

Cadazolid / ACT-179811

***Clostridium difficile*-associated diarrhea**

Protocol AC-061A303

A prospective, multicenter study to investigate the pharmacokinetics, safety, and efficacy of cadazolid versus vancomycin in pediatric subjects with *Clostridium difficile*-associated diarrhea

**LIST OF AMENDMENTS
(Summary of changes)**

Document History:

- Original Version	14 July 2015
- Global Amendments	
Amendment 1 (non-substantial)	05 November 2015
Amendment 2 (substantial)	27 October 2016
Amendment 3 (substantial)	01 March 2017

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Amendment 1 (non-substantial amendment), 05 November 2015

This amendment was made before protocol submission to any IRBs/IECs.

Amendment 2 (substantial amendment), 27 October 2016

Main reason for this Amendment: To align the global protocol with the European Medical Agency's decision on the cadazolid Paediatric Investigation Plan and with the FDA's requirements (as described below)

Description of change(s)	Amended protocol sections
Inclusion of neonates and infants below 6 months of age	Sections 1.3, 2.1, 3.1, 3.1.1, 3.2, 4.1, 4.2 & 4.3 Figure 1
The age cohort of subjects from 2 to < 12 years to be split into two new cohorts: subjects from 6 to < 12 years and from 2 to < 6 years resulting in a minor increase in sample size from 194 to 200 participants	Sections 3.1.1, 3.2, 4.1 & 10.5.1 Figure 1
Study timelines to be shortened by enrolling subjects in Part B of the study as soon as dosing recommendations are available for respective age groups based on Part A	Section 3.1.2 Figure 1, Figure 3
Addition of an Independent Data Monitoring Committee to review the data and make recommendations as to further dosing in the subsequent age cohorts or strata	Sections 3.1.1, 3.2, 3.4 & 8.3
Part B of the study to be blinded to an efficacy assessor	Sections 3.1.2, 3.3.2, 5.1.5 & 7.2.3.3
Palatability/acceptability to be studied in all subjects	Sections 2.4, 6.4.1, 7.4 & 10.3.5.7
Addition of a lower strength of oral cadazolid (50 mg)	Section 5.1.1, 5.1.3 & 5.16 Table 1

Amendment 3 (substantial amendment), 01 March 2017

Main reason for this Amendment: To address agreed changes in the response to Voluntary Harmonization Procedure (VHP) list of grounds for non-acceptance, dated 10 February 2017. The key requested modifications to the protocol included:

Description of change(s)	Amended protocol sections
Classification of the study as a Phase 2/3 instead of Phase 3, as the study includes a preliminary dose-finding part and a subsequent part assessing safety and efficacy	Title page, Section 3.1
Addition of a separate summary section of known potential risks and benefits for Cadazolid	Section 1.2.3
Updates of the exclusion criteria by adding the following criteria: <ul style="list-style-type: none"> - Exclusion of subjects with hypersensitivity to cadazolid and to any of the excipients of vancomycin); - Exclusion of subjects with mental disorders 	Section 4.4
Clarification on the effectiveness of double-barrier	Section 4.5.2

contraception methods and on the variability of acceptance of such contraception measures between concerned countries	
To clearly indicate that patients must be discontinued from the study in case of recognized fulminant or life-threatening Clostridium difficile-associated diarrhea	Section 5.1.8, 5.2.5 & 6.1.1.2



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Amendment 3	01 March 2017 (substantial)

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SIGNATURE PAGE FOR ACTELION PHARMACEUTICALS LTD

Hereinafter called Actelion

Treatment name / number

Cadazolid / ACT-179811

Indication

Clostridium difficile-associated diarrhea

Protocol number, study title

AC-061A303, A prospective, multicenter study to investigate the pharmacokinetics, safety, and efficacy of cadazolid versus vancomycin in pediatric subjects with *Clostridium difficile*-associated diarrhea

I approve the design of this study.

Title	Name	Date	Signature
Clinical Trial Physician			
Clinical Trial Statistician			

INVESTIGATOR SIGNATURE PAGE

Treatment name / number

Cadazolid / ACT-179811

Indication

Clostridium difficile-associated diarrhea

Protocol number, study title

AC-061A303, A prospective, multicenter study to investigate the pharmacokinetics, safety, and efficacy of cadazolid versus vancomycin in pediatric subjects with *Clostridium difficile*-associated diarrhea

I agree to the terms and conditions relating to this study as defined in this protocol, the Case Report Form (CRF), and any other protocol-related documents. I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a protocol deviation, including any ancillary studies or procedures performed on study subjects (other than those procedures necessary for the wellbeing of the subjects).

I agree to conduct this study in accordance with the Declaration of Helsinki principles, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and applicable regulations and laws. I will obtain approval by an independent ethics committee or institutional review board (IEC/IRB) prior to study start and signed informed consent from all parents/ legally designated representatives of the subjects included in this pediatric study. If an amendment to the protocol is necessary, I will obtain approval by an IEC/IRB and ensure approval by regulatory authorities has been obtained before the implementation of changes described in the amendment, and I will re-consent the subjects (if applicable). I will allow direct access to source documents and study facilities to sponsor representative(s), particularly Clinical Research Associate(s) (CRA[s]) and auditor(s), and agree to inspection by regulatory authorities or IEC/IRB representative(s). I will ensure that the study treatment(s) supplied by the sponsor is/are being used only as described in this protocol. I will ensure that all subjects or legally designated representatives have understood the nature, objectives, benefits, implications, risks and inconveniences for participating in this study. During the conduct of the study, I will constantly monitor the risk/benefit balance for an individual subject. I confirm herewith that the sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to health authorities worldwide.

Country	Site number	Town	Date	Signature
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**Principal
Investigator**

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse event
AUC _t	Area under the concentration-time curve from zero to time t
b.i.d.	Twice daily
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CCR	Clinical Cure Rate
CDAD	<i>Clostridium difficile</i> -associated diarrhea
CDC	Center for Disease Control
CDI	<i>C. difficile</i> infection
CFR	Code of Federal Regulations
CI	Confidence interval
CL	Confidence limit
CLSI	Clinical and Laboratory Standards Institute
C _{max}	Maximum plasma concentration
CRA	Clinical Research Associate
CRF	Case report form - paper or electronic
CRO	Contract Research Organization
CV	Coefficient of variation
DBP	Diastolic blood pressure
EDTA	Ethylene diamine tetraacetic acid
EIA	Enzyme Immuno-Assay
EMA	European Medicines Agency
EMLA	Eutectic Mixture of Lidocaine and Prilocaine
EOS	End-of-Study
EOT	End-of-Treatment
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
FAS	Full Analysis Set
FDA	Food and Drug Administration

FMT	Fecal microbiota transplant
FSFV	First subject-First visit
GCP	Good Clinical Practice
GDH	Glutamate Dehydrogenase
HR	Heart rate
i.v.	Intravenous
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IDSA	Infectious Diseases Society of America
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISF	Investigator Site File
LAR	Legally Authorized Representative
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOQ	Limit of quantification
LSLV	Last subject-Last visit
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimal inhibitory concentration
MIC ₅₀	MIC required to inhibit the growth of 50% of organisms
MIC ₉₀	MIC required to inhibit the growth of 90% of organisms
mITT	Modified intent-to-treat
MTF	Metronadizole treatment failure
NAAT	Nucleic Acid Amplification Test
NED	New episode of diarrhea
PBPK	Physiologically Based Pharmacokinetic
PCR	Polymerase chain reaction
PI	Principal investigator

PK	Pharmacokinetic
PKS	Pharmacokinetic Set
PPS	Per-protocol Set
q.i.d	Four times a day
ROD	Resolution of Diarrhea
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SBP	Systolic blood pressure
SCR	Sustained Cure Rate
SD	Standard deviation
SE	Standard error
SHEA	Society for Healthcare Epidemiology of America
SI	Standardized International
SIV	Site initiation visit
SOC	System organ class
SOP	Standard operating procedure
SS	Safety Set
t_{\max}	Time to C_{\max}
UBM	Unformed bowel movement
USPI	US Prescribing Information
VHP	Voluntary Harmonisation Procedure
VRE	Vancomycin-resistant enterococci
WHO	World Health Organization

SUBSTANTIAL GLOBAL AMENDMENT 3

Amendment rationale

This amendment applies to global protocol AC-061A303 Version 3 dated 27 October 2016. The resulting amended global protocol is Version 4 dated 1 March 2017.

