Microbiome Statistical Analysis Plan for the US Food and Drug Authority

Protocol Number and Title:	**ECO-RESET: A Phase 2B, Randomized, Double-Blind, Placebo-Controlled, Multiple Dose, Multicenter Study to Assess Efficacy and Safety of SER-287 in Adults with Active Mild-to-Moderate Ulcerative Colitis (SERES 201)**
Protocol Version and Date:	**Amendment 3** **8-October-2019**
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I confirm that I have reviewed this document and agree with the content.

APPROVALS
Seres Therapeutics
[redacted]

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GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
CRO	Contract Research Organization
CSP	Clinical Study Protocol
CSR	Clinical Study Report
eCRF	Electronic Case Report Forms
NGS	Next Generation Sequencing
SAP	Statistical Analysis Plan
mbSAP	Microbiome Statistical Analysis Plan
RNA-seq	RNA sequencing
TLF/s	Tables, Lists, and Figures
WMS	Whole Metagenomic Sequencing
Δm	Fold-change in a metabolite
$\Delta S_{obs\_spore\_dose}$	A per-subject measure of the magnitude of engraftment (ie, total species) where the dose is defined as being species detected in any SER-287 drug lot.
GSEA	Gene set enrichment analysis
UC	Ulcerative colitis

1. PURPOSE

This Microbiome Statistical Analysis Plan (mbSAP) details the statistical methodology to be used in analyzing secondary and exploratory microbiome endpoint study data and generate Tables, Listings and Figures (TLFs). It describes the variables and populations, anticipated data transformations and manipulations, and other details of the analyses not provided in the clinical SAP. This mbSAP addresses planned analysis and presentation of microbiome, transcriptomic, and metabolomic changes in subjects over the course of the study. The analysis of safety and clinical efficacy is described in the Clinical Statistical Analysis Plan, which is a separate document. The mbSAP will be finalized prior to database lock and describes the statistical analysis as it is foreseen when the study is being planned. Any deviations from the planned analyses will be described and justified in the final clinical study report (CSR).

This mbSAP is based on SER-287-201 Protocol Amendment 3 dated 08 October 2019 and its associated electronic case report forms (eCRF) Version 3.0, dated 06 May 2019.

This mbSAP is prepared for the United States Food and Drug Authority (FDA). A separate mbSAP will be provided for other regulatory authorities.

2. TIMINGS OF ANALYSES

2.1. AT THE END OF THE INDUCTION TREATMENT PERIOD

Seres will conduct an unblinded analysis of the secondary and exploratory microbiome endpoints when all planned subjects in the study are enrolled, have completed their Induction Treatment (Week 11 [Visit 7]) or have terminated from the study prematurely prior to Week 11 [Visit 7], and have been evaluated for the primary/key secondary efficacy endpoints. Analyses of the microbiome endpoints (described herein) will be performed following completion of the clinical endpoints (described in the Clinical SAP). Unblinded microbiome endpoint tables, listings and figures (TLFs) will be generated at this time. Limited personnel at Seres will have access to the unblinded TLFs.

Preprocessing and quality assessment of genomic, transcriptomic and metabolomic data may be conducted as data are generated and made available to Seres by qualified contract research organizations (CRO) to ensure that data are of appropriate quality and content to support secondary and exploratory endpoints. All data will first be analyzed in a blinded manner to ensure quality, and then unblinded to conduct the per mbSAP analysis. The mbSAP analyses described herein will be included in the CSR, or amended to the CSR as data, analyses, and reports are available.

2.2. AT THE END OF THE MAINTENANCE TREATMENT PERIOD

Seres will conduct an unblinded analysis of the exploratory microbiome endpoints when all subjects who were re-randomized in the Maintenance arm have been evaluated for the exploratory efficacy endpoints and have completed their Maintenance treatment (Week 37 [VisitM-7]) or have terminated the study prematurely prior to Week 37 [Visit M-7]. Unblinded microbiome efficacy endpoint summary tables will be generated at this time. Limited personnel at Seres will have access to the unblinded tables, listings and figures (TLFs).

Preprocessing and quality assessment of genomic, transcriptomic and metabolomic data may be conducted as data are generated and made available to Seres by qualified CRO to ensure that data are of appropriate quality and content to support exploratory endpoints. All data will first be analyzed in a blinded manner to ensure quality, and then unblinded to conduct the per mbSAP analysis. The mbSAP analyses described herein will be included in the CSR, or amended to the CSR as data, analyses, and reports are available.

2.3. AT THE END OF THE OPEN-LABEL TREATMENT PERIOD

Summary TLFs for relevant microbiome efficacy endpoint analyses for all subjects who entered the Open-Label Extension arm and have completed their Open-Label extension treatment (Week 0 [Visit OL-1] through Week 11 [Visit OL-5]) or have terminated the study prematurely prior to Week 11 [Visit OL-5] will be generated after final database lock. Preprocessing and quality assessment of genomic, transcriptomic and metabolomic data may be conducted as data are generated and made available to Seres by a qualified CRO to ensure that data are of appropriate quality and content to support exploratory endpoints. The mbSAP analyses described herein will be included in the CSR, or amended to the CSR as data, analyses, and reports are available.

3. STUDY OBJECTIVES

A comprehensive list of study objectives is provided in the CSP and Clinical SAP. The study objectives that are specifically addressed in this mbSAP are listed below.

3.1. OTHER SECONDARY OBJECTIVES

- To evaluate the engraftment of SER-287 bacteria from each SER-287 treatment arm into the intestinal microbial community over time

3.2. EXPLORATORY OBJECTIVES

- To evaluate changes in the composition of the intestinal microbiome over time
- To evaluate changes in signatures of host and microbial functional responses over time

4. ENDPOINTS

A comprehensive list of endpoints is provided in the CSP and Clinical SAP. The endpoints that are specifically addressed in this Microbiome SAP are listed below.

4.1. OTHER SECONDARY EFFICACY ENDPOINTS

- Engraftment of SER-287 bacteria over time

4.2. EXPLORATORY EFFICACY ENDPOINTS

- Composition of the intestinal microbiome through the end of the Maintenance treatment and Open-Label treatment periods
- Signatures of host and microbial functional responses through the end of the Maintenance treatment and Open-Label treatment periods

5. STUDY DESIGN

5.1. BRIEF DESCRIPTION

SERES-201 is a Phase 2B randomized, double-blind, placebo-controlled, multiple dose, multicenter study designed to evaluate the efficacy, safety and microbiome alterations associated with a regimen of pre-treatment with vancomycin followed by treatment with two (2) dose levels of SER-287, in adult subjects, age 18-80, with active mild-to-moderate UC. Microbiome alterations encompass engraftment of SER-287 dose species, changes in microbiome composition and changes in microbiome function.

The primary objective of the study is to assess the efficacy of once-daily dosing of SER-287 (Induction Dose) for 10 weeks, after pre-treatment with vancomycin, to achieve Clinical Remission in subjects with active mild-to-moderate ulcerative colitis. In addition, the study will evaluate multiple dosing regimens of SER-287, the safety and efficacy of SER-287, subjects' microbiome dynamics throughout treatment and post-treatment effects of SER-287.

