Multiple Ascending Dose Phase 1 Clinical Study of Safety, Tolerability and Pharmacokinetics of CRS3123, a Narrow Spectrum Agent with Minimal Disruption of Normal Gut Microbiota

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Abstract

CRS3123 is a novel small molecule that potently inhibits methionyl-tRNA synthetase of *Clostridioides difficile*, inhibiting *C. difficile* toxin production and spore formation.

CRS3123 has been evaluated in a multiple ascending dose placebo-controlled Phase 1 trial. Thirty healthy subjects, ages 18 to 45, were randomized into three cohorts of 10 subjects each, receiving either 200, 400 or 600 mg of CRS3123 (eight subjects per cohort) or placebo (two subjects per cohort) by oral administration twice daily for 10 days. CRS3123 was generally safe and well tolerated with no serious adverse events (SAEs), or severe treatment-emergent adverse events (TEAEs) reported. All subjects completed their assigned treatment and follow-up visits, and there were no trends in systemic, vital signs or laboratory TEAEs. There were no QTcF interval changes or any clinically significant changes in other ECG intervals or morphology.

CRS3123 showed limited but detectable systemic uptake; although absorption increased with increasing dose, the increase was less than dose proportional. Importantly, the bulk of the oral dose was not absorbed, and fecal concentrations were substantially above the MIC₉₀ value of 1 µg/ml at all dosages tested. Subjects receiving either of the two lower doses of CRS3123 exhibited minimal disruption of normal gut microbiota after ten days of twice-daily dosing.

CRS3123 was inactive against important commensal anaerobes including Bacteroides, Bifidobacteria and commensal Clostridia. Microbiome data showed favorable differentiation compared to other CDI therapeutics. These results support further development of CRS3123 as an oral agent for the treatment of CDI.
Introduction

*Clostridioides difficile* infection (CDI) is currently classified as an urgent threat by the CDC as the leading cause of antibiotic-associated diarrhea among hospitalized patients (1, 2) and the most common cause of healthcare-associated infections in the U.S. (3, 4). CDI is caused by Gram-positive, spore-forming and toxin-producing strains of *C. difficile* bacteria which are ubiquitous in the environment. The disruption of colonic epithelium is mediated by toxins A and B (TcdA and TcdB), with TcdB being the main virulence factor in CDI (5-7). Predisposition to CDI is caused by disruption of healthy intestinal microbiota, typically following exposure to broad-spectrum antibiotics (8), chemotherapeutic agents and/or radiation treatment (9) leading to decreased colonization resistance. Infection with hypervirulent and drug-resistant strains of *C. difficile*, such as ribotypes 027 and 078, is generally associated with more severe disease (10, 11).

Treatment options for CDI remain limited. Metronidazole and vancomycin, which currently account for the vast majority of prescriptions for CDI, are widely regarded as suboptimal therapies due to their broad spectrum and the associated high (20-40%) recurrence rate (12-15). The incidence of primary CDI has risen substantially over the last two decades, but the increase in recurrence rates has emerged as an even more alarming issue; recurrence necessitates re-treatment which is often associated with increasingly poor prognosis, driven by an increasing probability of recurrence with each episode (16-19).

Until recently, vancomycin was the only FDA-approved antibiotic for the treatment of CDI. Its widespread use has led to growing concerns about selection for vancomycin resistance, especially among Enterococci (VRE) and Staphylococci (VRSE, VISA, VRSA). To protect vancomycin from overuse (20), metronidazole, which has never been approved for the treatment
of CDI, has been used as an off-label alternative to vancomycin for mild-to-moderate CDI (21, 22). Now clearly established as an inferior treatment option, both in terms of primary cure and recurrence rates (1, 23, 24), metronidazole is no longer recommended in the latest IDSA/SHAE guidelines as the initial therapy for non-severe or severe CDI in adults, except in special cases (1). The latest drug to receive approval for CDI, fidaxomicin, has somewhat narrower spectrum and consequently lower overall recurrence rate compared to vancomycin, but only for infections caused by non-epidemic strains (25, 26).

Potential alternatives to antibiotic treatment of CDI have received considerable attention recently, such as the use of fecal microbiota transplant (FMT) and TcdB-binding monoclonal antibody bezlotoxumab (7, 27, 28). Although such treatments have shown efficacy as adjuncts to antibiotic therapy in reducing the incidence of recurrence, antibiotics remain the primary treatment option for the initial episode of CDI, as well as for the first recurrences (1). Therefore, the need for narrow-spectrum antibacterial agents that have the potential to further reduce recurrence remains high.

C. difficile bacteria in stationary phase remain metabolically active and continue to produce toxins. As a class, protein synthesis inhibitors may have an advantage over other antibiotic classes that interfere with metabolic pathways less active in stationary phase bacteria, such as inhibitors of DNA replication, RNA synthesis, or cell wall synthesis.

CRS3123 is a fully synthetic small molecule antibacterial drug candidate with a novel mechanism of action that potently inhibits type 1 methionyl-tRNA synthetase (MetRS), an essential component of protein translation (29, 30). CRS3123 is active only in bacteria that express type 1 MetRS, which include C. difficile, C. perfringens, as well as many aerobic Gram-positive bacteria, such as Staphylococci, Enterococci and Streptococci. Bacterial strains that
express type 2 MetRS virtually no susceptibility to CRS3123 and include most Gram-negative bacteria and many constituents of the normal intestinal microbiota such as Bacteroides, Bifidobacteria, Actinobacteria and Lactobacilli (31). As a protein synthesis inhibitor, CRS3123 inhibits C. difficile toxin production and sporulation, and is superior to vancomycin in protecting hamsters against recurrence in an in vivo model of CDI (29, 30). In addition, CRS3123 has exhibited low systemic absorption following oral dosing in pre-clinical testing, as well as in a first-in-human single ascending dose (SAD) study at doses ranging from 100 mg to 1,200 mg, in which the drug was also safe and well-tolerated (32). Here, we report the results of a randomized, double-blind, placebo-controlled, multiple ascending dose (MAD) phase 1 clinical trial in healthy subjects who received 200 mg, 400 mg or 600 mg twice-daily oral dose of CRS3123 or placebo for ten days, the intended dose frequency and duration of therapy in CDI patients. We show that CRS3123 was generally safe and well tolerated at all doses tested, with limited but detectable systemic uptake and high fecal concentrations. We also show limited impact of CRS3123 on the intestinal microbiota of healthy subjects participating in this study based on the evaluation of fecal microbiome using 16S rDNA sequencing. The studies conducted to date warrant the evaluation of CRS3123 in the patient population as a promising treatment for primary and recurrent CDI.
Materials and Methods

Study drug. Drug CRS3123 was provided by Crestone, Inc in the form of dihydrochloride salt (CRS3123.2HCl) and formulated into 200 mg gelatin capsules by SRI International (Palo Alto, CA). Drug product stability specifications were met beyond the study period (stable for at least 60 months storage at room temperature monitored according to ICH Guidance for Industry Q1A(R2) Stability Testing of New Substances and Products).