The main reason for this amendment is to address agreed changes in the responses to Voluntary Harmonisation Procedure (VHP) list of grounds for non-acceptance, dated 10 February 2017.

The key requested modifications to the protocol include:

- The classification of the study, as the study includes a preliminary dose-finding part and a subsequent part assessing safety and efficacy.
- The addition of a separate summary section of known potential risks and benefits for cadazolid, for the investigator to form his/her own opinion about the benefit/risks to the patient in the current trial.
- Updates of the exclusion criteria (i.e., exclusion of subjects with hypersensitivity to cadazolid and to any of the excipients of vancomycin) for safety purposes.
- The exclusion of subjects with mental disorders, as this population is even more vulnerable in children.
- The clarification on the effectiveness of double-barrier contraception methods and on the variability of acceptance of such contraception measures between concerned countries.
- Updates on the section 'Premature discontinuation of study treatment' to clearly indicate that patients must be discontinued from the study in case of recognized fulminant or life-threatening *Clostridium difficile*-associated diarrhea, and to add clarifications on the statements.

Not related to VHP requests, some clarifications have been made regarding the dispensation of vancomycin oral solution, clarifications regarding some microbiology tests have been added, minor clarifications and inconsistencies have been addressed and some administrative changes have been updated.

Changes to the protocol

Two versions of the amended protocol will be prepared: 1) a clean version and 2) a Word comparison document, showing deletions and insertions in comparison to the previous protocol version.

Amended protocol sections

The main sections of the protocol affected by this amendment are listed below. Where applicable, the same changes have also been made to the corresponding sections of the protocol synopsis:

- 1.2.3 Summary of benefits and risks
- 2.4 Other objectives
- 3.1 Study design
 - 3.1.3.3 Follow-up period (28 to 32 days)
- 3.2 Study design rationale
- 4.1 Subject population description
- 4.4 Exclusion criteria
- 4.5.2 Acceptable methods of contraception
- 5.1.1.1 Dosing in Part A
- 5.1.6.3 Study treatment dispensing
- 5.1.8 Premature discontinuation of study treatment
- 5.2.5 Auxiliary medicinal products / auxiliary therapy
- 6.1.3.1 *C. difficile* culture and susceptibility
- 6.1.3.2 Vancomycin-Resistant *Enterococci* culture and susceptibility
- 6.1.3.3 Change in Intestinal flora
- 6.4.1 Palatability and acceptability
- 7.2.1.3 *C. difficile* test to confirm subject eligibility and in case of Recurrence
- 7.2.2.2 Site Stool Diary
- 7.2.3.1 Stool sampling for microbiology
- 7.2.3.2 *C. difficile* test in case of suspected Recurrence (removed)
- 7.2.3.2 *C. difficile* strain identification and susceptibility
- 7.2.3.3 VRE quantitative culture and susceptibility

-
- 7.2.3.4 Intestinal flora analysis
 - 7.2.5. Laboratory assessments
 - 9.2.5 Reporting procedures
 - 10.2.1.6 Other efficacy variables
 - 10.2.1.7 Susceptibility of VRE
 - 10.2.1.8 Change in intestinal flora composition from baseline up to EOS
 - 10.3.2.5 Analysis of the other efficacy variables
 - 12.12 Reporting of study results and publication

Summary of previous amendments

Amendment	Date	Main reason(s)
1	5-Nov-2015	Correct the EudraCT number
2	27-Oct-2016	To align the global protocol with the cadazolid Paediatric Investigation Plan (PIP) European Medicines Agency's (EMA) decision and to align with FDA requirements

PROTOCOL SYNOPSIS AC-061A303

TITLE	A prospective, multicenter study to investigate the pharmacokinetics, safety, and efficacy of cadazolid versus vancomycin in pediatric subjects with <i>Clostridium difficile</i> -associated diarrhea.
OBJECTIVES	<p>Primary objectives</p> <p>The primary objective of Part A is to determine the cadazolid dose in children from birth to < 18 years of age by investigating the safety, efficacy, and the systemic and fecal pharmacokinetics (PK).</p> <p>The primary objective of Part B is to assess the safety and efficacy of cadazolid in children from birth to < 18 years of age as compared with vancomycin.</p> <p>Secondary objectives</p> <p>Secondary objectives of Part A are to assess the efficacy of cadazolid in terms of Clinical Cure, Sustained Clinical Cure, and Recurrence.</p> <p>Secondary objectives of Part B are to assess the efficacy of cadazolid in terms of Sustained Clinical Cure, Recurrence, time to Recurrence, and time to resolution of diarrhea (ROD) as compared to vancomycin.</p> <p>PK and other objectives</p> <p>PK and other objectives are described in Section 2.3 and Section 2.4.</p>
DESIGN	<p>Prospective, multicenter Phase 2/3 study in children from birth to < 18 years of age with <i>Clostridium difficile</i>-associated diarrhea (CDAD).</p> <p>The study consists of two parts. In each part, the treatment period will consist of 10 days of treatment followed by a 28 to 32-day Follow-up period.</p> <p>Part A is a multicenter, open-label, dose-finding part, for which safety, PK and efficacy will be investigated in pediatric subjects treated with cadazolid twice daily (b.i.d.) for 10 days.</p> <p>At least 24 subjects will be enrolled sequentially by descending</p>

	<p>cohort age.</p> <p>Three adolescent subjects aged 12 to < 18 years will be enrolled first.</p> <p>After these adolescent subjects have completed Part A, enrolment will be temporarily put on hold and safety, PK and efficacy data will be reviewed by an Independent Data Monitoring Committee (IDMC) before 3 children aged 6 to < 12 years will be enrolled. Another 3 adolescent subjects will be enrolled to have an adequate sample size for PK assessments in this cohort.</p> <p><i>This approach allows the IDMC to review the data and make recommendations as to further dosing. Details are described in the IDMC charter.</i></p> <p>After these first 3 children aged 6 to < 12 years have completed Part A, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 children aged 2 to < 6 years will be enrolled.</p> <p>After the first 3 children aged 2 to < 6 years have completed Part A, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 infants aged 3 months to < 2 years will be enrolled.</p> <p><i>If the IDMC requires additional data to conclude on a dosing recommendation, an additional 3 children aged 6 to < 12 years and/or 2 to < 6 years may be enrolled.</i></p> <p>After the 3 infants aged 3 months to < 2 years have completed Part A, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 subjects aged from birth to < 3 months will be enrolled. Another 3 infants aged 3 months to < 2 years will be enrolled to collect adequate PK and safety data in this cohort.</p> <p>The final 3 subjects aged from birth to < 3 months will be enrolled after safety, PK and efficacy data of the first subjects of the same age have been reviewed by the IDMC.</p> <p>The flow of recruitment is displayed in Figure 1. The study design of Part A is depicted in Figure 2.</p> <p>Part B is multicenter, randomized, assessor-blinded,</p>
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	<p>parallel-group, active-controlled for which safety and efficacy data will be investigated in pediatric subjects treated with cadazolid b.i.d. or vancomycin for 10 days. At least 176 subjects will be randomized in a 3:1 ratio to cadazolid or vancomycin. The randomization will be stratified by age stratum.</p> <p>Part B will be initiated when:</p> <ul style="list-style-type: none"> • Data review from the 6 adolescents in Part A is complete and a dosing recommendation for adolescents is available AND • Safety and efficacy results from the 2 adult Phase 3 studies (AC-061A301 and AC-061A302) have been analyzed. <p>Randomization in each subsequent stratum will start when a dosing recommendation from the corresponding age group is available based on Part A.</p>
<p>PERIODS</p>	<p>There will be three periods in Parts A and B.</p> <p>Screening Period (up to 48 hours): Starts with the signature of the Informed Consent Form (ICF) and ends before the subject's enrolment in Part A or randomization in Part B (within 48 h of the signature of the ICF) on Day 1.</p> <p>Treatment Period (10 days of treatment): Starts on Day 1 with the first dose of study treatment once the subject is enrolled in Part A or randomized in Part B and ends on the day of the last dose of study treatment (End-of-Treatment [EOT]).</p> <p>The EOT visit will take place at Visit 3 on Day 10 or earlier in case of premature permanent discontinuation of study treatment. See Footnote 1 in Table 2 (Part A) or Footnote 1 in Table 3 (Part B), for special situations.</p> <p>Follow-up Period (28 to 32 days): Starts after the last dose of study treatment and ends between Day 38 and Day 42 (Visit 5). The End-of-Study (EOS) visit will take place at Visit 5.</p>