Subjects will be randomized to one (1) of three (3) study arms (Table 1), each of which contain a six-day pre-treatment period (vancomycin or matching placebo) and a 10-week Induction Treatment period (SER-287 or matching placebo).

Table 1: SERES-201 Induction Treatment Arms

Arm	Pre-Treatment (QID for 6 days)	Induction Treatment (QD for 10 weeks)
A	Placebo	Placebo
B	Vancomycin	SER-287 Induction Dose
C	Vancomycin	SER-287 Step-Down Induction Dose

[redacted]

Subjects will be assessed for Clinical Remission status following 10 weeks of Induction Treatment.

- Remitters will be re-randomized to one (1) of two (2) Maintenance Treatment study arms (Table 2):
    - Arm D, treatment with placebo, to assess durability of Clinical Remission, and serve as a control
    - Arm E, treatment with a SER-287 Maintenance Dose, to assess Maintenance of Clinical Remission with a once-weekly dosing regimen
- Non-Remitters will be eligible to enter the Open-Label Extension arm, Arm F (Table 2).

All subjects will be followed for long-term safety for 26 weeks following their last treatment dose. The study duration from Screening through last follow-up, will be up to 67 weeks.

Table 2: SERES-201 Post-Induction Treatment Arms

Arm	Study Phase	Pre-Treatment (QID for 6 days)	Treatment	Frequency of Treatment	Treatment Duration
D	Maintenance Treatment	N/A	Placebo	Once-Weekly	26 weeks
E		N/A	SER-287 Maintenance Dose	Once-Weekly	26 weeks
F	Open-Label Extension	Vancomycin	SER-287 Induction Dose	Once-Daily	10 weeks

[redacted]

5.2. SUBJECT SELECTION

Detailed lists of inclusion and exclusion criteria are given in Section 4.7 of the Clinical SAP.

5.3. DETERMINATION OF SAMPLE SIZE

Details regarding the determination of sample size are given in Section 4.8 of the Clinical SAP.

5.4. METHOD OF ASSIGNING SUBJECTS TO STUDY TREATMENT

Subject identification, methods of assigning patients to study treatment arms are described in Section 4.9 and of the Clinical SAP.

5.5. BREAKING THE STUDY BLIND

After the mbSAP is final and the primary study period (Week 0 [Visit 3] to Week 11 [Visit 7]) data is declared complete and final, the study blind codes will be broken for the Induction period efficacy analyses. The Maintenance Treatment period will remain blinded until all data collected through M-Week 37 (Visit M-7) has been entered, cleaned and declared complete and final. Only limited personnel at Seres and the unblinded statistical and programming team will have access to the treatment code when the study blind is broken at the end of the Induction phase and at the end of the Maintenance phase. The remaining study team personnel (majority), PIs and subjects will remain blinded until the study is completed. A detailed unblinding plan will be implemented concurrently with the clinical SAP and mbSAP analyses

Due to the time lag involved in generating microbiome data, clinical results by treatment group, but not subject by subject unblinded data, will be available prior to start of any microbiome analyses for each phase of the study. Samples will be processed in a fashion to ensure unbiased analysis of all populations. Specifically, blinded genomic, metabolomic, and transcriptomic data will be analyzed at the end of each phase of the study (i.e., Induction, Maintenance). Data for each treatment phase will be processed through predefined computational pipelines after the database lock to generate microbiome, metabolomic, and transcriptomic profiles. During these data generation and processing steps, the study blind will be maintained through the use of dummy randomization codes.

Final TLFs for all subjects at the end of each treatment phase will be generated using true treatment codes upon unblinding, but before subjects have completed the following treatment phase or long-

term safety follow-up. Since the study is still ongoing, only a limited number of study team members will have access to the true treatment code at subject level.

Microbiome genomic, metabolomic, and host transcriptomic datasets used for secondary and exploratory endpoints analyses will be generated [redacted]. Data transfer from the vendors to Seres will occur via secure channels (*e.g.*, encrypted and password-protected hard drive, secure cloud transfer, etc.). All TLFs will be stored in a secure network location, which only the unblinded genomic data team at Seres will be able to access. A change log will be maintained on the primary data files to ensure that they are not modified. If any samples need to be re-sequenced due to issues of poor quality, this will be noted in the final analysis.

5.6. ASSESSMENTS AND PROCEDURES

For a schedule of assessments and procedures (Induction treatment, Maintenance treatment for remitters and Open-Label extension arm for non-remitters), please refer to the Schedule of Events section of the Clinical SAP. The schedule of assessments for microbiome endpoints are summarized below, Table 3.

5.6.1. Schedule of Assessments for Microbiome Data Collection

This mbSAP documents the analysis and presentation of microbiome compositional and functional changes. For clarity, we present below the schedule of assessments (Table 3) filtered to show only samples collected for the endpoints addressed in this document.

Metagenomic, metabolomic, and host transcriptomic data will be generated for the Induction phase. Metagenomic data will also be generated for the Maintenance and Open-label phases.

In the Maintenance Phase, if clinical remission is significantly greater ($p<0.05$) in the SER-287 arm (Arm E) compared to Placebo (Arm D) as assessed in the Clinical SAP, metabolomics and host transcriptomics data will be generated from the last scheduled visit (Week 37) or the last visit prior to Week 37 with a collected sample. In the Open Label phase, if at least 29% of subjects achieve Clinical Remission, as defined in the Clinical SAP, metabolomics and host transcriptomics data will be generated for the last time point in the Open Label phase (Week 11). Metabolomics and host transcriptomics data may also be generated for the Maintenance and Open-label phases at sponsor's discretion.

Table 3. Schedule of sample collections and data types to be generated for microbiome endpoint analysis.

Visit Number	Week Number	Study Phase	Period	Sample Type	Metagenomics	Metabolomics	Transcriptomics
1	Week -4 to -1	Induction	Screening	Stool	x	x	
2	Week -4 to -1	Induction	Screening	Biopsy			x
3	Week 0	Induction	Pre-Treatment				
4	Week 1	Induction	Treatment	Stool	x	x	
5	Week 3	Induction	Treatment	Stool	x	x	
6	Week 7	Induction	Treatment	Stool	x	x	
7	Week 11	Induction	Treatment	Stool	x	x	
				Biopsy			x
M-1	M-Week 12	Maintenance	Treatment				
M-2	M-Week 16	Maintenance	Treatment	Stool	x		

M-3	M-Week 20	Maintenance	Treatment	Stool	x		
M-4	M-Week 24	Maintenance	Treatment	Stool	x		
M-5	M=Week 28	Maintenance	Treatment	Stool	x		
M-6	M-Week 32	Maintenance	Treatment	Stool	x		
M-7	M-Week 37	Maintenance	Treatment	Stool	x	*	
				Biopsy			*
OL-1	OL-Week 0	Open-Label	Pre-Treatment				
OL-2	OL-Week 1	Open-Label	Treatment	Stool			
OL-3	OL-Week 3	Open-Label	Treatment	Stool	x		
OL-5	OL-Week 11	Open-Label	Treatment	Stool	x	*	
				Biopsy			*

x = Definitive sample analysis; * = Potential sample analysis pending trial outcome targets as outlined in Section 5.6.1.

