[11]	X	X					X				X		X
OWL lipidomic profile[11]		X					X				X		X

Blood sample for PBMC isolation[6,11]		X					X				X		
Study Period	**Screening Period**	**Part 1** Mandatory AXA1957 or Placebo Administration Period (13 weeks)						**Part 2[17]** Optional AXA1957 Extension Period (up to a maximum of 12 weeks)				**Follow-up Period** (2 weeks)	Early Term[1]
	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 W1 (±3 days)	Visit 3 W3 (±5 days)	Visit 4 **W 7** (±5 days)	Visit 5 W 10 (± 5 days)	Visit 6 **W13** (± 5 days)	Visit 7 W15 (±5 days)	Visit 8 **W19** (± 5 days)	Visit 9 W22 (±5 days)	Visit 10 **W25** (± 5 days)	Follow-Up[14] (±3 days)	
Blood sample for amino acid concentrations[12]		X					X				X		X
Urinalysis (quantitative)	X	X					X				X		X
Lifestyle guidance & monitoring		X	X	X	X	X	X	X	X	X	X	X	X
4-day baseline diet and exercise diary[15]	X												
Diet/Exercise lifestyle questionnaire[10]		X	X	X	X	X	X	X	X	X	X	X	X
Study food product administration diary[8]		X	X	X	X	X	X	X	X	X	X	X	
VAS[9]		X		X	X	X	X	X	X	X	X		
Dispense Study Food Product[13]		X		X	X	X	X[13]	X	X	X			
Study food product accountability		X	X	X	X	X	X	X	X	X	X		X
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess eligibility and willingness of subject to participate in Part 2							X						
Weekly Phone Calls[8]		X	X	X	X	X	X	X	X	X	X		

Abbreviations: ECG=electrocardiogram, MRI=magnetic resonance imaging, NFALD=nonalcoholic fatty liver disease OWL= one way liver, PBMC=peripheral blood mononuclear cells, RUQ=right upper quadrant, VAS=visual analog scale, W=week.
[1]Subjects who discontinue early in Part 1 or Part 2 will be asked to complete all assessments as indicated. Subjects who discontinue early and who have had the multiparametric MRI scan within 1 week of the date of discontinuation will not be required to repeat the early termination MRI.

[2] Physical examination will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose, throat, and neck, cardiovascular, musculoskeletal, and neurological systems.

[3]Vital signs include sitting systolic and diastolic blood pressure, heart rate, respirations and temperature. Blood pressure should be obtained after the subject has been sitting calmly for at least 5 minutes

[4]Serum pregnancy test at screening visit and urine pregnancy test at all other visits.

[5]The Screening MRI scan should occur approximately within 7 days prior to randomization and must NOT occur until each of the inclusion criteria #1 through #7 have been confirmed. Subjects are required to be fasting for at least 4 hours prior to all MRI scans. Scans should be scheduled based on scanner availability which may not correlate with the same day as the study clinic visit. MRIs at Visits 7 and 13 are not required to occur the same day as the study clinic visit, but must occur within the ± 5 day visit window. Every attempt should be made to schedule scans at roughly the same time of the day to reduce diurnal fluctuation in daily liver lipid levels.

[6]PBMC isolation required on Day 1 and W13 and Week 25 (extension period)

[7]See Protocol Table 5– Clinical Laboratory Evaluations for a list of biomarkers to be collected at the indicated time points. A retained sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

[8]The study dietician (or other qualified staff) will confirm that subjects are administering their assigned study food product accurately, and answer any questions on usual Lifestyle Recommendations for Adolescents with NAFLD (See protocol Appendix 1)

[9]Subjects will complete a hunger and satiety VAS at the indicated time points biweekly and must return completed VAS worksheets for review by study site qualified staff at each study visit.

[10]Subjects are expected to follow the study Guidance and Lifestyle Recommendations for Adolescents with NAFLD (Protocol Appendix 1). The Diet and Exercise questionnaire will be a tool for the study dietician (or other qualified staff) to help monitor adherence to the guidance throughout the study. The questionnaire is easy to use and requires a simple “yes/no” response.