	Subject participation in the study will be up to 44 days.
PLANNED DURATION	The overall planned study duration is an estimated 48 months from First Subject First Visit (FSFV) in Part A to Last Subject Last Visit (LSLV) in Part B.
SITE(S) / COUNTRY(IES)	Approximately 70 sites in 20 countries, including the US, Canada, and Europe.
SUBJECTS / GROUPS	<p>Overall, at least 200 subjects will be enrolled:</p> <p>Approximately 156 subjects treated with cadazolid (at least 24 subjects in Part A and approximately 132 subjects in Part B), and approximately 44 subjects treated with vancomycin (in Part B). This corresponds to 176 randomized (3:1) in Part B.</p> <p>Part A:</p> <p>At least 24 subjects will be enrolled in five age cohorts:</p> <ul style="list-style-type: none">• Cohort A: From 12 to < 18 years (at least 6 subjects)• Cohort B: From 6 to < 12 years (at least 3 subjects; 3 additional subjects may be added to this cohort if recommended by the IDMC)• Cohort C: From 2 to < 6 years (at least 3 subjects; 3 additional subjects may be added to this cohort if recommended by the IDMC)• Cohort D: From 3 months to < 2 years (at least 6 subjects)• Cohort E: From birth to < 3 months (at least 6 subjects) <p>Subjects who discontinue the treatment before the PK sampling day will be replaced within the same cohort.</p> <p>Part B:</p> <p>At least 176 subjects will be randomized in a 3:1 ratio to cadazolid or vancomycin. Randomization will be stratified by age stratum:</p> <ul style="list-style-type: none">• Stratum F: From 12 to < 18 years (at least 20 subjects)• Stratum G: From 6 to < 12 years (at least 30 subjects)• Stratum H: From 2 to < 6 years (at least 30 subjects)• Stratum I: From 3 months to < 2 years (at least 20 subjects)• Stratum J: From birth to < 3 months (no minimum number)

INCLUSION CRITERIA	<p>Parts A and B:</p> <ol style="list-style-type: none">1. Signed informed consent by parents or legally authorized representatives (LAR) and assent by the child according to local requirements prior to initiation of any study-mandated procedure.2. Male or female from birth to < 18 years.3. A female of childbearing potential is eligible only if the following applies:<ol style="list-style-type: none">a. Negative pregnancy test at Screening.b. Agreement to undertake another pregnancy test 28–32 days after the last dose of study treatment.c. Agreement to use an adequate method of birth control described in Section 4.5 from Screening up to at least 30 days after study treatment discontinuation.4. Subject is diagnosed with CDAD. As a minimum there must be a positive detection, within 72 h prior to enrolment/randomization, of either<ol style="list-style-type: none">a. Enzyme Immuno-Assay (EIA) for glutamate dehydrogenase (GDH) plus EIA toxin A/B in stool orb. EIA for GDH plus Nucleic Acid Amplification Test/ Polymerase Chain Reaction for toxin A/B in stool orc. Positive cell cytotoxicity assay ord. Toxigenic cultureand:<ol style="list-style-type: none">e. For subjects from birth to < 2 years: Watery diarrhea within the 48 h period prior to enrolment/randomization.f. For subjects from 2 years to < 18 years: ≥ 3 unformed bowel movements (UBMs) in a 24 h period within the 48 h period prior to enrolment/randomization.
EXCLUSION CRITERIA	<p>Parts A and B:</p> <ol style="list-style-type: none">1. Positive Rotavirus test for subjects < 5 years.2. Fulminant or life-threatening CDAD. If in the judgment of the investigator there is a suspicion of fulminant or life-threatening CDAD, the presence of any of the following criteria during the 72 h period prior to enrolment/randomization and related to the fulminant or life-threatening CDAD episode excludes the potential subject from the study:

	<ol style="list-style-type: none">a. Septic shock based on the investigator judgmentb. Peritonitisc. Ileusd. Toxic megacolone. Significant dehydration based on investigator judgmentf. White blood cells count > 30.0 10⁹/Lg. Core body temperature > 40 °C <ol style="list-style-type: none">3. More than one previous episode of CDAD in the 3-month period prior to enrolment/randomization.4. Antimicrobial treatment active against CDAD administered within 24 h prior to Screening except for metronidazole treatment failures (MTF).5. Subjects with body weight < 3 kg.6. Life expectancy less than 30 days from any cause.7. Inflammatory bowel disease, chronic abdominal pain, or chronic diarrhea of any etiology.8. Planned treatment with forbidden concomitant medications.9. Fecal microbiota transplant (FMT), immunoglobulin therapy, or any investigational drug to prevent or treat CDAD within 1 month period (or 5 half-lives in case of investigational drug, whichever is longer) prior to enrolment/randomization.10. Subject has received an investigational therapy within 1 month prior to enrolment/randomization except primary therapy for cancer with non-novel mechanisms of action and that do not affect the assessment of diarrhea.11. Monoclonal antibodies against <i>C. difficile</i> within 6 months prior to enrolment/randomization.12. Previous vaccination against <i>C. difficile</i>.13. Known hypersensitivity or contraindication to cadazolid, oxazolidinones, quinolones, or drugs of the same class, or any of their excipients.14. Females who are breastfeeding, or pregnant or planning to become pregnant during the study.15. Any circumstances or conditions, which, in the opinion of the investigator, may affect the subject's full participation in the study, or compliance with the protocol.16. Known mental disorders.
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	<p>Part B: All listed above exclusion criteria and the following additional exclusion criteria: 17. Known hypersensitivity or contraindication to vancomycin or any of its excipients. 18. Subject was screened for Part A.</p>																								
<p>STUDY TREATMENTS</p>	<p>Investigational treatment Oral cadazolid provided as granules in five strengths (250, 200, 150, 100, and 50 mg) for oral suspension taken b.i.d. with or without food.</p> <p>Part A: For subjects ≥ 12 years to < 18 years: 250 mg b.i.d. for 10 days. Subjects from birth to < 12 years: anticipated starting doses are based on weight categories (as shown in the table below). Doses may be adjusted depending on PK and safety results of the previously enrolled subjects.</p> <table border="1" data-bbox="618 1094 1442 1377"> <thead> <tr> <th>Weight (kg)</th> <th>Cadazolid dose per day (mg/day)</th> <th>Cadazolid dose per dosing (mg/dose)</th> <th>Total volume administered per dosing (mL)</th> </tr> </thead> <tbody> <tr> <td>3 to ≤ 5</td> <td>100</td> <td>50</td> <td>5</td> </tr> <tr> <td>> 5 to ≤ 10</td> <td>200</td> <td>100</td> <td>10</td> </tr> <tr> <td>> 10 to ≤ 15</td> <td>300</td> <td>150</td> <td>15</td> </tr> <tr> <td>> 15 to ≤ 20</td> <td>400</td> <td>200</td> <td>20</td> </tr> <tr> <td>> 20</td> <td>500</td> <td>250</td> <td>25</td> </tr> </tbody> </table> <p>Part B: Cadazolid b.i.d. for 10 days; dose to be determined based on PK and safety results from Part A.</p> <p>Comparator Vancomycin for 10 days either as oral capsule of 125 mg four times a day (q.i.d.), or as oral solution at a dose of 40 mg/kg/day divided into 3 or 4 doses (max. 500 mg per day).</p>	Weight (kg)	Cadazolid dose per day (mg/day)	Cadazolid dose per dosing (mg/dose)	Total volume administered per dosing (mL)	3 to ≤ 5	100	50	5	> 5 to ≤ 10	200	100	10	> 10 to ≤ 15	300	150	15	> 15 to ≤ 20	400	200	20	> 20	500	250	25
Weight (kg)	Cadazolid dose per day (mg/day)	Cadazolid dose per dosing (mg/dose)	Total volume administered per dosing (mL)																						
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> 10 to ≤ 15	300	150	15																						
> 15 to ≤ 20	400	200	20																						
> 20	500	250	25																						
<p>CONCOMITANT THERAPY</p>	<p>Prohibited medications 1. Antimicrobial treatment active against <i>Clostridium difficile</i> or FMT up to EOS (Visit 5) unless provided for Clinical Failure or Recurrence.</p>																								