Study subjects and design. This study was conducted in full accordance with the Declaration of Helsinki; Good Clinical Practices (GCP) at Quintiles Phase One Services in Overland Park, KS. Subjects were randomized in a 4:1 fashion (8 active: 2 placebo) in each cohort to receive 200 mg, 400 mg, or 600 mg CRS3123 or matching placebo every 12 hours for 10 days (https://clinicaltrials.gov/ct2/show/NCT02106338). Subjects fasted 1 hour before and 2 hours after administration of the study product with a minimum of 8 oz. (240 ml) of water. Water and non-caffeinated drinks (except grapefruit juice) during the study were not restricted. Informed consent was obtained before any study related procedures and clinical evaluations were performed. All 30 randomized subjects completed the study; 24 subjects received CRS3123 and 6 subjects received placebo. There were no premature withdrawals or discontinuations. Each subject in this study provided a written informed consent prior to any study related procedure. After a screening period of up to 35 days, eligible subjects were admitted to the study center for baseline evaluations on Day -1. On the morning of Day 1, eligible subjects were randomized to receive either the study drug or placebo orally every 12 hours through Day 10. Subjects remained inpatient at the study center until discharge on Day 12. During the inpatient period, subjects were monitored for safety and pharmacokinetics (PK). Subjects were followed for 2 additional outpatient visits at the study center, on Days 18±2 and 29±2.
Safety assessment and monitoring. Subjects were monitored closely with serial physical examinations, blood evaluations, and 12-lead ECGs. Subjects underwent safety laboratory testing including measurement of blood cell counts (white blood cell [WBC] with differential, hemoglobin, platelets), serum chemistry (sodium, potassium, chloride, calcium, bicarbonate, glucose, creatinine, blood urea nitrogen [BUN], creatine kinase [CK], aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], total bilirubin, protein, albumin, amylase, and lipase) and urinalysis (protein, blood and glucose). Study halting criteria had been established and were reviewed by an Independent Safety Monitor (ISM) and the SMC prior to proceeding with dosing within a cohort or to the next cohort.

The safety population included all subjects who received at least one dose of the study drug. The safety and tolerability of CRS3123 was evaluated by the sequential review of reported adverse events (AEs), changes from baseline in physical examination findings, vital signs measurements, safety laboratory tests (hematology, clinical chemistry, urinalysis), and key 12-lead electrocardiogram (12-lead ECG) findings. All safety assessments were summarized with descriptive statistics or frequency counts.

The period of observation for AE reporting began on Day 1 at the time of study drug administration and continued through the final visit. The following guidelines were used to quantify intensity: mild (events required minimal or no treatment and did not interfere with the subject’s daily activities), moderate (events resulted in a low level of inconvenience or concerned with the therapeutic measures; moderate events may have caused some interference with functioning), severe (events interrupted a subject’s usual daily activity and required systemic drug therapy or other treatment; severe events are usually classified as incapacitating), and life-
threatening (any adverse drug experience that, in the view of the Investigator, placed the subject at immediate risk of death from the reaction as it occurred).

Relationship to study drug was assessed by the investigators based on evidence to suggest a causal relationship between the study drug and the AE. All AEs were coded to System Organ Class (SOC) and Preferred Term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA), Version 17.0. A treatment-emergent AE (TEAE) was defined as any AE that occurred during the study observation period (i.e., from Day 1 at the time of study drug administration through the final visit). An AE with a partial onset date was classified as a TEAE if the reported partial onset date could not definitely be identified as occurring before the date of study drug administration. Any AE with a missing onset date or missing time on day of study drug administration was classified as a TEAE. To count the overall number of subjects who experienced each TEAE and calculated an occurrence rate, a subject experiencing multiple occurrences of an AE was only counted once for that PT. Similarly, if a subject experienced multiple TEAEs within the same SOC, that subject was counted once to calculate an occurrence rate for the SOC. To count the number of subjects who experienced each TEAE by severity classification (mild, moderate, severe, life-threatening) and calculate an occurrence rate, a subject experiencing multiple occurrences of an AE of the same severity was counted once in that particular severity classification for that PT. If a subject experienced multiple occurrences of an AE of differing severity, only the most severe occurrence within each PT was counted.

If any significant safety signals were encountered, the Investigator was to notify the Division of Microbiology and Infectious Diseases (DMID) who would then determine the need for an ad hoc Safety Monitoring Committee (SMC) review. For the purposes of this study, “ad hoc” simply
means an unscheduled SMC review which could have been held at any time throughout the study per DMID’s discretion. If deemed appropriate by halting rules and/or DMID’s discretion, enrollment was to be halted for an ad hoc SMC meeting. Decision to resume the study after halting was to be determined by DMID in consideration of the SMC recommendations. The dose for Cohort B and study progression to Cohorts B and C required a full (planned) SMC review of all safety data obtained through Day 18. If the safety data review of the 200 mg dose had resulted in an unfavorable safety profile, the study design allowed for the reduction of the dose to 100 mg for Cohort B and Cohort C would not be dosed. However, no unfavorable safety profile was observed and the dosing of Cohort B at 400 mg and the Cohort C at 600 mg was performed as planned.

The sample size for this study was not based on statistical considerations. Since safety testing of study drug was the primary objective of this study, consideration was given to the probability of detecting AE rates in 3 dosing groups of 10 subjects; each were logistically practical to provide sufficient information while minimizing the total number of subjects exposed to study drug.

There were no formal statistical hypotheses being tested in this study. With 8 active subjects in a cohort, an event rate of 24.5% or higher could be detected with 80% power.

**Study Enrollment.** Study enrolled men and non-pregnant women 18 to 45 years of age, between June – December 2014. The subjects had to meet the following criteria to be included in the study: general good health, negative serum pregnancy test, negative alcohol and drug use screening, and body mass index (BMI) of <35 kg/m², agreement by subjects with reproductive potential to use an adequate method of contraception during the study and for 4 weeks after the initiation of study drug administration, and agreement to avoid strenuous exercise for at least
72 hours prior to initial study drug administration and the scheduled follow-up visits on Days 18 and 29.

Subjects with the following conditions were excluded from the study: hypertension, pulmonary disease; asthma, diabetes mellitus, autoimmune disorder, history of malignancy (except low-grade skin cancer, i.e., basal cell carcinoma, which was surgically cured), chronic renal, hepatic, or pulmonary disease or gastrointestinal tract condition that could interfere with the absorption of the study drug, history of known CDI, cardiac rhythm abnormalities, prolonged QT interval, or ovarian cysts. Also excluded were subjects with laboratory values outside the expanded ranges for the following tests: blood cell counts, serum chemistry, and urinalysis. In addition to these exclusion criteria, vulnerable populations were excluded from the study (https://clinicaltrials.gov/ct2/show/NCT02106338).

**Blinding.** The investigator and subjects remained blinded to individual subjects’ treatment assignments during the entire duration of the study. Blinding was not broken until all subjects had completed the final study visit, all PK analyses were completed, the database had been monitored and locked and all results transferred for analysis.