6. ANALYSIS POPULATIONS

There will be four (4) analysis populations used in the mbSAP listed below.

6.1. MICROBIOME MODIFIED INTENT-TO-TREAT (MCITT) POPULATION

The mcITT population is a subset of the mITT-1_IP population defined in the clinical SAP. The mcITT population will consist of all randomized subjects with a baseline evaluation for the induction phase, who have active mild-to-moderate UC when the induction phase starts, receive any amount of study drug during the induction phase, and have an evaluable stool sample collected at baseline and at least one (1) evaluable stool sample collected after the start of dosing. Evaluable stool samples are defined as samples that pass the sample acceptability and quality control criteria for WMS (see Section 7.2). Subjects will be analyzed based on the treatment to which they are randomized.

6.2. MICROBIOME AS-TREATED INDUCTION PHASE (MBAT_IP) POPULATION

The mbAT_IP population will consist of all randomized subjects with a baseline evaluation for the induction phase, who have active mild-to-moderate UC when the induction phase starts, receive any amount of study drug during the induction phase, and have an evaluable stool sample collected at baseline and at least one (1) evaluable stool sample collected after the start of dosing. Evaluable stool samples are defined as samples that pass the sample acceptability and quality control criteria for any of WMS, Metabolomics, or Transcriptomics data types (see Section 7.2). Subjects will be analyzed based on the treatment received, rather than the treatment to which they were randomly assigned.

6.3. MICROBIOME AS-TREATED MAINTENANCE PHASE (MBAT_MP) POPULATION

The mbAT_MP population will consist all subjects who were mITT-1 population during the induction phase, enter the maintenance phase, are re-randomized in the maintenance phase, receive any amount of study drug during the maintenance phase, and have an evaluable stool sample collected at baseline and at least one (1) evaluable stool sample collected after the start of dosing during the maintenance phase. Evaluable stool samples are defined as samples that pass the sample acceptability and quality control criteria for any of WMS, Metabolomics, or

Transcriptomics data types (see Section 7.2). Subjects will be analyzed based on the treatment received, rather than the treatment to which they were randomly assigned.

6.4. MICROBIOME AS-TREATED OPEN-LABEL PHASE (MBOL) POPULATION

The mbOL population is a subset of the mITT-1_OL population defined in the clinical SAP. The mbOL population will consist of all subjects who were mITT-1 population during the induction phase, assigned to the open-label phase (as indicated in the "Status" CRF page), receive any amount of study drug during the open-label phase, and have an evaluable stool sample collected at baseline and at least one (1) evaluable stool sample collected after the start of dosing during the open-label phase. Evaluable stool samples are defined as samples that pass the sample acceptability and quality control criteria for any of WMS, Metabolomics, or Transcriptomics data types (see Section 7.2).

7. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

7.1. PLANNED COMPARISONS FOR MICROBIOME ENDPOINTS

Unless otherwise stated, all the comparisons below will be performed for the indicated study phase. Specific metrics and hypotheses are outlined in Sections 10.3. and 10.4.

7.1.1. Treatment-associated comparisons

Treatment-associated comparisons will assess the effects of SER-287 on microbiome measures, based on the following comparisons.

Induction phase:

- Treatment Arms B and C separately compared to placebo (Arm A). Compares individual dosing regimens to placebo.
- Treatment Arms B and C pooled together compared to placebo (Arm A). Compares both arms with SER-287 treatment to placebo.
- Treatment Arms B vs C. Compares the Induction dose and the step-down Induction dose.

Maintenance phase:

- Treatment (Arm E) compared to placebo (Arm D). Compares Maintenance dosing to placebo.

Open-label phase:

- For engraftment and microbiome composition metrics, no hypothesis testing will be performed. Instead, descriptive statistics will be reported.
- For stool metabolite composition and host mucosal functional response, hypothesis tests will be performed to assess change from baseline.

7.1.2. Outcome-associated comparisons

Outcome-associated comparisons will assess microbiome correlates of Clinical Remission and Endoscopic Improvement (as defined in the Clinical SAP). These comparisons will assess if

microbiome measures affect the likelihood of these clinical outcomes. For both Clinical Remission and Endoscopic Improvement, the following comparisons will be performed.

Induction phase:

- Subjects achieving the clinical outcome compared to subjects not achieving the clinical outcome within SER-287 treatment arms (Arms B and C).

[redacted]

7.2. SAMPLE ACCEPTABILITY CRITERIA AND QUALITY CONTROL

Samples are collected according to the time period described in the Schedule of Events (Table 3). Failure to meet quality thresholds (detailed below) will result in removal of a sample from analysis. Deviations in sample handling may result in removal of a sample from analysis.

7.2.1. Whole metagenomic sequencing

DNA will be extracted from stool samples at Seres via a qualified automated process on verified equipment. WMS on the extracted DNA will be performed [redacted]. Sample quality control will be performed by the vendor and subject to their quality control procedures. Additional details regarding specific sample quality control procedures will be obtained in a statement of work.

To ensure comparability between different subject samples, each sample will be subsampled to equalize the number of reads before subsequent analyses. If samples are sequenced multiple times (e.g., with multiple sample preparation methods; see Section 8.2.1), they will be merged to obtain one microbiome profile per sample; subsampling will occur either before or after profiles are merged and will be pre-specified in the analysis protocol. Before unblinding, the distribution of the number of reads per sample will be assessed; based on this distribution, a subsampling

level will be selected that maximizes the number of included samples while also maintaining a depth sufficient to characterize the microbiome profile. Any sample with a number of reads below this base level will be discarded from further analysis.

7.2.2. Metabolomics

Metabolomic samples will be processed, and quality control will be performed [redacted]. Sample quality control will be performed by the vendor and subject to their quality control procedures. Additional details regarding specific sample quality control procedures will be obtained in a statement of work.

7.2.3. Transcriptomics

Transcriptomics samples will be processed [redacted]. Sample quality control will be performed by the vendor and subject to their quality control procedures. Additional details regarding specific sample quality control procedures will be obtained in a statement of work.

7.3. STATISTICAL ANALYSIS -- GENERAL METHODS

- Continuous variables will be summarized using the number of subjects with evaluable data, mean, standard deviation (SD), median, minimum and maximum.
- Categorical variables will be summarized using the number of observations (n), frequency and percentage of subjects. All percentages will be presented as one-decimal point, unless otherwise specified. Percentages equal to 100 will be presented as 100% and percentages will be presented as 0% for zero frequencies. Unless stated otherwise, the percentages will be based on the number of non-missing observations.
- Any calculated p-values will be presented to 3 decimal places; p-values less than 0.001 will be presented as ‘p<0.001’ and p-values greater than 0.999 will be presented as ‘p>0.999’.
- For statistical analyses involving hypothesis testing across multiple related variables (i.e., bacterial species, stool metabolites, and host transcripts), adjusted p-values will additionally be reported to estimate the false discovery rate (FDR). Any adjusted p-value will be presented to 3 decimal places; adjusted p-values less than 0.001 will be presented as ‘p<0.001’ and adjusted p-values greater than 0.999 will be presented as ‘p>0.999’.
- Unless otherwise stated, all formal tests of hypotheses will be conducted at the two-sided level of significance with alpha=0.05.

