A Phase 1b Safety Study of SER-287, a Spore-Based Microbiome Therapeutic, for Active Mild to Moderate Ulcerative Colitis

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Phase 1b trial: Daily dosing had greatest engraftment and clinical remission (40% SER-287 vs 0% placebo)
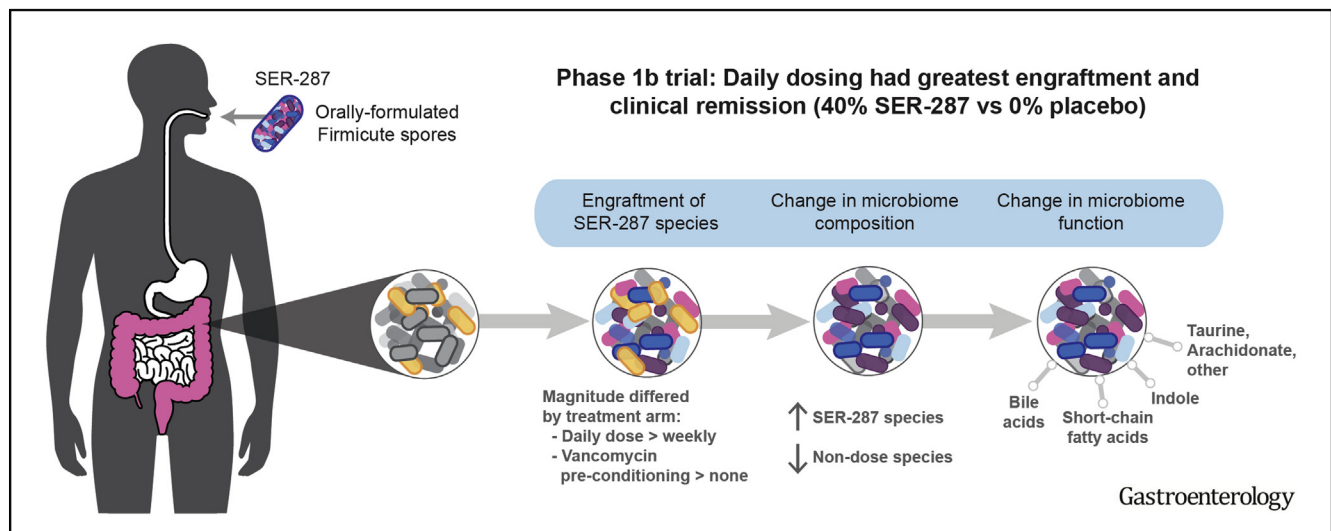
BACKGROUND & AIMS: Firmicutes bacteria produce metabolites that maintain the intestinal barrier and mucosal immunity. Firmicutes are reduced in the intestinal microbiota of patients with ulcerative colitis (UC). In a phase 1b trial of patients with UC, we evaluated the safety and efficacy of SER-287, an oral formulation of Firmicutes spores, and the effects of vancomycin preconditioning on expansion (engraftment) of SER-287 species in the colon. **METHODS:** We conducted a double-blind trial of SER-287 in 58 adults with active mild-to-moderate UC (modified Mayo scores 4–10, endoscopic subscores ≥1). Participants received 6 days of preconditioning with oral vancomycin (125 mg, 4 times daily) or placebo followed by 8 weeks of oral SER-287 or placebo. Patients were randomly assigned (2:3:3:3) to groups that received placebo followed by either placebo or SER-287 once weekly, or vancomycin followed by SER-287 once weekly, or SER-287 once daily. Clinical end points included safety and clinical remission (modified Mayo score ≤2; endoscopic subscores 0 or 1). Microbiome end points included SER-287 engraftment (dose species detected in stool after but not before SER-287 administration). Engraftment of SER-287 and changes in microbiome composition and associated metabolites were measured by analyses of stool specimens collected at baseline, after preconditioning, and during and 4 weeks after administration of SER-287 or placebo. **RESULTS:** Proportions of patients with adverse events did not differ significantly among groups. A higher proportion of patients in the vancomycin/SER-287 daily group (40%) achieved clinical remission at week 8 than patients in the placebo/placebo group (0%), placebo/SER-287 weekly group (13.3%), or vancomycin/SER-287 weekly group (17.7%) ($P = .024$ for vancomycin/SER-287 daily vs placebo/placebo). By day 7, higher numbers of SER-287 dose species were detected in stool samples from all SER-287 groups compared with the placebo group ($P < .05$), but this difference was not maintained beyond day 7 in the placebo/SER-287 weekly group. In the vancomycin groups, a greater number of dose species were detected in stool collected on day 10 and all subsequent time points through 4 weeks post dosing compared with the placebo group ($P < .05$). A higher number of SER-287 dose species were detected in stool samples on days 7 and 10 from subjects who received daily vs weekly SER-287 doses ($P < .05$). Changes in fecal microbiome composition and metabolites were associated with both vancomycin/SER-287 groups. **CONCLUSIONS:** In this small phase 1b trial of limited duration, the safety and tolerability of

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SER-287 were similar to placebo. SER-287 after vancomycin was significantly more effective than placebo for induction of remission in patients with active mild to moderate UC. Engraftment of dose species was facilitated by vancomycin preconditioning and daily dosing of SER-287. ClinicalTrials.gov ID NCT02618187.

Keywords: Inflammatory Bowel Disease; Gastrointestinal Microbiome; Microbe-Associated Metabolites; Microbiome Therapeutics.

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The gastrointestinal microbiome is a diverse ecosystem that provides essential functions for the host and represents a novel therapeutic target for UC. In "healthy" adults in the Human Microbiome Project, the microbiome is dominated by bacteria in 2 phyla, the Firmicutes and Bacteroidetes.[1–3] Spore-forming Firmicutes, particularly the families *Clostridiaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, have an important role in gut homeostasis through production of metabolites, which enhance gastrointestinal barrier and mucosal immune functions.[4] Although the pathogenesis of disease remains unclear, changes in the gastrointestinal microbiome and associated metabolites may be important.[5–7] UC patients with active disease exhibit lower microbial diversity with depletion of beneficial Firmicutes and relative expansion of pro-inflammatory *Enterobacteriaceae*.[8] Inflammatory responses are elicited by *Enterobacteriaceae* via lipopolysaccharide signaling though Toll-like receptors.[9] Microbiome compositional changes are associated with alterations in microbe-associated products, such as short-chain fatty acids (SCFA), secondary bile acids (BAs), and tryptophan metabolites, implicated in barrier function, mucin production, and colonic inflammation.[5] Temporal shifts in microbial composition and their metabolites have been postulated to precipitate disease flares.[6,10,11] Modulating the microbiome and the microbial triggers of inflammation and immune activation may provide an improved therapeutic option with limited off-target effects.

Although microbiome modulation with antibiotics or probiotics has not been an effective therapeutic strategy in UC,[12] data from placebo-controlled trials support a benefit for multidose fecal microbiota transplantation (FMT),[13] although the mechanism of action is not fully understood. Patients randomized to FMT had increased microbial diversity and a shift toward similarity to the donor microbiome[13–15] compared with placebo recipients. Importantly, the microbial taxa associated with clinical remission included spore-forming Firmicutes, such as *Lachnospiraceae*, *Ruminococcaceae*, and *Eubacteriaceae*.[8,13,15]

The potential role of the microbiome in UC pathogenesis, coupled with FMT efficacy data, motivated our development of SER-287 as a novel therapeutic. Although FMT contains the full spectrum of stool components to modify the microbiome, our approach fractionates spore-forming bacteria to specifically target Firmicutes relevant to gastrointestinal homeostasis.[4] Gram-positive Firmicute spores are

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Firmicutes bacteria produce metabolites that help maintain the intestinal barrier and mucosal immunity. Firmicutes and their associated metabolites are reduced in intestinal microbiota of patients with ulcerative colitis (UC).

NEW FINDINGS

In a phase 1b trial, administration of SER-287 (Firmicutes spores) following vancomycin preconditioning induced clinical remission in a significantly higher proportion of patients with mild to moderately active UC than placebo. The safety and tolerability of SER-287 were similar to those of placebo.

LIMITATIONS

This was a small study of limited duration (12 weeks)—larger studies are needed.

IMPACT

SER-287 may induce remission by changing the composition and function of the gut microbiome.

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isolated and enriched from stool of rigorously screened healthy donors by ethanol treatment and purification. This process also removes potential pathogens, such as vegetative bacteria, viruses, and 99% of other nonspore components, producing a more refined and safer therapeutic. Spores, which are intrinsically resistant to gastric acid, are formulated into oral capsules. Bacteria that germinate from these spores develop into metabolically active vegetative bacteria and colonize the colon, a process termed *engraftment*.

Our hypothesis was that SER-287 may reduce colonic inflammation by inducing compositional changes in the microbiome and related metabolites favorable to gastrointestinal homeostasis. We conducted a phase 1b randomized double-blind, placebo-controlled trial to evaluate the efficacy and safety of SER-287 in patients with active mild to moderate UC. To further evaluate the mechanism of action, the engraftment of SER-287 bacteria and their relationship to broader changes in the gastrointestinal microbiome and related metabolites was evaluated. We also investigated whether vancomycin preconditioning of the gastrointestinal microbiome could enhance engraftment of SER-287 bacteria. We hypothesized that clearance of Gram-positive bacteria with vancomycin can enhance engraftment of SER-287 bacteria by decreasing competition for nutrients and space.

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Methods

Study Design

We conducted a randomized, double-blind, placebo-controlled, multiple-dose trial at 20 US sites. The protocol was approved by Investigational Review Boards and patients gave written informed consent. The trial was funded by Seres Therapeutics and designed in conjunction with the academic advisors.

Patient Population

Eligible patients were ≥18 years of age with active mild to moderate disease as defined by a total modified Mayo score (TMMS) of 4–10 and an endoscopic subscore ≥1 (mild disease TMMS 4–6; moderate disease TMMS 7–10). During the screening period, medical history and symptom severity were collected. Disease activity and severity was assessed by the TMMS, which includes endoscopy, patient-reported outcomes of stool frequency and rectal bleeding, and a physician global assessment.

Treatment-naïve patients and those who had failed mesalamine formulations, thiopurines, or biologics were eligible to enroll. After randomization, patients were required to remain on a stable dose of mesalamine, oral corticosteroids (≤15 mg daily of prednisone or equivalent), or thiopurines. Biologic therapies, probiotics, or treatments delivered via enema or suppository were not permitted. The washout period for biologics was 3 months and for probiotics and rectal therapies was approximately 14 days. Inclusion and exclusion criteria and allowable medications are provided in the Supplementary Material.

Randomization and Treatments

The trial design is shown in Supplementary Figure 1. Patients were randomly assigned, in a 2:3:3:3 ratio, to 4 arms that incorporated 2 treatment phases. The first phase consisted of preconditioning with vancomycin (125 mg 4 times daily) or matching placebo for 6 days. The second phase consisted of administration of SER-287 (4 capsules containing 1×10^7 colony-forming units) once daily or once weekly or matching placebo once daily for 8 weeks. Subjects who received once weekly SER-287 took placebo capsules on days 2–7 weekly to maintain the blind. Randomization was performed centrally with an interactive web response system and was balanced across drug product lots (Supplementary Material).

Manufacture and Characterization of SER-287

Our donor screening and manufacturing program has been reviewed by the US Food and Drug Administration and is consistent with their recent publication.[16] Three donors underwent extensive health examination, including personal and family medical history and laboratory screening for chemistries; hematology; urinalysis; and blood and stool viral, bacterial, and parasite testing before donating stool. Donors completed physical examinations, questionnaires, and laboratory testing during and after the donation period before material was released for manufacturing.

Stool donations underwent 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. Three lots of SER-287 were prepared from 3 different donors' materials. All stool donations for a lot came from a single donor and all subjects received SER-287 made from a single lot. Nonspore matter was reduced by >200-fold during purification to <5 mg/dose, with final product purity approximately 10%–20% spores by weight. No contaminating bioburden was detected in the final product (assay limit of detection 20 colony-forming units/g). The SER-287 lots used in the trial were characterized by direct whole metagenomic shotgun sequencing and taxonomic profiling (Supplementary Material). Firmicutes genera identified in lots are in Supplementary Table 1.

Clinical End Points

Clinical evaluations were performed during the screening phase and at the end of the 8-week treatment phase (Supplementary Figure 1). Endoscopy was scored by central readers who were unaware of the treatment assignment, study visit number, or clinical information (https://www.robartsinc.com/). We evaluated clinical remission (TMMS ≤2 and an endoscopic subscore ≤1), endoscopic improvement (decrease in the endoscopic subscore ≥1), and clinical response (decrease of ≥3 points in TMMS from baseline plus either a decrease of ≥1 point in rectal bleeding subscore or an absolute rectal bleeding subscore of 0 or 1 at week 8.

Safety was assessed from the preconditioning phase through the end of the long-term safety phase. Assessments performed from randomization and for 4 weeks after completion of study treatment included laboratory values and adverse events classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities.

Statistical Methods

Primary end points were safety and changes in microbiome composition. Clinical remission, endoscopic improvement, and clinical response were secondary end points. The safety population comprised all patients who received at least 1 dose of study drug (vancomycin, SER-287, or placebo). The intent-to-treat population comprised all patients who were randomized. Descriptive statistics were used to summarize baseline characteristics. For the analysis of clinical remission, the proportions of patients achieving clinical remission at week 8 was compared between each SER-287 arm and placebo using a 2-sided Fisher exact test; a P value <.05 was considered significant. Asymptotic 95% confidence intervals for the rate differences were also estimated. In these analyses, an outcome of "failure" was assigned to patients who discontinued treatment before study day 48, received a rescue medication for UC flare, or who lacked the necessary data for determination of the relevant end point. No adjustment for multiple comparisons was made, as the secondary efficacy end points were intended to provide only preliminary data on efficacy. A similar approach was taken for analysis of the proportions of patients with clinical and endoscopic response. The rates of treatment-emergent adverse events were described by treatment group. The randomization plan, which included 15 patients into each of the SER-287 arms and 10 patients into the placebo/placebo arm, was determined to be sufficient to provide initial assessment of a potential treatment effect on efficacy, safety, and the microbiome.

Microbiome and Metabolomics Evaluations

We evaluated baseline microbiome composition, engraftment of SER-287 bacteria, and broader changes in the gastrointestinal microbiome and metabolites using metagenomic shotgun sequencing and mass spectrometry (metabolomics) of stool samples collected before treatment and post treatment. Details are provided in the Supplementary Material; the number of patients with evaluable metagenomic shotgun sequencing data from both baseline and post-treatment samples is reported in Supplementary Table 2.

Baseline microbiome composition and post-treatment changes were characterized by calculating α-diversity and β-diversity. α-Diversity is reported as the number of unique species detected in a single sample.[17] β-diversity, which measures compositional similarity between samples, was calculated using the binary Jaccard index.[17,18] The binary Jaccard metric measures similarity based on presence or absence of shared species, and ranges in value from 0 to 1, with 1 indicating 2 samples are identical.

No correction for multiple hypothesis testing was performed as this phase 1b trial was exploratory; the microbiome data collected were intended to provide preliminary insights on engraftment and microbiome change.

Baseline microbiome composition. For baseline composition, we compared 2 summary measures, α-diversity and *Enterobacteriaceae* abundance, in our study population to an external cohort of UC patients and controls from the Integrative Human Microbiome Project (HMP2).[6,19] Controls included people who presented for age-related colorectal cancer screening or patients with gastrointestinal symptoms without inflammatory bowel disease (IBD).

Engraftment of drug product species. Two approaches were taken to assess engraftment of drug product species (ie, "dose-species").

In the first prespecified analysis, we evaluated the prevalence of SER-287 dose-species in patients at baseline (screening endoscopy visit before treatment) and on day 0 (post-vancomycin preconditioning, before SER-287 dosing) and days 3, 7, 10, 14, 56, and 84 (post treatment with SER-287). We defined engrafting species as SER-287 dose-species detected post treatment that were not detected at baseline. A Mann-Whitney 2-sided test was performed comparing the number of engrafting species in patients of 1 arm with patients of another.

In the second post-hoc analysis, the change in compositional similarity between all spore-forming species in the patient microbiomes and the SER-287 dose from baseline to post treatment was quantified using the Jaccard β-diversity metric. This second complementary approach was performed to avoid underestimation of engraftment by not including species overlapping with drug product, which were present at baseline.

Changes in overall microbiome composition. The impact of SER-287 on overall changes in microbiome composition was assessed in a prespecified analysis by evaluating the distance between patients' baseline and post-treatment samples using a Jaccard β-diversity metric. A Mann-Whitney 2-sided test was performed comparing patients across arms.

Changes in microbiome composition were additionally assessed post-hoc by evaluating the proportion of patients in whom species were gained or lost at 8 weeks post treatment relative to baseline after SER-287 or placebo. These compositional changes represent engrafting species from the dose, in addition to species not detected in the dose.

The impact of SER-287 treatment on *Enterobacteriaceae* species was evaluated post-hoc. Individual species abundances were summed to determine the relative abundance of *Enterobacteriaceae* as a fraction of all species detected. Patients with paired baseline and 8-week stool samples were assessed, and fold-changes in *Enterobacteriaceae* relative abundance post treatment were tested using a Wilcoxon signed-rank test.