[11] Subjects are required to fast approximately 8 hours prior to their clinic visits on days when blood draws are obtained. On those days, the morning administration of their assigned study food product should be held until blood draws are completed. Subjects may consume their assigned study food product after the blood draw; site staff should inspect the venipuncture site(s) prior to subject’s discharge.

[12] On Day 1, Week 13, and Week 25, subjects will arrive at the study site after having fasted approximately 8 hours. They will have a fasted blood draw (T=0), will be administered their assigned study food product at the study site, and then approximately T=1-2 hours later, another blood sample will be collected for plasma amino acid concentrations. The exact clock times of the T=0 (pre-administration) and T=1-2 hr (post-administration) samples should be recorded in the source documents.

[13] AXA1957 will be provided to all subjects entering Part 2, there is no placebo.

[14]The Follow-Up Visit will occur 2 weeks after Visit 6 for subjects participating in Part 1 only. The Follow-Up Visit will occur 2 weeks after Visit 10 for those participating in Part 2.

[15] Diary must be filled out over 4 days which should include 2 weekdays and 2 weekend days. Subjects will record to the best of their ability all the typical foods/beverages they eat/drink on those days, and their usual physical activity routines to establish a baseline lifestyle pattern

[16]Randomization will occur only after eligibility is confirmed (including MRI criteria).

[17] A gap between Part 1 and Part 2 of up to 1 week is permitted.

5 DETERMINATION OF SAMPLE SIZE

As this is food product study, no formal sample size calculations will be conducted. A sufficient number of subjects will be screened to have approximately total 30 subjects complete the study (20 for AXA 1957, 10 for Palcebo). 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.

6 GENERAL ANALYSIS CONSIDERATIONS

The statistical analyses will be reported using summary tables and listings. The International Council for Harmonisation (ICH) numbering convention will be used for all tables and listings. No statistical tests will be conducted on the data. Efficacy data will not be analyzed.

Continuous variables will be summarized with means, SD, medians, minimums, maximums, and number of non-missing cases.

Categorical variables will be summarized by counts and by percentage of subjects in corresponding categories. Percentages are based on the total category count excluding the missing category if not otherwise mentioned. Percentages showing a rate relative to the total number of subjects in this group are given in certain tables (eg, AE tables). Footnotes will specify the percent basis in those cases.

All summary tables will be presented by study group.

Individual subject data obtained from the eCRFs, external vendors, central clinical laboratory and any derived data will be presented by subject in data listings. Listings will be sorted by study group assigned in Part 1, subject, date, visit, and time.

All analyses and tabulations will be performed using SAS® software Version 9.4 or higher. Tables, listings, and figures will be presented in RTF format.

The process for SAS program validation and quality control (QC) for programs and outputs is documented in the Synteract working instruction "SAS Programming Quality Control."
Study-specific QC requirements can be found in Appendix D: SAS Programming QC Requirements.

Conventions for handling partial dates and missing dates for AEs and prior and concomitant medications are given in Appendix B: Missing or incomplete dates of AEs. Listings will present the dates in their original format (without any imputation).

For all analyses, unscheduled visits will not be included for summarization and will only be included in the listings. Unscheduled visits will be used in the determination of baseline values, when applicable.

7 NOTATION OF TREATMENT GROUPS, VISITS AND BASELINE

Notation of treatment groups

The following notation of study groups will be used throughout the report, the overall group will only be on specified tables, e.g. Baseline tables.

Full notation (as used in the study protocol)	*Notation as used throughout all tables, listings and figures*
AXA1957 Placebo	AXA1957 Placebo

Visit terminology

Visit	*Notation as used throughout all tables, listings and figures*
SCREENING PERIOD	Screening
RANDOMIZATION	Randomization
VISIT 1 DAY 1	Day 1
VISIT 2 WK 1	Week 1
VISIT 3 WK 3	Week 3
VISIT 4 WK 7	Week 7
VISIT 5 WK 10	Week 10
VISIT 6 WK 13	Week 13
VISIT 7 WK 15	Week 15
VISIT 8 WK 19	Week 19
VISIT 9 WK 22	Week 22
VISIT 10 WK 25	Week 25
EARLY TERM	Early Termination
FOLLOW-UP	Follow-up

Days are measured from date of study food product administration.