- All relevant subject data will be included in listings and sorted by treatment, Subject ID, and visit, as applicable, for all randomized subjects.
- Unscheduled or repeat assessments will not be included in summary tables unless specified otherwise but will be included in the subject listings.
- All tables, listings and figures will include footers that identify the name of the program that created the item, together with the date and time on which it was created. Headers will include the total number of pages that the presentation contains and, for each page, the number of the page within the presentation.

7.4. KEY DEFINITIONS

7.4.1. Study Day

For the induction phase, Study Day 1 is defined as the first dosing day of induction study drug. Subsequent days are numbered consecutively (Day 2, Day 3, etc.). Before the day of study drug administration, study days are numbered sequentially with negative values (i.e., Day -1, Day -2, etc.). There is no Day 0.

[redacted]

[redacted]

7.4.2. Baseline values

For each study phase, baseline values will be taken as the last assessments within 30 days before dosing with any study drug for the specified phase. The baseline values for different phases of the study are defined as follows, unless otherwise specified:

- For the Induction phase, baseline values are between Day -30 and Day 1. That is, baseline values are assessments done at screening or pre-dose assessments on Day 1 and within 30

days of Day 1, using the time point that is designated as the sample acquired at the Visit 1 screening timepoint closest to but prior to dosing of any Induction treatment study drug.

[redacted]

[redacted]

7.4.3. SER-287 drug product species definitions

SER-287 is a formulation of bacterial Firmicute spores that are enriched and purified from human stool. Briefly, stool donations undergo Good Manufacturing Process-compliant manufacturing steps including clearance of non-spore forms of bacteria, fungi, parasites and viruses via solvent treatment and by sequential purification steps.

[redacted]

[redacted]

7.5. MISSING DATA

Every effort will be made to collect all data at specified time points, according to the schedule of study events. Missing data will not be imputed.

7.6. VISIT WINDOWS

Samples collected at scheduled visits will be assigned to nominal visits if they fall within the visit windows delineated in Table 4 for that scheduled visit. The study visits scheduled after randomization should occur at the times delineated in the Schedule of Events section of the protocol amendment 3. Samples collected at unscheduled or end of treatment visits will not be assigned to nominal visits.

Table 4: Visit Windows for mbSAP

Visit Number in CSP	Week Number in CSP	Visit window for mbSAP
1	Week -4 to -1	Days -30 to 1
4	Week 1 +/- 1 day	Days 2-11
5	Week 3 + 3 day	Days 13-35
6	Week 7 + 3 day	Days 40-60
7	Week 11 + 3 day	Days 65-100
Visit M-2	M-Week 16 + 3 days	Days 14_MP-42_MP
Visit M-3	M-Week 20 + 3 days	Days 43_MP-70_MP
Visit M-4	M-Week 24 + 3 days	Days 71_MP-98_MP
Visit M-5	M-Week 28 + 3 days	Days 99_MP-126_MP
Visit M-6	M-Week 32 + 3 days	Days 127_MP-154_MP
Visit M-7	M-Week 37 + 3 days	Days 155_MP-196_MP
Visit OL-2	OL-Week 1 +/- 1 day	Days 2_OL-11_OL

Visit OL-3	OL-Week 3 + 3 days	Days 13_OL-35_OL
Visit OL-5	OL-Week 11 + 3 days	Days 65_OL-100_OL

7.7. MULTIPLICITY ADJUSTMENTS

Adjustments for multiple testing will be made for hypotheses involving tests over multiple microbial species, metabolites, and transcripts; adjustments will be made within a given endpoint and timepoint and not across multiple timepoints or endpoints. The false discovery rate (FDR) at a given p-value cutoff will be controlled based on the Benjamini-Hochberg procedure; both p-values and corrected p-values will be reported.

8. ENDPOINTS FOR MICROBIOME ANALYSIS

8.1. MICROBIOME ENDPOINTS – BACKGROUND AND OVERVIEW

SER-287 is a formulation of bacterial Firmicute spores that are enriched and purified from human stool. The basic mechanism of action is thought to require engraftment of bacteria derived from SER-287 into the gastrointestinal tract of patients. We quantify engraftment, which is a measure of SER-287 pharmacokinetics (PK), based on whole metagenomic sequencing of both patient stool samples and SER-287 drug product. *Engraftment of SER-287 is assessed as a secondary endpoint and further characterized in exploratory endpoints.*

Engraftment of SER-287 is then hypothesized to result in: a) compositional change in the microbiome, b) functional change in microbiome, including change in the fecal metabolome, and c) functional change in the host, including change in the host transcriptome. Each of these changes that follow engraftment are aspects of SER-287 pharmacodynamics (PD) and will be assessed as exploratory endpoints.

Engraftment of SER-287 can result in other compositional changes in the microbiome through microbe-microbe and microbe-host interactions. This can manifest as some species becoming more prevalent while others become less prevalent. These broader *compositional changes in the microbiome will be assessed based on whole metagenomic sequencing (WMS) of patient stool in exploratory endpoints*.

Microbial metabolism is a mechanism by which the microbiome can affect host inflammation and barrier integrity through multiple disease-relevant pathways. Production and consumption of metabolites by gut microbes changes the concentration of these metabolites in the intestinal lumen and host tissue. Metabolites that are modulated by gastrointestinal microbes can subsequently impact host inflammation and epithelial barrier integrity (Brestoff and Artis, 2013). The fecal metabolome of patients will be characterized by LC-MS and LC-MS/MS methods. *Change in the concentration of microbially-modulated metabolites in patient fecal samples will be assessed in exploratory endpoints*.

The change in the molecular environment of the gastrointestinal tract due to change in the microbiome and metabolome in turn affects host gene expression. Some bacterially-modulated metabolites can directly regulate host gene expression; furthermore, bacterial antigens affect host gene expression. Thus, through several mechanisms, SER-287 is expected to alter gene expression in intestinal tissue. The host transcriptome of patients will be assessed by RNA-seq

from patient colonic biopsies and *change in host transcriptome will be assessed in exploratory endpoints.*

In summary, a sequence of events is thought to underlie the therapeutic effect of SER-287 starting from engraftment of SER-287 bacteria, and followed by broader compositional change in the microbiome, change in the fecal metabolome, and change in the host transcriptome. Each of these events will be separately assessed in this mbSAP.