**Analytical methodology.** PK method development, validation and sample testing for plasma, feces and urine was performed at KCAS, LLC (Shawnee, KS). Plasma, urine and fecal concentrations of CRS3123 were determined by means of validated, sensitive, and specific high-performance liquid chromatography/tandem mass spectrometric (HPLC MS/MS) assays. The lower limit of quantification of CRS3123 in plasma, feces, and urine was 10.0 ng/ml, 60.0 ng/g, and 100.0 ng/ml, respectively. The validated urine PK assay measured total free CSR3123. Since the majority of urinary CRS3123 was excreted as glucuronide conjugates (32), an
enzymatic cleavage step using glucuronidase was added to the process to convert the glucuronide conjugates to free CRS3123 for the urine analysis.

A selective, accurate, and reproducible analytical method using LC-MS/MS for the quantitation of CRS3123 in human fecal homogenate was linear over a range of 10.0 to 2000 ng/ml (fecal homogenate) or 60.0 to 12,000 ng/g (fecal material). Since 11 fecal samples collected on day 10 exceeded the upper limit, this method was amended by validating it for an extended range of 50.0 to 3000 µg/ml (fecal homogenate) or 300 to 18,000 µg/g (fecal material).

**Pharmacokinetics.** The PK population consisted of all subjects who received active study drug (CRS3123) and had at least one measured CRS3123 concentration at a scheduled PK time point after start of study drug administration.

Pharmacokinetic parameters for plasma were derived using noncompartmental methods with Phoenix® WinNonlin® Version 6.4, (Pharsight Corporation, Certara L.P., Princeton, New Jersey, US). PK parameters for urine and feces were derived using SAS® Version 9.4, (SAS Institute, Inc., Cary, North Carolina). All statistical calculations and reporting were performed using SAS® Version 9.4. Graphics were prepared with SAS® Version 9.4, or SigmaPlot® 12.5 (Systat Software, Inc., San Jose, California, US).

**Plasma PK.** Plasma concentrations of CRS3123 throughout the 12-hour dosing interval on PK days (Day 1 and Day 10) were summarized using descriptive statistics for each treatment. One-way analysis of variance models (ANOVA) were used to assess any differences in appropriate PK parameters between adjacent dose-levels following single dose (Day 1) and multiple doses (Day 10). An ANOVA model on the log-transformed PK parameters [AUC(0-12) and C_max on Day 1; AUC(0-tau) and C_max on Day 10] with fixed effect for treatment were performed separately for the single dose analysis and multiple dose analysis. From these models,
the least-squares (LS) means together with 95% CIs for each dose and the LS means with corresponding 90% CIs for all adjacent dose pairs were calculated. Transformed back from the logarithmic scale, geometric LS means with corresponding 90% CIs were provided for each dose and adjacent dose ratios following both single and multiple dosing. PK accumulation was presented using descriptive statistics and achievement of steady state was assessed graphically.

Dose proportionality of CRS3123 PK parameters following single dose \([\text{AUC}_{(0-12)}\text{ and } \text{C}_{\text{max}}\text{ on Day 1}]\) and multiple dose \([\text{AUC}_{(0-\tau)}\text{ and } \text{C}_{\text{max}}\text{ on Day 10}]\) over the administered dose range were quantified statistically using a power model \([\text{AUC}_{(0-12)}\text{ and } \text{C}_{\text{max}}\text{ on Day 1}; \text{AUC}_{(0-\tau)}\text{ and } \text{C}_{\text{max}}\text{ on Day 10}]=\alpha \cdot \text{dose}^\beta\) for each PK parameter on log scale as the dependent variable and the logarithm of the dose as the independent variable. The model parameters (slope and intercept) were estimated using least-squares regression. Point estimates and corresponding 2-sided 90% CIs for the slope parameter (\(\beta\)) and the intercept parameter (\(\alpha\)) were provided. A minimum of three values per dose level were required for a given parameter to estimate dose proportionality with the power model. Dose proportionality was concluded if the 90% CI for \(\beta\) fell within \([\ln(0.8)/\ln(R), \ln(1.25)/\ln(R)]\) where \(R\) was the observed dose range for the study (600 mg/200 mg).

**Urine PK.** A listing of individual PK urine sample collection start and stop dates/times, volume of urine excreted over each planned collection interval, CRS3123 concentration in each urine collection, amount of CRS3123 excreted in each urine collection, and the fraction of dose excreted unchanged in urine was listed for each urine collection by treatment. Urine samples for all 3 cohorts were stabilized by the bioanalytical laboratory upon receipt, for those samples which were not stabilized at the study center.
Fecal PK. Twenty-four-hour fecal collection was initiated for all subjects following Dose 1 and Dose 19 of study drug for measurement of CRS3123 concentrations in feces. A listing of individual PK fecal sample collection start and stop dates/times, fecal weight excreted for each planned collection, CRS3123 concentration in each fecal collection, and amount of CRS3123 excreted in each fecal collection was provided for each fecal collection for each treatment. The bioanalytical method was amended to allow for re-analysis of samples originally outside the linear range.

The following parameters were calculated from the fecal data: amount of unchanged drug excreted in feces (ng) following Doses 1 and 2 \( (A_{e,feces}) \) or Doses 19 and 20 \( (A_{e,feces,ss}) \) calculated as \([\text{fecal concentration} \times \text{fecal weight}]\) (in the case of multiple fecal samples, \(A_{e,feces}\) and \(A_{e,feces,ss}\) was determined as the sum of the amount calculated in each fecal sample collected during the 24-hour collection period), and fraction of dose excreted unchanged in feces \( (f_{e,feces,ss}) \) over a 24-hour interval after multiple dosing (%), calculated as \(A_{e,feces,ss}\) divided by Dose 19 + Dose 20. Due to the 12-hour dosing interval utilized in this study, \(f_{e,feces,ss}\) was only calculated after multiple dosing on Day 10 when CRS3123 would be expected to be at or near steady state. These data were summarized using descriptive statistics. A subject listing of individual fecal PK parameters for each treatment was provided for Day 1 and for Day 10.

Microbiome analysis. Fecal microbiota profiles were measured for each sample as each operational taxonomic unit species present and the relative abundance of that species, based on a region of 469 bp encompassing the V3 and V4 hypervariable regions of the 16S rRNA gene, following protocols developed at the Department of Microbiology and Immunology, University of Maryland School of Medicine Institute for Genome Sciences, Baltimore, MD (33-35).