Study days corresponding to measurements are calculated as:

- Assessment date – date of study food product administration + 1, if assessment date is on or after the date of study food product administration.
- Assessment date – date of study food product administration, if measurement date is before the date of study food product administration.

Baseline

Unless specified otherwise, baseline will be defined as the last non-missing value prior to the start of study food product or placebo administration.

For Study Part 1, the baseline is defined as the last nonmissing value prior to the first dose of study food product administration. For the cases when no time was collected, the value collected on Visit 1 will be taken as the baseline since the first administration will be supervised at site and the assessments will be taken before the

study food administration. For the subjects who enroll in Part 2, the Part 2 baseline is defined as the last non-missing value by the Visit 6. Data collected during Part 2 will only be included in the listings.

If there are multiple assessments that qualify as baseline and are collected on the same scheduled date, the average of these values will be used for baseline.

8 ANALYSIS SETS

- Safety Analysis Set: will include all subjects who receive at least 1 administration of either placebo or AXA1957. Any subject who is screened but who does not receive study food product will not be included in study reporting.

  The group assignment in the Safety Analysis Set will use actual study food product administration, which is defined as the sample dispensed to the subject on the first day. Since the primary objective for this study is safety and tolerability, the primary analysis population will be the safety analysis set.

9 STUDY POPULATION

9.1 Subject Disposition

Subject disposition information will be summarized for all enrolled subjects by randomized study group and overall. Summaries will include: the number of screened, enrolled subjects, the number of subjects in the Safety Analysis Set, and the number and percentages of subjects that

- completed the mandatory Part 1
- discontinued Part 1 and the primary reason for discontinuation
- enrolled in Part 2
- completed Part 2
- discontinued Part 2 and the primary reason for discontinuation

Reasons for early termination will also be summarized and percentages are calculated using the total number of early terminated subjects as denominators.

9.2 Protocol Deviations

Major protocol deviations that could potentially affect the physiological effect or safety conclusions of the study will be identified prior to database lock. Major protocol deviations may include, but are not limited to:

- Randomly assigned subjects who did not satisfy selected inclusion and exclusion criteria;
- Randomly assigned subjects who developed withdrawal criteria during the study but were not withdrawn;

- Subjects who received the wrong study group or incorrectamount;
- Subjects who received an excluded concomitant treatment;
- Failure to comply with good clinical practice guidelines;
- Subject who did not have at least one postbaseline Magnetic Resonance Imaging measurement and 80% -125% compliance rate of the assigned study food product administration in any 2 close visits where MRI procedure is performed

A listing of all protocol deviations including the deviation designation (major or minor), category, if deviation significant, deviation details and the action taken will be presented in a data listing. Any COVID-19-related protocol deviations will be flagged in the listing.

9.3 Subject Characteristics

9.3.1 Demographic and Baseline Characteristics

The demographic and baseline characteristics will be summarized in the Safety Analysis Set.

The demographic characteristics include age collected as years on consent date, sex, race and ethnicity. The baseline characteristics include baseline height (cm), baseline weight (kg), body mass index (BMI; kg/m^2), baseline waist circumference (cm) and childbearing potential.

The baseline characteristics of the fatty liver include fat ROI pooled median (%) and cT1 whole median (ms).

Descriptive statistics will be presented for age, height, weight, BMI, fat ROI pooled median (%), cT1 whole median (ms) and other continuous variables. Frequency counts and percentages will be presented for sex, ethnicity, race and other categorical variables based on the total number of subjects in the Safety Analysis Set.

All demographic and baseline information will be listed according to product administration group by subject.

Baseline Fibroscan or right upper quadrant ultrasound data will only be listed.

9.3.2 Medical History

Medical history will be coded using the MedDRA version 22.0. The number and percentage of subjects with any medical history will be summarized overall and for each system organ class (SOC) and preferred term (PT). Percentages will be calculated based on number of subjects in the Safety Analysis Set.

By subject medical history data including specific details will be presented in a listing.

9.3.3 Prior and Concomitant Medications

Prior and concomitant medication verbatim terms in the eCRFs will be mapped to Anatomical/Therapeutic/Chemical (ATC) class and Preferred Names using the World Health Organization Drug Dictionary Enhanced (WHO DDE) Drug Reference List (Version Mar.1.2019) B3 format.

