STATISTICAL ANALYSIS PLAN

Protocol Number: ACX-362E-201

ACX-362E for Oral Treatment of *Clostridioides difficile* Infection: A Phase 2A Open-Label Segment Followed by a Phase 2B Double-Blind Vancomycin-Controlled Segment

Phase 2B

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List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
CDI	*Clostridioides difficile* Infection
ECC	Extended Clinical Cure
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EOT	End of Treatment
EQ-5D-5L	European Quality of Life - Five Dimension
ICF	Informed Consent Form
ITT	Intent-to-Treat
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetic(s)
PP	Per Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCC	Sustained Clinical Cure
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TOC	Test of Cure
UBM	Unformed Bowel Movement
US	United States

1.0 INTRODUCTION

This document details the analysis plan for the study entitled "ACX-362E for Oral Treatment of *Clostridioides difficile* Infection: A Phase 2A Open-Label Segment Followed by a Phase 2B Double-Blind Vancomycin-Controlled Segment". It describes the proposed efficacy and safety analyses, including planned summary tables and by-subject data listings. This statistical analysis plan (SAP) concerns Phase 2B. An earlier SAP describes the analyses of Phase 2A.

Clostridioides difficile (*C. difficile*) is an anaerobic, spore-forming, Gram-positive bacterium. It is the causative agent of *C. difficile* infection (CDI), an increasingly common, potentially life-threatening disease. *C. difficile* can live harmlessly in the colon, but in the presence of an antibiotic administered for another condition, overgrowth may occur, replacing normal bacterial flora and resulting in CDI and or *C. difficile*-associated disease (Nelson et al., 2017).

The epidemiology of *C. difficile* has changed with the emergence of the North American pulsed-field gel electrophoresis type 1 strain, and use of more sensitive assays (Lessa et al., 2015). *Clostridioides difficile* infection affects approximately 500,000 patients in the United States (US) each year, and is associated with 15,000 to 30,000 deaths (McDonald et al., 2018). In fact, 65,000 hospital acquired hospital-onset cases, 50,000 hospital acquired post-discharge cases, and 263,000 nursing home-onset cases, result in $4 billion in excess costs to the healthcare system annually (Clostridium Infections – Pipeline Review, 2012). Hospitalizations for CDI among nonpregnant adults has doubled from 2000 through 2010 and are projected to continue to increase in 2011 and 2012 (Lessa et al., 2015).

Although the discontinuation of predisposing antibiotic therapy and treatment with metronidazole, oral vancomycin, or the recently approved drug fidaxomicin (Dificid®), are effective in management of CDI, the development of new alternative therapeutic measures will be of great benefit. Not only is the incidence of hospitalizations and deaths associated with *C. difficile* rising (Lessa et al., 2015; Louie et al., 2012; Zilberberg et al., 2008), but some strains may become hypervirulent and increasingly resistant to cephalosporins and fluoroquinolones (Razavi et al., 2007).

Current treatment strategy includes discontinuation of predisposing antibiotic and oral administration of vancomycin or fidaxomicin. If access to vancomycin or fidaxomicin is limited, metronidazole may be used for an initial episode of non-severe CDI only (McDonald et al., 2018).

While vancomycin continues to show good clinical efficacy for *C. difficile*, there is an increasing incidence of serious illness because of vancomycin-resistant enterococci in the US, and use of oral vancomycin (as well as oral metronidazole) has been shown to promote overgrowth of this pathogen (Al-Nassir et al., 2008). Use of vancomycin to treat *C. difficile* is also associated with unacceptably high recurrence rates. Fidaxomicin (Dificid®), although newly approved for treatment of CDI, is a treatment option if the risk of recurrence is high, but not for complicated CDI (Smits et al., 2016).

Several additional products are under development, and include small molecules, natural products, vaccines, and fecal transplants (Clostridium Infections – Pipeline Review, 2012; McDonald et al., 2018).