Sample collection, storage, and DNA extraction. Fecal samples were collected at nine time
points during the study for fecal microbiome analysis: twice before first dose and on Days 1, 4, 7, 9, 12, and approximately 24 hours prior to follow-up visits on Day 18 and Day 29. The subject was allowed to collect and bring the first fecal sample when admitted (collected within 24 hours of admission and kept refrigerated until collected by staff). DNA was extracted from 150 mg of archived stool samples stored at -80°C for 16S rRNA gene amplification and sequencing. Cell lysis was initiated by adding 50 μl lyzosyme (10 mg/ml), 6 μl of mutanolysin (25,000 U/ml; Sigma-Aldrich) and 3 μl of lysostaphin (4,000 U/ml in sodium acetate; Sigma-Aldrich) and 41 μl of TE50 buffer (10 mM Tris-HCl, 50 mM EDTA, pH 8.0). Following a 1-hour incubation at 37°C, 10 μl proteinase K (20 mg/ml), 100 μl 10% SDS, and 20 μl RNase A (20 mg/ml) were added to the mixture and incubated for 1 hour at 55°C. Microbial cells were lysed by mechanical disruption using a bead beater (FastPrep instrument, Qbiogene) set at 6.0 m/s for 30 seconds. DNA was purified from the lysate using the QIAsymphony robotics platform, consistently providing between 5 and 20 μg of high-quality and amplifiable whole genomic DNA. Following DNA quantitation via the Quant-iTTM PicoGreen® dsDNA assay kit (Life Technologies), samples were diluted to a 5 ng/μl concentration.

Amplification and sequencing of the 16S rRNA gene. PCR reactions with dual barcoded 16S rRNA universal primers (319F/806R) were set up using a semi-automated platform with a ViaFlo 96 high-performance 96-channel pipettor and performed on BioRad Tetrad 2 instruments with up to 576 dual index barcoding (33). Amplification was achieved with Phusion High-Fidelity polymerase (New England BioLab) and 50 ng of template DNA in a total reaction volume of 25 μl. The cycling parameters were 30 sec of denaturation at 98°C, followed by 30 cycles of 15 sec at 98°C (denaturing), 15 sec at 58°C (annealing) and 15 sec at 72°C (elongation), with a final extension at 72°C for 60 sec. Controls included a no-template control.
for each barcode combination, extraction controls, and a DNA sample of known composition (positive control). The presence of amplicons was confirmed by gel electrophoresis on a 2% E-gel 96. PCR products were normalized using the SequaPrep Normalization Kit (Life Technologies, Carlsbad, CA) and pooled in equimolar amounts (25 ng) in a single tube.

Amplification primers and reaction buffer were removed from the pooled amplicon mixtures using the AMPure Kit (Agencourt). The purified amplicon mixtures (up to 576 per pool) were sequenced on an Illumina MiSeq instrument using the 300 bp paired-end protocol (MiSeq 300PE) at the Genomics Resource Center (GRC) at the Institute for Genome Sciences, University of Maryland School of Medicine.

Bioinformatic analysis. For sequence quality control, assembly and taxonomic assignments, a custom bioinformatics pipeline (33) was applied that relies on QIIME software (36), UCHIME (37) and the RDP Naive Bayesian classifier (38). Baseline for fecal microbiome assessments was defined as the mean of the pre-dose assessments on Day 1. Presence was defined as positive abundance. Presence of each operational taxonomic unit (OTU) species and the shift in presence/absence occurrence from baseline were tabulated by treatment. Shifts were categorized as no change, appearance, and disappearance.

Additional 16S rRNA data analysis was performed at the Division of Biomedical Informatics and Personalized Medicine, University of Colorado. Joined and quality filtered sequences sharing >97% sequence similarity were binned at operational taxonomic units (OTUs). The OTUs were assigned taxonomical classification by SortMeRna (39) and sumaclust (40) to the Greengenes 13.8 database within QIIME 1.9 (36). Differences in community composition between placebo and treatment groups at each day was performed by weighted UniFrac (41) and PERMANOVA.
Results

Here, we report the results of the multiple ascending dose, single center, randomized, placebo-controlled, double-blind study Phase 1 study of CRS3123.

Demographics

Thirty healthy male and female subjects, ages 18 to 45, were randomized into three ascending dose cohorts; 200, 400 and 600 mg twice-daily for ten days (Cohorts A, B and C) (Figure 1). No subjects reported clinically significant medical history findings. Overall, more than 60% of the enrolled subjects were males (66.7%), and the overall mean age of the subjects was 30 years (Figure 1, Table S1).

Pharmacokinetics

Plasma. After first dose of CRS3123 on Day 1, median \( t_{\text{max}} \) ranged from 2 to 3 hours and geometric mean \( t_{1/2} \) ranged from 3 to 4 hours across the 3 dose groups (Figure 2A). Geometric mean CRS3123 AUC\( (0-12) \) and \( C_{\text{max}} \) following first dose administration increased approximately 2.3-fold and 1.9-fold, respectively, with a 3-fold increase in dose from 200 mg to 600 mg (Figure 3, A and B). Plasma CRS3123 PK parameters on Day 1 following a single dose are summarized by treatment in Table 1 and Table S2.

Following 10 days of twice-daily administration, median \( t_{\text{max,ss}} \) ranged from 1 to 2 hours and geometric mean \( t_{1/2,ss} \) ranged from 5 to 6 hours across the three CRS3123 dose groups (Figure 2B). Across the dose range studied, geometric mean RAUC ranged from 1.1 to 1.6 and geometric mean RC\( _{\text{max}} \) ranged from 1.1 to 1.3 (Table S3). Based on the mean plasma trough concentration-time profiles (Figure 2C), there was minimal accumulation of CRS3123 following multiple twice-daily dosing for 10 days. This finding is consistent with the relatively short \( t_{1/2,ss} \).
in relationship to the 12 hour dosing interval. Plasma CRS3123 PK parameters on Day 10 following multiple dosing are summarized in Table 1 and Table S3. The increase in plasma exposure was less than proportional to the increase in dose across the dose range studied, at both Day 1 and Day 10. Dose proportionality of CRS3123 PK parameters following single dose (AUC\(_{(0-12)}\) and C\(_{\text{max}}\) on Day 1) and multiple dose (AUC\(_{(0-tau)}\) and C\(_{\text{max,ss}}\) on Day 10) over the administered dose range were quantified statistically using a power model

\[
([\text{AUC}_{(0-12)} \text{ and } C_{\text{max}} \text{ on Day 1}; \text{AUC}_{(0-tau)} \text{ and } C_{\text{max,ss}} \text{ on Day 10}] = \alpha \cdot \text{dose}^\beta)
\]

for each PK parameter on log scale as the dependent variable and the logarithm of the dose as the independent variable. The slope (\(\beta\)) estimates were well below one, indicating a less than proportional increase in exposure.

**Fecal PK.** Following single dose and multiple dose administration of CRS3123, a substantial fraction of administered CRS3123 was retained in the GI tract and excreted in feces (Table 1, Table S4). Based on the fecal concentrations observed on Day 10, mean \(f_{\text{e,feces,ss}}\) (\%) values under steady-state conditions was at least one-third, one-half, and two-thirds of the twice-daily doses of 200 mg, 400 mg and 600 mg, respectively (Table 1, Table S4). Since this was not a mass balance study and the fecal sample size varied by over two orders of magnitude between subjects (ranging from 1.74 to 194.7g during the 24 hour collection period), these \(f_{\text{e,feces,ss}}\) values are approximate, yet consistent with fecal elimination being the major route of elimination for CRS3123. High levels in feces were observed at all three dose groups, with median values of 2,115 µg/g (range 966 – 4,430), 5,390 µg/g (range 2,920 – 8,700) and 8,280 µg/g (range 2,200 – 14,000) following 200, 400 and 600 mg twice-daily doses (Figure 4).