	<ol style="list-style-type: none">2. Other medication active against CDAD (e.g., cholestyramine, probiotics) up to EOS (Visit 5).3. Initiation of treatment with opiates or change in dose or regimen up to 2 days after EOT.4. Anti-peristaltic medications, kaolin, pectin and charcoal containing anti-diarrheal medication up to EOS (Visit 5). <p>Auxiliary medicinal products / auxiliary therapy</p> <p>At any time during the study treatment, if there is progressively worsening diarrhea, persistent fever > 38.3 °C, evidence of fulminant (severe-complicated) or life-threatening CDAD including the development of hypotension, septic shock, ileus, megacolon, or peritoneal signs, or hypersensitivity to study treatment, the investigator must consider the subject as Treatment Failure.</p> <p>In case of Clinical Failure or Recurrence, an antimicrobial treatment active against CDAD (or alternatively, FMT) should be initiated as recommended per local guidelines and/or physician judgment. Subject considered as a treatment failure must discontinue study treatment (but be followed up to EOS [Visit 5]).</p>
ENDPOINTS	<p>Primary efficacy endpoints</p> <p>There is no primary efficacy endpoint in Part A.</p> <p>The primary efficacy endpoint for Part B is Clinical Cure assessed by the blinded efficacy assessor and recorded in the CRF based on the following criteria:</p> <ul style="list-style-type: none">• Less than 3 UBMs (or no watery diarrhea if subject is < 2 years) per day for 2 consecutive days between first dose of study treatment up to EOT (inclusive), AND• Subject remains well up to EOT + 2 days (inclusive) based on investigator judgment, AND• No need for additional antimicrobial treatment active against CDAD between first dose of study treatment up to EOT + 2 days (inclusive).

	<p>Secondary efficacy endpoints</p> <p>Key secondary efficacy endpoints in Part A are Clinical Cure [as described in Section 6.1.1.1], Sustained Clinical Cure [Section 6.1.2.2], and Recurrence [Section 6.1.2.1] assessed by the investigator.</p> <p>Key secondary efficacy endpoints in Part B are Sustained Clinical Cure [Section 6.1.1.2], Recurrence [Section 6.1.2.1], time to Recurrence [Section 6.1.2.3], and time to ROD [Section 6.1.2.4] assessed by the blinded efficacy assessor.</p> <p>Other efficacy endpoints</p> <p>Other efficacy endpoints are described in Section 6.1.3.</p> <p>Safety endpoints</p> <p>Safety assessments will be evaluated during treatment (up to EOT) and the Follow-up period (up to EOS) after last dose of study treatment:</p> <ul style="list-style-type: none">• Deaths up to EOS• Serious adverse events (SAEs), adverse events (AEs) up to EOS.• Treatment-emergent AEs and SAEs up to 7 days after EOT.• AEs leading to premature discontinuation of study treatment.• Change from baseline** up to 7 days after EOT in vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], heart rate [HR]) and body weight.• Change from baseline** up to 7 days after EOT in hematology and blood chemistry.• Marked abnormalities in vital signs (SBP and DBP) up to 7 days after EOT (treatment-emergent).• Marked abnormalities in hematology, and blood chemistry up to 7 days after EOT (treatment-emergent). <p><i>**Baseline is defined as the last assessment prior to study treatment start.</i></p> <p>PK endpoints</p> <p>The primary endpoint of Part A will be based on plasma (pre-dose, and 1 h, 2 h, 4 h, and 12 h post-dose) and fecal concentrations of cadazolid.</p> <ul style="list-style-type: none">• The following endpoints will be derived by
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	<p>non-compartmental analysis of cadazolid plasma concentration-time profiles obtained at Visit 3.</p> <ul style="list-style-type: none"> ○ C_{max}, time to C_{max} (t_{max}), and area under the concentration-time curve during a dose interval (AUC_{τ}). ● Cadazolid fecal concentration will be measured at Visit 3. <p>In Part B, the cadazolid plasma concentration 2 h post-dose at Visit 3 (and fecal PK concentration if a stratum is administered a cadazolid dose that was not tested in subjects of the corresponding cohort in Part A) will be evaluated.</p> <p>Other endpoints</p> <p>Palatability of cadazolid (Part A and Part B) and vancomycin (Part B) formulations will be assessed at Day 1 and at Visit 3 using a 5-point facial hedonic scale.</p> <p>Acceptability of cadazolid and vancomycin formulations will be assessed through a 3-point categorical scale at Day 1 and at Visit 3.</p>
ASSESSMENTS	Refer to the schedule of assessments in Table 2 and Table 3.
STATISTICAL METHODOLOGY	<p>Analysis Sets</p> <p>Screened Analysis Set (SCRAS)</p> <p>This analysis set, SCRAS, includes all subjects who were screened and received a subject number.</p> <p>Full Analysis Set (FAS)</p> <p>The FAS includes all enrolled subjects assigned to a study treatment, whether or not they have received study treatment. Two FAS including subjects from Part A and from Part B, respectively, will be defined (FAS_A and FAS_B).</p> <p>Safety Set (SS)</p> <p>The SS includes all subjects who received at least one dose of study treatment in Part A or in Part B.</p> <p>Modified ITT Analysis Set (mITT)</p> <p>The mITT analysis set includes subjects in the FAS who have received at least one dose of study treatment and have a confirmed diagnosis of CDAD. Two mITT including subjects from Part A and from Part B, respectively, will be defined</p>