Ibezapolstat (ACX-362E) has activity in vitro and in vivo against *C. difficile* (Xu et al., 2011; Dvoskin et al., 2012). The crisis in antibiotic resistance of Gram-positive bacteria prompted the development of novel antibacterials through structure–activity relationship studies of replication-specific DNA polymerase IIIC. It is from among these compounds that antibacterial compounds against *C. difficile* with potent in vitro activity have been discovered. Among these is ibezapolstat, which also shows potent in vivo activity in the established Syrian golden hamster model of CDI (Kokkotou et al., 2008; Dvoskin et al., 2012).

ACX-362E is an inhibitor of the *C. difficile* DNA polymerase IIIC and is highly selective for *C. difficile* compared with other Gram-positive anaerobes and aerobic bacteria. ACX-362E, 2-(3,4-dichlorobenzyl)-7-(2-[morpholinyl]ethyl)guanine, is a small molecule, poorly bioavailable and active orally in the hamster model. In vitro, ACX-362E inhibited numerous *C. difficile* strains, with a minimum inhibitory concentration required to inhibit the growth of 90% of organisms value of 4 µg/mL, and without inhibiting *Bifidobacteria* and *Lactobacillus spp*., important "good" intestinal anaerobic bacteria, or Gram-negative bacteria. These results suggest that development of ACX-362E as an oral treatment for CDI in human patients will result in a novel, first-in-class drug to treat this emerging infectious disease.

Subjects in this study who receive ACX-362E will be administered 450 mg orally every 12 hours for 10 days. ACX-362E is administered orally because it has been shown that ACX-362E has low oral availability with low systemic absorption and also has a unique specificity for *C. difficile* that does not affect the normal good flora of the colon, an important advantage to prevent recurrence.

2.0 STUDY OBJECTIVES

The objectives for Phase 2B of this study are as follows:

2.1 Primary Objectives

1. Assess CDI clinical cure rates 2 days after the end of treatment (EOT)
2. Evaluate the safety and tolerability of ACX-362E administered every 12 hours for 10 days in the treatment of CDI

2.2 Secondary Objectives

1. Determine the fecal concentrations of ACX-362E in subjects with CDI during the course of treatment
2. Assess incidence of sustained clinical cure (SCC) at 28 ± 2 days after EOT

2.3 Exploratory Objectives

1. Compare the effects of ACX-362E versus vancomycin on relative and quantitative changes to the fecal microbiome
2. Assess times to resolution of diarrhea during the treatment period
3. Assess times to hospital discharge during the treatment period
4. Assess the impact of ACX-362E treatment on subject reported quality of life and resource utilization
5. Assess the incidence of extended clinical cure (ECC) at 56 ± 2 days and 84 ± 2 days after EOT in subjects participating in the extended follow-up period

3.0 STUDY DESIGN

3.1 Overview

This Phase 2, multicenter, combined segment open-label single-arm segment followed by a double-blind, randomized, active-controlled clinical segment is designed to evaluate ACX-362E in the treatment of CDI.

Segment 2A of this trial is an open-label cohort of approximately 20 subjects in approximately 5 study centers in the US and Canada. If safety, tolerability, and efficacy are considered acceptable by the Trial Oversight Committee, Segment 2A will be followed by a double-blind, randomized, active-controlled trial (Segment 2B) with an additional cohort of at least 72 subjects.

In Segment 2B, subjects with CDI will be enrolled and randomly assigned in a 1:1 ratio to either ibezapolstat 450 mg orally every 12 hours with food or to vancomycin 125 mg orally every 6 hours in up to 30 centers in the United States and Canada. Treatment will be administered for 10 days and subjects will be followed for 28 ± 2 days for recurrence. Blinding in Segment 2B will be maintained by over-encapsulation of both drugs and dosing every 6 hours in all subjects. Subjects randomized to ibezapolstat will receive 2 doses per day of ibezapolstat and 2 doses per day of placebo. Additionally, subjects recruited from selected sites will participate in an extended follow-up period consisting of observation visits at 56 and 84 days after treatment to evaluate the long-term impact of ibezapolstat on the microbiome and on disease recurrence. At these selected sites, consecutive subjects will be offered the opportunity to participate in the extended follow-up period until approximately 16 subjects are recruited.