Metabolic changes in stool. Using both targeted metabolomics of SCFAs and BAs and global metabolomics, we evaluated post-hoc how microbe-associated metabolites correlated with SER-287 treatment and clinical remission. Analyses were performed on 5 SCFAs and 9 BAs from the targeted panels and 1328 metabolites identified by global metabolomics. Changes in metabolite abundance post treatment were assessed based on fold-changes between patients' baseline and post-treatment samples at 8 weeks post dosing using a Wilcoxon signed-rank test. Direct statistical comparison with placebo was not performed due to the low number of patients with paired samples. Association of metabolite concentrations with clinical remission was assessed at the clinical efficacy end point 8 weeks post dosing. A 2-sided Mann-Whitney test was performed to assess whether metabolites differed in remitters vs nonremitters across arms.

Results

Patient Demographics and Baseline Characteristics

Table 1 and Supplementary Figure 2 show the patient disposition and demographics. Eighty-five patients were screened and 58 enrolled at 20 US sites within 17 months. Thirty-nine patients (67.2%) had left-sided disease and 19 patients (32.8%) had extensive disease. Mean time since diagnosis for the overall population was 12.2 years.

Most patients (91.4%) had failed at least 1 UC therapy. Forty-five (77.6%) were receiving stable UC medications at study entry, which included mainly mesalamine formulations (65.5%); 9 patients (15.5%) were receiving more than 1 medication (eg, mesalamine, an immunonomodulator, and/or a corticosteroid). Six patients (10.3%) had prior exposure to biologic therapy (ie, tumor necrosis factor antagonists, anti-integrins, or both). Thirteen patients (22.4%) were not receiving UC medications.

Safety

SER-287 was generally safe and well-tolerated across treatment arms (Table 2). Adverse events were mild (67.0%) or moderate (33.0%). One subject from the vancomycin/SER-287 daily dosing arm with a history of depression had a serious adverse event of worsening depression, deemed unrelated to treatment, which did not lead to treatment discontinuation. During the preconditioning phase, 7 patients had adverse events, most of which were gastrointestinal: 3 received placebo and 4 received vancomycin (Supplementary Table 3).

During the treatment phase and the subsequent 4 weeks, the most commonly reported adverse events were

Table 1. Demographic and Baseline Characteristics

Characteristic	Placebo/placebo (n = 11)	Placebo/SER-287 weekly (n = 15)	Vancomycin/ SER-287 weekly (n = 17)	Vancomycin/ SER-287 daily (n = 15)	All subjects (n = 58)
Age, y, mean	45.8	46.5	47.9	47.8	47.1
Female sex, n (%)	7 (63.6)	9 (60.0)	7 (41.2)	8 (53.3)	31 (53.4)
Extent of disease, n (%)					
Left-sided UC	8 (72.7)	10 (66.7)	12 (70.6)	9 (60.0)	39 (67.2)
Extensive UC	3 (27.3)	5 (33.3)	5 (29.4)	6 (40.0)	19 (32.8)
Smoking history, n (%)	3 (27.3)	5 (33.3)	5 (29.4)	6 (40.0)	19 (32.8)
Severity of UC, n (%)					
Mild	3 (27.3)	6 (40.0)	9 (52.9)[a]	6 (40.0)	24 (41.4)
Moderate	8 (72.7)	9 (60.0)	7 (41.2)[a]	9 (60.0)	33 (56.9)
Mayo score at study entry, mean	7.3	6.8	6.4	6.9	6.8
UC medication(s) at study entry, n (%)	9 (81.8)	13 (86.7)	11 (64.7)	12 (80.0)	45 (77.6)
Mesalamine	7 (63.6)	11 (73.3)	9 (52.9)	11 (73.3)	38 (65.5)
Immunomodulator	2 (18.2)	4 (26.7)	2 (11.8)	1 (6.7)	9 (15.5)
Steroid	3 (27.3)	2 (13.3)	0	3 (20.0)	8 (13.8)
Combination therapy	3 (27.3)	3 (20.0)	0	3 (20.0)	9 (15.5)
Endoscopy score at baseline, n (%)					
1	1 (9.1)	3 (20.0)	5 (29.4)	3 (20.0)	12 (20.7)
2	5 (45.4)	7 (46.7)	7 (41.2)	9 (60.0)	28 (48.3)
3	5 (45.4)	5 (33.3)	5 (29.4)	3 (20.0)	18 (31.3)
Time since UC diagnosis, y, mean	11.52	12.43	11.84	12.74	12.17

[a]One subject in vancomycin/SER-287 weekly group with TMMS = 3 at entry is not included.

gastrointestinal disorders, including abdominal pain, constipation, diarrhea, flatulence, and nausea. Rates of gastrointestinal adverse events were lowest among patients who received daily dosing of SER-287 (6.7%) compared with placebo/SER-287 weekly (46.7%), vancomycin/SER-287 weekly (41.2%), and placebo/placebo (45.5%). The rate of

infections among those who received daily dosing of SER-287 was similar to placebo (Supplementary Table 4).

Treatment discontinuation due to gastrointestinal adverse events occurred in 1 patient (6.7%) in the placebo/SER-287 weekly arm; 2 patients (11.8%) in the vancomycin/SER-287 weekly arm, and none of the patients

Table 2. Adverse Events

Variable	Placebo/placebo (n = 11)	Placebo/SER-287 weekly (n = 15)	Vancomycin/SER-287 weekly (n = 17)	Vancomycin/SER-287 daily (n = 15)
All AEs	7 (63.6)	9 (60.0)	14 (82.4)	8 (53.3)
Serious AEs	0	0	0	1 (6.7)
AEs of special interest	0	1 (6.7)	0	0
Severity of AEs				
Mild	3 (27.3)	7 (46.7)	6 (35.3)	3 (20.0)
Moderate	4 (36.4)	2 (13.3)	8 (47.1)	5 (33.3)
Treatment-related AEs	1 (9.1)	4 (26.7)	7 (41.2)	2 (13.3)
AEs leading to discontinuation of study drug	0	1 (6.7)	2 (11.8)	0

NOTE. Values are n (%).
AE, adverse event.

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on the SER-287 daily dosing or placebo arms (Table 2). One patient in the placebo/SER-287 weekly arm (6.7%) discontinued treatment due to a UC flare (deemed unrelated to study drug by the investigator), and 1 patient (5.9%) in the vancomycin/SER-287 weekly arm and 1 patient in the vancomycin/SER-287 daily arm (6.7%) discontinued the study prematurely due to perceived lack of efficacy (Supplementary Figure 2).

Disposition of the Patients and Treatment Adherence

Overall, 84.5% of patients completed induction therapy, including 10 of 11 (90.9%) who received placebo/placebo; 13 of 15 (86.7%) who received placebo/SER-287 weekly; 14 of 17 (82.3%) who received vancomycin/SER-287 weekly; and 12 of 15 (80.0%) who received vancomycin/SER-287 daily. Mean adherence with study drug ranged from 84.5% to 86.9% of all dispensed capsules taken across treatment arms.

Efficacy

Patients assigned to the vancomycin/SER-287 daily arm achieved a higher clinical remission rate at week 8 (40%) than those assigned to placebo/placebo (0%), placebo/SER-287 weekly (13.3%), and vancomycin/SER-287 weekly (17.7%) (Figure 1A). Remission in the vancomycin/SER-287 daily arm was significantly higher than the placebo/placebo arm (Fisher exact test, $P = .024$). Greater remission was observed in vancomycin/SER-287 daily vs vancomycin/SER-287 weekly, suggesting dose-dependence. Endoscopic improvement rates were numerically greater in the SER-287 arms but not significantly different than placebo (Figure 1B). The outcomes for the 2 weekly dosing regimens of SER-287 were similar. No significant differences in clinical response (Figure 1C) were observed in any of the 3 SER-287 treatment arms compared with placebo. No significant differences in fecal calprotectin and C-reactive protein data were observed across arms at 8 weeks.

Characteristics of the Baseline Microbiome

In comparing the baseline values of our study population with controls from HMP2,[7] no significant differences in α-diversity were seen (Kruskal-Wallis, $P > .05$). Median abundance of *Enterobacteriaceae* in our study patients trended higher than the UC cohort in HMP2 and significantly higher than the non-IBD healthy cohort (median 0.39%, 0.17%, and 0.02%, respectively; Mann-Whitney $P = .003$).

In comparing patients with mild or moderate disease, α-diversity did not significantly differ. In moderate patients, *Enterobacteriaceae* abundance was higher than in mild patients (Mann-Whitney $P = .038$); moderate patients also had more genera considered proinflammatory (eg, *Klebsiella, Proteus)*. Twelve of 51 patients (24%) had no detectable *Enterobacteriaceae*; most (75%) had mild UC. In contrast, of the 39 patients (76%) with detectable *Enterobacteriaceae*, most (63%) had moderate disease.

Engraftment of SER-287 Drug Product Species

Engraftment occurred rapidly by day 7, with a significant increase in engrafting species in all SER-287 arms compared with placebo (Figure 2, Supplementary Table 5). Beyond day 7, the distinction between the placebo/placebo and placebo/SER-287 weekly arms was not significant. In contrast, the favorable effect of antibiotic preconditioning on engraftment was clearly apparent by day 10 for both weekly and daily SER-287 administration and sustained through 4 weeks post-dosing (Figure 2, $P < .05$ for all comparisons, Supplementary Table 5). In the vancomycin preconditioning arms, a significantly greater number of engrafting species were observed at days 7 and 10 in the SER-287 daily dosing arm compared with the SER-287 weekly dosing arm ($P < .05$, Supplementary Table 5). This observation suggests that engraftment is both facilitated by vancomycin preconditioning and dose-dependent (Figure 2, Supplementary Table 5). Engraftment at days 7 and 10 was also greater in remitters than nonremitters in all arms combined (Mann-Whitney, $P = .001$ and $P = .025$); however, this association was not significant within any individual arm or at later time points.

In the second assessment of engraftment, based on β-diversity, we observed a similar favorable impact of vancomycin-preconditioning and SER-287 dosing frequency as patient spore composition became most similar to SER-287 drug product species in the vancomycin/SER-287 daily arm (Supplementary Figure 3). We saw no difference in engraftment as assessed by β-diversity between the placebo/placebo and placebo/SER-287 weekly arms. We also did not observe significant differences in engraftment across drug lots by either measure of engraftment.

Effects of SER-287 Treatment on Overall Microbiome Composition

Across all species, α-diversity (the number of unique species) did not significantly increase in any arm ($P > .05$ for all arms). However, α-diversity of spore-forming species significantly increased, and was durable in the vancomycin/SER-287 daily dosing arm, consistent with greater engraftment (Supplementary Table 6). Through 8 weeks, β-diversity was significantly different from baseline only in the arms with SER-287/vancomycin preconditioning indicating changes in overall microbiome composition (Supplementary Table 7).

We next sought to characterize compositional changes in the microbiome at finer resolution based on gain and loss of individual species post treatment (Figure 3). In the placebo/placebo arm, microbiome changes are due to natural fluctuations.[19] Compared with placebo/placebo, minimal compositional shifts were observed in the placebo/SER-287 weekly arm consistent with low magnitude engraftment. In contrast, significant changes were observed in both vancomycin preconditioning arms with a trend toward greater change in the SER-287 daily-dosing arm. To further characterize these changes, we separately evaluated the proportion of species increasing or decreasing in prevalence. More species were gained (Mann-Whitney, $P = .022$ and $P =$
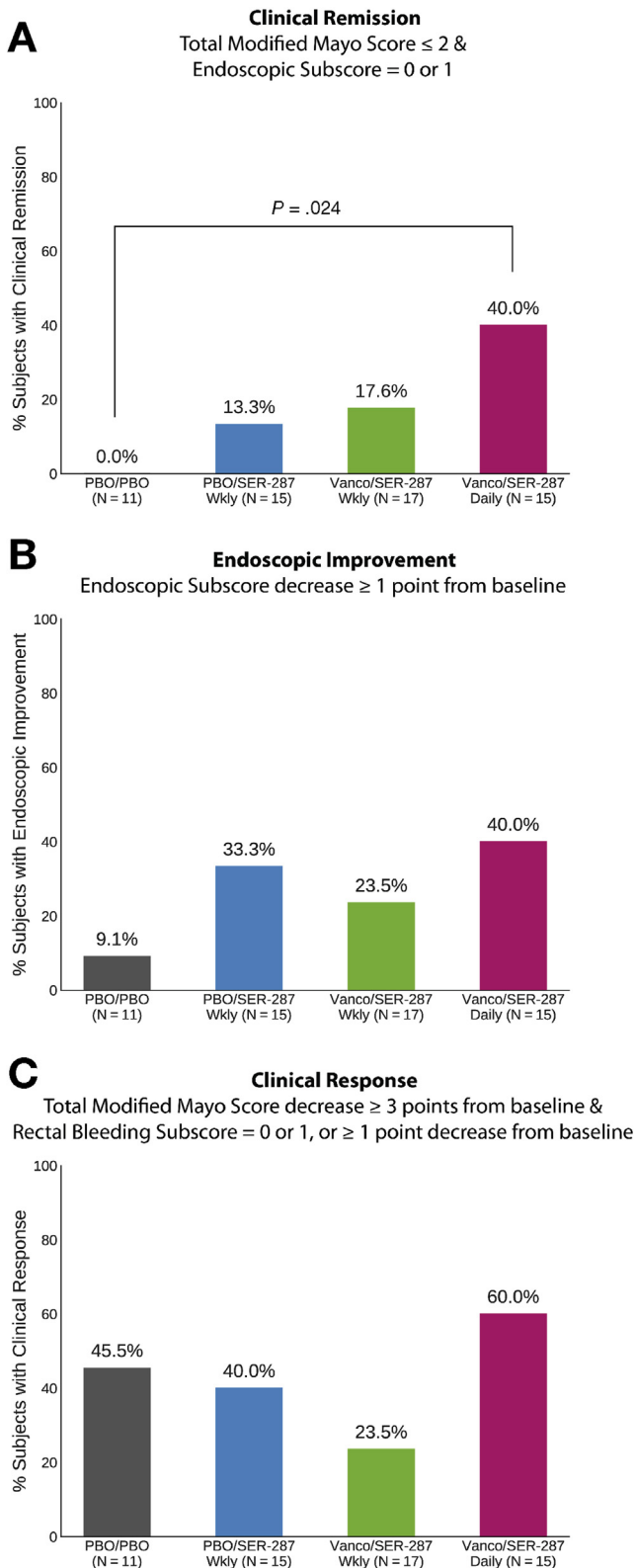
A **Clinical Remission**
Total Modified Mayo Score ≤ 2 &
Endoscopic Subscore = 0 or 1

B **Endoscopic Improvement**
Endoscopic Subscore decrease ≥ 1 point from baseline

C **Clinical Response**
Total Modified Mayo Score decrease ≥ 3 points from baseline &
Rectal Bleeding Subscore = 0 or 1, or ≥ 1 point decrease from baseline

Figure 1. Proportion of patients achieving end points of clinical remission (*A*), endoscopic improvement (*B*), and clinical response (*C*) at week 8 in intent-to-treat population. Two-sided Fisher exact *P* value indicated when <.05. PBO, placebo; Vanco, vancomycin; Wkly, weekly.

.014 for vancomycin/SER-287 weekly and vancomycin/SER-27 daily arms, respectively) and lost (Mann-Whitney, *P* = .004 and *P* = .006 for vancomycin/SER-287 weekly and vancomycin/SER-287 daily arms, respectively) in the vancomycin-preconditioning arms than in the placebo/placebo arm.

Genera of newly detected species were predominantly spore-formers found in SER-287, such as *Clostridium, Gemminger, Dorea, Roseburia, Blautia,* and *Faecalibacterium* (Figure 3, *blue bars*). Genera decreasing post treatment were predominantly nonspore formers, such as *Veillonella, Streptococcus,* and *Bacteroides* (Figure 3, *gray bars*). Taken together, global changes in the microbiome were associated with engraftment of drug product species (Supplementary Table 1) and additional changes related to nondrug product species.

We evaluated the effect of SER-287 on *Enterobacteriaceae* abundance at 8 weeks post treatment among patients with *Enterobacteriaceae* detected at baseline (n = 29). We observed the greatest decrease of *Enterobacteriaceae* relative abundance compared with baseline in vancomycin preconditioning arms (median decreases –0.09% and –0.58% for SER-287 weekly and daily, respectively) and a slight increase in placebo preconditioning arms (median increases +0.36% and +0.03% for placebo and SER-287 weekly, respectively) (Supplementary Figure 4 and Supplementary Table 8). Furthermore, an association was observed between a decline in *Enterobacteriaceae* and clinical remission (*P* =.02; vancomycin/SER-287 weekly + daily arms, Supplementary Table 9).