	<p>(mITT_A and mITT_B).</p> <p>Per-Protocol Analysis Set (PPS)</p> <p>The PPS will be prepared only for subjects in Part B and will include any subjects from the mITT without any major protocol deviations. The PPS will be fully defined in the Statistical Analysis Plan (SAP).</p> <p>Efficacy analyses will be performed on the mITT and the PPS analysis sets.</p> <p>PK Sets (PKS)</p> <p>Part A</p> <p>The plasma PK Set A (PKS_A) comprises all subjects treated with cadazolid included in the FAS who were able to provide at least evaluable pre-dose, 2 h and 12 h post-dose samples.</p> <p>The stool PK Set A1 (PKS_A1) comprises all subjects treated with cadazolid included in the FAS_A who were able to provide an evaluable stool sample.</p> <p>Part B</p> <p>The plasma PK Set B (PKS_B) comprises all subjects treated with cadazolid included in the FAS who were able to provide an evaluable 2 h PK sample (\pm 1 h).</p> <p>The stool PK Set B1 (PKS_B1) comprises all subjects treated with cadazolid included in the FAS_B who were required and able to provide an evaluable stool sample.</p> <p>Description of Statistical Analysis</p> <p>The objective of the study is to evaluate PK, safety and efficacy.</p> <p>Overall testing strategy</p> <p>As planned efficacy analyses are exploratory, no formal hypothesis testing is planned and consequently there is no overall testing strategy.</p> <p><u>General statistical methods</u></p> <p>Descriptive statistics will be used to summarize all endpoints. Listings will be prepared on the FAS_A and FAS_B analysis</p>
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	<p>sets.</p> <p>For the analyses conducted using data from part A, the results will be presented by age cohort, unless otherwise specified.</p> <p>For the analyses conducted using data from part B, the results will be presented by treatment group (cadazolid or vancomycin), unless otherwise specified.</p> <p>The full details of the analyses will be provided in the SAP that will be finalized before database closure.</p> <p><u>Safety variables</u></p> <p>All safety variables will be summarized with descriptive statistics using the SS.</p> <ul style="list-style-type: none">• AEs up to EOS.• SAEs up to EOS.• Treatment-emergent AEs up to 7 days after EOT.• Treatment-emergent SAEs up to 7 days after EOT.• AEs leading to permanent discontinuation of study treatment. <p>All AEs and SAEs will be coded using the MedDRA dictionary version 19 or higher.</p> <p>All AEs will be listed by treatment group, age cohort, and subject in chronological order (using onset date).</p> <p>Deaths</p> <p>The primary reason for death as reported in the Death electronic case report form will be listed and summarized in a frequency table, by treatment group.</p> <p>Vital Signs and Laboratory parameters</p> <p>Descriptive summary statistics by visit and treatment will be provided for absolute values and changes from baseline in SBP, DBP, HR, body temperature, and body weight as well as in all laboratory parameters. The number of subjects with at least one marked abnormality up to EOT + 7 days (treatment-emergent) for the vital signs and laboratory parameters, will be summarized with counts and percentages. Treatment-emergent abnormality is defined as an abnormality post first dose study</p>
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	<p>treatment not present at baseline. The analyses of laboratory values will be performed on Standardized International (SI) units. If laboratory values are received in a different unit, they will be converted to SI before being analyzed.</p> <p>Treatment exposure and compliance</p> <ul style="list-style-type: none">• Total study treatment dose (mg) is defined as the sum of all doses received during the treatment period.• Duration of exposure to treatment for cadazolid and vancomycin (days) is defined as the time elapsing between study treatment initiation and discontinuation, inclusive.• Compliance will be described in terms of the percent of doses taken out of planned doses. <p>Treatment exposure and compliance variables will be summarized descriptively for Part A and Part B.</p> <p><u>Efficacy variables</u></p> <p>The following efficacy variables will be evaluated:</p> <p>Clinical Cure Rate (CCR) and Sustained Cure Rate (SCR)</p> <p>Clinical Cure Rate (%) and Sustained Cure Rate (%) will be presented with their 95% 2-sided confidence limits (CLs) by treatment arm, in Part A on the mITT_A, and in Part B on the mITT_B and the PPS_B analysis set.</p> <p>Time to Resolution of Diarrhea (ROD) and Time to Recurrence</p> <p>Time to ROD and Time to Recurrence will be analyzed using Kaplan-Meier estimation and presented graphically for Part B on the mITT_B.</p> <p><u>Other variables</u></p> <p>Palatability and acceptability</p> <p>Palatability and acceptability of cadazolid and vancomycin formulations at Day 1 and at Visit 3, rated on a 5-point facial hedonic scale and a 3-point categorical scale, respectively, will be summarized per treatment using counts and percentages.</p>
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	<p>PK parameters</p> <p>Part A:</p> <p>PK endpoints: the PKS_A will be used.</p> <p>Concentration-time data of cadazolid in plasma:</p> <ul style="list-style-type: none">• Individual concentration-time data will be listed by age cohort, dose, subject, and time.• Concentration-time data will be summarized by dose presenting the number of observations, arithmetic mean, median, minimum, maximum, standard deviation (SD), standard error (SE), % coefficient of variation (%CV), and 95% CI of the arithmetic mean. <p>Cadazolid fecal concentrations:</p> <p>Fecal PK endpoints: the PKS_A1 will be used.</p> <ul style="list-style-type: none">• Individual fecal concentration will be listed by age group, dose and subject.• Fecal concentrations will be summarized by dose presenting the number of observations, arithmetic mean, geometric mean, median, minimum, maximum, SD, SE, %CV, 95% CI of the arithmetic and the geometric means, and the fold over MIC₉₀ for the geometric mean and for the observed minimum. <p>Derived plasma PK parameters of cadazolid:</p> <ul style="list-style-type: none">• Individual PK parameters (C_{max}, t_{max}, and AUC_{τ}) will be listed by dose and by subject.• C_{max}, t_{max}, and AUC_{τ} will be summarized by dose with arithmetic mean, geometric mean, minimum, median, maximum, SD, SE, coefficient of variation (CV) in %, and 95% CI of the arithmetic and geometric means (<i>for t_{max} the geometric mean and its 95% CI will not be calculated</i>) <p>Part B:</p> <p>PK endpoint: for cadazolid plasma concentration: The PKS_B will be used.</p> <p>Cadazolid plasma concentrations:</p> <ul style="list-style-type: none">• Individual concentration at 2 h will be listed by dose and by subject.
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	<ul style="list-style-type: none">Concentrations at 2 h will be summarized by dose presenting the number of observations, arithmetic mean, geometric mean, median, minimum, maximum, SD, SE, %CV, and 95% CI of the arithmetic and the geometric means. <p>Cadazolid fecal concentrations (if a stratum is administered a cadazolid dose that was not tested in subjects of the corresponding cohort in Part A):</p> <ul style="list-style-type: none">Fecal PK endpoints: the PKS_B1 will be used. The fecal concentrations will be listed and summarized as for Part A.
SAMPLE SIZE ASSUMPTIONS	Approximately 156 pediatric subjects treated with cadazolid at the proposed duration of treatment, 24 in Part A and 132 in Part B. Assuming that systemic absorption in children is as low as in adults, a database of approximately 156 pediatric subjects is adequate to characterize the safety profile of cadazolid, including gastrointestinal tolerability side effects that are the most likely with a non-absorbed medication and which occurred at a rate of approximately 4–5% in the adult Phase 2 study. Assuming that a treatment-emergent AE is not observed, there is 95% confidence that the upper bound of the true rate is at most 2%.
STUDY COMMITTEES	Independent Data Monitoring Committee (IDMC) An IDMC has overall responsibility for safeguarding the interests of subjects by monitoring PK, safety and efficacy data obtained in the study and making appropriate recommendations based on the reported data, thus ensuring that the study is being conducted with the highest scientific and ethical standards. The IDMC will be fully operational prior to enrolment of the first subject into the study. The composition and operation of the IDMC is described in the IDMC charter.

PROTOCOL

1 BACKGROUND

1.1 *Clostridium difficile*-associated diarrhea

1.1.1 Introduction

Clostridium difficile-associated diarrhea (CDAD), also known as *C. difficile* infection (CDI), is an infectious disease of the gastrointestinal tract. CDI usually occurs in patients with a history of antibiotic use that allows *C. difficile* to grow and elaborate virulent toxins that can cause intestinal inflammation and diarrhea.