For all subjects, the diagnosis of CDI will be based on unexplained and new onset of diarrhea (≥ 3 unformed bowel movements (UBMs) in a 24-hour period) and a positive result using a US Food and Drug Administration-approved test to detect free (*C. difficile*) toxins in stools. Baseline safety evaluations will consist of physical examination, vital signs, routine laboratory (hematology, clinical chemistry, and urinalysis) testing, and ECG.

Subjects will be evaluated for treatment response and safety (adverse events (AEs), physical examination, vital signs, laboratory tests, and 12-lead ECG) at designated time points according to Table 1. Clinical cure of CDI will be defined as survival and the resolution of diarrhea in the 24-hour period immediately before EOT that is maintained for 48 hours post EOT without a requirement for additional CDI treatment. All subjects, regardless of outcome after 10 days of treatment, will be followed for recurrence until Day 38; recurrence is defined as a new episode of diarrhea (≥ 3 UBMs in a 24-hour period) with a positive free toxin test result (using a Sponsor-approved diagnostic test) that in the opinion of the Investigator requires retreatment with an antibacterial for *C. difficile*.

Stool samples for microbiome testing will be collected at the time points outlined in Table 1, frozen, and sent to the central laboratory for analysis. An additional sample will be collected in the event of any suspected recurrence post test of cure (TOC), regardless of the outcome from the 10 days of treatment. Samples will also be collected in the event of any suspected recurrence at 56 and 84 days after EOT for those subjects participating in the extended follow-up period. Evaluation of changes to the microbiome at the TOC visit as well as overgrowth by vancomycin-

resistant *Enterococcus* and multi-drug resistant organisms will be performed using Next Gen metagenomics sequencing and quantitative polymerase chain reaction.

For pharmacokinetic (PK) analysis, fecal concentrations of ACX-362E will be determined for the stool samples collected at time points outlined in Table 1. Blood samples to measure plasma levels of ACX-362E in approximately 50% of subjects (i.e., approximately 36 subjects) in Segment 2B will be drawn 2 hours and 4 hours after the morning dose at time points specified in Table 1.

The schedule of assessments for this study is presented in Table 1.

Table 1: Schedule of Assessments

Study Phase	Screening	Treatment					Follow-Up				Recurrence	Extended Follow-Up[i]	
Assessment	Screening Visit[a]	Baseline[a]	Check In	Check In	Check In	End of Treatment	Test of Cure	Check In	Check In	End of Study	Suspected Recurrence	Obs Visit	Obs Visit
Day	Day -2 to Day 1	Day 1	Day 3	Day 5	Day 8	Day 10	Day 12 (≥ 48 hrs post last dose)	Day 20	Day 30	Day 38	Test of Cure to End of Study[j]	Day 66	Day 94
Visit Window			±1 Day	±1 Day	±1 Day	±1 Day	+1 Days	±2 Days	±2 Days	±2 Days	<48 hrs of Reporting	±2 Days	±2 Days
Visit Format	Clinic	Clinic	Tel.	Clinic	Tel.	Tel.	Clinic	Tel.	Tel.	Clinic	Clinic	Clinic	Clinic
Segment 2A/2B													
Informed consent	X												
Inclusion/exclusion criteria	X												
CDI diagnosis including free toxin test[b]	X										X		
Demographics	X												
Medical & medication histories including detailed CDI history	X												
Physical examination[c]	X												
Height	X												
Vital signs		X		X			X[d]						
Safety laboratory tests[e]		X		X			X						
Pregnancy test[f]	X						X						
Electrocardiogram		X		X									
Dosing		←	—	—	—	→							
Investigator assessment of clinical response							X			X			