Effect of SER-287 Treatment on Microbe-Associated Metabolites

For SCFA and BA concentrations we assessed change post treatment within arms and association with remission across arms (see Methods; Supplementary Tables 10 and 11). The SCFA hexanoate was positively associated with both SER-287 (*P* = .02 in vancomycin/daily dosing arm; Figure 4*A*) and clinical remission (*P* = .05; Figure 4*B*). Butyrate did not significantly change post treatment, but there was a trend for an increase in the ratio of butyrate to propionate concentrations in the vancomycin/daily dosing arm (*P* = .07). We also examined secondary BA lithocholic acid (LCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA). We did not observe a significant change in LCA and DCA post treatment, but UDCA was negatively associated with both SER-287 treatment (*P* = .047 in the vancomycin/daily dosing arm; Figure 4*A*) and clinical remission (*P* = .043; Figure 4*B*). LCA and DCA increases tended to positively associate with clinical remission (*P* = .053 and *P* = .09, respectively), with an observed increase in the ratio of LCA/UDCA.

Global liquid chromatography-tandem mass spectrometry metabolomics were used to identify metabolites associated with SER-287 treatment and remission. Of the 1328 metabolites in our data set, we conducted 3 analyses. First,
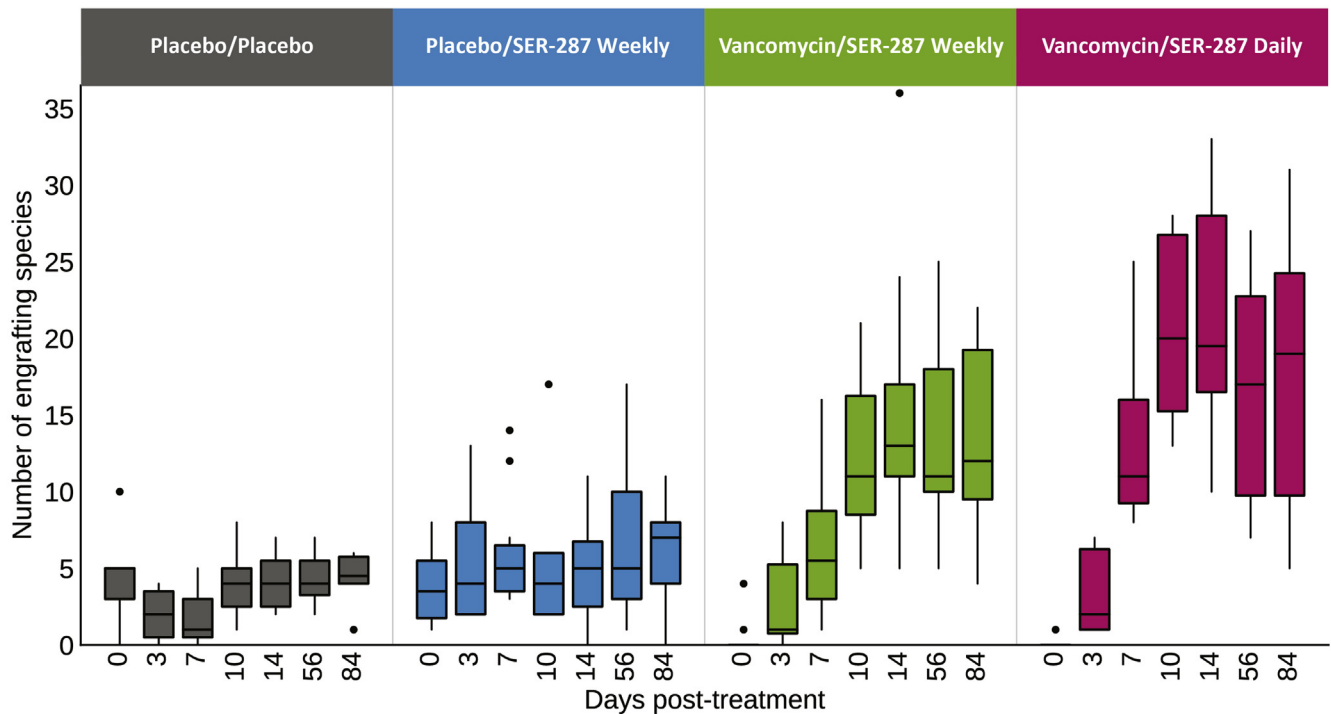
Figure 2. Engraftment of SER-287 species is dose-dependent and facilitated by vancomycin preconditioning. *Boxplots* display median (*horizontal line*), 25th and 75th percentiles (*box edges*), range of nonoutlier observations (*whiskers*), and outlier observations (*dots*; >1.5 times interquartile range). Significance values are in Supplementary Table 5.

we examined changes in metabolite concentrations relative to pretreatment baseline (Supplementary Table 12). We focused our analysis on the vancomycin-preconditioning arms where SER-287 engraftment was observed at the 8-week time point; in total, 166 metabolites changed (13 increased; 153 decreased; $P < .05$). Second, we examined the association of metabolites with outcome (Supplementary Table 13); 186 metabolites differentiated remitters from nonremitters (57 increased; 129 decreased; $P < .05$). Lastly, 38 metabolites were associated with both vancomycin/SER-287 treatment and remission; of these, 3 metabolites increased and 35 decreased. All 3 metabolites that increased are amino-acid derivatives: N-acetylarginine and 3 derived from tryptophan (indole and 3-methylindole). The 35 metabolites that decreased include lipids (eg, sphingolipids, acyl-carnitines, and arachidonate), dinucleotides, taurine-derived metabolites (taurine and N-acetyltaurine), and 5 unidentified compounds. Three of the 38 metabolites are microbe-associated and affect inflammatory pathways, specifically indole, taurine, and arachidonate (Figure 4C and D), and several others are host-derived lipids and acyl-carnitines known to be perturbed in IBD.[6,20–22]

Discussion

This first clinical trial of an oral microbiome drug, SER-287, provides evidence of a favorable safety profile and preliminary insights into drug pharmacology and multiple mechanisms of action. In keeping with our hypothesis, SER-287 bacteria engrafted and modulated the microbiome and

microbe-associated metabolites of functional relevance to UC. In this small study, we also have preliminary data of an efficacy signal of SER-287 supporting a role for microbiome therapeutics in the treatment of active mild to moderate UC.

The safety profile of SER-287, which was the primary safety end point, was similar to placebo. The most commonly reported adverse events were gastrointestinal disorders, which were less frequent in the SER-287 daily dosing arm relative to other arms, including placebo. Favorable tolerability might be expected given that SER-287 is composed of Firmicutes, considered important to gastrointestinal health.[1,3] Safety confirmation in larger trials would establish this therapeutic approach as particularly suitable for monotherapy for milder UC patients or in combination with other agents. Oral vancomycin preconditioning was well-tolerated relative to placebo, consistent with its well-known safety profile.

In this small phase 1b study, the daily SER-287 regimen was the most effective regimen as assessed by all prespecified efficacy end points. The use of centrally read endoscopy, a modality that reduces bias in assessment of clinical remission, and was likely critical to determining a benefit of SER-287.[23] Although clinical response rates did not significantly differ between placebo and SER-287 arms, the placebo response rate was high, typical of UC trials conducted in mild to moderately active disease[24]; it is noteworthy that this highly subjective outcome is no longer recommended as a primary end point.[24,25] Clinical remission and endoscopic improvement were highly similar between the 2 weekly SER-287 regimens, with and without vancomycin preconditioning, suggesting that the direct
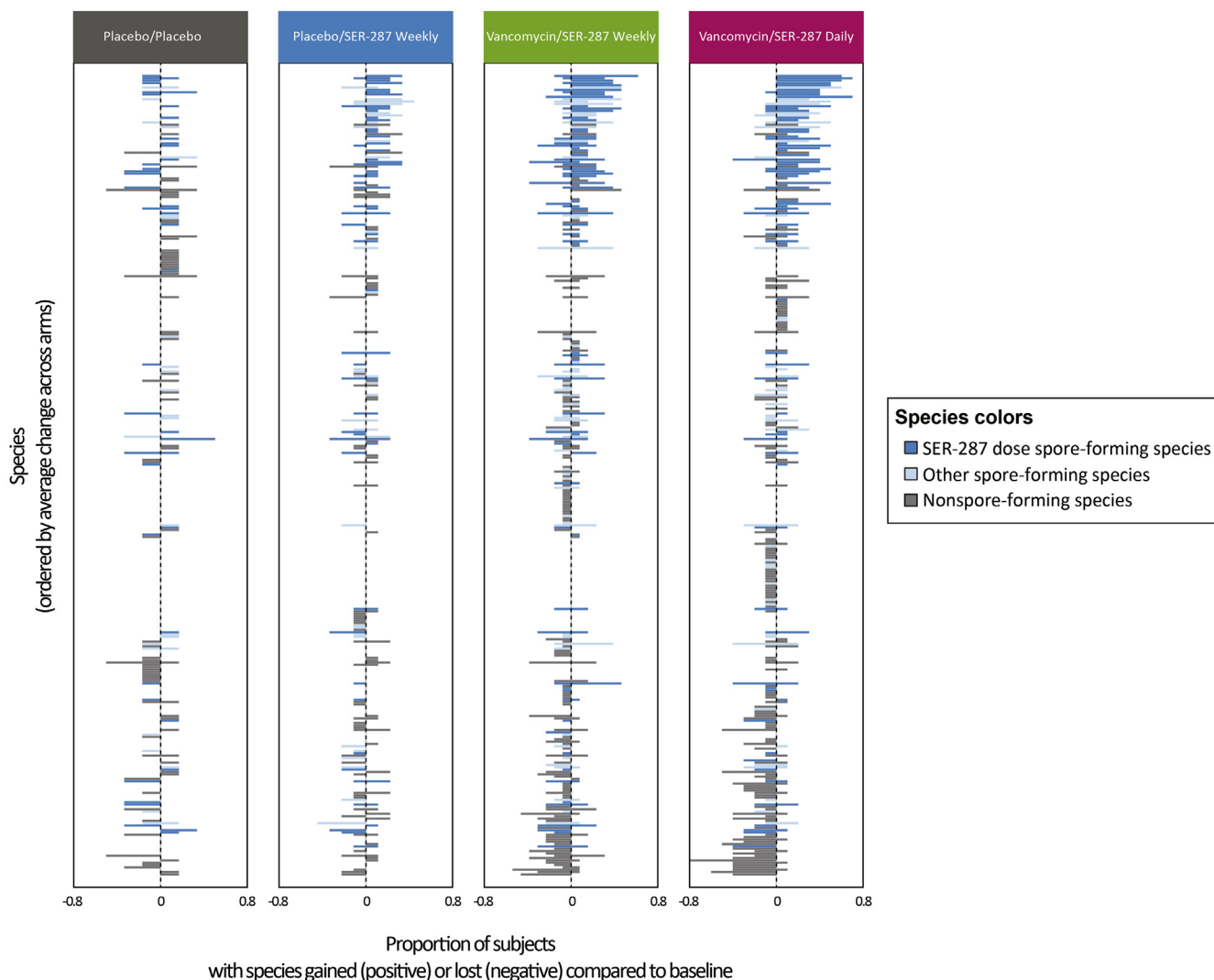
Figure 3. Vancomycin preconditioning followed by SER-287 results in a broad shift in overall composition of spore and nonspore microbial species 8 weeks post treatment. The proportion of patients in each arm that gained (positive values) or lost (negative values) a given species compared with baseline is shown (*x-axis*). Species (*y-axis*; n = 344) are ordered by average change across arms; species without change in any subject are not depicted (n = 15). *Bar colors* indicate spore-forming species detected in SER-287 (*dark blue*), other spore-forming species not detected in SER-287 (*light blue*), and other non–spore-forming species (*gray*). SER-287 dose species gained compared with baseline are the same species represented in Figure 2.

therapeutic effect of vancomycin was limited. Likewise, we suspect that the efficacy of the daily dosing arm was driven by greater SER-287 drug exposure, although we cannot definitively rule out any impact of vancomycin without a placebo comparator.

We propose engraftment as a key mechanism of action of a live microbiome biotherapeutic. This proposal is based on the hypothesis that a microbiome drug is more likely to have a therapeutic effect if there is measurable engraftment of drug product, which evokes measurable changes in the overall composition of the gut microbiome, associated metabolites, and other functional changes in the host. We propose that engraftment kinetics of a microbiome therapeutic are comparable to small molecule drug pharmacokinetics.

Most ecosytems are stable and generally resistant to colonization. Thus, the ability to engraft is dependent on disruption of the microbial environment.[26,27] The decision to use an antibiotic as preconditioning therapy was informed by human studies showing the disruptive impact of these drugs on the gut microbial community[28] and preclinical studies supporting increased engraftment after antibiotic treatment. For example, in the context of recurrent *Clostridium difficile* infection, antibiotic-induced reductions in gut microbial diversity[29] can be restored through engraftment of orally dosed bacteria.[30] Oral vancomycin was specifically evaluated as a preconditioning regimen based on its well-known safety profile; lack of systemic absorption; its spectrum of antibacterial activity; and the hypothesis that reduction of pre-existing Gram-
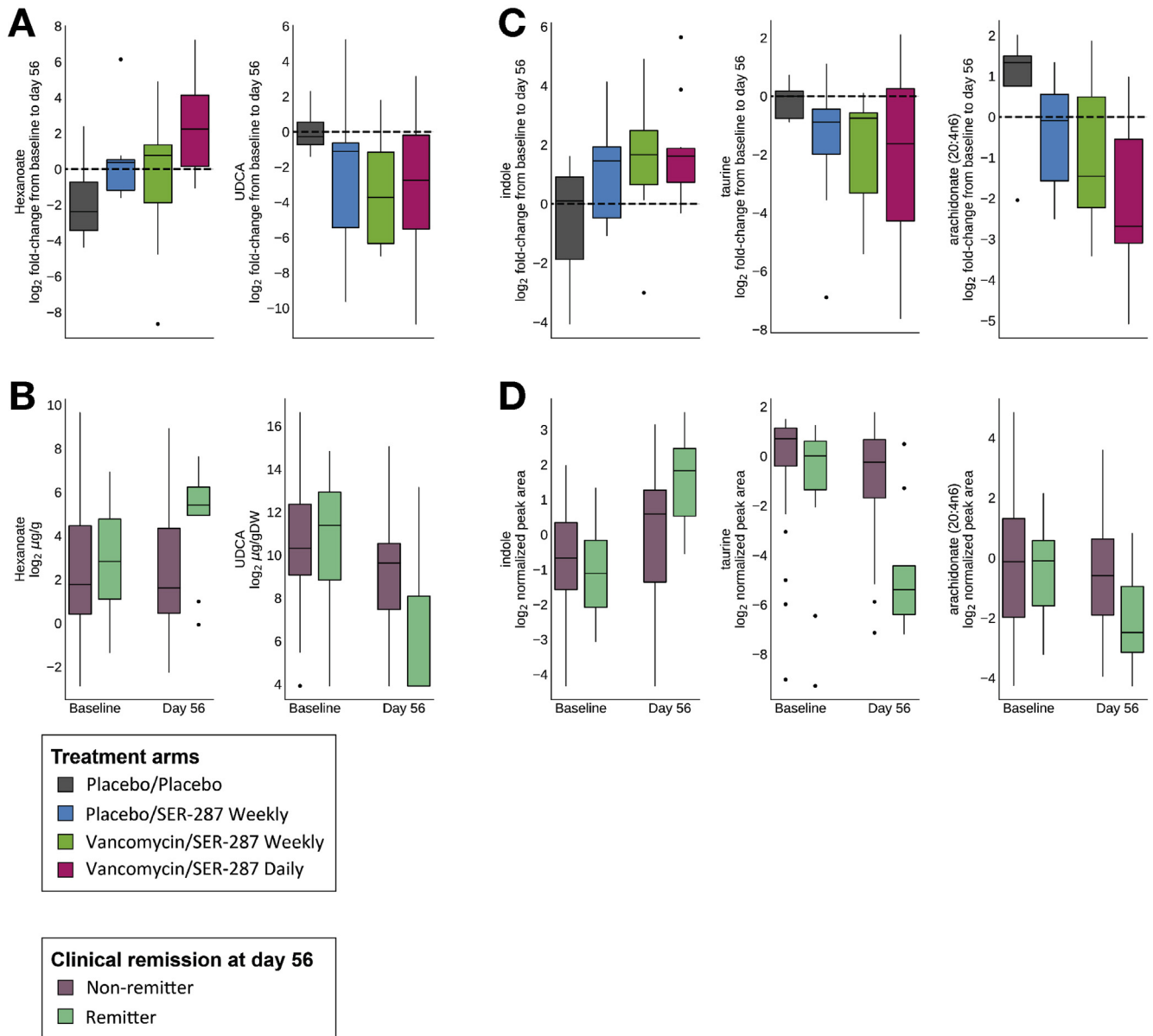
Figure 4. Microbe-associated metabolites associated with SER-287 treatment and clinical outcome. Metabolite levels in fecal samples were assessed via targeted (*A, B*) and global (*C, D*) metabolomics. In (*A*) and (*C*), metabolite fold-changes are shown by treatment arm; *black horizontal dashed line* indicates whether the metabolite is higher or lower compared with baseline. For (*B*) and (*D*), metabolite abundance is shown by remission status. Significance values are provided in Supplementary Tables 10–13.

positive bacteria would create an ecological niche for engraftment of Gram-positive SER-287 species. The 2 SER-287 weekly dosing arms affirm that vancomycin pre-conditioning facilitated engraftment, although we were not able to definitively link engraftment to efficacy. The rules of engraftment may also be influenced by drug dosing frequency.[27] Daily dosing of SER-287 led to greater engraftment at earlier time points compared with weekly dosing, consistent with a dose-dependent effect. Although, we did not observe differences in engraftment or clinical outcome across drug product lots, further evaluation in future trials will confirm whether or not SER-287 is associated with a donor effect.