C. difficile is a spore-forming Gram-positive anaerobic bacillus present throughout the environment. Acid-resistant spores are ingested and passed through the stomach to germinate in the small bowel and eventually colonize the colon where they can be a harmless component of normal gut flora. Some strains of *C. difficile* elaborate toxins, termed A and B, that cause disease by invading epithelial cells, altering their cytoskeleton and resulting in a disruption of the epithelial barrier. These toxins then invade the intestinal mucosa where they also function as potent immunostimulatory molecules promoting inflammation [Poutanen 2004, Kelly 2008]. The resulting CDI includes a wide spectrum of clinical presentations ranging from diarrhea to pseudomembranous colitis, to a fulminant, relapsing, and/or fatal colitis [Dallal 2002, Voelker 2010].

1.1.2 Epidemiology

The etiology, pathogenesis, evolution of infection, clinical manifestations, and treatment of CDI are similar in adults and children, however, the epidemiologic data in the pediatric population are very limited.

Children are born with a minimal intestinal microbiome that is unable to resist *C. difficile* colonisation. Consequently, rates of *C. difficile* colonisation in children < 1 year of age is at least 70% [Khalaf 2012] and with toxigenic strain colonisation is at least 50% [Cerquetti 1995, Jangi 2010]. Subsequently, *C. difficile* colonization decrease to adult levels in children aged 3–5 years old due to the development of a more adult microbiome able to resist *C. difficile* colonization and/or the progressive maturation of the immune system as demonstrated by the rise in serum antibodies to toxin A and B [Enoch 2011].

The incidence of CDI in children is lower than in adults [Lessa 2015]. The estimated number of CDI cases in children 1–17 years old in the US in 2011 was 16,900 (95% confidence interval [CI] 13,200–20,800) with an incidence per 100,000 persons of 24.2 (95% CI 18.7–29.7) [Lessa 2015]. The incidence per 100,000 persons in children 1-17 years old for community-associated CDI and healthcare associated CDI were 17.9 (95% CI 14.1–21.4) and 6.3 (95% CI 4.6–8.3), respectively [Lessa 2015]. Recent

data suggest that CDI is an increasingly prevalent infection in children although the age-specific epidemiology of CDI in the pediatric population remains poorly studied [Khanna 2013, Kim 2008, Zilberberg 2010]. Whether the changing epidemiology is due to the emergence of the epidemic hypervirulent strains is unclear.

Recognized risk factors for children acquiring CDI included antimicrobial therapy, use of proton pump inhibitors, repeated enemas, use of diapers, prolonged nasogastric tube insertion, gastrostomy and jejunostomy tubes, underlying bowel disease (e.g., Hirschsprung's disease), gastrointestinal tract surgery, renal insufficiency, and impaired humoral immunity. Published data suggest that a larger proportion of pediatric than adult CDI cases are community-associated infections [Lessa 2015, Zilberberg 2010], and that many of these cases lack the traditional risk factor of exposure to antimicrobial drugs though recent studies suggest higher antibiotic exposure (> 70%) and higher healthcare exposure than previously recognized [Morinville 2005, Khanna 2013].

1.1.3 Current CDAD therapies

Metronidazole and vancomycin are the mainstays of antibiotic therapy for adult and pediatric CDI and the choice of agent depends on the episode type (first episode or recurrence) and the severity of illness. As in adults, an estimated 20–30% of children experience a recurrence following antibiotic therapy for CDI though rates as high as 67% have been described [Khanna 2013, McFarland 2000, Morinville 2005].

Oral metronidazole is the drug of choice in children for initial treatment of the first episode of CDI and for first recurrence of mild-moderate CDI. It is rapidly absorbed from the upper small intestine and, although high levels are achieved in tissue, lower levels are found in the lower intestine. Metronidazole is associated with side effects, including nausea, metallic taste, emesis, extremity numbness, convulsive seizures, and neuropathy.

Oral vancomycin is the drug of choice for severe disease and second recurrence and those children who have not responded to metronidazole. It is not absorbed to a great extent after oral administration and achieve high gut concentrations; therefore infrequent side effects are noted. Clinically significant serum concentrations, especially with the use of higher than standard doses, have been reported in some patients. Concerns about the increasing frequency of vancomycin-resistant enterococci have limited its use.

Several factors, including the 027/BI/NAP1 epidemic hypervirulent strain and the recurrence rate, have contributed to the overall morbidity and mortality of CDI and have resulted in the re-emergence of *C. difficile* as a major global health problem. New drug therapy that reduces recurrence rates and spares the gut microbiome, or improves outcomes for patients infected with the epidemic hypervirulent strain or those with severe disease, remains a significant unmet medical need.

Fidaxomicin, an antibiotic belonging to the macrocyclic class of anti-bacterials, was approved in the EU and in US in 2011 for the treatment of CDAD in patients ≥ 18 years of age. It has proven to be comparable to vancomycin with regard to cure rates, and importantly, has demonstrated higher Sustained Cure Rate (SCR) [Louie 2011, Cornely 2012]. The decrease in recurrence rate is thought to be due to the reduced attenuation of colonic flora with fidaxomicin treatment compared with vancomycin treatment [Tannock 2010]. However, fidaxomicin's SCR against the virulent 027/BI/NAP1 strain was not better than vancomycin. The safety and efficacy of fidaxomicin in children aged below 18 years has not yet been established, but clinical trials are ongoing in the pediatric population.

In addition to other antimicrobials undergoing evaluation, alternative novel approaches including fecal microbiota transplant, monoclonal antibodies, and vaccines are under investigation for the treatment and prevention of CDI.

1.2 Study treatment(s)

1.2.1 Cadazolid

This section is a brief summary of available data about cadazolid that is relevant to the study. For more detailed information, please see the Investigator's Brochure (IB) [Cadazolid IB].

Cadazolid is a novel antibiotic with bactericidal activity against *C. difficile*, including hypervirulent and moxifloxacin-resistant strains such as 027/BI/NAP1. Minimal inhibitory concentrations (MICs) of cadazolid against clinical isolates of *C. difficile* range from 0.06 to 0.5 $\mu\text{g/mL}$. Cadazolid is a potent inhibitor of bacterial protein synthesis and strongly inhibits toxin synthesis in cultures of toxigenic *C. difficile* strains in a manner superior to vancomycin and metronidazole. In contrast to vancomycin, *in vitro* experiments demonstrate that cadazolid prevents sporulation in vegetative cells at sub-MIC concentrations.

Single oral doses of 30–3000 mg in the single-ascending dose study (AC-061-101) and multiple oral doses of 300–3000 mg twice daily (b.i.d.) for 10 days in the multiple-ascending dose study (AC-061-102) have been investigated in healthy subjects. In addition, an open-label study (AC-061-103) investigated a single oral dose of 3000 mg to subjects with severe CDAD. All oral doses of cadazolid were well tolerated. Systemic cadazolid exposures were extremely low in all studies with negligible quantities of drug detected in the urine. A maximum plasma concentration of 6.88 ng/mL was observed in the multiple-ascending dose study at a 3000 mg dose and the highest maximum plasma concentration (C_{max}) observed in study AC-061-103 was 7.25 ng/mL. Almost the entire oral dose was recovered as unchanged drug in the feces.

A double-blind, randomized Phase 2 study (AC-061A201) investigated the efficacy and safety of three doses of cadazolid (250 mg, 500 mg, and 1000 mg, orally, b.i.d.) and vancomycin (125 mg orally, four times a day [q.i.d.]) for 10 days in 84 adult subjects with CDAD. All oral doses of cadazolid were well tolerated. All cadazolid doses provided Clinical Cure Rates (CCR) similar to or higher than vancomycin at test-of-cure 24–72 h after the last dose of study treatment. Numerically higher or similar SCRs due to lower recurrence rates were observed in all cadazolid doses compared to vancomycin [Louie 2013]. Very low systemic exposure was also observed with the maximal individual plasma concentration of 18.9 ng/mL in the cadazolid 1000 mg b.i.d. dose group. Finally, the gut microbiome was less impacted by cadazolid treatment compared to vancomycin.