Study Phase	**Screening**	**Treatment**					**Follow-Up**				**Recurrence**	**Extended Follow-Up[i]**	
Assessment	**Screening Visit[a]**	**Baseline[a]**	**Check In**	**Check In**	**Check In**	**End of Treatment**	**Test of Cure**	**Check In**	**Check In**	**End of Study**	**Suspected Recurrence**	**Obs Visit**	**Obs Visit**
Day	**Day -2 to Day 1**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 10**	**Day 12 (≥ 48 hrs post last dose)**	**Day 20**	**Day 30**	**Day 38**	**Test of Cure to End of Study[j]**	**Day 66**	**Day 94**
Visit Window			**±1 Day**	**±1 Day**	**±1 Day**	**±1 Day**	**+1 Days**	**±2 Days**	**±2 Days**	**±2 Days**	**<48 hrs of Reporting**	**±2 Days**	**±2 Days**
Visit Format	**Clinic**	**Clinic**	**Tel.**	**Clinic**	**Tel.**	**Tel.**	**Clinic**	**Tel.**	**Tel.**	**Clinic**	**Clinic**	**Clinic**	**Clinic**
Concomitant medications	←	—	—	—	—	—	—	—	—	—	→		
Adverse events	←	—	—	—	—	—	—	—	—	—	→		
Subject diary	←	—	—	—	—	—	—	—	—	—	→		
Stool samples for future analyses[g] (microbiome and fecal drug concentration analyses)	X		X	X	X	X	X	X	X	X	X		
Blood sample for pharmacokinetics[h]		X		X									
Segment 2B only													
Randomization per IWRS		X											
EQ-5D-5L	X						X			X	X	X	X
Medical Resource Utilization Questions	X		X	X	X	X	X	X	X	X	X		
Stool samples for future analyses (microbiome analyses)												X	X

Abbreviations: CDI, *Clostridioides difficile* infection; IWRS, Interactive Web Response System; Obs, observation; Tel., telephone.

[a] May occur the same day as the baseline visit. Written informed consent for the study will be obtained from all subjects before any protocol specific procedures are performed.

[b] Any Sponsor-approved free toxin test may be used.

[c] A complete physical at screening; limited physical examination at other times as clinically indicated.

[d] Perform at Day 38 or earlier (at an unscheduled visit) if subject experiences a confirmed recurrence.

[e] Safety laboratory tests will include biochemistry, hematology, and urinalysis.
[f] Only in women of childbearing potential. Serum test will be done at Screening; urine test will be done at the site at other specified time points.
[g] If subject does not attend clinic then arrangements must be made to collect stool sample from subject's home. Sample must be collected before first dose of study drug. Stool samples should be kept cool and brought to the clinic within 24 hours after collection.
[h] Pharmacokinetic blood samples will be collected 2 hours (± 15 minutes) and 4 hours (± 30 minutes) after first dose of the day. In Segment 2B, pharmacokinetic blood samples will be collected in approximately 50% of subjects (i.e., approximately 36 subjects).
[i] Randomized Segment 2B subjects recruited from selected sites will have the opportunity to participate in an extended follow-up period to Day 94.
[j] EOS includes observation visits Day 66 and Day 94 for those subjects participating in the extended follow-up period.

3.2 Method of Assigning Subjects to Treatment

On Day 1, subjects will be randomly allocated to kits containing blister packs of either ibezapolstat or vancomycin and will be assigned a randomization number according to an interactive web response system (IWRS). The randomization schedule will be produced by staff outside the study team and kept secure from blinded study staff until after the study database is locked. Site staff will give the kits containing the blister packs to hospital staff while the subject is in the hospital (if needed), or to the subject to take home for self-dosing.

3.3 Blinding

Segment 2B is a double-blind study. Ibezapolstat, placebo, and vancomycin capsules will be identical in physical appearance by over-encapsulation, packaged into blister packs with each dose labeled, and will be administered 4 times a day so that neither study staff nor the subjects will know to which treatment the subject was assigned. Subjects randomized to vancomycin will receive 3 capsules (1 vancomycin capsule and 2 placebo capsules) to mimic the 3 ibezapolstat 150 mg capsules every 12 hours (e.g., 12 AM and 12 PM) and a single vancomycin capsule (e.g., 6 AM and 6 PM) every 12 hours. The Investigator, study staff, Sponsor, Trial Oversight Committee, and the subject will remain blinded to study treatment throughout the study.