- Prior medications are medicationsthat started and ended before the study food product administration.
- Concomitant medications are defined as any medication used prior to Screening with stop date at/or after date of first study medication, that are ongoing from Screening/baseline, or that are taken at/after date of first study medication intake.
- If the start or stop date is incomplete and the allocation to previous or concomitant is not clear, the medication will be considered to be concomitant.

All medications will be listed along with a flag to indicate whether the medication was a prior medication or a concomitant medication.

10 PHYSIOLOGICAL EFFECT ANALYSES

Physiological effects data will only be listed.

11 PHARMACOKINETIC ANALYSES

Pharmacokinetic analyses will be described in a separate plan.

12 SAFETY ANALYSES

12.1 Product Administration Compliance and Exposure

The study product administration data will be listed only.

12.2 Adverse Events

All AE summaries will be restricted to study food product-emergent AEs, where study food product-emergent AE is defined as any AE with onset (or worsening of a pre-existing condition) after the first administration of the study food product. The algorithms in Appendix B will be applied to missing and incomplete start and stop dates when identifying the study food product-emergent AEs. If it cannot be determined whether the AE is study food product-emergent due to a partial onset date, then it will be counted as such. The Part 2 study food product-emergent AEs for subjects from Part 1 placebo group is defined as any AE with onset (or worsening of a pre-existing condition) after the Visit 6. The study food product-emergent AEs will be summarized for Part 1. The AE occurs in Part 2 will only be included in listing with a column indicating which Part the AE occurred in. Verbatim terms in the eCRFs will be mapped to PT and SOC using MedDRAversion 22.0.

Each AE summary list as below will be displayed by Part 1 study group and overall. Adverse events summaries will be constructed displaying AEs in decreasing order of frequency under the AXA1957 group according to the numbers of subjects reporting the AE (not the number of events) in SOC and PT within each SOC.

- Overall summary (number of AEs, study food product-emergent AEs, related study food product-emergent AEs, serious AEs (SAEs), severe study food product-emergent AEs, study food product-emergent AEs leading to study product discontinuations, interruption or product administration amount reduction and study food product-emergent AEs leading to death).
- Summary table of study food product-emergent AEs by MedDRA SOC and PT.
- Summary table of study food product-emergent AEs by MedDRA SOC, PT and highest severity. At each level of subject summarization, a subject is classified according to the highest severity if the subject reported 1 or more events.
- Summary table of study food product-emergent AEs by MedDRA SOC, PT, and closest relationship to study food product (Related/Not Related). Related AEs are those reported as "Definitely Related"or "Possibly Related," and unrelated AEs are those reported as "Unlikely Related." At each level of subject summarization, a subject is classified according to the closest relationship if the subject reported 1 or more events.

AEs with missing severity and relationship will be kept as missing and will be summarized as a separate group in corresponding tables.

All AEs, SAEs, all deaths regardless of causality and all AEs leading to study discontinuation, interruption or reduction of study product will be presented in separate listings.

12.3 Clinical Laboratory Evaluation

International System (SI) units and normal ranges are provided by the Central Laboratory for the examined parameters (serum chemistry, hematology, lipid panels). Laboratory shift tables will be provided for all laboratory parameters where low/normal/high can be ascertained to assess changes in laboratory values from baseline to post baseline. Normal ranges provided by the laboratory should be used in the analysis. Laboratory shift tables will only be provided for Study Part 1.

Detailed subject listings of all safety laboratory data collected during the study will be provided. Laboratory values outside normal limits will be identified in the subject listing with flags for low (L) and high (H).

Coagulation and urinalysis results will not be summarized but will be provided in a data listing.

12.4 Vital Signs

All vital sign results will be listed for the Safety Analysis Set.

12.5 Physical Examination

Since abnormal physical exams will be summarized as adverse events, no separate summary tables will be presented. All physical exam results will be listed for the Safety Analysis Set.

12.6 Electrocardiogram

All 12-lead ECG data by subject will be presented in a listing.

12.7 Other Safety Evaluations

Assessment results of hunger and satiety visual analog scale (VAS) self-assessment, diet/exercise lifestyle questionnaire, fibroscan or right upper quadrant (RUQ) ultrasound, Biosmarkers, fasting lipid profile, Metabolic Panel I, pyruvic acid, plasma glucose/insulin, peripheral blood mononuclear cell (PBMC), lifestyle counseling, telephone call, and pregnancy test results will be provided in listings.