SER-287 engraftment was associated with gains and losses of both spore-forming and non–spore-forming species. Post-treatment genera gained included Firmicutes related to dose-species, such as *Clostridium, Gemminger, Dorea, Roseburia, Blautia,* and *Faecalibacterium,* implicated in colonocyte health, epithelial gut integrity, and regulation of immune tolerance.[31] We only observed a significant increase in the α-diversity of spore-forming Firmicutes in the SER-287 daily dosing arm. Additionally, declines in *Enterobacteriaceae* abundance, including pro-inflammatory species, such as *Klebsiella pneumoniae* and *Proteus mirabilis*,[9,32] were associated with remission in the 2 SER-287 treatment arms after vancomycin preconditioning. The clinical

significance of this finding is unclear, given the limited sample size, the heterogeneity of *Enterobacteriaceae* detection at baseline and the magnitude of the changes. Collectively, these observations support that modulation of the microbiome was due to replacement of pre-existing spore-formers with drug product species in addition to changes in species not in the dose. These data suggest that total diversity may be less informative than the specific species that are interacting and functioning within the microbial network.[33,34] In support of that concept, baseline α-diversity was not associated with disease severity, whereas baseline *Enterobacteriaceae* abundance was higher in moderate subjects. We propose that these compositional changes in non–dose-related species represent 1 significant aspect of the mechanism of action and pharmacodynamics of SER-287.

Changes in microbe-associated metabolites are another dimension of SER-287 pharmacodynamics. In post-hoc analyses, we focused on metabolites that impact epithelial barrier integrity and signaling of anti-inflammatory pathways. Consistent with the HMP2 IBD cohort,[6] we did not observe significant increases in abundances of butyrate or secondary BAs DCA and LCA in SER-287 recipients. Notably, we did see changes in ratios of metabolites, such as butyrate to propionate and LCA/UDCA, suggesting that SER-287 can lead to shifts in the functional pathways utilized with clinical benefit. The metabolites with the strongest associations with both SER-287 treatment and clinical remission are microbe-associated metabolites with relevance to gut homeostasis, such as hexanoate, indole, taurine, and arachidonate. Microbial products of tryptophan metabolism, such as indole, can signal aryl hydrocarbon receptor in the gut and impact mucin production, epithelial barrier function, and immune signaling.[5] Arachidonic acid is a precursor of prostaglandins that can impact neutrophil migration across the epithelium.[35,36] Notably, the constellation of metabolite associations with SER-287 or clinical remission aligns with emerging data from several UC cohort studies[6,20–22] (Supplementary Tables 10–13). We hypothesize that these associations reflect modulation of multiple metabolic pathways by SER-287 and contribute to its mechanism of action. Although we did not observe a direct overall correlation of SER-287 engraftment with clinical outcomes in this small safety study, we suspect that metabolic correlations are likely a more sensitive indicator of the pharmacodynamics of a microbiome drug; this may become clearer in our phase 2 trial.

SER-287 offers advantages over other microbiome-modulating approaches, such as single-strain therapeutics and FMT. Single-strain therapeutics are unlikely to provide the spectrum of functional activity necessary for efficacy. In contrast, FMT represents a broad spectrum of microbes, but due to minimal processing can also include harmful pathogens. Bacterial infections transmitted through contaminated FMT have, uncommonly, led to deaths and hospitalizations.[37,38] However, because most FMT use has been outside the clinical trial setting, the safety profile of FMT remains unknown.[39] The US Food and Drug Administration has also alerted providers of the potential risk of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) transmission via FMT due to prolonged fecal shedding.[40] The COVID-19 (coronavirus disease 2019) pandemic is a reminder that FMT will always be vulnerable to emerging infections when pathogens are unrecognized until after transmission.[41,42] In addition, FMT carries risk of transmission of proinflammatory strains and potentially carcinogenic bacteria.[9,43] In contrast, SER-287 is a multifunctional consortia of bacterial spores purified under stringent manufacturing processes. These processes isolate the microbial components essential to gut homeostasis, inactivating bacteria, parasites, fungi, and viruses, providing a reliable safety net to mitigate risk.[30]

Strengths of this trial included its placebo-controlled design, affording an opportunity to examine the impact of a therapeutic intervention on the UC microbiome. Blinded central reads of endoscopic assessments enhance confidence in the outcomes.[23] The collective and consistent observations relative to clinical outcomes, engraftment, microbiome composition, and microbe-associated metabolites are consistent with the biologic activity of SER-287. One limitation was its relatively small sample size, which precludes definitive conclusions about efficacy and safety. A further limitation of our trial was the absence of vancomycin preconditioning followed by placebo. This arm was not included due to concerns about a potential bloom of proinflammatory *Enterobactericeae* after antibiotics alone,[44,45] and the potential risk of *Clostridioides difficile* infection.[46] One study of 27 UC subjects who were randomly assigned to antibiotic preconditioning followed by FMT compared with antibiotics alone is particularly informative.[46] *C difficile* infection occurred in 30% of subjects in the antibiotic alone arm, which the authors attributed to the low diversity state that ensued. In addition, reduction of beneficial Firmicutes and expansion of proinflammatory Proteobacteria were observed, which might explain the absence of any clinical remitters in this arm compared with 24% randomized to FMT. Because patients with UC are at increased risk of *C difficile* infection with worse clinical outcomes than those without underlying UC, we chose not to include a vancomycin preconditioning/placebo arm in our phase 2 trial based on these concerning data. In addition, vancomycin monotherapy is not recommended by Society guidelines.[12]

These prospective trial data support the favorable impact of SER-287 on the gut microbiota and their associated metabolites through multiple physiologic pathways, with important implications for UC management. SER-287 may represent a new therapeutic paradigm for UC, with potential advantages with respect to safety and convenience of oral dosing. Based on these results, daily SER-287 after vancomycin preconditioning is undergoing additional evaluation in a phase 2b trial (ClinicalTrials.gov ID NCT03759041).

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at

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Supplementary Methods

Complete Inclusion and Exclusion Criteria

To be eligible for enrollment, a subject was required meet all of the following criteria before undergoing any study-related procedures:

1. Signed informed consent;

2. Male or female, 18 years or older;

3. UC diagnosed by routine clinical, radiographic, endoscopic and pathologic criteria;

4. Active mild to moderate UC as determined by lower endoscopy (flexible sigmoidoscopy or colonoscopy) within approximately 3 days of randomization to study, defined as:

   a. TMMS of 4–10, inclusive

   b. Modified Mayo endoscopic subscore of $\geq$1, with evidence of mucosal lesions

   c. At least 15 cm of disease from anal verge

5. If female, subject is nonlactating, and either:

   a. Not of childbearing potential, defined as post-menopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.

   b. Of childbearing potential and is practicing at least 1 highly effective method of birth control, including the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of these methods for the duration of the study.

6. If male and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

Any of the following was regarded as a criterion for exclusion from the trial:

1. Fever >38.3°C;

2. Known or suspected toxic megacolon and/or known small bowel ileus;

3. Known history of Crohn's disease;

4. Subjects with serum albumin $\leq$2.5 g/dL at baseline;

5. Cytomegalovirus polymerase chain reaction positive from blood plasma at screening;

6. Known stool tests positive for ova and/or parasites, or positive stool culture, within the 30 days before enrollment;

7. Subjects on cyclosporine or triple immunosuppression. Triple immunosuppression will include any 3 of the following classes of drugs taken in combination: steroids (ie, prednisone, budesonide, budesonide MMX), immunosuppressant (ie, methotrexate, azathioprine, 6-mercaptopurine), and/or other immunosuppressant (ie, tacrolimus, mycophenolate mofetil [CellCept]);

8. Biologic medication (infliximab, adalimumab, golimumab, certolizumab, vedolizumab, ustekinumab, or natalizumab) use within 3 months before screening;

9. Known active malignancy, except for basal cell skin cancer or squamous cell skin cancer;

10. Subjects with previous colectomy, ostomy, J-pouch, or previous intestinal surgery (excluding cholecystectomy, appendectomy);

11. Subjects with known history of celiac disease or gluten enteropathy;

12. Subjects with *C difficile*–positive stool toxin test performed by the central laboratory at screening visit;

13. Antibiotic use within 1 month before randomization;

14. Expected to receive antibiotics within 8 weeks of signing the informed consent form (ie, for planned/anticipated procedure).

15. Received an investigational drug within 1 month before study entry;

16. Received an investigational antibody or vaccine within 3 months before study entry;

17. Previously enrolled in a SER-109 or SER-287 study;

18. Received an FMT within 6 months before study entry;

19. Poor concurrent medical risks with clinically significant comorbid disease such that, in the opinion of the investigator, the subject should not be enrolled including:

    a. Subjects with decompensated liver cirrhosis (Child-Pugh class B or C) or uncontrolled liver disease

    b. History of bone marrow transplantation

    c. Known hypogammaglobulinemia

    d. Known severe immunodeficiency

    e. Underlying liver function test (screening alanine aminotransferase or aspartate aminotransferase) abnormalities >2× upper limit of normal

    f. Absolute neutrophil count <500 cells/mm^{3}.

20. Subjects with anatomic or medical contraindications to lower endoscopy (flexible sigmoidoscopy or colonoscopy), including but not necessarily limited to toxic megacolon; gastrointestinal fistulas; immediate postoperative status from abdominal surgery; severe coagulopathy; large or symptomatic abdominal aortic aneurysm; or any subject where study physician deems subject at significant risk of complications of lower endoscopy (flexible sigmoidoscopy or colonoscopy);

21. Unable to stop steroid or mesalamine enemas or suppositories before screening visit;

22. Unable to stop opiate treatment, unless on a stable dose with no increase in dose planned for the duration of the study;

23. Unable to stop probiotics before screening visit;

24. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with medical monitor);

25. Unable to comply with the protocol requirements;

26. Any condition that, in the opinion of the investigator, might interfere with study objectives;

27. Known allergy or intolerance to oral vancomycin; and

28. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.

Permitted Concomitant Medications

The complete list of concomitant medications permitted on study included:

- Oral aminosalicylates (aminosalicylates taken for at least 6 weeks, with a stable dose for ≥2 weeks before screening required)

- Immunomodulators: 6-mercaptopurine, azathioprine, and methotrexate (stable dose for ≥12 weeks before screening required)

- Prednisone ≤15 mg (stable dose for ≥2 weeks before screening required)

- Budesonide ≤6 mg (stable dose for ≥2 weeks before screening required)

- Budesonide MMX ≤9 mg (stable dose for ≥2 weeks before screening required)

- Opiate treatment (stable dose or short-term use is permitted)

- Short term use of nonsteroidals/nonsteroidal anti-inflammatory drugs

- Long-term use of low-dose aspirin (81 mg)

Subjects receiving any of the above permitted concomitant medications at the time of study entry were required to maintain a stable dosage throughout the study (except for drugs allowed for short term use as noted above), unless investigator judgment required increase, reduction, or discontinuation for safety concerns or medical necessity. Dosing changes due to safety concerns or medical necessity were a cause to discontinue subjects from the study.

Clinical End Points and Statistics: Ulcerative Colitis Flares

UC flares were considered adverse events of special interest defined as a 2-point increase in the partial Mayo score, documented at 2 contiguous visits after initiation of study drug plus worsening clinical status warranting a change in UC treatment, as determined by the principal investigator. During the subsequent 22-week follow-up period, occurrence of a UC flare was defined by a change in UC medication, as reported by the patient.

Stool Sample Collection

Subject stool samples were collected at baseline (before vancomycin preconditioning); on day 0 (post-vancomycin preconditioning, before SER-287 dosing); and on days 3, 7, 10, 14, 56, and 84 (post treatment with SER-287). For analyses involving comparisons of baseline with post-treatment microbiome, all subjects with available paired samples are included (Supplementary Table 2).

Whole Metagenomic Shotgun Sequencing

Bacterial DNA was extracted from 200 μL or 0.2 g of subject stool and dose samples using the MagAttract PowerSoil DNA Kit (Qiagen, Hilden, Germany). Libraries were sequenced on the Illumina HiSeq to a target depth of 5 gigabases for subject samples and 10 gigabases for SER-287 lots.

Samples were processed per standard HMP-2 data processing guidelines (https://hmpdacc.org/hmp/resources/). Read pair deduplication was implemented using a custom Python function to remove polymerase chain reaction and sequencing artifacts. Adapter sequences and low-quality sequence data was trimmed using trimmomatic.[1] Finally, reads were aligned to human and artificial reference sequences using bowtie2[2] to remove unwanted sequence data (eg, human DNA or artificial DNA). Raw sequencing data have not been deposited in public databases to protect the intellectual property of Seres Therapeutics.

Taxonomic Profiling of Metagenomic Shotgun Sequencing Samples

Species taxonomic profiling of metagenomic shotgun sequencing reads for both SER-287 lots and subject stools was performed using the MetaPhlAn2 software.[3,4] The curated public reference database used for MetaPhlAn2 includes species-level genomic markers. In addition to these publicly available bacterial species genomes, we added

spore-forming genomes from our proprietary Seres strain collection to increase detection of spore-forming species underrepresented in public databases. For all analyses, species were classified as being spore-forming or not spore-forming according to a proprietary, curated annotation of species experimentally demonstrated to form spores or identified in the literature as spore-forming species.

Before generation of final taxonomic profiles, all samples were subsampled to the same sequencing depth to account for any correlation between sequencing depth and observed species richness. A subsampling depth of 150,000 reads mapped to the MetaPhlAn2 reference database was used. Any samples with <150,000 mapped reads were omitted from downstream analysis. The ensuing output from the MetaPhlAn2 taxonomic profiling pipeline is referred to as the "microbiome profile" of a sample.

Sample Preparation for Metabolomics

Short-chain fatty acids. Sample preparation and data generation was performed by Metabolon Inc (Durham, NC). Samples were maintained at –80°C until processed. Samples were analyzed for SCFAs by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Metabolon Method TAM135). Human stool samples are spiked with stable labeled internal standards and are subjected to protein precipitation with methanol. After centrifugation, an aliquot of the supernatant is derivatized to form the corresponding SCFA hydrazides. The reaction mixture is diluted, and an aliquot is injected onto an Agilent 1290/AB Sciex QTrap 5500 LC-MS/MS system (Agilent, Santa Clara, CA) equipped with a C18 reverse-phase ultra high-performance liquid chromatography (UPLC) column. The mass spectrometer is operated in negative mode using electrospray ionization (ESI). The peak area of the individual analyte product ions is measured against the peak area of the product ions of the corresponding internal standards. Quantitation is performed using a weighted linear least squares regression analysis generated from fortified calibration standards prepared immediately before each run. Metabolite concentrations are reported in units of microgram of metabolite per gram of stool.

Bile acids. Sample preparation and data generation was performed at Seres Therapeutics. Samples were maintained at –80°C until processed. Three sample aliquots were prepared from thawed samples. Aliquot 1 was weighed and spiked with labeled standards at a final concentration of 5 μM/mg before any further extraction. Aliquots 1 and 2 were homogenized in 10× v/w 50% methanol, incubated on ice for 1 hour and further extracted with an equal volume of cold acetonitrile. Extracted samples were spun down, filtered, and analyzed by targeted liquid chromatography mass spectrometry. Aliquot 2 was spiked with labeled BA standards post extraction, before analysis by LC-MS. Aliquot 3 was weighed, dried overnight in an oven at 105°C, and weighed again to determine the water content of the sample. LC-MS analysis was carried out using an Agilent 1260 series Liquid Chromatograph coupled to a Bruker Compact qTOF mass spectrometer (Bruker, Billerica, MA). BAs were

separated using a Microsolv C18 bidentate column (Microsolv, Eatontown, NJ) with a gradient of water and acetonitrile. Data were processed using Bruker Compass DataAnalysis software and quantified using calibration curves generated with commercially available pure standards. All data were expressed relative to sample dry weight (ng/mg). Spike in controls were used to determine extraction efficiency of bile acids of interest. Additional controls to ensure quality and consistency across samples and runs were included with every batch analysis on the LC-MS.

Global metabolomics. Sample preparation and data generation was performed by Metabolon Inc. Samples were maintained at –80°C until processed. Proteins were precipitated with methanol (Glen Mills GenoGrinder 2000; Glen Mills Inc, Clifton, NJ) followed by centrifugation to remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites. The resulting extract was divided into 5 fractions: 2 for analysis by 2 separate reverse-phase UPLC-MS/MS methods with positive ion mode ESI; 1 for analysis by reverse-phase/UPLC-MS/MS with negative ion mode ESI; 1 for analysis by hydrophilic interaction UPLC-MS/MS with negative ion mode ESI, and 1 sample was reserved for backup. All methods utilize a Waters ACQUITY UPLC and a Thermo Scientific (Waltham, MA) Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization source and Orbitrap mass analyzer operated at 35,000 mass resolution. The scan range varies slightly between methods, but covers approximately 70–1000 m/z. Compounds were identified based on 3 distinct parameters: Retention index, accurate mass, and MS/MS spectrum in comparison with Metabolon's proprietary library of purified standards and recurrent unknown entities. Peaks were quantified using area under the curve, adjusted for variations across runs and scaled as necessary for further analysis. Missing values were assumed to be below the level of detection for that biochemical with the instrumentation used and were imputed to the observed minimum for that particular biochemical across all samples.