**Urine PK.** We have demonstrated previously that a small fraction of the orally administered dose of CRS3123 is metabolized into glucuronide conjugates detectable in urine (32). Therefore,
we have converted the glucuronide conjugates into parent CRS3123 prior to urine analysis, so that the reported values reflect the sum of the free (parent) drug and its glucuronide metabolites. Renal elimination of CRS3123 as free drug and glucuronide conjugates was minimal and accounted for less than 2% of the administered dose. Following a single dose on Day 1, approximately 1% of the dose was excreted in urine across the 3 dose groups as free drug and glucuronide conjugates. Following multiple dosing for 10 days, between 1% and 2% of the dose was excreted in urine across the 3 dose groups as free drug and glucuronide conjugates (Table 1, Table S5).

Safety Assessment
At all three doses tested, CRS3123 administered orally twice-daily for 10 days was generally safe and well tolerated with no deaths, other SAEs, or severe TEAEs reported. No subjects were withdrawn from the study due to TEAEs. There were no trends in systemic, vital signs or laboratory TEAEs. The majority of TEAEs reported in the study were of mild severity. There was no prolongation of the QTcF interval or any clinically significant changes in other ECG intervals or morphology. Treatment-related AEs were defined as TEAEs for which there was reasonable evidence to suggest a causal relationship between the study product and the AE. There were no treatment-related trends evident in the observed or change from baseline mean laboratory values and no clinically-significant physical examination findings in this study. Among the treatment-related AEs, the proportion of subjects in placebo (33.3% subjects) reporting at least one treatment-related AE was higher compared to the active groups (12.5% subjects). No subject in Cohort A (200 mg CRS3123) reported any treatment-related AEs. Two subjects in Cohort B (400 mg CRS3123) reported at least one treatment-related AE: subject 2003 reported a mild treatment-related AE of diarrhea on Day 2 which resolved the same day, and...
subject 2004 reported a mild treatment-related AE of dysgeusia on Day 4 which resolved the following day. One subject (3004) in Cohort C had a mild treatment-related AE of increased ALT on Day 6 which resolved 12 days later. Subject 1010 (Cohort A, placebo) had a mild treatment-related AE of increased lipase on Day 10, and subject 2006 (Cohort B, placebo) reported a mild treatment-related AE of diarrhea on Day 5; both these treatment-related AEs resolved the following day. Summary of all TEAEs and treatment-related AEs by system organ class and preferred term for each treatment (safety population) is presented in Tables 2 and 3, respectively.

**Microbiome analysis**

Of 266 stool samples processed for 16S rRNA gene sequencing, 248 samples resulted adequate sequence data, yielding 2.1 million high-quality 16S rRNA gene V3-V4 regions sequence reads that were used for taxonomic assignment.

Analysis of community composition showed a significant difference between all treatment groups and the placebo arm at day 9 (point of highest difference) in a dose dependent manner (median UniFrac distance 0.361, p=0.006; 0.440, p=0.027; 0.663, p=0.013; for 200 mg, 400 mg, 600 mg respectively; Figure 5). However, all differences between the groups decreased by day 29 of the trial (median UniFrac distance 0.404, p=0.006; 0.4480, p=0.177; 0.470, p=0.077; for 200 mg, 400 mg, 600 mg respectively 2½ weeks after cessation of antibiotics). Increases in proteobacteria were minimal at 200 mg treatment (maximum of 1.6% compared to pretreatment value of 0.8%) and limited to <10% relative abundance at the 600 mg treatment.

The proportion of the major phyla in stool showed minimal changes over time in the 200 mg dosing group and was not different compared to the placebo group. In the 400 mg and 600 mg BID dosing groups, the relative abundance of Firmicutes decreased, while other phyla such as...
Bacteriodetes and Actinobacteria increased. Importantly, no phyla were lost during treatment with CRS3123 (Figure 5) at any dose and the composition was back to baseline levels within 7 days of stopping the treatment. At the genus level, no significant changes were observed after nine days of twice daily dosing of CRS3123 in any of the highly abundant intestinal genera with the exception of the target genus *Clostridium* (Figure 6A). CRS3123 did not impact commensal anaerobes including *Bacteroides* and members of *Clostridium* cluster XIVa and IV (*e.g.*, *Coprococcus*, *Dorea*, *Roseburia*, *Ruminococcus*). The data obtained in microbiome analysis of healthy subjects during CRS3123 treatment are in good agreement with the expected spectrum of activity based on the MetRS phylogeny shown Figure 6B.

**Discussion**

The study described in this report was preceded by a single ascending dose study of CRS3123, the first study in which a MetRS inhibitor was administered orally to humans, in single oral doses of 100 mg, 200 mg, 400 mg, 800 mg, and 1200 mg (32). In this first-in-human study, CRS3123 was found to be safe and well tolerated with no dose-limiting toxicities and no serious adverse events (SAEs) reported. The primary objective of the current study was to determine the safety and tolerability of escalating doses of CRS3123 following twice-daily oral administration to healthy adults over a period of ten days. The secondary objectives were to determine the plasma, urine and fecal concentrations and systemic exposure of CRS3123 after multiple oral doses and the exploratory objective was to survey the effect of CRS3123 on fecal microbiome. CRS3123 administered as oral multiple ascending dose was generally safe and well-tolerated with no SAEs, or severe TEAEs reported. No subjects were withdrawn from the study due to TEAEs. There were no trends in systemic, vital signs or laboratory TEAEs. The majority of
Absorption of CRS3123 was rapid with median $t_{\text{max}}$ occurring 2 to 3 hours following a single dose and 1 to 2 hours following multiple twice-daily dosing. The geometric mean $t_{1/2}$ ranged from approximately 3 to 4 hours following a single dose and from 5 to 6 hours following multiple twice daily dosing. There was essentially no accumulation with multiple twice daily dosing which is consistent with this relatively short $t_{1/2}$ of CRS3123. In addition, the increase in CRS3123 exposure was less than proportional to the increase in dose across the dose range studied.