13 CHANGES TO PROTOCOL-SPECIFIED ANALYSES

Note the following modifications and/or clarifications to the methodology specified in the protocol.

Due to the early closure of the study, the statistical analysis will only be conducted on the baseline and safety data. No table or listings will be created for the physiological effects data.

REFERENCES

US Department of Health and Human Services (DHHS), Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry ICH E9 Statistical principles for clinical trials. September 1998 [cited 2018 Aug 03]. Available from: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073137.pdf

14 APPENDICES

Appendix A: Presentation of Data and Programming Specifications

General

- Specialized text styles, such as bold, italics, borders, shading, superscripted, and subscripted text will not be used in tables, figures, and data listings unless they add significant value to the table, figure, or data listing.
- Only standard keyboard characters are to be used in tables and data listings.
- Special characters, such as nonprintable control characters, printer-specific, or font-specific characters, will not be used on a table, figure, or data listing.
- Hexadecimal character representations are allowed (eg, μ, α, ß).
- All footnotes will be left justified and at the bottom of a page. Footnotes must be used sparingly and must add value to the table, figure, or data listing.

Tables

- Formal organization of tabulations may be changed during programming, if appropriate, eg, tables for the different variables may be combined into a single table, or tables with more than 1 variable may be split into several tables.
- Means and medians will be presented to 1 more decimal place than the raw data. Standard deviations will be presented to 2 more decimal places than the raw data. Minimums and maximums will be reported with the same number of decimal places as the raw data.
- Percentages will be presented to the tenths place.
- For frequency counts of categorical variables, categories whose counts are zero will be displayed for the sake of completeness. For example, if none of the Subjects discontinue due to "lost to follow-up," this reason will be included in the table with a count of 0. Categories with zero counts will not have zero percentages displayed.
- Lower and upper CI values must be presented to 1 decimal place more than the raw/derived data (ie, to the same number of decimal places as the mean).
- Percentiles (eg, 25%, 75%) must be presented to 1 decimal place more than the raw/derived data.
- For all inferential analyses, *P* values will be rounded to 4 decimal places (or at the highest level of precision) with a leading zero (0.0001). *P* values less than 0.0001 will be presented as "<0.0001."
- The last footnotes will be

  "Source: xxx", where xxx indicates the source table number(s) if applicable (in case aggregated results like mean or median are plotted) or the source listing(s) (in case individual responses are plotted) and/or source dataset(s) (eg, ADaM) and "PROGRAM SOURCE: ...\\xx.sas, DATA CUT OFF DATE: DDMMMYYYY, RUN DATE: DDMMYY hh:mm", where extract date is the datestamp of the data snapshot used.

Listings

- Formal organization of the listing may be changed during programming, if appropriate, eg, additional variables may be included, change in the column order, or the listing may be split into multiple parts due to space constraints.
- If not otherwise specified, all data listings will be sorted by sequence/treatment, center, Subject number, visit, and date/time, as appropriate.

- All date values will be presented in a SAS date (eg, 29AUG2001) format.
- All observed time values will be presented using a 24-hour clock in the HH:MM:SS format (eg, 01:35:45 or 11:26). Seconds will only be reported if they were measured as part of the study.
- The last footnote will be
  "PROGRAM SOURCE: ...\\xx.sas, DATA CUT OFF DATE: DDMMMYYYY, RUN DATE: DDMMYY hh:mm", where extract date is the date tamp of the data snapshot used.

Appendix B: Missing or incomplete dates of AEs

The most conservative approach will be systematically considered. If the AE onset date is missing/incomplete, it is assumed to have occurred during the study food product administration phase (ie, considered a study product-emergent AE) except if the partial onset date or other data, such as the stop date, indicates differently. Similarly, a medication with partial start and stop dates could be considered as both a prior and concomitant treatment.