Sample Selection and Taxonomic Profiling of Integrative Human Microbiome Project Data

Metadata for the Integrative Human Microbiome Project were downloaded from the link https://ibdmdb.org/tunnel/products/HMP2/Metadata/hmp2_metadata.csv. Data were accessed March 14, 2018. Only metagenomics samples with available sequencing files were considered (N = 1338). These represented multiple visits from 106 unique subjects, including non-IBD (n = 50), UC (n = 30), and Crohn's disease (n = 50) cohorts. For each unique subject, the sample corresponding to the earliest visit number was selected. In cases where multiple samples were attributed the same visit number, the sample with the lowest human read count was selected.

Each sample was then processed using the same steps as the SERES-101 study samples, including all preprocessing

steps (read deduplication, trimming, and human read filtering; see whole metagenomic shotgun sequencing). Samples were also subsampled to the same depth as the SERES-101 study samples for comparability (150,000 mapped reads) before taxonomic profiling with MetaPhlAn2. Samples with <150,000 mapped reads were omitted from further analyses.

After all processing steps, a total of 97 samples were available for HMP2 comparative analyses, including samples from 25 non-IBD, 26 UC, and 46 Crohn's disease unique subjects.

Long-Term Ulcerative Colitis Flares

In a post-hoc assessment, the number of flares was low in all arms. The following number of reported UC flares were observed during a 26-week period by treatment arm: 5 (33.3%) in the placebo/weekly SER-287 arm; 1 (5.9%) in the vancomycin/weekly SER-287 arm; 2 (13.3%) in the vancomycin/daily SER-287 arm; and 3 (27.3%) in the placebo/placebo arm. Among the 11 patients who achieved clinical remission on the SER-287 dosing arms, there were no reported UC flares, as assessed by a change of UC medications during the 26-week long-term safety follow-up phase.

Supplementary Table 10. Bile Acid and Short-Chain Fatty Acid Change From Baseline to 8 Weeks Post Treatment.

NOTE. Change in metabolite abundance post treatment was assessed based on $\log_2$ fold-change between subjects' baseline and post-treatment samples; a Wilcoxon signed-rank test was performed, to assess whether the fold-changes are centered around 0. The number of subjects with baseline and post-treatment SCFA data is: n = 4 for placebo/placebo, n = 8 for placebo/SER-287 weekly, n = 11 for vancomycin/SER-287 weekly, n = 11 for vancomycin/SER-287 daily. The number of subjects with baseline and post-treatment BA data is: n = 3 for placebo/placebo, n = 8 for placebo/SER-287 weekly, n = 9 for vancomycin/SER-287 weekly, n = 11 for vancomycin/SER-287 daily.

Supplementary Table 11. Bile Acid and Short-Chain Fatty Acid Association With Clinical Remission

NOTE. Association of metabolite abundance with clinical remission was assessed at 8 weeks post treatment. A 2-sided Mann-Whitney U test was performed to assess whether the abundance of metabolites significantly differed in remitters compared with nonremitters; subjects in all treatment arms are considered. There are n = 29 nonremitters and n = 9 remitters with SCFA data post treatment. There are n = 27 nonremitters and n = 9 remitters with BA data post treatment.

Supplementary Table 12. Global Metabolomic Change From Baseline to 8 Weeks Post Treatment

Change in metabolite abundance post-treatment was assessed based on $\log_2$ fold-change between subjects' baseline and post-treatment samples; a Wilcoxon signed-rank test was performed to assess whether the fold-changes were centered around zero. All metabolites are reported with $P < .05$ for vancomycin/SER-287 daily dosing and vancomycin/SER-287 weekly dosing arms combined; metabolites with names starting with "X – " indicate compounds of unknown identity; asterisks indicate putative metabolite identities. The number of subjects with baseline and post-treatment data is: n = 4 for placebo/placebo, n = 8 for placebo/SER-287 weekly, n = 11 for vancomycin/SER-287 weekly, n = 11 for vancomycin/SER-287 daily.

Supplementary Table 13. Global Metabolomics Association With Clinical Remission

NOTE. Association of metabolite abundance with clinical remission was assessed at 8 weeks post treatment. A 2-sided Mann-Whitney U test was performed to assess whether the abundance of metabolites significantly differed in remitters (n = 9) compared with nonremitters (n = 29); subjects in all treatment arms are considered. No correction for multiple hypothesis testing was performed. All metabolites are reported with $P < .05$; metabolites with names starting with "X –" indicate compounds of unknown identity; asterisks indicate putative metabolite identities.

Supplementary References

1. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 2014; 30:2114–2120.
2. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat. Methods 2012;9:357–359.
3. Segata N, Faust K, Izard J, et al. Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol 2012;8:e1002606.
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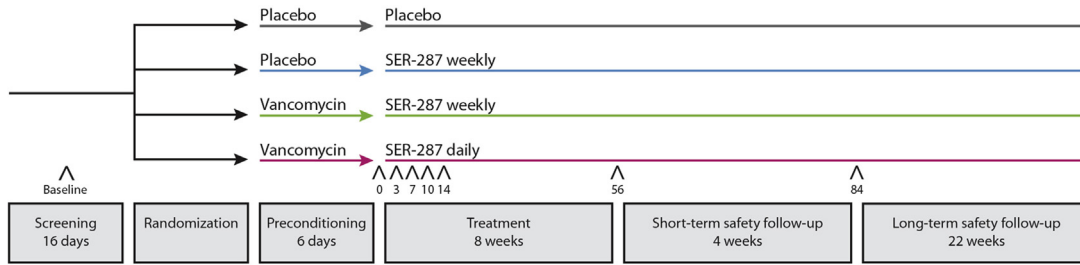
Supplementary Figure 1. Study Schematic. *Carats* (-) indicate approximate times of stool sampling at baseline (screening endoscopy visit before any treatment) and on days 0 (post-vancomycin preconditioning, before SER-287 dosing), 3, 7, 10, 14, 56, and 84 post-treatment with SER-287. Final samples were collected at the end of treatment, at the end of the short-term safety follow-up period and/or at any early termination visit.
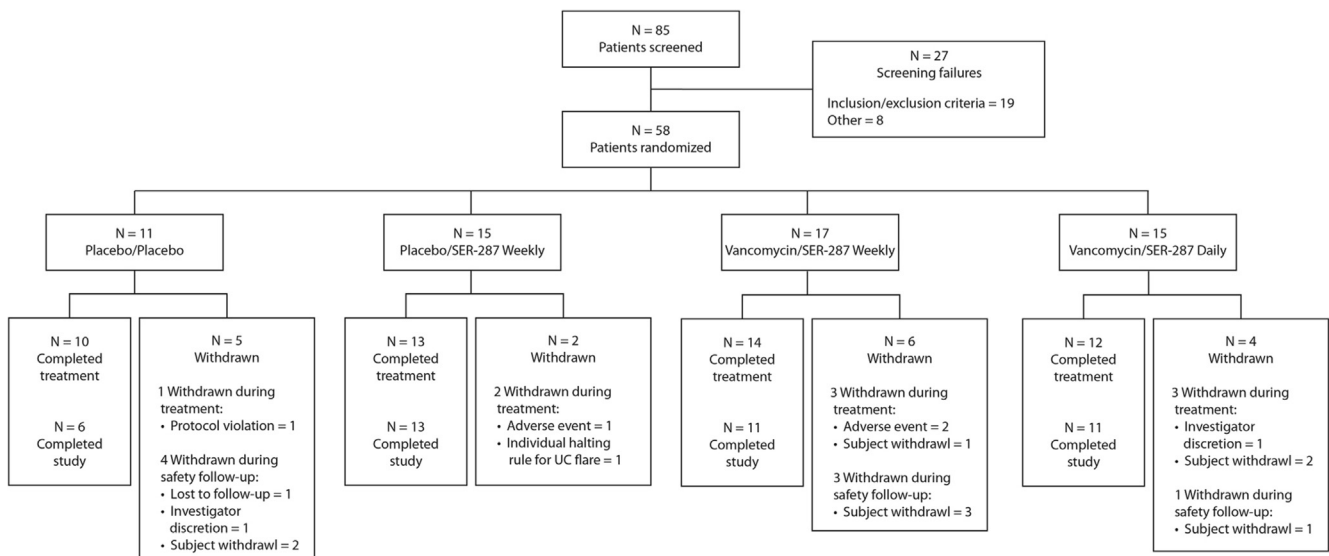
Supplementary Figure 2. Consolidated Standards of Reporting Trials diagram.
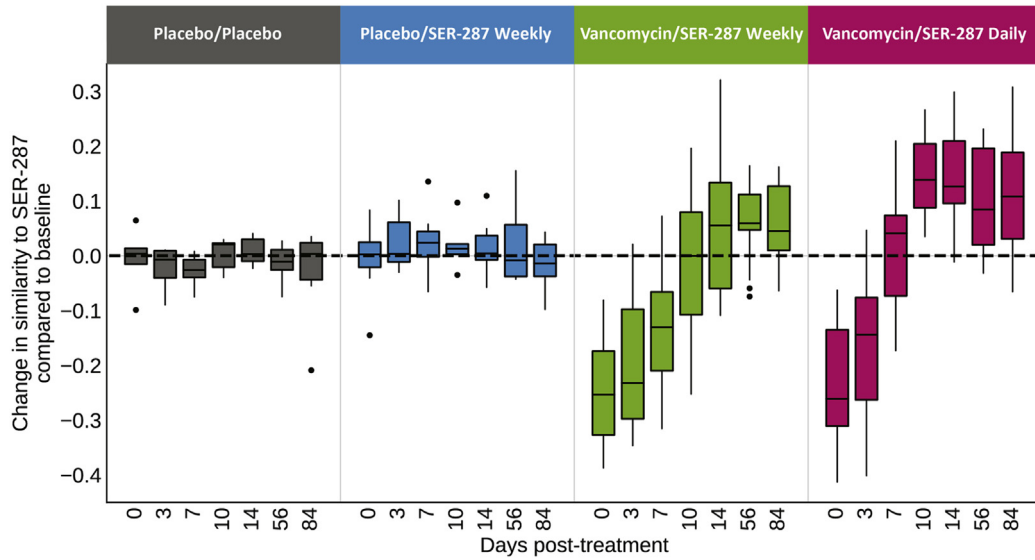
Supplementary Figure 3. Engraftment of SER-287 species is dose-dependent and facilitated by vancomycin preconditioning; preconditioned patient microbiome samples become more similar to SER-287 dose composition after rebound from vanco- mycin. Similarity between a subject's microbiome and SER-287 dose composition is quantified with a binary Jaccard similarity coefficient of spore-forming species in subject samples and SER-287 doses; the change in similarity for each subject and timepoint is relative to that subjects' baseline sample (*y-axis*) across time (*x-axis*). *Black horizontal dashed line* indicates the threshold where a subjects' spore fraction is more (>0) or less (<0) similar to SER-287 post-treatment. Initial preconditioning with vancomycin moves patient samples further from SER-287 dose composition; by day 10 in the vancomycin/SER-287 daily dosing arm, the patient spore-forming microbiome becomes more similar to SER-287 and remains so through the 8-week dosing period (day 56) and the long-term follow-up at day 84 (*P* < .05, Wilcoxon signed-rank test, all time points); in the vancomycin/SER-287 weekly dosing arm, only the day 56 time point was statistically significant *P* < .05, consistent with a dose-dependent effect.
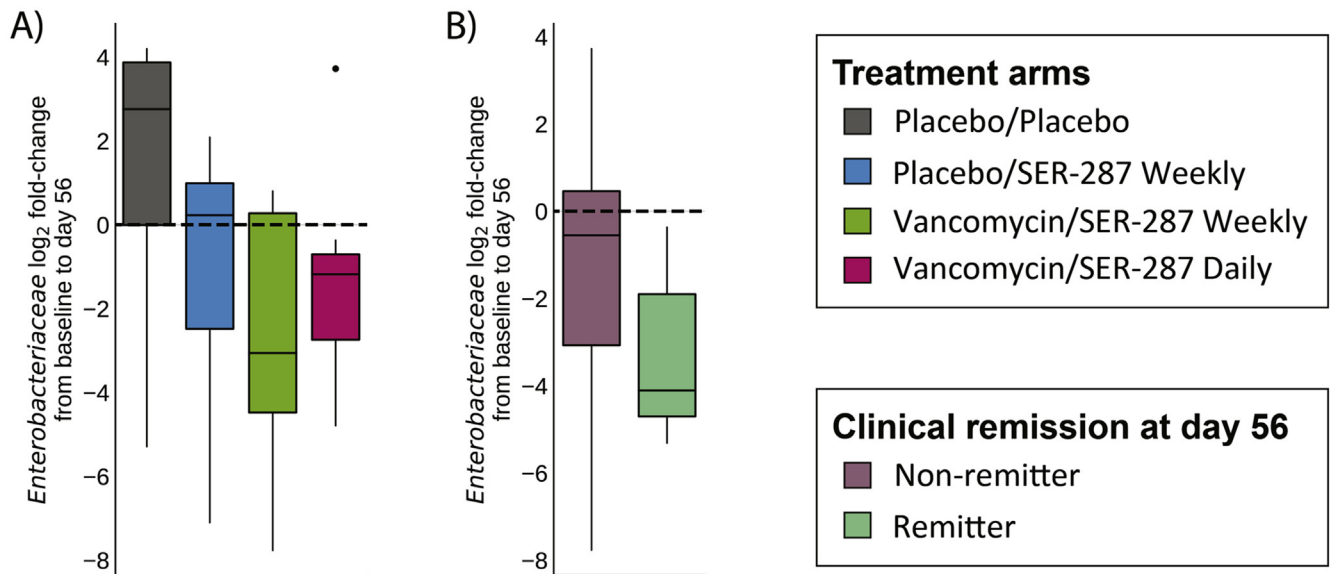
Supplementary Figure 4. *Enterobacteriaceae* species abundance tends to decrease with vancomycin pre-conditioning and SER-287 with remitters having a larger decrease in *Enterobacteriaceae* compared with non-remitters. (A) Fold-change in *Enterobacteriaceae* species abundance relative to baseline is shown 8 weeks post treatment (day 56) for subjects in each arm; *boxplots* are colored by treatment arm. (B) *Enterobacteriaceae* fold-change is shown across remitters and nonremitters for subjects in vancomycin preconditioning arms only; *boxplots* are colored by clinical remission status.

Supplementary Table 1. Spore-Forming Bacterial Taxa Identified in SER-287 Lots

Phylum	Class	Order	Family	Genus	Drug lots, n
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*	Unclassified *Christensenellaceae*	2
			Clostridiaceae	*Clostridium*	3
			Eubacteriaceae	*Anaerofustis, Eubacterium*	1
				[Eubacterium]	2
			Lachnospiraceae	*Acetatifactor, Coprococcus*	1
				Desulfotomaculum	2
				[Clostridium], [Coprococcus], [Eubacterium], [Faecalicatena], [Ruminococcus], Anaerostipes, Blautia, Dorea, Eisenbergiella, Fusicatenibacter, Lachnospira, unclassified *Lachnospiraceae, Lactonifactor, Marvinbryantia, Roseburia*	3
			Peptostreptococcaceae	*Intestinibacter*	3
			Ruminococcaceae	*Harryflintia*	1
				[Clostridium], [Ruminococcus], Agathobaculum, Faecalibacterium, Fusicatenibacter, Gemmiger, Massilimaliae, unclassified *Ruminococcaceae, Ruminococcus, Subdoligranulum*	3
	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Catenibacterium, Holdemanella, Solobacterium*	1
				Erysipelatoclostridium	2
				[Clostridium], Holdemania, Turicibacter	3

NOTE. The 3 lots of SER-287 that were used in the clinical trial were characterized by direct whole metagenomic shotgun sequencing. The list of genera with spore-forming species identified in any of the 3 lots is indicated; bracketed genera are pending reassignment in National Center for Biotechnology Information taxonomy (https://www.ncbi.nlm.nih.gov/taxonomy); unclassified family names within the genera column indicate species without genus assignments. Rows in the table are organized according to the hierarchical taxonomy. The last column indicates the number of drug lots in which the genera were identified.

Supplementary Table 2. Number of Subjects With Evaluable Metagenomic Shotgun Sequencing Data From Both Baseline and Post-Treatment Samples

Days post treatment	Placebo/ placebo	Placebo/SER-287 Weekly	Vancomycin/SER-287 Weekly	Vancomycin/SER-287 Daily
0	5	8	12	10
3	7	7	12	8
7	7	11	14	10
10	7	5	12	10
14	7	10	13	10
56	6	9	13	10
84	6	9	8	8

NOTE. Indicated is the number of subjects within each arm (columns) at the indicated time point (rows) with both baseline and post-treatment samples with metagenomic shotgun sequencing data.