The assessments in the mbSAP are either treatment- or outcome-associated comparisons (Section 7.1). Treatment-associated comparisons will assess the effect of treatment arm on microbiome PK and PD measures. Outcome-associated comparisons will assess if microbiome PK or PD measures are associated with Clinical Remission and Endoscopic Improvement. Collectively, these two sets of comparisons aim to identify PK and PD effects of SER-287 that may affect the likelihood of clinical outcomes and may underlie the mechanism of action of SER-287.

8.2. MICROBIOME ENDPOINTS – MOLECULAR AND BIOINFORMATIC METHODS

8.2.1. Stool microbiome taxonomic profiling

We will assess the bacterial taxonomic composition of SER-287 drug product lots and subject stool samples using whole metagenomic sequencing (WMS). WMS is a widely used method for profiling microbiomes which involves shotgun genomic sequencing of bulk DNA obtained from samples of interest. [redacted] The resulting WMS data can be used to identify specific species, subject to limitations due to assay sensitivity and the availability of complete bacterial genomes required for constructing reference databases.

[redacted]

To ensure comparability between different subject samples, each sample will be subsampled to equalize the number of reads before subsequent analyses based on methods outlined in Section 7.2.1. Any subject sample with a number of reads below this base level will be discarded from further analysis. Outputs from this computational pipeline will be referred to as the "microbiome profile" (i.e., the relative abundance of bacterial species) of a sample.

8.2.2. Stool metabolomics profiling

The fecal metabolome of patients will be characterized by LC-MS and LC-MS/MS through global profiling and targeted profiling of short-chain fatty acids. In targeted metabolomic profiling the concentrations of a specific set of metabolites is determined with well-defined limits of quantification. In global profiling, hundreds of metabolites across diverse classes are identified and quantitated in an unbiased manner, but the quantitative concentrations are not determined; instead, peaks are quantified using area-under-the-curve. Compounds will be identified based on retention index, mass and/or MS/MS spectrum in comparison to a library of purified standards and/or recurrent unknown entities. Determined concentrations (for targeted profiling) and peak areas (for global profiling) will be referred to as the "metabolomic profile" of a sample.

8.2.3. Host mucosal transcriptome profiling

The transcriptome of patients will be characterized by whole transcriptome sequencing using next-generation sequencing technology (NGS). RNA-Seq data from the mucosal biopsy samples from each subject will be generated. Total RNA will be extracted from biopsies and reverse transcribed to generate cDNA libraries that are sequenced using an Illumina sequencing platform. Sequence data will be processed for quality with well-established sequencing criteria and reads will be aligned to a current reference human genome. Following alignment to the human reference genome, mapped gene counts will be computed. Outputs from this

computational pipeline (i.e., mapped read counts for each detected human transcript) will be referred to as the "transcriptome profile" of a sample.

8.3. SECONDARY ENDPOINT: SER-287 ENGRAFTMENT IN INDUCTION PHASE

Engraftment of SER-287 into subject microbiomes is measured relative to subject baseline samples, as defined in Section 7.4.2. Baseline samples in this analysis represent subject microbiome composition prior to antibiotic pre-conditioning and treatment with SER-287. As SER-287 is derived from healthy, screened donor stool, some subset of SER-287 species are expected to be present in some subject baseline samples. Thus, microbiome engraftment will be measured by the number of spore-forming species detected in SER-287 that are absent in subject baseline samples, and present post-treatment, [redacted]

[redacted]

8.4. EXPLORATORY ENDPOINTS: EFFECTS OF TREATMENT WITH SER-287 ON THE INTESTINAL MICROBIOME AND MICROBIAL FUNCTIONAL RESPONSES

8.4.1. SER-287 engraftment

As outlined above, the magnitude of SER-287 engraftment will be assessed in the Induction phase using the *spore dose* drug product definition (outlined in Section 7.4.3) as a secondary endpoint. [redacted]

[redacted]

8.4.2. Microbiome species composition and microbial functional response

Engraftment of SER-287 spore-forming species is expected to mediate broader changes in the microbiome. In addition to engrafting species, the presence of other species (i.e., spore-forming bacterial species not detected in SER-287 and non-spore-forming species) are expected to change post-treatment. Some of these species are expected to appear after the start of treatment, or to disappear after the start of treatment. The gain and loss of species as a whole forms a broader characterization of how SER-287 treatment both directly and indirectly affects microbiome compositional change compared to the natural flux of species gains and losses that occur in placebo subjects.

The change in the composition of the microbiome is in turn expected to alter patients' fecal metabolome. Production and consumption of metabolites by gut microbes changes the concentration of these metabolites in the intestinal lumen. To assess this microbial functional response, we will evaluate changes in concentrations of metabolite levels in fecal samples due to SER-287 treatment.

8.4.2.1. Frequency of species newly appearing post-treatment

Newly appearing species will be defined for each subject and post-treatment time point as those species which are absent in a subject's baseline sample and present post-treatment. These include both engrafting species and other species that newly appear post-treatment.

To assess whether the frequency of newly appearing species differs across arms, we will first generate a 2x2 contingency table for each species based on the presence or absence of that species in subjects' stool across the prespecified pairwise comparisons of treatment groups at a particular time point. The contingency table will have two categories: species absent at baseline and present post-treatment, and species absent at baseline and absent post-treatment. For each species, we will then calculate a two-sided p-value based on a Fisher's Exact test to assess if the frequency of newly appearing species differs across arms at the given time point. FDR q-values will also be reported to correct for multiple hypotheses within each time point separately. Species detected in fewer than 5 subjects at a given time point across all treatment groups will not be assessed at that time point.

We will test the following hypotheses at Week 1, Week 3, Week 7 and Week 11 for the mbAT_IP population and at M-Week 16, M-Week 20, M-Week 24, M-Week 28, M-Week 32,

and M-Week 37 for the mbAT_MP population for pairwise comparisons detailed in Section 7.1.1:

- H_0: The frequency of a newly appearing species does not differ between treatment arms.
- H_A: The frequency of a newly appearing species does differ between treatment arms.

Descriptive statistics will be provided for newly appearing species for the mbOL population at OL-Week 3 and OL-Week 11; in the absence of a placebo arm in the Open-Label Phase, no p-values will be reported.

8.4.2.2. Frequency of species newly absent post-treatment

Newly absent species will be defined for each subject and post-treatment time point as those species which are present in a subject's baseline sample and absent post-treatment.

To assess whether the frequency of inhibited species differs across arms, we will first generate a 2x2 contingency table for each species based on the presence or absence of that species in subjects' stool across pairwise comparisons of treatment groups. The contingency table will have two categories: species present at baseline and absent post-treatment, and species present at baseline and present post-treatment. For each species, we will then calculate a two-sided p-value based on a Fisher's Exact test to assess if the frequency of newly absent species differs across arms at a given time point. FDR q-values will also be reported to correct for multiple hypotheses within each time point separately. Species detected in fewer than 5 subjects at a given time point not be assessed at that time point.