The following algorithms will be applied to missing and incomplete start and stop dates:

Start dates

- If the day portion of the start date is missing, then the start date will be estimated to be equal to the date of first dose of study drug, provided the start month and year are the same as the first dose of study drug and the stop date is either after the first dose of study drug or completely missing. Otherwise, the missing day portion will be estimated as "01."
- If both the day and month portions of the start date are missing, then the start date will be estimated to be equal to the date of first dose of study drug, provided the start year is the same as the first dose of study drug and the stop date is either after the first dose of study drug or completely missing. Otherwise, the event will be assumed to start on the first day of the given year (eg, ??-???-2013 is estimated as 01-JAN-2013).
- If the start date is completely missing and the stop date is either after the dose of study drug or completely missing, the start date will be estimated to be the day of study drug dosing. Otherwise, the start date will be estimated to be the first day of the same year as the stop date. All other non-AE and nonconcomitant medication day calculations where only partial dates are available will be handled as follows: the first day of the month will be used in the calculations if the day part of a start date is missing while January 1 will be employed if both the month and day parts of a start date are missing.

Stop dates

- If only the day of resolution is unknown, the day will be assumed to be the last of the month (eg, ??-JAN-2013 will be treated as 31-JAN-2013).
- If both the day and month of resolution are unknown, the event will be assumed to have ceased on the last day of the year (eg, ??-???-2013 will be treated as 31-DEC-2013).
- If the stop date is completely missing or the event is continuing, the event will be assumed to be after first dose of study drug and will be imputed using the last known date on the study.

Appendix C: Standard calculations

Variables requiring calculation will be derived using the following formulas:

- **Days** – A duration expressed in days between 1 date (date1) and another later date (date2) is calculated using the formulas noted below:
  duration in days = date2 – date1 + 1
- **Months –** A duration expressed in months is calculated using the INTCK function of SAS as follows: months=intck('month','date1'd,date2'd, 'continuous').
- **Years –** A duration expressed in years between 1 date (date1) and another later date (date2) is calculated as follows:
  duration in years = intck('year,'date1'd,date2'd, 'continuous').
- **Height –** Height entries made in inches (in) are converted to centimeters (cm) using the following formula: height (cm) = height (in) × 2.54.
- **Weight** – Weight entries made in pounds (lb) are converted to kilograms (kg) using the following formula: weight (kg) = weight (lb)/2.2046.
- **Temperature –** Temperature entries in degrees Fahrenheit are converted to degrees centigrade using the following formula:
  temp (degrees centigrade) = 5/9 × [temp (degrees Fahrenheit) – 32].
- **Change from baseline** – Change from baseline will be calculated as:
  Change = postbaseline value – baseline value.
- **Percent change from baseline –** Change from baseline will be calculated as:
  Percent change from baseline = (postbaseline value – baseline value)/baseline value × 100.

Appendix D: SAS Programming QC Requirements

Derived datasets are independently programmed by two programmers. The separate datasets produced by the 2 programmers must match 100%. Detailed specifications for the derived datasets are documented in the study analysis dataset specifications provided to the client at study conclusion.

Tables and listings are independently reprogrammed by a second programmer.
Listings are checked for consistency against corresponding tables, figures, and derived datasets.

The entire set of TLs is checked for completeness and consistency prior to its delivery to the client by the lead biostatistician and a senior level, or above, reviewer.

Appendix E: List of Tables, Figures, and Listings

The following proposal for Section 14 and 16.2 of the CSR is completed according to ICH E3 guidelines. The ICH heading numbers and description are in **bold**. Minor changes from this planned index do not need to be amended in the SAP.

Formal organization of tabulations may be changed during programming, if appropriate, (eg, tables for the different variables may be combined into a single table, or tables with more than 1 variable may be split into several tables).