Following single and multiple dose administration of CRS3123, the majority of unchanged CRS3123 was retained in the GI tract and excreted in feces, while renal elimination of CRS3123 as free drug and glucuronide conjugates was minimal and accounted for less than 2% of the administered dose. The observation that high concentrations of CRS3123 are found in feces (well above 1,000 µg/g for all three doses tested) is important for the development of new agents for the treatment of CDI, since the lower GI tract is the site of the infection. Aside from efficacy potential, which is determined by the MIC$_{90}$ value ($=1$ µg/ml for CRS3123), equally important are the levels of the drug in relation to the MPC value, which ranges from 16 - 128 µg/ml, depending on the strain of *C. difficile* (29). As a class, tRNA synthetase inhibitors have been associated with either pre-existing high-level resistance (as is the case of isoleucyl-tRNA synthetase inhibitor mupirocin resistance driven by a phylogenetically distinct enzyme encoded by the *mupA* gene), or rapid development of resistance during clinical development of epetraborole, a leucyl-tRNA synthetase inhibitor previously in development as a systemic agent for the treatment of urinary tract infections (42, 43). Fortunately, high-level resistance imparted

TEAEs reported in the study were of mild severity. There was no prolongation of the QTcF interval or any clinically significant changes in other ECG intervals and ECG morphology.
by an entirely different MetRS isoform does not appear to exist in *C. difficile*, based on testing of more than 108 clinical isolates. This is worth noting, since some Gram-positive bacteria, notably *S. pneumoniae* and *B. anthracis*, are known to express both type 1 and type 2 MetRS enzymes, leading to lack of susceptibility to all compounds in the same chemical series as CRS3123 (29).

For suppression of emergence of strains with reduced susceptibility resulting from selection of spontaneous mutants, maintaining intra-intestinal levels of drug well above the MPC throughout the dosing regimen is a key consideration, which based on the measured CRS3123 concentrations in the feces reported this study, is readily achievable.

Overall, the PK characteristics observed in human subjects suggest a promising profile for a CDI therapeutic; these properties include 1) limited systemic exposure and efficient clearance, both of which mitigate potential systemic safety risks, 2) high concentrations achieved in the gut, well above the target MIC required for pharmacodynamic success, and 3) high potential to suppress emergence of resistance due to the high ratio of gut concentration *versus* MPC.

Narrow antibacterial spectrum is of paramount importance for allowing rapid regeneration of healthy intestinal microbiota following an episode of CDI. CRS3123 has a low propensity for disruption of normal intestinal microbiota based on phylogenetic analyses of the MetRS gene as well as on *in vitro* studies (31). However, given that levels of CRS3123 present in the gut are much higher than concentrations tested in bacterial cultures (up to 32 µg/ml (31)), the impact of CRS3123 on the levels of the microbiome in healthy subjects was uncertain. We have shown here that CRS3123 indeed has minimal impact on the gut microbiome of healthy subjects.

The percentage of subjects with *Clostridium* species isolated at baseline but not isolated at subsequent visits was greater in the CRS3123 treatment groups than the placebo group during the treatment phase of the study (Day 1 through Day 10). This provides preliminary evidence that...
CRS3123 has antimicrobial activity toward *Clostridium* species in healthy subjects. Individuals with chronic *C. difficile* often have very low diversity microbiomes that have increased abundances of facultative anaerobes such as Proteobacteria (44); we did not see an increase in Proteobacteria with the 200 mg and 400 mg dose of CRS3123 (Figure 5A).

A narrow spectrum of activity is a prerequisite for optimal CDI therapy, allowing the compromised gut microbiota to recover and reestablish a healthy microbiome that provides protection from recurrence in the weeks following treatment. This is clearly a shortcoming of vancomycin, a broad-spectrum agent which is often used to treat CDI but results in a recurrence in 25-30% of patients (25, 26). In fact, several recent drug candidates with broad spectrum of activity, such as surotomycin, LFF-571 and cadazolid, failed to show reduced recurrence rates compared to vancomycin during clinical trials, halting their further development. In contrast, fidaxomicin and one agent in clinical development (ridinilazole) have a narrower spectrum and have demonstrated benefits with regard to reducing recurrences after treatment of CDI (45, 46).

CRS3123 holds promise as the most narrow-spectrum agent against *C. difficile* yet, which is largely due to the phylogeny of the MetRS target. Gram-negative anaerobes in the gut microbiome, including *Bacteroides, Prevotella, Bifidobacterium, Porphyromonas* and *Actinomyces* spp harbor a MetRS type 2 enzyme resistant to CRS3123, which is consistent with the observed lack of *in vitro* or *in vivo* effect on these organisms. Among the Gram-positive anaerobes harboring the susceptible MetRS type 1 enzyme, several encode an additional MetRS type 2 (e.g., *Faecalibacterium spp.*) or yet another, phylogenetically distinct MetRS type 3 (e.g., *Eggerthella, Collinsella, Ruminococcus*) which protects these beneficial species from CRS3123.

Despite having only a type 1 MetRS, many other organisms of the normal gut microbiota were also non-susceptible, including representatives of *Clostridium* cluster XIVa and IV (*Dorea,*...
Lachnospira, Roseburia) which are important indicators of a healthy gut microbiome (47, 48), some Lactobacillus spp. and several other Gram-positives shown in Figure 6. These data are based on in vitro susceptibility results for Clostridium ramosum, bifidobacteria, Lactobacillus casei–rhamnosus–plantarum group and Gram-negative anaerobes (31) and on microbiota analysis of stool samples collected during this Phase 1 clinical trial. It is unknown whether resistance in these organisms is due to any of the subtle amino acid residue differences within their MetRS enzyme compared to the C. difficile MetRS and/or due to impaired uptake and/or efflux of the drug. It appears that the presence of MetRS type 1 is essential but not sufficient to confer susceptibility to CRS3123. In conclusion, CRS3123 demonstrated a substantially narrower spectrum compared to vancomycin and also to fidaxomicin, which are currently the only two FDA-approved drugs to treat CDI.

The results of this multiple ascending dose phase 1 study, together with an earlier single ascending dose phase 1 study, warrant the progression of testing of CRS3123 in patients with CDI.

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Figure 6. Effect of CRS3123 on prevalent genera (A) and phylogeny of MetRS in bacteria (B). These data are based on \textit{in vitro} susceptibility results (31) and on microbiota analysis of stool samples collected during this Phase 1 clinical trial.
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Figure Legends

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Table 1. CRS3123 PK Parameters Summary in Plasma, Stool and Urine.

| Pharmacokinetics parameters | 200 mg | 400 mg | 600 mg |
|-----------------------------|--------|--------|--------|
|                             | Day 1  | Day 10 | Day 1  | Day 10 | Day 1  | Day 10 |
| **Plasma**                  |        |        |        |        |        |        |
| AUC (ng·h/ml)\(^{ab}\)      | 1550 (64.1) | 2500 (41.3) | 2340 (57.6) | 3200 (29.4) | 3560 (64.1) | 4030 (55.6) |
| C\(_{max}\) (ng/ml)\(^{bc}\) | 352 (59.4) | 470 (32.3) | 507 (42.5) | 615 (32.4) | 654 (57.9) | 731 (47.9) |
| t\(_{max}\) (h)\(^{cd}\)    | 2.0 (0.5, 4.0) | 1.5 (1.0, 2.0) | 3.0 (2.0, 4.0) | 2.0 (1.0, 2.0) | 2.0 (1.0, 4.0) | 1.0 (1.0, 2.0) |
| t\(_{1/2}\) (h)\(^{cd}\)    | 3.0 (2.7, 3.7) | 4.6 (4.1, 7.0) | 3.6 (2.9, 4.3) | 5.0 (3.4, 7.7) | 2.9 (2.6, 4.0) | 6.1 (5.4, 7.3) |
| **Stool**                   |        |        |        |        |        |        |
| C\(_{stool}\) (µg/g)\(^{df}\) | NC | 2115 (966, 4430) | NC | 5390 (2920, 8700) | NC | 8280 (2200, 14000) |
| t\(_{stool}\) (h)\(^{dh}\)  | NC | 24.9 (1.9, 68.8) | NC | 52.0 (3.6, 87.6) | NC | 43.1 (11.0, 125.8) |
| **Urine**                   |        |        |        |        |        |        |
| A\(_e, urine\) (µg)\(^{fg}\) | 2300 (1550, 3060) | 3380 (2840, 4920) | 4170 (2380, 8220) | 5260 (4110, 7260) | 4250 (2860, 8900) | 6630 (3320, 9880) |
| t\(_{e, urine}\) (h)\(^{fh}\) | 1.2 (0.78, 1.5) | 1.7 (1.3, 2.5) | 1.1 (0.60, 2.1) | 1.3 (1.0, 1.8) | 0.71 (0.48, 1.5) | 1.1 (0.55, 1.7) |