We will test the following hypotheses at Week 1, Week 3, Week 7 and Week 11 for the mbAT_IP population and at M-Week 16, M-Week 20, M-Week 24, M-Week 28, M-Week 32, and M-Week 37 for the mbAT_MP population for pairwise comparisons detailed in Section 7.1.1:

- H_0: The frequency of a newly absent species does not differ between treatment arms.
- H_A: The frequency of a newly absent species does differ between treatment arms.

Descriptive statistics will be provided for newly absent species for the mbOL population at OL-Week 3 and OL-Week 11; in the absence of a placebo arm in the Open-Label Phase, no p-values will be reported.

8.4.2.3. Stool metabolite composition

The fecal metabolome of patients will be characterized by LC-MS and LC-MS/MS through global profiling and targeted profiling of short-chain fatty acids at baseline and post-treatment. For each detected metabolite, we will assess the log2 fold-change of metabolite abundance, Δm, from baseline compared to post-treatment. Undetected metabolites in samples will be imputed to the minimum observed level across all samples before fold-change calculation.

To assess whether the fold-change in metabolite abundance from baseline, Δm, differs between prespecified treatment arm comparisons, we will use a linear model:

$$\Delta m \sim \boldsymbol{\beta}_{Arm}Arm$$

FDR q-values will also be reported to correct for multiple hypotheses within each time point separately.

We will test the following hypotheses at Week 3 and Week 11 for the mbAT_IP population and at M-Week 37 for the mbAT_MP population (pending trial outcome targets outlined in Section 5.6.1) for pairwise comparisons detailed in Section 7.1.1:

- H_0: The fold-change of metabolites (Δm) post-treatment does not differ between treatment arms.
- H_A: The fold-change of metabolites (Δm) post-treatment does differ between treatment arms.

In the Open-Label phase, pending trial outcome targets outlined in Section 5.6.1 we will assess if the fold-change in metabolite abundance from baseline to OL-Week 11, Δm, is centered around zero using a linear model for the mbOL population. FDR q-values will also be reported to correct for multiple hypothesis correction within each time point separately.

For all comparisons, in addition to reporting statistics for all measured metabolites, we will report the results for the following metabolites and ratios of metabolite concentrations that are specifically hypothesized to be affected by SER-287:

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8.4.3. Host mucosal functional response

Microbial species and metabolites that change as a result of treatment by SER-287 interface with the host intestinal mucosal layer and can directly or indirectly affect host gene expression. We will evaluate changes in gene expression from baseline to the end of treatment between subjects in SER-287 treatment arms versus placebo. Specifically, we will evaluate the change in aggregate in genes in specific pathways relevant to disease as well as in genes identified as being either positively or negatively associated with UC in the iHMP study (Lloyd-Price et al., 2019).

8.4.3.1. Differential host gene expression and pathway enrichment

To assess whether there are specific host genes associated with treatment with SER-287, we will compare the change in gene expression of subjects from baseline to post-treatment between prespecified treatment arm comparisons. [redacted]

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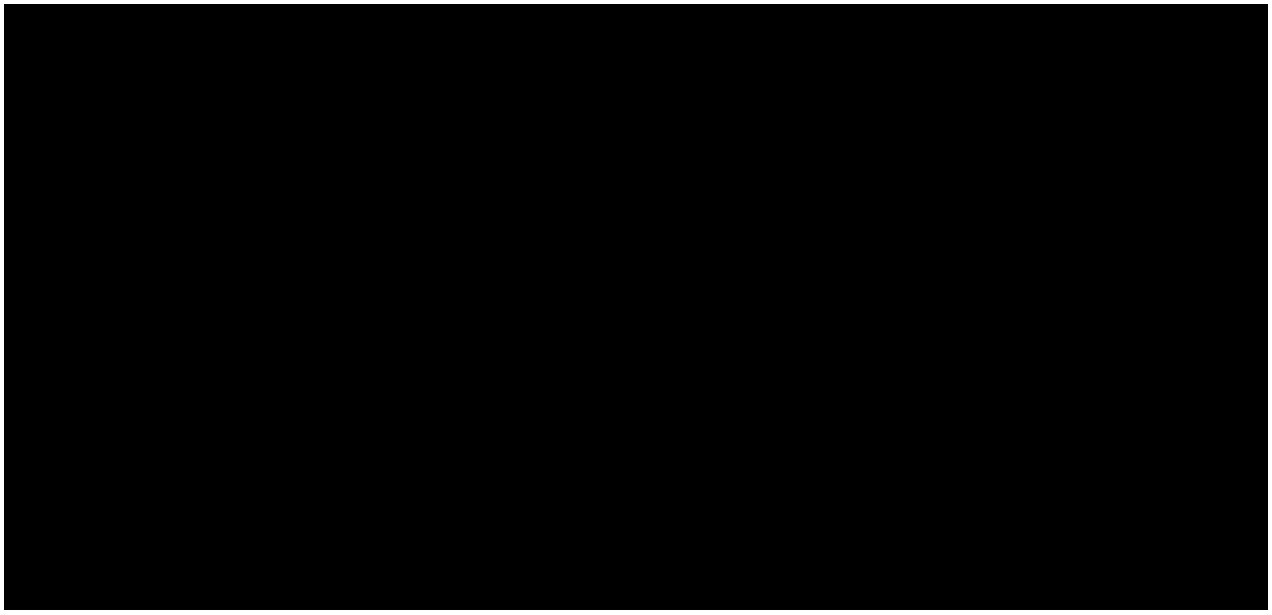

We will then test the association between treatment with SER-287 and the aggregate change in expression of genes in pre-specified gene sets (Table 6) with gene set enrichment analysis (GSEA). GSEA tests whether a predefined set of genes shows statistically significant differences between groups, which can be more powerful in detecting differences when a functionally related set of genes change in expression together, but individual genes may not reach the threshold of significance. GSEA will be run using the 'fgsea' R package.

Genes relevant to disease will be composed of two types of gene sets: 1) Gene sets defined in prespecified KEGG [Kyoto Encyclopedia of Genes and Genomes] (Kanehisa et al., 2019) pathways of interest; and 2) Genes which are significantly differentially expressed between subjects with ulcerative colitis and non-IBD subjects in the iHMP study (Lloyd-Price et al., 2019), applying the same linear model described above. For the latter (2), genes will also be partitioned into two categories: Those that are higher in UC vs. non-IBD subjects (N = 2901, FDR < 0.05), and those that are lower in UC vs. non-IBD subjects (N = 3924, FDR < 0.05). These will be tested as separate pathways.

For all gene sets, the set of shared genes between this list and those detected after quality filtering in study samples will be used in the GSEA analysis.

We will test the following hypotheses at Week 11 for the mbAT_IP population and at M-Week 37 for the mbAT_MP population (pending trial outcome targets outlined in Section 5.6.1) for pairwise arm comparisons detailed in Section 7.1.1:

- H_0: Gene set expression does not shift differentially from baseline to post-treatment between prespecified treatment arm comparisons
- H_A: Gene set expression shifts differentially from baseline to post-treatment between prespecified treatment arm comparisons

We will test the following hypotheses at OL-Week 11 for the mbOL population (pending trial outcome targets outlined in Section 5.6.1):

- H_0: Gene set expression does not shift differentially from baseline to OL-Week 11
- H_A: Gene set expression does not shift differentially from baseline to OL-Week 11

In addition to gene set statistics, statistics for individual genes within gene sets with FDR<0.05 will be reported.