1.2.2 Vancomycin

Among the current mainstays of therapy for the treatment of pediatric CDI, oral vancomycin has been chosen as the comparator. It is the comparator for the ongoing cadazolid Phase 3 adult clinical trials and has been the comparator of choice in many contemporary adult clinical trials. Vancomycin has been proven efficacious in mild-moderate disease in the setting of clinical trials and is approved in many countries for the treatment of CDI without any qualifier on the severity of disease.

Emerging clinical evidence suggests that vancomycin is a better therapy than metronidazole. The use of oral vancomycin is recommended as the treatment of choice for severe CDI in adults by both ESCMID and SHEA/IDSA guidelines and for recurrent episodes of CDI in many countries [Bauer 2009, Cohen 2010], because of relatively high failure rates of metronidazole in recent reports and a slower clinical response to metronidazole compared with oral vancomycin treatment [Wilcox 1995, Musher 2005, O'Brien 2007, Zar 2007]. The recent tolevamer trials in which both vancomycin and metronidazole were used as control arms also suggested lower clinical success rates in metronidazole subjects compared to vancomycin subjects (72.7% versus 81.1%; Odds Ratio [95% CI]: 1.681 [1.114, 2.537], $p = 0.0134$) [Johnson 2012].

Vancomycin is a licensed antibiotic for the treatment of adults with CDAD in the US [Vancomycin USPI] and in Europe [Vancomycin SPC]. The recommended pediatric dose of vancomycin is 40 mg/kg/day in 3–4 divided doses (maximum, 2 g/day) for 7–10 days [Vancomycin USPI, Vancomycin SPC]. The safety and effectiveness in patients <18 years of age have not been established [Vancomycin USPI]. This high-dose vancomycin (maximum, 2 g/day) may result in higher systemic exposure and require the need for serum concentration monitoring, and is currently recommended for children with severe complicated or fulminant disease who have not improved on the standard dose of vancomycin (maximum, 500 mg/day) that is recommended for children with severe CDI [Cohen 2010, Crews 2015, Wood 2014].

Therefore, the comparator in this clinical trial is vancomycin. Vancomycin is provided either as oral capsules of 125 mg q.i.d. or as an oral liquid of 40 mg/kg/day in 3-4 divided doses (maximum, 500 mg/day) for 10 days.

See also Section 1.1.3 for information on vancomycin in the treatment of pediatric CDI.

1.2.3 Summary of benefits and risks

Summary of benefits

The AC-061A303 study will be performed in pediatric subjects with CDAD.

CDAD results from overgrowth of toxin-producing strains in the colon following disturbance of the normal protective enteric flora. The etiology, pathogenesis, evolution of infection, clinical manifestations, and treatment of *C. difficile* infections are similar in adults and children.

As in adults, there exists in the pediatric population a need for compounds with: (1) a novel mechanism of action, (2) better efficacy (i.e., lower recurrence rates and higher sustained cure rates [including in infections due to hypervirulent strains], reduced time to resolution of diarrhea, minimal effect on normal gut flora, and low potential for resistance development), and (3) potentially better safety in relation to systemic adverse reactions.

Cadazolid is a novel antibiotic with potent and bactericidal activity against *C. difficile*, including hypervirulent strains such as ribotypes 027 and 078. In *in vitro* studies, cadazolid was highly active against all strains of *C. difficile* tested, with MICs ranging from 0.03 to 0.5 µg/mL and MIC_{90s} of 0.125 to 0.5 µg/mL. Cadazolid is a potent inhibitor of bacterial protein synthesis and strongly inhibits toxin synthesis in cultures of toxigenic *C. difficile* strains in a manner superior to vancomycin and metronidazole. In contrast to vancomycin, *in vitro* experiments demonstrate that cadazolid prevents sporulation in vegetative cells at sub-MIC concentrations.

A double-blind, randomized Phase 2 study (AC-061A201) investigated the efficacy and safety of three doses of cadazolid (250 mg, 500 mg, and 1000 mg, orally, b.i.d.) and vancomycin (125 mg orally, q.i.d.) for 10 days in 84 adult subjects with CDAD. All oral doses of cadazolid were well tolerated. The observed effect of all doses of cadazolid on clinical cure rate was similar to, or higher than, with vancomycin. The observed sustained cure rate was higher for all doses of cadazolid than for vancomycin, suggesting a lower recurrence rate. While cadazolid decreased *C. difficile* viable counts and spore counts, its effect on other bacterial groups was minimal and either similar or less pronounced than for vancomycin.

Summary of risks

Based on *in vitro* data, cadazolid demonstrates a low propensity to induce spontaneous resistance development. No cross-resistance with fluoroquinolones (moxifloxacin) was found in *C. difficile*, enterococci or *Bacteroides* spp., and the propensity to induce linezolid resistance by cadazolid was also extremely low.

Intravenous (i.v.) administration of cadazolid over 14 days in dogs was associated with a reversible increase of liver enzymes without histological correlation at the highest dose tested (10 mg/kg/day). Intravenous administration of cadazolid did not show any potential for teratogenicity in rats or rabbits. The no-observed-effect level for maternal toxicity and embryo-fetal development was 10 mg/kg/day after daily i.v. administration of cadazolid from Days 6 to 17 of gestation (C_{\max} 6200 g/mL) in rats and 3 mg/kg/day after daily i.v. administration of cadazolid from Days 6 to 18 of gestation (mean C_{\max} 1450 ng/mL) in rabbits. For pre- and post-natal development including reproductive performance of the F1 generation (non-treated offspring) of pregnant dams (rats), the no-observed-adverse-effect level was 30 mg/kg/day after daily i.v. administration from Day 6 of gestation to Day 20 of lactation (mean C_{\max} 6520 ng/mL). The increase of liver enzymes in dogs and the observations in the pre- and post-natal developmental studies in rats are considered to be of negligible safety concern for the intended clinical use, as they occurred at systemic exposure several orders of magnitude higher than that observed after oral administration in healthy subjects and subjects with CDAD.

To date, 116 subjects (48 healthy subjects and 68 subjects with mild-to-severe CDAD) have been exposed to cadazolid at doses up to 3000 mg b.i.d., administered for 10 days.

Systemic absorption of cadazolid in subjects treated in these clinical studies was extremely low. A C_{\max} of 6.88 ng/mL was observed in the multiple-ascending dose study at a 3000 mg dose, and the highest C_{\max} observed in study AC-061-103 was 7.25 ng/mL. The majority of the oral dose (arithmetic mean: 81–93%) was recovered as unchanged drug in feces in healthy subjects (AC-061-101 and AC-061-102).

In Phase 1 and 2 studies, cadazolid was well tolerated. In the Phase 2 study, the majority of adverse events (AEs) were reported for no more than one subject in any individual dose group, and the incidence of AEs in the cadazolid groups showed no dose-dependent effect. Most of the treatment-emergent AEs were non-serious and of mild-to-moderate intensity. In this Phase 2 study, the incidence of AEs considered as treatment-related by the investigator was 13.6–25.0% across the cadazolid groups. These events included flatulence, headache and pruritus, each occurring in at least two cadazolid-treated subjects, and a report of bacterial disease carrier. In the vancomycin group, the incidence of AEs considered as treatment-related by the investigator was 31.8%.

Based on data from three completed Phase 1 studies, one Phase 2 study, and two ongoing blinded Phase 3 studies, no adverse drug reactions with cadazolid were identified.