According to the randomization schedule as indicated in the Schedule of Assessments (Table 1), the Investigator or designee will obtain the kit number from the IWRS for the subject. The pharmacist or designee will provide the assigned kit number to the subject. No study site personnel, subjects, Sponsor personnel, or Sponsor designees will be unblinded to treatment assignment throughout the duration of the study unless unblinding is required. If an Investigator becomes unblinded to a given subject's study treatment, that subject will continue in the study and undergo all protocol-required procedures for the determination of safety and efficacy.

In the event that emergency unblinding is required for a given subject because of AEs or concerns for the subject's safety or wellbeing, the Investigator may break the randomization code for the subject via the IWRS, by which system the unblinding will be captured. The Investigator is responsible for notifying the Medical Monitor and/or Sponsor of such an event within 24 hours. The unblinding and its cause will also be documented in the eCRF.

3.4 Determination of Sample Size

Sample size for Segment 2B was calculated based on the following specifications:

1. Non-inferiority study
2. Endpoint is clinical cure at the TOC visit
3. One-sided normal approximation test
4. Alpha = 0.05
5. Allocation to treatment = 1:1
6. True cure rate = 0.80 in both arms
7. Non-inferiority margin = 0.25
8. Power = 80%
9. Sample size adjusted for one interim analysis based on the method of Pocock

A sample size of 36 subjects per treatment group, or 72 subjects total, will satisfy these

specifications.

3.5 Changes to the Protocol-Specified Analyses

The protocol states that at least 72 subjects are planned for Segment 2B of this study; however, study enrollment was stopped after 32 subjects were enrolled and randomized.

The protocol indicates that the primary analysis of efficacy will be based on the Intent-to-Treat (ITT) Population and that a secondary analysis of the primary and secondary efficacy endpoints will be based on the Per Protocol Population. Instead, the primary analysis of efficacy will be based on the Per Protocol Population, and a secondary analysis of the primary and secondary efficacy endpoints will be based on the ITT Population.

The protocol specifies that time to hospital discharge (for hospitalized subjects only) and medical resource utilization data are exploratory efficacy endpoints. These proposed endpoints are being dropped.

The protocol states that a single interim analysis will be performed when the first 36 Segment 2B subjects in the ITT Population (one half of the planned sample size) have data for the primary efficacy endpoint of clinical cure at the TOC visit. This will not be done.

The protocol indicates that the null and alternative hypotheses for the primary efficacy endpoint are as follows:

$H_0: \pi_{1,T} \leq \pi_{1,C} - \delta$
and
$H_1: \pi_{1,T} > \pi_{1,C} - \delta,$

where $\pi_{1,T}$ and $\pi_{1,C}$ are the true proportions of subjects with clinical cure at the TOC visit for the ibezapolstat 450 mg and vancomycin 125 mg treatments, respectively, and δ is the non-inferiority margin, which was to be 0.25. No hypothesis test will be performed.

The protocol states that, for the secondary efficacy endpoint, the null hypothesis that the true proportions are equal for the 2 treatments will be tested using a two-sided Fisher's exact test at the 0.05 significance level. No hypothesis will be performed.

The protocol states "The number and percentage of subjects having a treatment emergent AE (TEAE) in each System Organ Class (SOC) and having each individual type of AE (Preferred Term) will be presented. TEAEs will also be summarized at the event level by SOC/Preferred Term and severity. This will be done for all TEAEs, all serious TEAEs, and all TEAEs leading to discontinuation from the study." However, this will be done only for TEAEs and not for serious TEAEs or TEAEs leading to discontinuation from the study.

4.0 EFFICACY AND SAFETY ENDPOINTS

4.1 Primary Efficacy Endpoint

The primary endpoint of this study is clinical cure at the test of cure (TOC) visit, which is defined as survival and the resolution of diarrhea in the 24-hour period immediately before EOT that is maintained for 48 hours post EOT without a requirement for additional CDI treatment. Diarrhea is defined as ≥ 3 unformed bowel movements (UBMs) in a 24-hour period; < 3 UBMs is considered as resolution of diarrhea. A UBM is defined as a Type 5, 6, or 7 bowel movement on the Bristol Stool Chart.