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8.5. EXPLORATORY ENDPOINTS: ASSOCIATION BETWEEN CLINICAL OUTCOME AND THE INTESTINAL MICROBIOME AND HOST AND MICROBIAL FUNCTIONAL RESPONSES

8.5.1. SER-287 engraftment

The basic mechanism of action of SER-287 is thought to require engraftment of bacteria derived from SER-287 into the gastrointestinal tract of patients. We will assess if the magnitude of engraftment is associated with clinical outcome.

8.5.1.1. Magnitude of engraftment

We will evaluate the association of $\Delta S_{obs\_spore\_dose}$ with clinical outcomes across arms. This measure will provide insight as to whether the magnitude of engraftment at particular timepoints are predictive of clinical outcome.

We will perform logistic regression with arm and baseline richness of dose species in subject stool samples, $S_{obs\_spore\_dose\_baseline}$, as covariates:

$$\text{Logit(Outcome)} \sim \boldsymbol{\beta}_{\Delta S}\Delta S_{obs\_spore\_dose} + \boldsymbol{\beta}_{Arm}\text{Arm} + \boldsymbol{\beta}_{Baseline}S_{obs\_spore\_dose\_baseline}$$

We will test the following hypotheses for each time point at Weeks 3, 7 and 11 for the mbAT_IP and OL-Week 3 and OL-Week 11 for the mbOL populations pending trial outcome targets outlined in Section 7.1.1:

- H_0: The magnitude of engraftment is not associated with clinical outcome (i.e., $\boldsymbol{\beta}_{\Delta S} = 0$).
- H_A: The magnitude of engraftment is associated with clinical outcome (i.e., $\boldsymbol{\beta}_{\Delta S} \neq 0$).

8.5.2. Microbiome species composition and microbial functional response

Changes in both microbiome species and metabolites composition are hypothesized to underlie SER-287 mechanism of action. We will assess if the presence of specific species and abundance of specific metabolites are associated with clinical outcome.

8.5.2.1. Microbiome species composition

We will evaluate the association of specific species with subject clinical outcome across arms. This measure will provide insight as to whether specific species (either engrafting from SER-287 or changing as a result of treatment) are associated with clinical outcome.

To assess whether specific species are associated with clinical outcome, we will perform logistic regression for individual species observed in subject stool samples using treatment arm and baseline observation of the species in subject stool samples as covariates:

$$\text{Logit(Outcome)} \sim \boldsymbol{\beta}_{Arm}\text{Arm} + \boldsymbol{\beta}_{obs\_spp\_post}I_{obs\_spp\_post} + \boldsymbol{\beta}_{obs\_spp\_baseline}I_{obs\_spp\_baseline}$$

where Outcome = binary variable indicating clinical outcome status, Arm = treatment arm, $I_{obs\_spp\_post}$ = binary variable indicating if species was observed post-treatment, $I_{obs\_spp\_baseline}$ = binary variable indicating if species was observed at baseline.

Species detected in fewer than 5 subjects at a given time point will not be assessed at that time point. FDR q-values will also be reported to correct for multiple hypotheses within each time point separately.

We will test the following hypotheses for each time point at Week 1, Week 3, Week 7 and Week 11 for the mbAT_IP population and OL-Week 3 and OL-Week 11 for the mbOL population pending trial outcome targets outlined in Section 7.1.1:

- H_0: The presence of a species post-treatment is not associated with clinical outcome (i.e., $\boldsymbol{\beta}_{obs\_spp\_post} = 0$).
- H_A: The presence of a species post-treatment is associated with clinical outcome (i.e., $\boldsymbol{\beta}_{obs\_spp\_post} \neq 0$).

8.5.2.2. Stool metabolite composition

We will assess whether the concentration of metabolites in stool is associated with clinical outcome following treatment with SER-287. In order to assess whether $\log_2$ fold-changes of metabolite abundances, Δm, are associated with clinical outcome, we will perform logistic regression for each metabolite detected in any subject stool sample using treatment arm and $\log_2$ baseline metabolite abundance as covariates:

$$\text{Logit}(\text{Outcome}) \sim \boldsymbol{\beta}_{Arm}\text{Arm} + \boldsymbol{\beta}_{\Delta m}\Delta m + \boldsymbol{\beta}_{Baseline}m_{Baseline}$$

where Outcome = binary variable indicating clinical outcome status, Arm = treatment arm, Δm = $\log_2$ fold-change of metabolite abundance and $m_{Baseline}$ = $\log_2$ baseline metabolite abundance.

We will test the following hypotheses at Week 3 and Week 11 for the mbAT_IP population and OL-Week 11 for the mbOL population pending trial outcome targets outlined in Section 7.1.1:

- H_0: The fold-change of a metabolite (Δm) does not differ between responders and non-responders (i.e., $\boldsymbol{\beta}_{\Delta m} = 0$).
- H_A: The fold-change of a metabolite (Δm) does differ between responders and non-responders (i.e., $\boldsymbol{\beta}_{\Delta m} \neq 0$).

For all comparisons, in addition to reporting statistics for all measured metabolites, we will report the results for the following metabolites that are specifically hypothesized to be associated with clinical outcome (Table 7).

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8.5.3. Host mucosal functional response

Treatment-associated changes in both microbiome species composition and their complement of metabolites may also result in changes is host mucosal gene expression. We will evaluate changes in host mucosal gene expression are associated with clinical outcome.

8.5.3.1. Differential host gene expression and pathway enrichment

To assess whether there are specific host genes associated with clinical outcome, we will compare the change in gene expression from baseline to Week 11 of subjects achieving and not achieving a clinical outcome.

The same approach as detailed in Section 8.4.3.1 will be applied, with raw counts data will be transformed to log2-counts per million (LC_M) with R function using the 'voom' function of the R 'limma' package. To test whether shifts from baseline of individual genes are associated with clinical outcomes, we will apply "lmFit" and "eBayes" functions in the R 'limma' package. We will fit the mixed linear model as shown below, with subject as a random effect.

$$c = X_{Outcome-Timepoint}\beta_{Outcome-Timepoint} + X_{Arm-Timepoint}\beta_{Arm-Timepoint} + u_{Subj} + \varepsilon$$

where

- c is the voom-transformed gene expression value for a single sample

- $X_{Outcome-Timepoint}$ is a nominal variable for each combination of clinical outcome and Timepoint
- $\beta_{Outcome-Timepoint}$ is a vector of the fixed effects
- $X_{Arm-Timepoint}$ is a nominal variable for each combination of Arm and Timepoint
- $\beta_{Arm-Timepoint}$ is a vector of the fixed effects
- u_{Subj} is the vector of random effects
- ε is the random error term

For each transcript, we will evaluate the change in expression from baseline between pre-specified treatment arm comparisons by evaluating the following contrast:

$$(\beta_{Remission-PostTx} - \beta_{Remission-Baseline}) - (\beta_{Nonremission-PostTx} - \beta_{Nonremission-Baseline}).$$

We will then test the association between clinical outcomes and the aggregate change in expression of pre-specified gene sets (Table 8) with gene set enrichment analysis (GSEA), as described in Section 8.4.3.1. For all gene sets, the set of shared genes between this list and those detected after quality filtering in study samples will be used in the GSEA analysis.