\(^a\) Geometric mean (%CV)  
\(^b\) AUC\((0-12)\) for Day 1, AUC\((0-tau)\) for Day 10  
\(^c\) C\(_{max}\) for Day 1, C\(_{max,ss}\) for Day 10  
\(^d\) Median (min, max)  
\(^e\) t\(_{max}\) for Day 1, t\(_{max,ss}\) for Day 10  
\(^f\) C\(_{stool}\) for Day 1, C\(_{stool,ss}\) for Day 10  
\(^g\) Amount excreted (A\(_e\)): A\(_e,(0-12)\) for Day 1, A\(_e,(0-12),ss\) for Day 10  
\(^h\) Fraction excreted (f\(_e\)): A\(_e,(0-12)\) for Day 1, A\(_e,(0-12),ss\) for Day 10  
NC, not calculated
Table 2. Summary of All Treatment-emergent Adverse Events by System Organ Class and Preferred Term for Each Treatment (Safety Population)

| System Organ Class/Preferred Term                                      | Placebo (N = 6) | Cohort A (200 mg Q12h) (N = 8) | Cohort B (400 mg Q12h) (N = 8) | Cohort C (600 mg Q12h) (N = 8) | All Active (N = 24) |
|----------------------------------------------------------------------|-----------------|---------------------------------|--------------------------------|--------------------------------|-------------------|
| Subjects with TEAEs                                                  | 6 (100.0%)      | 8 (100.0%)                      | 6 (75.0%)                      | 6 (75.0%)                      | 20 (83.3%)        |
| Ear and labyrinth disorders                                         | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Ear discomfort                                                      | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Gastrointestinal disorders                                          | 1 (16.7%)       | 0 (0.0%)                        | 2 (25.0%)                      | 0 (0.0%)                       | 2 (8.3%)          |
| Diarrhea                                                            | 1 (16.7%)       | 0 (0.0%)                        | 2 (25.0%)                      | 0 (0.0%)                       | 2 (8.3%)          |
| General disorders and administration site conditions                 | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Infusion site thrombosis                                            | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Infections and infestations                                         | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Upper respiratory tract infection                                    | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Injury, poisoning and procedural complications                       | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Arthropod bite                                                      | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 0 (0.0%)                       | 1 (4.2%)          |
| Investigations                                                      | 4 (66.7%)       | 5 (62.5%)                       | 4 (50.0%)                      | 6 (75.0%)                      | 15 (62.5%)        |
| Alanine aminotransferase increased                                   | 0 (0.0%)        | 1 (12.5%)                       | 0 (0.0%)                       | 2 (25.0%)                      | 3 (12.5%)         |
| Amylase increased                                                   | 0 (0.0%)        | 1 (12.5%)                       | 1 (12.5%)                      | 1 (12.5%)                      | 3 (12.5%)         |
| Aspartate aminotransferase increased                                 | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 2 (25.0%)                      | 2 (8.3%)          |
| Blood bilirubin increased                                           | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Blood calcium increased                                             | 0 (0.0%)        | 0 (0.0%)                        | 1 (12.5%)                      | 0 (0.0%)                       | 1 (4.2%)          |
| Blood potassium increased                                           | 0 (0.0%)        | 0 (0.0%)                        | 1 (12.5%)                      | 0 (0.0%)                       | 1 (4.2%)          |
| Blood pressure diastolic decreased                                   | 1 (16.7%)       | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Blood pressure diastolic increased                                   | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Blood pressure systolic decreased                                    | 1 (16.7%)       | 0 (0.0%)                        | 0 (0.0%)                       | 0 (0.0%)                       | 0 (0.0%)          |
| Blood pressure systolic increased                                    | 0 (0.0%)        | 0 (0.0%)                        | 0 (0.0%)                       | 1 (12.5%)                      | 1 (4.2%)          |
| Hemoglobin decreased                                                | 1 (16.7%)       | 2 (25.0%)                       | 1 (12.5%)                      | 1 (12.5%)                      | 4 (16.7%)         |
| Lipase increased                                                    | 2 (33.3%)       | 1 (12.5%)                       | 2 (25.0%)                      | 2 (25.0%)                      | 5 (20.8%)         |
| System Organ Class/Preferred Term                              | Placebo (N = 6) | Cohort A (200 mg Q12h) (N = 8) | Cohort B (400 mg Q12h) (N = 8) | Cohort C (600 mg Q12h) (N = 8) | All Active (N = 24) |
|---------------------------------------------------------------|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------|
| White blood cell count increased                             | 1 (16.7%)      | 0 (0.0%)                      | 0 (0.0%)                      | 0 (0.0%)                      | 0 (0.0%)          |
| Musculoskeletal and connective tissue disorders               | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |
| Myalgia                                                       | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |
| Nervous system disorders                                      | 2 (33.3%)      | 1 (12.5%)                     | 2 (25.0%)                     | 0 (0.0%)                      | 3 (12.5%)         |
| Dysgeusia                                                     | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |
| Headache                                                      | 2 (33.3%)      | 1 (12.5%)                     | 1 (12.5%)                     | 0 (0.0%)                      | 2 (8.3%)          |
| Psychiatric disorders                                         | 0 (0.0%)       | 1 (12.5%)                     | 0 (0.0%)                      | 0 (0.0%)                      | 1 (4.2%)          |
| Anxiety                                                       | 0 (0.0%)       | 1 (12.5%)                     | 0 (0.0%)                      | 0 (0.0%)                      | 1 (4.2%)          |
| Respiratory, thoracic and mediastinal disorders               | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 1 (12.5%)                     | 2 (8.3%)          |
| Oropharyngeal pain                                            | 0 (0.0%)       | 0 (0.0%)                      | 0 (0.0%)                      | 1 (12.5%)                     | 1 (4.2%)          |
| Throat irritation                                             | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |
| Skin and subcutaneous tissue disorders                       | 0 (0.0%)       | 0 (0.0%)                      | 2 (25.0%)                     | 0 (0.0%)                      | 2 (8.3%)          |
| Dry skin                                                      | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |
| Erythema                                                      | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                     | 0 (0.0%)                      | 1 (4.2%)          |