4.2 Secondary Efficacy Endpoint

The secondary efficacy endpoint of this study is sustained clinical cure (SCC), which is defined as a clinical cure at the TOC visit (i.e., at least 48 hours post EOT) and no recurrence of CDI within the 28 ± 2 days post EOT. Recurrence is defined as a new episode of diarrhea (≥ 3 UBMs in a 24-hour period) with a positive toxin result, using a Sponsor-approved *C. difficile* free toxin test and, in the opinion of the Investigator, requiring retreatment with an antibacterial agent for *C. difficile*.

4.3 Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Microbial endpoints: Quantitative changes in relevant fecal bacterial communities and microbial diversity will be assessed. Changes will be assessed during the treatment period and post EOT.
- Time to resolution of diarrhea, defined as the time from outset of treatment to the first formed bowel movement not followed within the next 24 hours by a UBM.
- Quality of life assessments including EQ-5D-5L.
- ECC, defined as a clinical cure at the TOC visit (i.e., at least 48 hours post EOT) and no recurrence of CDI within the 56 ± 2 days post EOT (ECC56) and 84 ± 2 days post EOT (ECC84). Recurrence is as defined for the secondary efficacy endpoint.

This statistical analysis plan does not address the analysis of microbial endpoints.

4.4 Safety Endpoints

Safety endpoints for all subjects include the nature, frequency, and severity of AEs, including serious adverse events (SAEs); changes from baseline in findings on physical examination, vital sign measurements, safety laboratory tests (hematology, chemistry, urinalysis); and ECG findings.

4.5 Pharmacokinetic (PK) Endpoints

The secondary PK endpoint is systemic exposure of ACX-362E, which will be determined by measuring plasma ACX-362E concentrations at specified time points following dose administration; fecal concentrations of ACX-362E will be measured at specified study visits.

The PK analysis is not part of this analysis plan.

5.0 STATISTICAL CONSIDERATIONS

5.1 General Methodology

The statistical analysis of the data obtained from this study will be performed using SAS® version 9.4 or higher.

Data collected in this study will be documented using summary tables and subject data listings. Continuous variables will be summarized using descriptive statistics, specifically the sample size, mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized using frequencies and percentages. For continuous data, the minimum and the maximum will use the same decimal accuracy as the raw data. The mean, median, and standard deviation will use one more decimal place than the raw data. For categorical data, percentages will be reported to one decimal place. Summary statistics will be presented by treatment group.

Data listings will be sorted by treatment group and patient ID.

5.2 Adjustments for Covariates

No adjustments for covariates will be made.

5.3 Handling of Dropouts and Missing Data

Multiple imputation methods will be used for the imputation of missing data for the primary and secondary efficacy endpoints for the ITT Population. For each treatment group and each imputed dataset, subjects with missing data will have a value for the endpoint imputed randomly based on the Bernoulli probability distribution with probability of success equal to the observed probability of success for the endpoint (clinical cure or sustained clinical cure) in the given treatment group. PROC MIANALYZE of SAS® will be used to combine the results from the imputed datasets to produce an overall estimate of the true success rate for each treatment group and the other desired statistics as specified in Section 9.1 and 9.2. Based on what is known to date for the observed, blinded data, there is one subject who has missing values for the primary and secondary efficacy endpoints. This subject dropped out of the study without receiving any study drug and has no follow-up data.

No other imputation for missing data will be performed.

5.4 Interim Analyses and Data Monitoring

No interim analyses will be performed.

5.5 Multicenter Study

This is a multicenter study. It is planned to enroll at least 72 subjects at up to 30 study centers in the United States (US) and Canada for Segment 2B.

5.6 Multiple Comparisons / Multiplicity

No adjustment for multiple comparisons/multiplicity is needed since no hypothesis testing is to be done.

5.7 Examination of Subgroups

No subgroup analyses will be performed.

5.8 Baseline

Baseline is defined as the last value prior to the start of study medication.

6.0 ANALYSIS POPULATIONS

6.1 Enrolled Population

The Enrolled population will include all subjects in Segment 2B who sign the informed consent form (ICF).