Supplementary Table 3. Treatment-Emergent Adverse Events by System Organ Class and Preferred Term: Preconditioning Phase, Safety Population

Variable	Placebo pretreatment (n = 26)	Vancomycin pretreatment (n = 32)
Subjects with at least 1 TEAE	3 (11.5)	4 (12.5)
Gastrointestinal disorders	2 (7.7)	3 (9.4)
Constipation	1 (3.8)	1 (3.1)
Diarrhea	1 (3.8)	1 (3.1)
Fecal incontinence	0	1 (3.1)
Flatulence	0	1 (3.1)
Infections and infestations	0	1 (3.1)
Candida infection	0	1 (3.1)
Musculoskeletal and connective tissue disorders	1 (3.8)	0
Back pain	1 (3.8)	0
Muscle spasms	1 (3.8)	0
Psychiatric disorders	1 (3.8)	0
Insomnia	1 (3.8)	0
Skin and subcutaneous tissue disorders	0	1 (3.1)
Rash	0	1 (3.1)

TEAE, treatment-emergent adverse event.

Supplementary Table 4. Treatment-Emergent Adverse Events by System Organ Class and Preferred Term: Treatment Phase and Short-Term Safety Phase, Safety Population

Variable	Placebo/ placebo (n = 11)	Placebo/ SER-287 weekly (n = 15)	Vancomycin/ SER-287 weekly (n = 17)	Vancomycin/ SER-287 daily (n = 15)	All subjects receiving any dose of SER-287 (n = 47)
Subjects with at least 1 TEAE	7 (63.6)	9 (60.0)	13 (76.5)	7 (46.7)	29 (61.7)
Gastrointestinal disorders	5 (45.5)	7 (46.7)	7 (41.2)	1 (6.7)	15 (31.9)
Abdominal distension	0	0	1 (5.9)	0	1 (2.1)
Abdominal pain	1 (9.1)	3 (20.0)	4 (23.5)	0	7 (14.9)
Abdominal pain upper	0	1 (6.7)	0	0	1 (2.1)
Abnormal feces	0	0	1 (5.9)	0	1 (2.1)
Bowel movement irregularity	0	0	2 (11.8)	0	2 (4.3)
Colitis ulcerative	1 (9.1)	1 (6.7)	1 (5.9)	0	2 (4.3)
Constipation	0	1 (6.7)	0	1 (6.7)	2 (4.3)
Diarrhea	2 (18.2)	1 (6.7)	1 (5.9)	0	2 (4.3)
Diarrhea hemorrhagic	1 (9.1)	0	0	0	0
Dyspepsia	1 (9.1)	0	0	0	0
Flatulence	0	0	2 (11.8)	0	2 (4.3)
Frequent bowel movements	0	1 (6.7)	0	0	1 (2.1)
Gastroesophageal reflux disease	0	1 (6.7)	0	0	1 (2.1)
Mucous stools	0	0	1 (5.9)	0	1 (2.1)
Nausea	0	3 (20.0)	1 (5.9)	1 (6.7)	5 (10.6)
Esophagitis	0	0	1 (5.9)	0	1 (2.1)
Rectal discharge	0	1 (6.7)	0	0	1 (2.1)
Vomiting	0	1 (6.7)	1 (5.9)	0	2 (4.3)
General disorders and administration site conditions	1 (9.1)	0	3 (17.6)	1 (6.7)	4 (8.5)
Asthenia	0	0	1 (5.9)	0	1 (2.1)
Chest pain	0	0	1 (5.9)	0	1 (2.1)
Influenza-like illness	0	0	1 (5.9)	0	1 (2.1)
Edema peripheral	0	0	0	1 (6.7)	1 (2.1)
Pyrexia	1 (9.1)	0	0	0	0
Immune system disorders	0	0	1 (5.9)	0	1 (2.1)
Hypersensitivity	0	0	1 (5.9)	0	1 (2.1)
Infections and infestations	3 (27.3)	1 (6.7)	5 (29.4)	4 (26.7)	10 (21.3)
Cellulitis	0	0	1 (5.9)	0	1 (2.1)
Nasopharyngitis	0	0	1 (5.9)	1 (6.7)	2 (4.3)
Oral candidiasis	1 (9.1)	0	0	0	0
Otitis externa	1 (9.1)	0	0	0	0
Pneumonia	0	0	0	1 (6.7)	1 (2.1)
Pyelonephritis	0	1 (6.7)	0	0	1 (2.1)
Rhinitis	0	1 (6.7)	0	1 (6.7)	2 (4.3)
Sinusitis	0	0	1 (5.9)	0	1 (2.1)
Tooth infection	1 (9.1)	0	0	0	0
Upper respiratory tract infection	0	0	1 (5.9)	2 (13.3)	3 (6.4)
Urinary tract infection	0	1 (6.7)	0	0	1 (2.1)
Viral upper respiratory tract infection	0	0	1 (5.9)	0	1 (2.1)
Injury, poisoning and procedural complications	2 (18.2)	0	0	0	0
Contusion	1 (9.1)	0	0	0	0
Limb injury	1 (9.1)	0	0	0	0
Muscle injury	1 (9.1)	0	0	0	0
Investigations	0	0	1 (5.9)	0	1 (2.1)
Nitrite urine present	0	0	1 (5.9)	0	1 (2.1)
White blood cells urine positive	0	0	1 (5.9)	0	1 (2.1)
Metabolism and nutrition disorders	0	0	1 (5.9)	1 (6.7)	2 (4.3)
Decreased appetite	0	0	1 (5.9)	0	1 (2.1)
Hypokalemia	0	0	0	1 (6.7)	1 (2.1)

Supplementary Table 4. Continued

Variable	Placebo/ placebo (n = 11)	Placebo/ SER-287 weekly (n = 15)	Vancomycin/ SER-287 weekly (n = 17)	Vancomycin/ SER-287 daily (n = 15)	All subjects receiving any dose of SER-287 (n = 47)
Musculoskeletal and connective tissue disorders	0	2 (13.3)	1 (5.9)	2 (13.3)	5 (10.6)
Back pain	0	2 (13.3)	1 (5.9)	1 (6.7)	4 (8.5)
Muscle spasms	0	0	0	1 (6.7)	1 (2.1)
Nervous system disorders	0	0	1 (5.9)	3 (20.0)	4 (8.5)
Headache	0	0	1 (5.9)	3 (20.0)	4 (8.5)
Psychiatric disorders	0	0	0	1 (6.7)	1 (2.1)
Depression	0	0	0	1 (6.7)	1 (2.1)
Reproductive system and breast disorders	0	0	1 (5.9)	0	1 (2.1)
Pelvic pain	0	0	1 (5.9)	0	1 (2.1)
Respiratory, thoracic and mediastinal disorders	0	1 (6.7)	2 (11.8)	1 (6.7)	4 (8.5)
Cough	0	1 (6.7)	0	1 (6.7)	2 (4.3)
Nasal congestion	0	0	1 (5.9)	0	1 (2.1)
Rhinitis allergic	0	0	1 (5.9)	0	1 (2.1)
Skin and subcutaneous tissue disorders	0	0	1 (5.9)	2 (13.3)	3 (6.4)
Hidradenitis	0	0	0	1 (6.7)	1 (2.1)
Skin reaction	0	0	1 (5.9)	0	1 (2.1)
Swelling face	0	0	0	1 (6.7)	1 (2.1)

NOTE. Values are n (%).
TEAE, treatment-emergent adverse event.

Supplementary Table 5. Comparison of Number of Engrafting SER-287 Dose Species Across Arms

Days post treatment	P value (unadjusted)	Placebo/placebo mean	Placebo/SER-287 weekly mean
0	.768	4.600	3.875
3	.104	2.000	5.571
7	.009	1.857	6.091
10	.805	4.000	6.200
14	.730	4.143	4.800
56	.515	4.333	7.222
84	.372	4.333	5.889

Days post treatment	P value (unadjusted)	Placebo/placebo mean	Vancomycin/SER-287 weekly mean
0	.008	4.600	0.417
3	.863	2.000	2.583
7	.018	1.857	6.143
10	.001	4.000	12.583
14	.001	4.143	15.385
56	.002	4.333	13.615
84	.011	4.333	13.375

Days post treatment	P value (unadjusted)	Placebo/placebo mean	Vancomycin/SER-28 daily mean
0	0.006	4.600	0.100
3	0.411	2.000	3.375
7	0.001	1.857	13.500
10	0.001	4.000	20.600
14	0.001	4.143	21.300
56	0.002	4.333	16.700
84	0.007	4.333	18.000

Days post treatment	P value (unadjusted)	Placebo/SER-287 weekly mean	Vancomycin/SER-287 daily mean
0	<.001	3.875	0.100
3	.172	5.571	3.375
7	.002	6.091	13.500
10	.017	6.200	20.600
14	<.001	4.800	21.300
56	.012	7.222	16.700
84	.007	5.889	18.000

Supplementary Table 5. Continued

Days post treatment	P value (unadjusted)	Placebo/SER-287 weekly mean	Vancomycin/SER-287 weekly mean
0	.001	3.875	0.417
3	.049	5.571	2.583
7	.804	6.091	6.143
10	.044	6.200	12.583
14	.001	4.800	15.385
56	.020	7.222	13.615
84	.018	5.889	13.375

Days post treatment	P value (unadjusted)	Vancomycin/SER-287 weekly mean	Vancomycin/SER-287 daily mean
0	.658	0.417	0.100
3	.285	2.583	3.375
7	.003	6.143	13.500
10	.012	12.583	20.600
14	.067	15.385	21.300
56	.335	13.615	16.700
84	.317	13.375	18.000

NOTE. Pairwise arm comparisons are indicated. P value indicates 2-sided Mann-Whitney U test for the indicated treatment arm compared with placebo. See Supplementary Table 2 for the number of subjects.

Supplementary Table 6. Change in Spore-Forming Species Richness (α-Diversity) Post Treatment Compared With Placebo

Treatment group compared with placebo/placebo	Days post treatment	P value (unadjusted)	Placebo/placebo mean	Treatment group mean
Placebo/SER-287 weekly	0	.769	1.800	−1.500
	3	.158	−4.000	1.571
	7	.093	−3.143	1.545
	10	.622	1.286	0.000
	14	.219	2.714	0.000
	56	.766	0.167	2.333
	84	.935	−2.600	−3.286
Vancomycin/SER-287 daily	0	.008	1.800	−27.667
	3	.027	−4.000	−20.875
	7	.305	−3.143	4.600
	10	.001	1.286	21.556
	14	.002	2.714	17.900
	56	.037	0.167	12.100
	84	.048	−2.600	13.625
Vancomycin/SER-287 weekly	0	.012	1.800	−27.400
	3	.005	−4.000	−25.545
	7	.074	−3.143	−11.846
	10	.821	1.286	−1.636
	14	.361	2.714	8.769
	56	.313	0.167	5.615
	84	.065	−2.600	8.000

NOTE. P value indicates 2-sided Mann-Whitney U test for the indicated treatment arm compared with placebo. See Supplementary Table 2 for the number of subjects.

Supplementary Table 7. Change in Microbiome Composition (β-Diversity) Post-Treatment Compared With Placebo

Treatment group compared with placebo/placebo	Days post treatment	P value (unadjusted)	Placebo/placebo mean	Treatment group mean
Placebo/SER-287 weekly	0	.769	0.330	0.329
	3	.701	0.317	0.296
	7	.469	0.288	0.339
	10	.871	0.265	0.354
	14	.733	0.326	0.342
	56	.517	0.340	0.400
	84	.626	0.421	0.443
Vancomycin/SER-287 daily	0	.003	0.330	0.865
	3	.001	0.317	0.775
	7	.001	0.288	0.747
	10	.001	0.265	0.663
	14	.001	0.326	0.672
	56	.002	0.340	0.650
	84	.067	0.421	0.680
Vancomycin/SER-287 weekly	0	.002	0.330	0.906
	3	<.001	0.317	0.841
	7	<.001	0.288	0.775
	10	.001	0.265	0.718
	14	<.001	0.326	0.654
	56	.001	0.340	0.601
	84	.092	0.421	0.606

NOTE. β-diversity distance (binary Jaccard) from baseline for subjects in each treatment group at the given time point, compared with placebo. P value indicates 2-sided Mann Whitney U test for the indicated treatment arm compared with placebo. See Supplementary Table 2 for the number of subjects.

Supplementary Table 8. Change in *Enterobacteriaceae* Relative Abundance From Baseline to 8 Weeks Post Treatment Across Arms

Arms	No. of subjects	Median *Enterobacteriaceae* log$_2$FC	*P* value
Placebo/placebo	4	2.758	.875
Placebo/SER-287 weekly	8	0.229	.945
Vancomycin/SER-287 weekly	10	−3.061	.064
Vancomycin/SER-287 daily	7	−1.183	.156
Vancomycin/SER-287 weekly + daily	17	−1.377	.009

NOTE. The change in *Enterobacteriaceae* abundance was assessed at 8 weeks post treatment. Only subjects with baseline and 8-week stool samples and subjects with *Enterobacteriaceae* at baseline (n = 29) were included in analyses. Change in *Enterobacteriaceae* abundance post treatment was assessed using log$_2$ fold-change between subjects' baseline and 8-week post-treatment samples. A 2-sided Wilcoxon signed-rank test was performed to assess whether the fold-changes were centered around 0 for each arm individually and for vancomycin/SER-287 weekly and daily treatment arms combined.

Supplementary Table 9. Decrease In *Enterobacteriaceae* Abundance Is Associated With Clinical Remission

Arms	Clinical remission	No. of subjects	Median *Enterobacteriaceae* log$_2$ FC	*P* value
Vancomycin/SER-287 weekly + daily	Y	7	−4.108	.016
Vancomycin/SER-287 weekly + daily	N	10	−0.552	.322

NOTE. The association *Enterobacteriaceae* abundance with clinical remission was assessed at 8 weeks post treatment. Only subjects with baseline and 8-week stool samples and subjects with *Enterobacteriaceae* at baseline (n = 29) were included in analyses. Change in *Enterobacteriaceae* abundance post treatment was assessed using log$_2$ fold-change between subjects' baseline and 8-week post-treatment samples. A 2-sided Wilcoxon signed-rank test was performed to assess whether the fold-changes are centered around 0 for vancomycin/SER-287 weekly and daily treatment arms combined in remitters vs nonremitters.

Supplementary Table 10.

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
Glycocholic acid	0.530	2.101	-2.254	-0.310	-1.184	1.000	0.208	0.139	0.721	0.398
Taurocholic acid	-0.772	-0.669	-1.792	-2.117	-1.971	0.593	0.463	0.515	0.214	0.157
Glycochenodeoxycholic acid	0.079	1.721	-1.999	-0.848	-1.366	1.000	0.263	0.214	0.959	0.355
Taurochenodeoxychoic acid	4.798	-0.041	-3.557	-1.066	-2.187	0.109	1.000	0.214	1.000	0.287
Cholic acid	0.583	-1.843	-1.693	-1.374	-1.518	1.000	0.484	0.314	0.477	0.232
Chenodeoxycholic acid	0.223	-1.724	-2.714	-1.015	-1.779	1.000	0.161	0.086	0.594	0.058
Deoxycholic acid	-1.172	-1.286	-0.593	0.693	0.114	0.109	0.161	0.767	0.477	0.823
Lithocholic acid	-1.256	-0.419	-0.733	0.859	0.143	1.000	0.889	0.441	0.285	0.687
Ursodeoxycholic acid	0.283	-2.280	-3.436	-3.106	-3.255	1.000	0.093	0.021	0.047	0.003
Acetate	-0.185	0.124	-0.180	0.173	-0.003	0.465	0.779	0.445	1.000	0.543
Butyrate	-1.074	0.067	-0.597	1.065	0.234	0.144	0.779	0.075	0.328	0.067
Propionate	-0.480	0.288	-0.456	-0.520	-0.488	0.273	0.263	0.155	0.131	0.042
Valerate	-1.406	0.460	-0.599	-0.016	-0.307	0.715	0.575	0.534	0.131	0.168
Hexanoate	-1.274	0.505	-0.316	2.447	1.065	0.465	1.000	0.790	0.026	0.095