MedDRA = Medical Dictionary for Regulatory Activities; Q12h = Every 12 hours; TEAE = Treatment-emergent adverse event. System organ class and preferred term are from the Medical Dictionary for Regulatory Activities (MedDRA), Version 17.0. The number of subjects in each column could not be added because a subject may have had more than one adverse event. A subject experiencing multiple occurrences of the same adverse event was counted, at most, once per System Organ Class and Preferred Term for each treatment and once for “All Active.”
Table 3. Summary of Treatment-related Adverse Events by System Organ Class and Preferred Term for Each Treatment (Safety Population)

| System Organ Class/Preferred Term | Placebo (N = 6) | Cohort A (200 mg Q12h) (N = 8) | Cohort B (400 mg Q12h) (N = 8) | Cohort C (600 mg Q12h) (N = 8) | All Active (N = 24) |
|----------------------------------|----------------|-------------------------------|--------------------------------|-------------------------------|-------------------|
| Subjects with TEAEs             | 2 (33.3%)      | 0 (0.0%)                      | 2 (25.0%)                      | 1 (12.5%)                     | 3 (12.5%)         |
| Gastrointestinal disorders      | 1 (16.7%)      | 0 (0.0%)                      | 1 (12.5%)                      | 0 (0.0%)                      | 1 (4.2%)          |
| Diarrhea                        | 1 (16.7%)      | 0 (0.0%)                      | 1 (12.5%)                      | 0 (0.0%)                      | 1 (4.2%)          |
| Investigations                  | 1 (16.7%)      | 0 (0.0%)                      | 0 (0.0%)                       | 1 (12.5%)                     | 1 (4.2%)          |
| Alanine aminotransferase increased | 0 (0.0%)    | 0 (0.0%)                      | 0 (0.0%)                       | 1 (12.5%)                     | 1 (4.2%)          |
| Lipase increased                | 1 (16.7%)      | 0 (0.0%)                      | 0 (0.0%)                       | 0 (0.0%)                      | 0 (0.0%)          |
| Nervous system disorders        | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                      | 0 (0.0%)                      | 1 (4.2%)          |
| Dysgeusia                       | 0 (0.0%)       | 0 (0.0%)                      | 1 (12.5%)                      | 0 (0.0%)                      | 1 (4.2%)          |

MedDRA = Medical Dictionary for Regulatory Activities; Q12h = every 12 hours; TEAE = Treatment-emergent adverse event. System Organ Class and Preferred Term are from the Medical Dictionary for Regulatory Activities (MedDRA), Version 17.0. The number of subjects in each column could not be added because a subject may have had more than one adverse event. A subject experiencing multiple occurrences of the same adverse event was counted, at most, once per System Organ Class and Preferred Term for each treatment and once for “All Active”. Related TEAEs are defined as TEAEs assessed as related to study drug, or those for which the relationship was unknown or missing.
Assessed for eligibility (n = 95)

Failed screening (n = 57)
Eligible but not enrolled (n = 8)

Enrolled (n = 30)

10 subjects were randomized to each cohort, 8 received drug, 2 received placebo (total placebo = 6)

| Cohort A (200 mg) | Cohort B (400 mg) | Cohort C (600 mg) | Placebo | All subjects |
|-------------------|-------------------|-------------------|---------|-------------|
| Sex | Sex | Sex | Sex |
| Females (n = 4) | Females (n = 2) | Females (n = 2) | Females (n = 2) |
| Males (n = 4) | Males (n = 6) | Males (n = 6) | Males (n = 4) |
| Race | Race | Race | Race |
| White = 4 | White = 2 | White = 4 | White = 3 |
| African American = 4 | African American = 6 | African American = 4 | African American = 2 |
| American Indian = 1 |
| Age (years) Mean±SD = 28±9 Range = 19-44 | Age (years) Mean±SD = 28±7 Range = 22-44 |
| Height (cm) Mean±SD = 170±13 Range = 154-189 |
| Weight (kg) Mean±SD = 83.5±24.2 Range = 48.0-119.8 |
| Body Mass Mean±SD = 28.5±5.4 Range = 19.8±33.6 |

| Sex | Sex | Sex |
| Females (n = 2) | Females (n = 2) | Females (n = 2) |
| Males (n = 6) | Males (n = 6) | Males (n = 6) |
| Race | Race | Race |
| White = 4 | White = 2 | White = 4 |
| African American = 4 | African American = 6 | African American = 4 |
| Age (years) Mean±SD = 29±7 Range = 21-41 |
| Height (cm) Mean±SD = 170±9 Range = 156-181 |
| Weight (kg) Mean±SD = 87.7±13.0 Range = 66.0-108.1 |
| Body Mass Mean±SD = 30.2±2.5 Range = 26.4±33.7 |

| Sex | Sex |
| Females (n = 2) | Females (n = 2) |
| Males (n = 4) | Males (n = 4) |
| Race | Race |
| White = 3 | White = 4 |
| African American = 2 | African American = 4 |
| American Indian = 1 | African American = 4 |
| Age (years) Mean±SD = 35±7 Range = 25-43 |
| Height (cm) Mean±SD = 171±11 Range = 154-189 |
| Weight (kg) Mean±SD = 87.0±7.3 Range = 78.3-98.0 |
| Body Mass Mean±SD = 29.8±3.6 Range = 25.7-34.2 |

| Sex | Sex |
| Females (n = 10) | Females (n = 10) |
| Males (n = 20) | Males (n = 20) |
| Race | Race |
| White = 13 | White = 12 |
| African American = 16 | African American = 16 |
| American Indian = 1 | American Indian = 1 |
| Age (years) Mean±SD = 30±8 Range = 19-44 |
| Height (cm) Mean±SD = 171±11 Range = 154-189 |
| Weight (kg) Mean±SD = 83.6±15.2 Range = 48.0-119.8 |
| Body Mass Mean±SD = 28.4±4.2 Range = 18.5±34.2 |
AAC01395-19 - Figure 3

![Graphs showing individual and mean CRS3123 concentrations](https://example.com/graph.png)
Figure 4

Graph showing the concentration of CRS123 (µg/g) in stool across different doses of 200 mg, 400 mg, and 600 mg, with dashed lines indicating MPC window and MIC₉₀.
AAC01395-19 - Figure 5

(A) Weighted UniFrac Distance

(B) Weighted UniFrac Distance

(C) Weighted UniFrac Distance

(D) Weighted UniFrac Distance

[Y-axis: Weighted UniFrac Distance]

Days: 0, 1, 4, 7, 9, 12, 18, 29

Other, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Synergistetes, Verrucomicrobia

Legend:
- Verrucomicrobia
- Synergistetes
- Proteobacteria
- Firmicutes
- Bacteroidetes
- Actinobacteria
- Other