We will test the following hypotheses at Week 11 for the mbAT_IP population and at OL-Week 11 for the mbOL population pending trial outcome targets outlined in Section 5.6.1:

- H_0: Gene set expression does not shift differentially from baseline to Week 11 between responder and non-responder.
- H_A: Gene set expression shifts differentially from baseline to Week 11 between responder and non-responder.

The following is the list of pathways, including the iHMP gene sets, specifically hypothesized to be differentially expressed between responder and non-responder. The hypothesized directionality of the enrichment score is given comparisons of responder and non-responder, where positive indicates greater enrichment (higher expression) in the former and negative indicates greater enrichment in the latter.

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In addition to gene set statistics, statistics for individual genes within gene sets with FDR<0.05 will be reported.

9. CHANGES FROM ANALYSIS PLANNED IN PROTOCOL

9.1. MCITT POPULATION

The mcITT population was modified to be a strict subpopulation of the mITT-1_IP population; this way, only subjects that have mild-to-moderate UC and receive any amount of study drug are analyzed.

10. REFERENCES

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The Human Microbiome Project Consortium (2012). A framework for human microbiome research. Nature 486, 215–221.

Truong, D.T., Franzosa, E.A., Tickle, T.L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., and Segata, N. (2015). MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12, 902–903.

11. PROGRAMMING CONSIDERATIONS

All tables, data listings, figures (TLFs), and statistical analyses will be generated using R version 3.2.3 or later. Computer-generated table, listing and figure output will adhere to the following specifications.

11.1. GENERAL CONSIDERATIONS

- One program can create several outputs or a separate program can be created for each output at statistical programmer's discretion.
- Each output will be stored in a separate file.
- Output files will be delivered in Word format, rtf format, or pdf format.
- Numbering of TFLs will follow International Conference to Harmonisation (ICH) E3 guidance

11.2. TABLE, LISTING, AND FIGURE FORMAT

11.2.1. General

- All TLFs will be produced using the Courier New font, sized as appropriate
- Headers and footers for figures will be in Courier New font, sized as appropriate.
- Legends will be used for all figures with more than 1 variable, group, or item displayed.
- TLFs will be in black and white (no color), unless otherwise specified
- Specialized text styles, such as bolding, italics, borders, shading, and superscripted and subscripted text, will not be used in the TLFs, unless otherwise specified. On some occasions, superscripts 1, 2, or 3 may be used (see below).
- Only standard keyboard characters will be used in the TLFs. Special characters, such as non-printable control characters, printer-specific, or font-specific characters, will not be used. Hexadecimal-derived characters will be used, where possible, if they are appropriate to help display math symbols (e.g., μ). Certain subscripts and superscripts (e.g., cm^2, C_{max}) will be employed on a case-by-case basis.
- Mixed case will be used for all titles, footnotes, column headers, and programmer-supplied formats, as appropriate.

11.2.2. Headers

- All output should have the following header at the top left of each page:

  Seres Therapeutics, Inc.
  Protocol: SERES-287-201

- All output should have Page n of N at the top or bottom right corner of each page. TLFs should be internally paginated in relation to the total length (i.e., the page number should appear sequentially as page n of N, where N is the total number of pages in the table).
- The date (date output was generated) should appear along with program name and location as the last footer on each page.

11.2.3. Display Titles

- Each TLF should be identified by the designation and a numeral. (i.e., Table 14.1.1). ICH E3 numbering is strongly recommended but sponsor preferences should be obtained prior to final determination. A decimal system (x.y and x.y.z) should be used to identify TLFs with related contents. The title is centered. The analysis set should be identified on the line immediately following the title. The title and table designation are single spaced. A solid line spanning the margins will separate the display titles from the column headers. There will be 1 blank line between the last title and the solid line

  Table x.y.z
  First Line of Title
  Second Line of Title if Needed
  Analysis Set

11.2.4. Column Header

- Column headings should be displayed immediately below the solid line described above in initial upper-case characters.
- For numeric variables, include “unit” in column or row heading when appropriate.

11.2.5. Table Conventions

- Units will be included where available.
- Where percentages are presented in these tables, zero percentages will not be presented and so any counts of 0 will be presented as 0 and not as 0 (0%).
- Unless otherwise specified, the estimated mean and median for a set of values should be printed out to 1 more significant digit than the original values, and standard deviations should be printed out to 2 more significant digits than the original values. The minimum and maximum should report the same significant digits as the original values. For example:

N	XX
Mean	XXX.X
Std Dev	X.XX
Median	XXX.X
Minimum	XXX
Maximum	XXX

- P-values should be output in the format: “0.xxx”, where xxx is the value rounded to 3 decimal places. Any p-value less than 0.001 will be presented as <0.001. If the p-value should be less than 0.0001 then present as <0.0001. If the p-value is returned as >0.999 then present as >0.999
- Percentage values should be printed to one decimal place, in parentheses with no spaces, one space after the count (e.g., 7 (12.8%), 13 (5.4%)). Values that round down to 0.0 will be displayed as '<0.1'. Unless otherwise noted, for all percentages, the number of subjects

in the analysis set for the treatment group who have an observation will be the denominator. Percentages after zero counts should not be displayed and percentages equating to 100% should be presented as 100%.

11.2.6. Listing Conventions

- Listings will be sorted for presentation in order of treatment groups as above, subject number, visit/collection day, and visit/collection time.

11.2.7. Figure Conventions

- Unless otherwise specified, for all figures, study visits will be displayed on the X-axis and endpoint (e.g., treatment mean change from Baseline) values will be displayed on the Y-axis.

11.2.8. Footnotes

- A solid line spanning the margins will separate the body of the data display from the footnotes.
- All footnotes will be left justified with single-line spacing immediately below the solid line underneath the data display.
- Footnotes should always begin with “Note:” if an informational footnote, or 1, 2, 3, etc. if a reference footnote. Each new footnote should start on a new line where possible.
- Footnotes will be present on the page where they are first referenced and thereafter on each page of the table, unless the footnote is specific only to certain pages. Subject specific footnotes should be avoided.
- Footnotes will be used sparingly and must add value to the table, figure, or data listing. If more than six lines of footnotes are planned, then a cover page may be used to display footnotes, and only those essential to comprehension of the data will be repeated on each page.
- The last 2 lines of the footnote section will be a standard source that indicates the name of the program used to produce the data display, date the program was run, and the listing source (or data source for a listing) (i.e., ‘Program: myprogram.sas Listing source: 16.x.y.z’).

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