TABLES, FIGURES AND GRAPHS

Table Number	Table Title	Comment
14	**TABLES, FIGURES, AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT**	
14.1	**DEMOGRAPHIC DATA**	
14.1.1	Subject Disposition	All Subjects
14.1.2	Demographic and Baseline Characteristics	Safety Analysis Set
14.1.3	Medical History	Safety Analysis Set
14.1.4	Baseline Characteristics of the Fatty Liver	Safety Analysis Set
14.3	**Safety data**	
14.3.1	**Displays of Adverse Events**	
14.3.1.1	Overall Summary of Study Food Product-Emergent Adverse Events: Study Part 1	Safety Analysis Set
14.3.1.2	Study Food Product-Emergent Adverse Events by System Organ Class and Preferred Term: Study Part 1	Safety Analysis Set
14.3.1.3	Study Food Product -Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity: Study Part 1	Safety Analysis Set
14.3.1.4	Study Food Product -Emergent Adverse Events by System Organ Class, Preferred Term, and Closest Relationship to Study Food Product: Study Part 1	Safety Analysis Set
14.3.4	**Laboratory**	
14.3.4.1	Serum Chemistry – Shift from Baseline: Study Part 1	Safety Analysis Set
14.3.4.2	Haematology – Shift from Baseline: Study Part 1	Safety Analysis Set
14.3.4.3	Lipids – Shift from Baseline: Study Part 1	Safety Analysis Set

Section 16.2: **List of Data Listings**

ICH Listing Number	Listing Title	Comment
16.2	**SUBJECT DATA LISTINGS**	
16.2.1	**Discontinued Subjects**	
16.2.1.1.1	Subject Disposition: Study Part 1	All Subjects
16.2.1.1.2	Subject Disposition: Study Part 2	All Subjects
16.2.2	**Protocol deviations**	
16.2.2	Protocol Deviations	Safety Analysis Set
16.2.3	**Subjects excluded from the efficacy analysis**	
16.2.3	Inclusion/Exclusion Criteria	Safety Analysis Set
16.2.4	**Demographic data**	
16.2.4.1	Demographics	All Subjects
16.2.4.2	Medical history	Safety Analysis Set
16.2.4.3	Prior and Concomitant Medications	Safety Analysis Set
16.2.5	**Compliance and/or drug concentration data**	
16.2.5.1	Study Food Product Administration	Safety Analysis Set
16.2.6	**Physiological effect analyses**	
16.2.6.1	Liver Multi-Scan Screening	Safety Analysis Set
16.2.6.2	Liver Multi-Scan Enhanced	Safety Analysis Set
16.2.6.3	Lean Muscle Mass scan	Safety Analysis Set
16.2.6.4	Visceral Adipose Tissue scan	Safety Analysis Set
16.2.6.5	Liver volume	Safety Analysis Set
16.2.7	**Adverse events listings**	
16.2.7.1	Adverse Events	Safety Analysis Set
16.2.7.2	Serious Adverse Events	Safety Analysis Set
16.2.7.3	Adverse Events Leading to Study Discontinuation	Safety Analysis Set
16.2.7.4	Deaths	Safety Analysis Set
16.2.8	**Listing of individual laboratory measurements by subject, when required by regulatory authorities**	
16.2.8.1	Hematology	Safety Analysis Set
16.2.8.2	Chemistry	Safety Analysis Set
16.2.8.3	Coagulation	Safety Analysis Set
16.2.8.4	Urinalysis	Safety Analysis Set
16.2.8.5	Vital Signs	Safety Analysis Set
16.2.8.6	Physical Examination	Safety Analysis Set
16.2.8.7	Pregnancy Test	Safety Analysis Set
16.2.8.8	Electrocardiogram	Safety Analysis Set
16.2.8.9	Fibroscan or Right Upper Quadrant (RUQ) Ultrasound	Safety Analysis Set
16.2.8.10.1	Biomarkers - Metabolic, Inflammation and Fibrosis	Safety Analysis Set
16.2.8.10.2	Biomarkers - Enhanced Liver Fibrosis (ELF)	Safety Analysis Set

ICH Listing Number	Listing Title	Comment
16.2.8.10.3	Biomarkers - Apolipoproteins	Safety Analysis Set
16.2.8.11	Fasting Lipid Profile	Safety Analysis Set
16.2.8.12	Metabolic Panel	Safety Analysis Set
16.2.8.13	Pyruvic Acid, Plasma Glucose/Insulin, and Lactatel	Safety Analysis Set
16.2.8.14	Peripheral Blood Mononuclear Cells	Safety Analysis Set
16.2.8.15	Diet/Exercise Lifestyle Questionnaire	Safety Analysis Set
16.2.8.16	Hunger and Satiety Visual Analog Scale (VAS)	Safety Analysis Set
16.2.8.17	Lifestyle Counseling	Safety Analysis Set
16.2.8.18	Telephone Call	Safety Analysis Set