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
(15:0)-anacardic acid	0.00	0.31	-1.57	-0.62	-1.10	1.00000	0.31731	0.10881	0.17971	0.04311
(15:2)-anacardic acid	0.12	1.37	-3.83	-1.40	-2.61	0.65472	0.17971	0.04311	0.28505	0.01729
(16 or 17)-methylstearate (a19:0 or i19:0)	0.39	0.36	-0.86	-0.92	-0.89	0.46521	0.57540	0.09116	0.05046	0.00985
(3'-5')-adenylyladenosine*	-0.34	-0.05	-1.08	-1.04	-1.06	1.00000	1.00000	0.03815	0.13864	0.00740
(3'-5')-adenylylcytidine	-0.20	0.25	-0.65	-1.44	-1.04	0.31731	0.86577	0.26262	0.06632	0.02772
(3'-5')-adenylylguanosine*	-1.23	0.23	-1.30	-0.81	-1.05	0.10881	0.73532	0.02088	0.21352	0.01387
(3'-5')-adenylyluridine	-0.91	0.37	-0.83	-0.92	-0.87	0.28505	0.88864	0.13864	0.13864	0.03858
(3'-5')-guanylylcytidine	1.25	0.00	-1.28	-1.33	-1.30	0.17971	1.00000	0.13864	0.12349	0.03516
(3'-5')-guanylyluridine	0.00	0.54	-1.39	-0.89	-1.14	1.00000	0.75315	0.10974	0.17295	0.04683
(3'-5')-uridylylcytidine*	-0.15	-0.70	-0.59	-1.27	-0.93	0.31731	0.12349	0.26039	0.03815	0.02223
(3'-5')-uridylyluridine	-1.11	-0.32	-0.53	-1.14	-0.84	0.17971	0.48384	0.37426	0.03815	0.04753
1,2-dipalmitoyl-GPC (16:0/16:0)	0.97	-0.82	-0.78	-1.57	-1.17	0.71500	0.20758	0.28600	0.09116	0.04245
1-margaroylglycerol (17:0)	0.82	-0.38	-0.65	-1.17	-0.91	0.14413	0.16143	0.07537	0.00444	0.00138
1-oleoyl-GPG (18:1)*	1.21	0.09	0.58	1.17	0.88	0.27332	0.71500	0.44127	0.02840	0.04753
1-palmitoyl-2-linoleoyl-galactosylglycerol (16:0/18:2)*	-1.41	-0.32	1.93	0.40	1.17	0.28505	0.49896	0.02842	0.72128	0.04380
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.53	-0.34	-0.59	-1.79	-1.19	0.46521	0.23672	0.20262	0.04685	0.02277
1-ribosyl-imidazoleacetate*	0.30	-0.36	-0.25	-0.87	-0.56	0.65472	0.65472	0.28505	0.07962	0.03569
1-stearoyl-GPC (18:0)	0.98	-0.43	-1.01	-1.78	-1.39	0.46521	0.32699	0.21322	0.05046	0.02405
1-stearoyl-GPG (18:0)	0.19	-0.79	-1.13	-0.72	-0.92	0.71500	0.26262	0.10951	0.16881	0.03255
1-stearoyl-GPI (18:0)	0.02	-0.47	-1.19	-1.68	-1.44	1.00000	0.26262	0.15486	0.07537	0.01702
2'-deoxyadenosine	0.49	-0.49	-0.87	-1.57	-1.22	0.46521	0.75315	0.17307	0.01660	0.00791
2'-deoxyadenosine 5'-monophosphate	0.92	0.95	-1.10	-1.63	-1.37	0.10881	0.27332	0.13864	0.04086	0.01237
2'-deoxyinosine	1.37	-0.13	-0.45	-1.22	-0.84	0.14413	0.88864	0.32806	0.05046	0.03346
2-hydroxyoleate	-0.81	-0.56	-2.12	-0.67	-1.39	0.10881	0.67442	0.06188	0.33288	0.04202
2-stearoyl-GPE (18:0)*	0.33	-0.20	-0.83	-2.00	-1.41	1.00000	0.71500	0.23672	0.04685	0.02168

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
3-(4-hydroxyphenyl)propionate	-0.34	-1.05	-1.18	-1.89	-1.54	0.46521	0.48384	0.15486	0.02080	0.00815
3-aminoisobutyrate	-0.16	-2.10	-0.72	-1.40	-1.06	0.71500	0.01796	0.28600	0.05934	0.02983
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	0.91	0.16	-0.76	-1.24	-1.00	0.59298	0.89274	0.16143	0.04995	0.01306
3-hydroxy-2-ethylpropionate	-0.41	0.72	-1.20	-1.02	-1.11	1.00000	0.49896	0.17307	0.05061	0.03467
3-hydroxyphenylacetate	0.68	-0.77	-2.03	-1.10	-1.56	0.31731	0.07474	0.02840	0.12349	0.00748
3-sulfo-L-alanine	0.38	-1.54	-1.46	-0.77	-1.11	0.71500	0.01729	0.02842	0.01516	0.00128
4-hydroxychlorothalonil	0.53	-0.81	-0.83	-0.39	-0.61	0.27332	0.02771	0.06298	0.49896	0.04799
4-hydroxycinnamate	1.10	0.37	-0.74	-1.43	-1.08	0.14413	0.48384	0.21322	0.03285	0.01558
5-dodecenoate (12:1n7)	2.89	1.15	-0.66	-1.14	-0.90	0.06789	0.23672	0.26039	0.04086	0.03334
5alpha-androstan-3alpha,17alpha-diol monosulfate	-0.37	-0.45	-0.40	-0.58	-0.49	0.59298	0.50018	0.05934	0.16881	0.01688
5alpha-androstan-3alpha,17beta-diol monosulfate (1)	-0.15	-0.77	-1.14	-0.82	-0.98	0.59298	0.34545	0.06870	0.09289	0.01306
5alpha-androstan-3beta,17alpha-diol disulfate	-0.31	-0.29	-0.27	-0.22	-0.24	0.28505	0.50018	0.10974	0.16143	0.04947
5alpha-pregnan-3beta,20alpha-diol disulfate	-0.02	-0.92	-0.93	-2.06	-1.50	1.00000	0.32699	0.38627	0.01660	0.02063
N,N-dimethyl-5-aminovalerate	1.20	-1.57	-0.10	-2.29	-1.19	0.71500	0.09289	0.32806	0.05046	0.04245
N-acetyl-beta-alanine	-0.55	-0.91	-1.46	-0.38	-0.92	0.46521	0.49896	0.00765	0.50762	0.01427
N-acetyl-beta-glucosaminylamine	0.51	0.26	-0.28	-0.62	-0.45	0.46521	1.00000	0.32806	0.09260	0.04955
N-acetylarginine	-0.47	-0.60	0.49	1.29	0.89	0.14413	0.26262	0.18231	0.07537	0.01558
N-acetylaspartate (NAA)	-0.58	0.22	-1.07	-0.60	-0.83	0.46521	0.48384	0.09116	0.18231	0.04586
N-acetylhistidine	0.77	0.10	0.35	0.83	0.59	0.46521	0.88864	0.42360	0.03285	0.03925
N-acetylserine	0.80	0.09	-0.29	-1.01	-0.65	0.27332	0.77943	0.32806	0.07537	0.03925
N-acetyltaurine	0.31	-2.20	-2.35	-2.00	-2.17	1.00000	0.03569	0.02080	0.00691	0.00054
N-oleoyltaurine	-1.01	-0.93	-2.00	-0.65	-1.32	0.28505	0.34523	0.05061	0.50018	0.03033

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
N-propionylalanine	0.39	-0.58	-0.89	-0.80	-0.84	0.46521	0.40081	0.16881	0.07537	0.02731
N-stearoyltaurine	-1.24	-1.39	-1.67	-1.39	-1.53	0.10881	0.20758	0.01252	0.02799	0.00118
N1-Methyl-2-pyridone-5-carboxamide	0.28	-0.24	-0.98	-1.40	-1.19	0.59298	0.32699	0.24775	0.00765	0.01082
N6-carboxymethyllysine	-0.52	-1.89	-1.14	-0.97	-1.05	0.14413	0.09289	0.13067	0.13067	0.03626
STVLT	0.80	-0.46	-0.86	-1.18	-1.02	0.31731	0.31731	0.06789	0.17630	0.02623
TMP	1.74	0.92	-0.72	-1.89	-1.31	0.10881	0.68583	0.39802	0.05061	0.04373
X - 11299	0.11	0.00	-0.45	-0.28	-0.36	0.65472	1.00000	0.31731	0.06789	0.04311
X - 12849	1.16	0.16	-0.51	-1.09	-0.80	0.17971	0.31731	0.17971	0.10881	0.04311
X - 13255	-0.77	0.00	-0.54	-1.67	-1.11	0.31731	1.00000	0.10881	0.06789	0.01796
X - 13703	0.13	0.17	-0.34	-1.04	-0.69	0.31731	0.31731	0.17971	0.13801	0.04252
X - 13835	-1.50	-0.86	-1.28	-0.66	-0.97	0.14413	0.12349	0.02080	0.59371	0.04586
X - 14254	1.28	0.82	-1.54	-1.43	-1.49	0.46521	0.57540	0.04086	0.05046	0.00366
X - 14263	0.46	-0.56	-0.31	-0.65	-0.48	1.00000	0.32699	0.47691	0.01279	0.02616
X - 14416	-0.03	0.54	-1.46	-1.92	-1.69	0.65472	0.46307	0.24112	0.05046	0.04202
X - 14454	-1.30	0.24	-1.32	-1.24	-1.28	0.27332	0.61209	0.13067	0.02623	0.01558
X - 14662	2.07	0.00	-0.44	-0.77	-0.60	0.17971	1.00000	0.10881	0.17971	0.04311
X - 15806	0.90	0.12	-0.85	-1.16	-1.01	0.31731	0.89274	0.09097	0.13864	0.02618
X - 17162	-0.19	0.08	3.22	1.66	2.44	0.65472	0.86577	0.16881	0.21352	0.04862
X - 17869	-0.13	0.23	-0.36	-0.97	-0.67	0.65472	0.68583	0.31049	0.04640	0.03924
X - 17877	0.54	0.22	-1.16	-1.22	-1.19	0.65472	1.00000	0.02088	0.03569	0.00193
X - 17960	-1.21	0.33	-1.09	-0.33	-0.71	0.46521	0.77943	0.06188	0.42360	0.04951
X - 17983	0.88	0.83	-1.53	-2.38	-1.95	0.59298	0.34523	0.17630	0.01252	0.00748
X - 18938	1.07	-0.56	-1.22	-0.72	-0.97	0.06789	0.48384	0.09116	0.24775	0.03626
X - 19746	0.91	0.00	-0.33	-0.95	-0.64	0.31731	1.00000	0.17971	0.06789	0.02771
X - 19751	-0.63	-0.37	-0.28	-2.42	-1.35	0.46521	0.77943	0.78968	0.03285	0.03626
X - 20172	0.74	0.92	-1.29	-2.46	-1.87	0.71500	0.57540	0.15486	0.05046	0.01702

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
X - 20197	0.95	0.48	-1.20	-2.37	-1.78	0.46521	0.57540	0.13067	0.02623	0.00896
X - 20624	0.33	-1.58	-2.81	-1.13	-1.97	1.00000	0.12349	0.00691	0.06789	0.00123
X - 21467	1.17	0.60	-0.95	-1.75	-1.35	0.28505	0.31731	0.06789	0.06789	0.01172
X - 21788	0.93	-2.38	-3.16	-1.98	-2.57	0.46521	0.06870	0.01637	0.01172	0.00084
X - 22030	0.20	0.00	-1.51	-0.50	-1.00	0.65472	0.91651	0.11585	0.34545	0.04986
X - 23115	-3.07	0.33	-1.63	-1.08	-1.35	0.06789	1.00000	0.09116	0.10951	0.02027
X - 23581	0.88	-0.32	0.84	0.79	0.82	0.46521	0.57540	0.18231	0.13067	0.04245
X - 23585	-0.52	1.13	1.22	2.56	1.89	0.10881	0.09289	0.13067	0.05934	0.01897
X - 23639	0.90	-0.04	0.96	0.50	0.73	0.06789	0.88864	0.03285	0.38627	0.04202
X - 24243	-0.58	-0.89	-1.47	-0.70	-1.08	0.27332	0.40081	0.00335	0.28450	0.00784
X - 24608	0.77	0.15	-0.37	-0.46	-0.41	0.14413	0.57540	0.21322	0.01637	0.04245
X - 24699	1.32	-1.13	-1.02	-2.24	-1.63	0.71500	0.27332	0.67840	0.02840	0.03858
X - 24728	0.82	0.67	-0.28	-1.87	-1.07	0.10881	0.65472	0.17971	0.06789	0.02771
X - 24952	-0.17	0.20	-0.23	-0.26	-0.25	0.46521	0.32699	0.28600	0.06188	0.03085
X - 24983	0.80	-0.53	-0.69	-0.95	-0.82	0.14413	0.23672	0.06188	0.24775	0.03346
X - 24996	-1.17	0.51	-0.77	-0.44	-0.60	0.17971	0.31731	0.10881	0.10881	0.02771
X - 25053	-0.78	-0.27	0.05	-2.44	-1.19	0.46521	0.73532	0.95935	0.01279	0.04955
X - 25264	0.43	0.30	-0.72	-1.33	-1.03	0.65472	0.89274	0.17295	0.13941	0.03861
acetylagmatine	-0.06	0.90	1.71	1.58	1.65	0.59298	0.34545	0.10974	0.10974	0.03110
adenine	-0.50	-0.47	-0.79	-0.83	-0.81	0.27332	0.32699	0.06188	0.15486	0.02842
adrenate (22:4n6)	-0.98	-0.51	-1.27	-1.70	-1.49	0.27332	0.17295	0.05046	0.00691	0.00187
alpha-ketoglutarate	1.51	-0.21	-0.56	-0.62	-0.59	0.06789	0.77943	0.32806	0.04086	0.04245
arachidate (20:0)	-0.72	0.19	-1.38	-0.82	-1.10	0.06789	0.32699	0.01637	0.07537	0.00297
arachidonate (20:4n6)	0.51	-0.38	-1.01	-2.08	-1.54	0.71500	0.40081	0.09116	0.01279	0.00267
arachidoylcarnitine (C20)*	0.56	-0.01	-1.14	-1.06	-1.10	0.27332	0.67442	0.00444	0.02623	0.00038
arginine	0.92	0.31	1.11	1.83	1.47	0.46521	0.67442	0.28600	0.02623	0.02027

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
behenate (22:0)*	-1.44	0.30	-1.81	-1.20	-1.50	0.06789	0.88864	0.01279	0.00765	0.00029
behenoyl sphingomyelin (d18:1/22:0)*	1.06	0.35	-0.64	-1.18	-0.91	0.28505	0.89274	0.10974	0.06632	0.01387
behenoylcarnitine (C22)*	0.61	0.07	-0.72	-0.61	-0.66	0.06789	0.48384	0.00585	0.03285	0.00069
betaine	2.07	-0.07	-1.42	-1.62	-1.52	0.14413	0.26262	0.09116	0.05046	0.01082
cadaverine	-0.46	1.94	-1.99	-1.70	-1.85	0.14413	0.12349	0.07537	0.13067	0.02616
citrulline	0.35	-0.27	-0.40	-0.85	-0.63	1.00000	0.16143	0.32806	0.00993	0.00450
cytidine	-1.51	0.20	1.89	0.95	1.42	0.46521	0.88864	0.02623	0.28600	0.01558
dCMP	1.39	1.09	-1.35	-2.14	-1.75	0.10881	0.50018	0.07446	0.04086	0.00707
dihomolinoleate (20:2n6)	-0.98	0.02	-0.54	-1.25	-0.89	0.27332	1.00000	0.42360	0.01637	0.03085
dihomolinolenate (20:3n3 or 3n6)	-1.06	-0.54	-1.48	-2.23	-1.86	0.27332	0.32699	0.09116	0.00335	0.00098
dihydroorotate	1.81	-1.40	-1.15	-1.39	-1.27	0.06789	0.02506	0.28450	0.10951	0.04566
docosahexaenoate (DHA; 22:6n3)	0.68	0.05	-0.20	-2.09	-1.15	0.46521	1.00000	0.92915	0.00993	0.04586
docosapentaenoate (DPA; 22:5n3)	-0.33	-0.46	-0.88	-2.32	-1.60	0.71500	0.48384	0.28600	0.00444	0.00740
eicosanoylsphingosine (d20:1)*	-0.65	-1.28	-1.08	-0.69	-0.88	0.14413	0.04995	0.04086	0.15486	0.01187
eicosenoate (20:1n9 or 1n11)	-0.82	0.35	-0.94	-0.94	-0.94	0.06789	0.88864	0.03285	0.05046	0.00366
eicosenoylcarnitine (C20:1)*	-0.64	-0.57	-1.01	-0.43	-0.72	0.27332	0.26262	0.02080	0.37394	0.01858
enterodiol	-0.14	-1.68	-4.71	-2.00	-3.35	0.59298	0.17630	0.00506	0.13864	0.00170
erucate (22:1n9)	-1.10	0.29	-0.36	-1.06	-0.71	0.06789	0.57540	0.32806	0.06188	0.04245
erucoylcarnitine (C22:1)*	0.19	-0.35	-1.33	-0.98	-1.15	0.46521	0.48384	0.00506	0.04086	0.00102
gamma-glutamyl-epsilon-lysine	0.52	0.60	-0.46	-1.34	-0.90	0.46521	0.67442	0.21322	0.04086	0.01424
gamma-glutamylglycine	0.97	-0.82	-1.17	-0.73	-0.95	0.17971	0.17971	0.06298	0.13801	0.01860
gamma-glutamylmethionine	0.80	-1.25	-0.28	-1.59	-0.93	0.59298	0.09289	0.65664	0.00993	0.02842
gamma-glutamylvaline	0.52	-0.29	-0.67	-0.85	-0.76	0.10881	0.71500	0.20262	0.12349	0.03110
hydantoin-5-propionate	0.80	-1.83	-0.97	-1.69	-1.33	0.46521	0.02771	0.15486	0.02842	0.00869
hypoxanthine	-1.01	-0.03	-0.62	-1.24	-0.93	0.27332	1.00000	0.28600	0.06188	0.03346
indole	0.18	1.08	1.45	1.78	1.62	0.71500	0.09289	0.04086	0.00585	0.00069