6.2 Intent-to-Treat Population

The intent-to-treat (ITT) population will include all subjects who are randomized. Subjects will be analyzed based on their randomized assignment. The ITT population will be used for a secondary analysis of the primary and secondary efficacy endpoints.

6.3 Per Protocol (PP) Population

The PP Population will consist of patients in the ITT Population with no major protocol deviations. The determination of which patients have major protocol violations will be made in a blinded manner prior to database lock. For analyses based on the PP Population, patients will be grouped according to the treatment to which they were randomized. This population will be used for the primary analysis of the efficacy endpoints.

6.4 ITT Extension Population

The ITT Extension population will include all randomized subjects who participate in the extended follow-up period. Subjects will be analyzed based on their randomized assignment. The ITT Extension population will be used for the analysis of the ECC56 and ECC84 exploratory endpoints.

6.5 Safety Population

The Safety population will include all subjects who receive at least 1 dose of study drug. Subjects will be analyzed based on the treatment actually received. This population will be used for the analysis of safety.

7.0 SUBJECT DISPOSITION

The number of enrolled subjects, re-screened subjects, and screen failures will be presented. The number of randomized subjects (ITT Population) and the number and percentage of randomized subjects who are in the Per Protocol Population, the ITT Extension Population, and the Safety Population; who completed study treatment; who withdrew from study treatment, and their primary reason for early withdrawal; who completed the study; who withdrew early from the study treatment, and their primary reason for early withdrawal, will be summarized by treatment group.

The reason for screen failure will be summarized using frequencies and percentages for screen failures.

8.0 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Descriptive statistics will be presented by treatment group and overall for the continuous variables of age and height; frequencies and percentages will be presented for the categorical variables of sex, whether the patient is of child bearing potential (females only), race, and ethnicity.

The following data will each be summarized by treatment group using descriptive statistics for continuous variables and frequencies and percentages for categorical variables: medical history, current episode of CDI, treatment of current episode of CDI, previous episodes of CDI (prior to the current episode), and the Day 1 limited physical examination.

9.0 EFFICACY ANALYSES

9.1 Primary Efficacy Endpoint

The primary efficacy endpoint is clinical cure at the TOC visit, defined as survival and resolution of diarrhea in the 24-hour period immediately before EOT that is maintained for 48 hours post EOT without a requirement for additional CDI treatment.
After EOT, subjects with initial clinical cure will be followed for an additional 28 ± 2 days to assess the recurrence of CDI.

This endpoint will be summarized by treatment group using frequencies and percentages, together with an exact (Clopper-Pearson) 95% confidence interval for the true cure percentage. The 95% exact unconditional confidence interval will also be calculated for the difference between the 2 population proportions ($\pi_{1,C} - \pi_{1,T,}$ where $\pi_{1,C}$ and $\pi_{1,T}$ are the true proportions of subjects with clinical cure at the TOC visit for the vancomycin 125 mg and ibezapolstat 450 mg treatments, respectively.

In addition, the rate of clinical cure will also be summarized for ibezapolstat by frequency and percentage along with an exact 95% confidence interval pooling data from Phases A and B.

9.2 Secondary Efficacy Endpoint

The secondary efficacy endpoint is sustained clinical cure (SCC), which is defined below, and is determined for subjects who achieve initial clinical cure. The determination will be made at the Day 38 follow-up visit.

1. Recurrence is defined as ≥ 3 bowel movements with unformed stools in any 24-hour period with free *C. difficile* toxin A or B (or both) detected in a stool sample and, in the opinion of the Investigator, there is a need for CDI retreatment.
2. SCC is defined as initial clinical cure without subsequent recurrence within 28 ± 2 days post EOT.

This endpoint will be summarized by treatment group using frequencies and percentages, together with an exact (Clopper-Pearson) 95% confidence interval for the true SCC percentage. A 95% exact unconditional confidence interval will also be calculated for the difference between the 2 population proportions ($\pi_{2,C} - \pi_{2,T}$), where $\pi_{2,C}$ and $\pi_{2,T}$ are the true proportions of subjects with SCC for the vancomycin 125 mg and ibezapolstat 450 mg treatments, respectively.