Supplementary Table 11. Continued

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
lactosyl-N-behenoyl-sphingosine (d18:1/ 22:0)*	0.80	-1.10	-0.71	-1.43	-1.07	0.71500	0.20758	0.13067	0.05061	0.01524
lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	1.37	-0.72	-1.36	-2.05	-1.70	0.46521	0.20758	0.09116	0.03285	0.00551
lactosyl-N-palmitoyl-sphingosine (d18:1/ 16:0)	1.18	-0.65	-1.28	-1.76	-1.52	0.46521	0.26262	0.10951	0.04086	0.00896
leucylglycine	0.11	-0.58	-1.36	-1.32	-1.34	0.71500	0.46307	0.02799	0.07537	0.00740
lignoceroyl sphingomyelin (d18:1/24:0)	1.21	0.41	-0.39	-1.72	-1.05	0.59298	0.50018	0.51467	0.02182	0.02977
linolenoyl ethanolamide	-0.66	-1.28	-0.25	-2.34	-1.29	0.10881	0.26262	0.72128	0.00506	0.02063
linoleoyl ethanolamide	-0.91	-1.40	-0.97	-2.71	-1.84	0.14413	0.09289	0.28600	0.00506	0.00784
linoleoyl-arachidonoyl-glycerol (18:2/ 20:4) [2]*	2.44	-0.71	-1.23	-1.47	-1.35	0.14413	0.67442	0.11413	0.21322	0.04955
lysylleucine	-1.18	0.32	-1.04	-1.54	-1.29	0.10881	1.00000	0.21322	0.10951	0.04245
margarate (17:0)	0.05	0.27	-1.35	-1.35	-1.35	1.00000	1.00000	0.02623	0.03285	0.00215
margaroylcarnitine (C17)*	1.13	-0.12	-0.67	-0.90	-0.79	0.06789	0.88864	0.04086	0.09116	0.01424
nervonate (24:1n9)*	-1.21	0.12	-1.30	-1.11	-1.20	0.14413	0.88864	0.07537	0.04086	0.00671
nervonoylcarnitine (C24:1)*	0.26	0.00	-0.55	-0.37	-0.46	0.14413	0.88864	0.10951	0.16881	0.04202
nonadecanoate (19:0)	-0.14	0.64	-0.95	-1.28	-1.12	0.71500	0.16143	0.05046	0.01279	0.00155
oleate/vaccenate (18:1)	-0.51	-0.07	-1.12	-0.83	-0.98	0.46521	0.67442	0.03285	0.05046	0.00450
oleoyl ethanolamide	-1.08	-0.47	-0.99	-1.82	-1.40	0.14413	0.40081	0.18231	0.02080	0.00985
oleoylcarnitine (C18)	0.96	-0.61	-0.43	-1.60	-1.02	0.28505	0.57540	0.32806	0.01637	0.01702
orotidine	1.16	0.79	-1.09	-1.32	-1.20	0.10881	0.22492	0.04311	0.03569	0.00373
palmitate (16:0)	-0.10	0.18	-1.10	-0.75	-0.92	0.71500	0.77943	0.02080	0.06188	0.00173
palmitoleoyl ethanolamide*	-1.48	-0.92	-0.65	-1.78	-1.21	0.27332	0.17295	0.28450	0.01516	0.01260
palmitoyl sphingomyelin (d18:1/16:0)	0.96	-0.55	-0.65	-1.52	-1.09	0.46521	0.16143	0.15486	0.01637	0.00740
palmitoyl-arachidonoyl-glycerol (16:0/ 20:4) [2]*	1.13	-0.80	-0.95	-0.78	-0.86	0.46521	0.31049	0.05934	0.28600	0.04566

	Mean log2 fold-change					p-value				
	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily	Placebo/ Placebo	Placebo/ SER-287 weekly	Vancomycin/ SER-287 weekly	Vancomycin/ SER-287 daily	Vancomycin/ SER-287 weekly + daily
palmitoylcarnitine (C16)	1.70	-0.67	-0.44	-1.62	-1.03	0.06789	0.40081	0.32806	0.06188	0.03346
phenethylamine	2.86	-0.81	-0.90	-2.06	-1.48	0.14413	0.31049	0.50762	0.02842	0.03334
phenylpyruvate	2.67	-0.51	-0.96	-1.48	-1.22	0.06789	0.20758	0.24775	0.03285	0.02616
phytosphingosine	-0.71	-1.95	-0.84	-1.31	-1.07	0.27332	0.01172	0.06188	0.02080	0.00240
prolylglycine	1.44	-0.91	-0.52	-2.05	-1.29	0.06789	0.40081	0.47691	0.02623	0.03085
skatol	-0.11	1.37	0.61	0.74	0.68	0.31731	0.27332	0.10881	0.22492	0.03569
sphingomyelin (d18:1/24:1, d18:2/24:0)*	1.35	-0.70	-0.78	-1.77	-1.27	0.28505	0.23672	0.28600	0.03666	0.03255
sphingomyelin (d18:2/16:0, d18:1/16:1)*	1.49	-0.36	-0.80	-1.59	-1.20	0.17971	0.34545	0.18231	0.08583	0.02762
sphingomyelin (d18:2/24:1, d18:1/24:2)*	0.47	-1.21	-0.84	-2.20	-1.52	0.59298	0.17630	0.20262	0.02842	0.01524
stearate (18:0)	-0.29	0.32	-1.00	-0.75	-0.88	0.46521	0.57540	0.02080	0.05046	0.00267
stearoyl-arachidonoyl-glycerol (18:0/ 20:4) [2]*	1.53	-0.73	-1.24	-1.83	-1.53	0.27332	0.67442	0.09260	0.16881	0.04786
stearoylcarnitine (C18)	0.70	-0.25	-1.03	-1.30	-1.16	0.06789	0.67442	0.01637	0.01637	0.00069
taurine	0.03	-1.61	-1.82	-2.10	-1.96	1.00000	0.09289	0.00765	0.07537	0.00267
trans-nonadecenoate (tr 19:1)*	-2.32	-1.10	-0.24	-1.34	-0.79	0.27332	0.09097	0.48384	0.00444	0.01959
tryptamine	1.08	-0.64	-1.45	-2.40	-1.93	0.46521	0.57540	0.42360	0.03285	0.04245
tryptophan	0.87	-0.16	-0.59	-0.57	-0.58	0.14413	0.32699	0.10951	0.13067	0.02405
tryptophylglycine	0.54	0.46	-0.43	-1.45	-0.94	0.46521	0.61209	0.51467	0.02842	0.04014
tyrosol	-0.35	0.92	-1.30	-0.75	-1.02	1.00000	0.16143	0.06188	0.13067	0.01858
uridine-2',3'-cyclic monophosphate	-0.09	-0.53	-0.94	-1.72	-1.33	0.71500	0.26262	0.07537	0.02840	0.00359

Supplementary Table 12.

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
Glycocholic acid	1.141	0.236
Taurocholic acid	0.069	0.749
Glycochenodeoxycholic acid	1.007	0.319
Taurochenodeoxychoic acid	-0.494	0.704
Cholic acid	-0.101	1.000
Chenodeoxycholic acid	0.086	0.912
Deoxycholic acid	1.753	0.053
Lithocholic acid	1.979	0.093
Ursodeoxycholic acid	-2.763	0.043
Acetate	-0.115	0.391
Propionate	-0.347	0.571
Butyrate	-0.228	0.583
Valerate	1.669	0.114
Hexanoate	2.476	0.050

Supplementary Table 13.

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
N-acetylglycine	-0.891	0.03937
N-acetylserine	-1.216	0.02142
N-acetylthreonine	-2.193	0.01652
N-methylalanine	0.834	0.03926
N-acetylglutamate	1.020	0.02563
pyroglutamine*	-1.212	0.04279
1-methylhistidine	-1.540	0.02128
3-methylhistidine	-2.245	0.01341
imidazole propionate	-2.496	0.00315
4-imidazoleacetate	1.537	0.00907
5-aminovalerate	-1.498	0.01343
N-acetylphenylalanine	-1.205	0.00542
N-methylphenylalanine	1.838	0.01685
phenyllactate (PLA)	-1.311	0.02799
4-hydroxyphenylacetate	-1.482	0.01624
phenol sulfate	-3.136	0.02467
kynurenine	-1.473	0.00109
skatol	1.681	0.03145
indole	1.529	0.02563
N-acetylleucine	-1.435	0.00668
alpha-hydroxyisocaproate	-1.860	0.03053
1-carboxyethylisoleucine	-0.949	0.04858
ethylmalonate	0.922	0.02142
N-acetylvaline	-1.290	0.00200
1-carboxyethylvaline	-1.706	0.04029
2-hydroxy-3-methylvalerate	-1.522	0.02344
3-hydroxyisobutyrate	-1.191	0.03329
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)*	-1.078	0.04883
hypotaurine	-2.656	0.00591
taurine	-3.633	0.00282
N-acetyltaurine	-4.291	0.00281
norvaline	-1.735	0.01161
dimethylarginine (ADMA + SDMA)	-2.119	0.00158
N-acetylarginine	1.297	0.01106
N-acetylcitrulline	-1.143	0.01622
creatine	-2.917	0.01783
putrescine	-3.397	0.00148
N-acetyl-isoputreanine*	-3.141	0.00176
N1,N12-diacetylspermine	-2.093	0.01955

Supplementary Table 13. Continued

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
N-acetylputrescine	-2.277	0.01343
(N(1) + N(8))-acetylspermidine	-1.627	0.01002
cysteinylglycine disulfide*	-1.037	0.04858
2-hydroxybutyrate/2-hydroxyisobutyrate	-1.982	0.00252
histidylalanine	-1.844	0.01957
tryptophylglycine	-1.639	0.02337
valylglutamine	-1.175	0.04279
valylglycine	-1.413	0.01624
valylleucine	-1.389	0.02799
lactate	-3.075	0.00282
tricarballylate	0.997	0.04643
arachidonate (20:4n6)	-1.610	0.03939
3-methylglutarate/2-methylglutarate	2.006	0.00602
3-carboxyadipate	1.419	0.03939
hexadecanedioate (C16)	0.859	0.00315
butyrylglycine (C4)	-1.148	0.03389
palmitoylcarnitine (C16)	-2.363	0.02563
stearoylcarnitine (C18)	-1.395	0.02799
deoxycarnitine	-1.141	0.02344
2-hydroxynervonate*	2.706	0.01781
3-hydroxydecanoate	-1.310	0.00194
3-hydroxylaurate	-0.977	0.00668
5-hydroxyhexanoate	2.603	0.02281
behenoyl ethanolamide (22:0)*	1.574	0.01949
lignoceroyl ethanolamide (24:0)*	1.846	0.01953
choline	-1.154	0.01002
phosphocholine	-3.211	0.00474
trimethylamine N-oxide	-1.937	0.01343
1,2-dipalmitoyl-GPC (16:0/16:0)	-3.640	0.00027
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	-3.095	0.00068
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	-2.284	0.00542
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	-1.721	0.03622
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	-3.232	0.00096
1-palmitoyl-GPC (16:0)	-1.920	0.03327
1-stearoyl-GPC (18:0)	-3.063	0.00177
1-oleoyl-GPC (18:1)	-2.472	0.03690
1-linoleoyl-GPC (18:2)	-1.539	0.02333
1-stearoyl-GPE (18:0)	-2.492	0.00178

Supplementary Table 13. Continued

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
1-palmitoyl-GPS (16:0)*	-1.995	0.00223
1-stearoyl-GPS (18:0)*	-2.624	0.00224
1-stearoyl-GPG (18:0)	-1.655	0.03021
1-stearoyl-GPI (18:0)	-2.192	0.03939
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*	-2.393	0.00279
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	-1.407	0.04858
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	-1.932	0.02142
1-(1-enyl-oleoyl)-GPE (P-18:1)*	-2.389	0.01811
1-(1-enyl-stearoyl)-GPE (P-18:0)*	-1.664	0.02563
1-dihomo-linoleoylglycerol (20:2)	-1.385	0.04719
palmitoyl-myristoyl-glycerol (16:0/14:0) [2]	-0.924	0.02563
palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]*	-2.743	0.00047
stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]*	-2.583	0.00914
oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	-2.406	0.01467
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	-2.780	0.01038
glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	-1.553	0.03327
glycosyl-N-tetracosadienoyl-sphingosine (d18:1/24:2)*	-1.687	0.03055
lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	-4.159	0.00002
lactosyl-N-behenoyl-sphingosine (d18:1/22:0)*	-3.521	0.00015
lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	-4.576	0.00002
palmitoyl sphingomyelin (d18:1/16:0)	-2.485	0.00252
behenoyl sphingomyelin (d18:1/22:0)*	-2.144	0.00149
tricosanoyl sphingomyelin (d18:1/23:0)*	-2.079	0.01311
lignoceroyl sphingomyelin (d18:1/24:0)	-2.963	0.00162
sphingomyelin (d18:2/16:0, d18:1/16:1)*	-2.696	0.00697
sphingomyelin (d18:1/20:0, d16:1/22:0)*	-2.167	0.02077
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	-2.107	0.01439
sphingomyelin (d18:1/24:1, d18:2/24:0)*	-3.363	0.00133
sphingomyelin (d18:2/24:1, d18:1/24:2)*	-4.014	0.00049
coprostanol	2.398	0.03348
4-cholesten-3-one	1.682	0.03939
androsterone sulfate	-2.137	0.03117
tauro-beta-muricholate	-1.025	0.04858
6-oxolithocholate	1.381	0.02341
ursodeoxycholate sulfate (1)	-3.055	0.04633
xanthosine	-1.258	0.03471
adenine	1.401	0.01343
uridine-2',3'-cyclic monophosphate	-1.915	0.00162

Supplementary Table 13. Continued

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
3-ureidoisobutyrate	-2.958	0.00022
3-ureidopropionate	-2.075	0.00487
beta-alanine	-1.429	0.01106
cytidine 2',3'-cyclic monophosphate	-0.874	0.01311
cytosine	1.359	0.01612
2'-deoxycytidine	1.666	0.02139
3-aminoisobutyrate	-2.148	0.00105
methylphosphate	0.899	0.04643
(3'-5')-adenylylguanosine*	-1.455	0.00646
(3'-5')-adenylyluridine	-1.329	0.04193
(3'-5')-adenylyladenosine*	-1.526	0.01233
(3'-5')-guanylyluridine	-1.957	0.00913
gulonate*	-1.625	0.01696
gamma-tocotrienol	1.135	0.03053
pterin	1.024	0.02309
L-urobilin	2.768	0.03606
beta-cryptoxanthin	1.646	0.00541
pyridoxamine	1.250	0.01343
4-hydroxybenzoate	-2.095	0.01748
p-cresol	1.562	0.03618
paraxanthine	1.654	0.03792
1,3,7-trimethylurate	1.972	0.04487
2-piperidinone	-1.884	0.04279
ciliatine (2-aminoethylphosphonate)	1.180	0.04564
ergothioneine	-1.383	0.01394
sitostanol	1.983	0.00708
glutamyl-meso-diaminopimelate	1.661	0.00134
N-methylpipecolate	1.138	0.04279
N-carbamoylglutamate	1.129	0.02531
sulfate*	-1.281	0.04643
X - 12093	1.043	0.04990
X - 12199	1.993	0.00984
X - 13728	1.372	0.02904
X - 14626	-1.334	0.03568
X - 14838	1.878	0.01478
X - 15136	1.740	0.01957
X - 16060	1.407	0.02584
X - 17299	-3.213	0.02128

Supplementary Table 13. Continued

	Difference in average log2 concentration (Remitters - Non-remitters)	p-value
X - 17653	1.155	0.02892
X - 17654	1.364	0.01333
X - 17877	-1.083	0.03568
X - 18162	-1.429	0.02842
X - 20624	-1.538	0.02346
X - 21353	-1.153	0.02309
X - 21470	-2.407	0.02121
X - 21788	-2.927	0.00723
X - 21796	0.782	0.02563
X - 22030	1.229	0.03716
X - 22520	-1.598	0.04858
X - 23105	3.787	0.01313
X - 23438	1.191	0.03935
X - 23748	1.577	0.02318
X - 23753	1.335	0.02631
X - 23925	0.848	0.01883
X - 24177	-1.323	0.03939
X - 24243	-1.976	0.00351
X - 24246	-2.806	0.00437
X - 24359	-2.127	0.01323
X - 24408	-1.495	0.00906
X - 24413	-2.951	0.00542
X - 24425	1.208	0.04643
X - 24556	1.018	0.03053
X - 24697	3.213	0.02281
X - 24729	-0.941	0.03327
X - 24763	1.354	0.02977
X - 24829	2.099	0.00702
X - 24983	-2.170	0.00703
X - 25009	-1.829	0.01193
X - 25074	-1.783	0.00513
X - 25111	2.121	0.02329
X - 25182	-1.067	0.02142