In addition, the rate of sustained clinical cure will also be summarized for ibezapolstat by frequency and percentage along with an exact 95% confidence interval pooling data from Phases A and B.

9.3 Exploratory Endpoints

9.3.1 Time to Resolution of Diarrhea

Time to resolution of diarrhea, defined as the time from onset of treatment to the first formed bowel movement not followed within the next 24 hours by a UBM, will be summarized by treatment group using the Kaplan-Meier method, with estimates of the true resolution of diarrhea rate at Days 1, 5, 10, 12, and 40. The estimated median time to resolution of diarrhea, together with a 95% confidence interval, will also be presented.

9.3.2 Quality of Life Assessments

The EQ-5D-5L scores and the changes from baseline (screening) for each of the 5 dimensions and the EQ-5D-5L visual analog scale score will be summarized by treatment group and time point using descriptive statistics.

Medical resource utilization data will be listed.

9.3.3 ECC56 and ECC84

These endpoints will be summarized by treatment group using frequencies and percentages, together with exact (Clopper-Pearson) 95% confidence intervals for the true percentages. Also, 95% confidence intervals based on the normal approximation will be calculated for the differences between the 2 population proportions. These endpoints will be analyzed based on the ITT Extension Population.

10.0 SAFETY ANALYSES

Safety endpoints for all subjects include the nature, frequency, and severity of AEs; changes from baseline in findings on physical examination, vital sign measurements, safety laboratory tests (hematology, chemistry, urinalysis); and ECG findings.

10.1 Adverse Events

Treatment-emergent AEs (TEAEs) are defined as any AE with onset date after the date of the start of the study drug or any AE already present at baseline that worsens in severity. All reported AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). Events with missing onset dates will be considered to be treatment-emergent unless there is additional information indicating that the AE started before study treatment. Only TEAEs will be summarized.

The number and percentage of subjects with at least one TEAE, at least one treatment-related TEAE, at least one serious TEAE, and at least one TEAE leading to discontinuation from the study will be presented by treatment group. AEs that are definitely, probably, or possibly related, or for which the relationship is missing, will be considered treatment-related.

The number and percentage of subjects having a TEAE in each System Organ Class (SOC) and having each individual type of AE (Preferred Term) will be presented. TEAEs will also be summarized at the event level by SOC/Preferred Term and severity and by SOC/Preferred Term and relationship to the study drug.

SAEs will be listed.

10.2 Clinical Laboratory Tests

Clinical laboratory data (hematology and serum chemistry) will be summarized by treatment group and time point using descriptive statistics for both the actual values and the changes from baseline (Day 1). Also, the number and percentage of subjects with lab values in the normal range, outside the normal range without clinical significance, and outside the normal range with clinical significance will be presented.

Urinalysis data will be summarized by treatment group and time point using frequencies and percentages for categorical variables and descriptive statistics for continuous variables. A shift table will be presented showing changes in status (normal, abnormal without clinical significance, abnormal with clinical significance) from Baseline (Day 1) to Days 5 and 12.

10.3 Vital Signs

Vital signs data will be summarized by treatment group and time point using descriptive statistics for both the actual values and the changes from baseline (Day 1). Also, the number and percentage of subjects with vital sign values in the normal range and outside the normal range will be presented.

10.4 12-Lead ECG

Electrocardiogram data will be summarized by treatment group and time point using descriptive statistics for both the actual values and the changes from baseline. A shift table will be presented showing changes in status (normal, abnormal without clinical significance, abnormal with clinical significance) from Baseline (Day 1) to Day 5.

10.5 Concomitant Medications

Concomitant medications (medications initiated during the study period and medications initiated prior to the study period but continuing in the study period) will be summarized by treatment group using frequencies and percentages of subjects having a medication in each ATC3 class and having a medication with the given preferred term.

11.0 OTHER ANALYSES

Serum and urine pregnancy data will be summarized by treatment group using counts and percentages.

The investigator assessment of clinical response at Days 12 and 38 will be summarized by treatment group using frequencies and percentages.

Study drug compliance and accountability data will be provided in Listing 3.1.

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Appendix A: TABLE SHELLS

Appendix B: LISTING SHELLS