In this Phase 2/3 AC-061A303 study in pediatric subjects, all potential subjects with CDAD will undergo a medical check-up at study start to confirm eligibility. In both parts of the study, subjects will receive study treatment for 10 days and will be followed-up until 28–32 days after the last dose of study treatment, including interviews (face-to-face or by telephone) daily during treatment and twice weekly thereafter. At any time during the course of treatment the investigator may consider stopping the study drug and switching to alternative therapy for CDAD.

An Independent Data Monitoring Committee (IDMC) will monitor safety, pharmacokinetic (PK) and efficacy data obtained in the study and make appropriate recommendations (including but not limited to dose recommendations) based on the reported data, thus ensuring that the study is conducted with the highest scientific and ethical standards. The IDMC will be fully operational prior to enrollment of the first subject into the study.

Conclusion

Cadazolid is a novel antibiotic which has demonstrated activity against *C. difficile* in nonclinical *in vitro* and animal studies. High concentrations of cadazolid in the intestine and very low systemic absorption make it an attractive candidate for the treatment of subjects with CDAD.

Based on an assessment of the available nonclinical and clinical data there appears to be no prohibitive safety issues associated with a 10-day b.i.d. oral administration of cadazolid to pediatric subjects with CDAD.

Provided the protocol is adhered to, careful observation and medical management will minimize any associated risk in this study.

Overall, the benefit/risk assessment for subjects participating in the proposed AC-061A303 study in pediatric patients with CDAD is considered positive.

1.3 Purpose and rationale of the study

Cadazolid has demonstrated activity against *C. difficile* in pre-clinical *in vitro* and animal studies. High concentrations of cadazolid in the intestine and very low systemic absorption make it an attractive candidate for the treatment of CDAD in man. The results of the Phase 2 study in adults have suggested clinical response similar to or numerically higher than vancomycin, and higher SCRs than vancomycin. Based on the Phase 2 results, a cadazolid dose of 250 mg b.i.d. for 10 days was chosen for further evaluation in the adult Phase 3 program which is currently ongoing. The program consists of 2 identical pivotal trials (AC-061A301 and AC-061A302) designed to evaluate the

efficacy and safety of cadazolid versus vancomycin in adult subjects with mild to severe CDAD. The selected dose is expected to optimize the clinical response and to limit/reduce the impact on the gut microbiome, leading to improved Sustained Cure relative to vancomycin.

The overall purpose of this study is to provide reassurance on the safety and efficacy of cadazolid in children from birth to < 18 years of age. The specific purpose of each part of the study is to first provide safety, PK and efficacy data to determine the cadazolid dose (Part A) and then to investigate the safety and efficacy of cadazolid versus vancomycin (Part B).

This pediatric clinical trial is not powered to demonstrate efficacy in children from birth to < 18 years. It is expected that there will be no difference between the adult and the pediatric populations regarding the pharmacodynamic effects of cadazolid. The pediatric assessment of PK, safety, and clinical endpoint data in conjunction with efficacy data from two adequate and well controlled trials in adults will be used to evaluate the efficacy and the benefit/risk of cadazolid in pediatric patients.

2 STUDY OBJECTIVES

2.1 Primary objective(s)

The primary objective of Part A is to determine the cadazolid dose in children from birth to < 18 years of age by investigating the safety, efficacy and the systemic and fecal PK.

The primary objective of Part B is to assess the safety and efficacy of cadazolid in children from birth to < 18 years of age as compared with vancomycin.

2.2 Secondary objectives

Secondary objectives of Part A are to assess the efficacy of cadazolid in terms of Clinical Cure, Sustained Clinical Cure, and Recurrence.

Secondary objectives of Part B are to assess the efficacy of cadazolid in terms of Sustained Clinical Cure, Recurrence, time to Recurrence, and time to resolution of diarrhea as compared to vancomycin.

2.3 Pharmacokinetic objectives

For the PK objective in Part A, see Section 2.1.

The PK objective in Part B is:

- to evaluate cadazolid plasma concentration 2 h post-dose at Visit 3.

- to evaluate cadazolid fecal PK profile at Visit 3 (*only applicable if a stratum is administered a cadazolid dose that was not tested in subjects of the corresponding cohort in Part A*).

2.4 Other objectives

Other objectives are:

- to evaluate the palatability and acceptability of cadazolid and vancomycin formulations.
- to evaluate the susceptibility of *C. difficile* to cadazolid, vancomycin, linezolid, moxifloxacin, metronidazole, and fidaxomicin.
- to evaluate the Vancomycin-Resistant Enterococci (VRE) count and susceptibility to a panel of antibiotics including cadazolid and vancomycin.
- To evaluate the change in intestinal flora composition including *Lactobacilli* and *Bifidobacterium*.

3 OVERALL STUDY DESIGN AND PLAN

3.1 Study design

The study is a prospective, multicenter Phase 2/3 study in children from birth to < 18 years of age with CDAD.

The study consists of two parts. In each part, the treatment period will be 10 days of treatment followed by a 28 to 32-day Follow-up period.

Regular visits to assess efficacy and safety are scheduled at Screening, Day 1, Day 5 (± 1 day), Day 10 ($- 2$ days), Day 13 (± 1 day), and Day 40 (± 2 days). The visits at Day 5 and Day 13 can be performed on site or by telephone. Visits for new episodes of diarrhea will be scheduled if required. Protocol-mandated procedures and assessments will be performed according to Table 2 and Table 3.

3.1.1 Part A

Part A is a multicenter, open-label, dose-finding part, for which safety, PK, and efficacy will be assessed in pediatric subjects treated with cadazolid b.i.d. for 10 days. At least 24 subjects will be enrolled sequentially in five age cohorts:

Three adolescent subjects aged 12 to < 18 years will be enrolled first.

After the completion of these 3 adolescent subjects, enrolment will be temporarily put on hold and safety, PK and efficacy data will be reviewed by an IDMC before 3 children aged 6 to < 12 years will be enrolled. Another 3 adolescent subjects will be enrolled to have an adequate sample size for PK assessments in this cohort.

- *This approach allows the IDMC to review the data and make recommendations as to further dosing. Details are described in the IDMC charter.*

After the completion of the 3 children aged 6 to < 12 years, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 children aged 2 to < 6 years will be enrolled.

After the completion of the 3 children aged 2 to < 6 years, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 infants aged 3 months to < 2 years will be enrolled.

- *If the IDMC requires additional data to conclude on a dosing recommendation, an additional 3 children aged 6 to < 12 years and/or 2 to < 6 years may be enrolled.*

After the completion of the 3 infants aged 3 months to < 2 years, enrolment in that cohort will be temporarily put on hold and safety, PK and efficacy data will be reviewed by the IDMC before 3 subjects aged from birth to < 3 months will be enrolled. Another 3 infants aged 3 months to < 2 years will be enrolled to collect adequate PK and safety data in this cohort.

The final 3 subjects aged from birth to < 3 months will be enrolled after safety, PK and efficacy data of the first 3 subjects of the same age been reviewed by the IDMC.

The flow of recruitment is displayed in Figure 1. The study design of Part A is depicted in Figure 2.

3.1.2 Part B

Part B will be multicenter, randomized, assessor-blinded, parallel-group, active-controlled for which efficacy and safety data will be assessed in pediatric subjects treated with cadazolid or vancomycin for 10 days.

At least 176 subjects will be randomized in a 3:1 ratio to cadazolid or vancomycin. The randomization of subjects will be stratified by age groups, with strata corresponding to the age cohorts defined for Part A.

Randomization in Part B for adolescent subjects will start when:

- Data review from the 6 adolescents in Part A is complete and dosing recommendation for adolescents is available
- AND
- Safety and efficacy results from the 2 adult Phase 3 studies (AC-061A301 and AC-061A302) have been analyzed.

Randomization in each subsequent stratum will start when a dosing recommendation from the corresponding age cohort is available based on Part A [see also Figure 1].

The study design of Part B is depicted in Figure 3.

Figure 1 Flow of recruitment

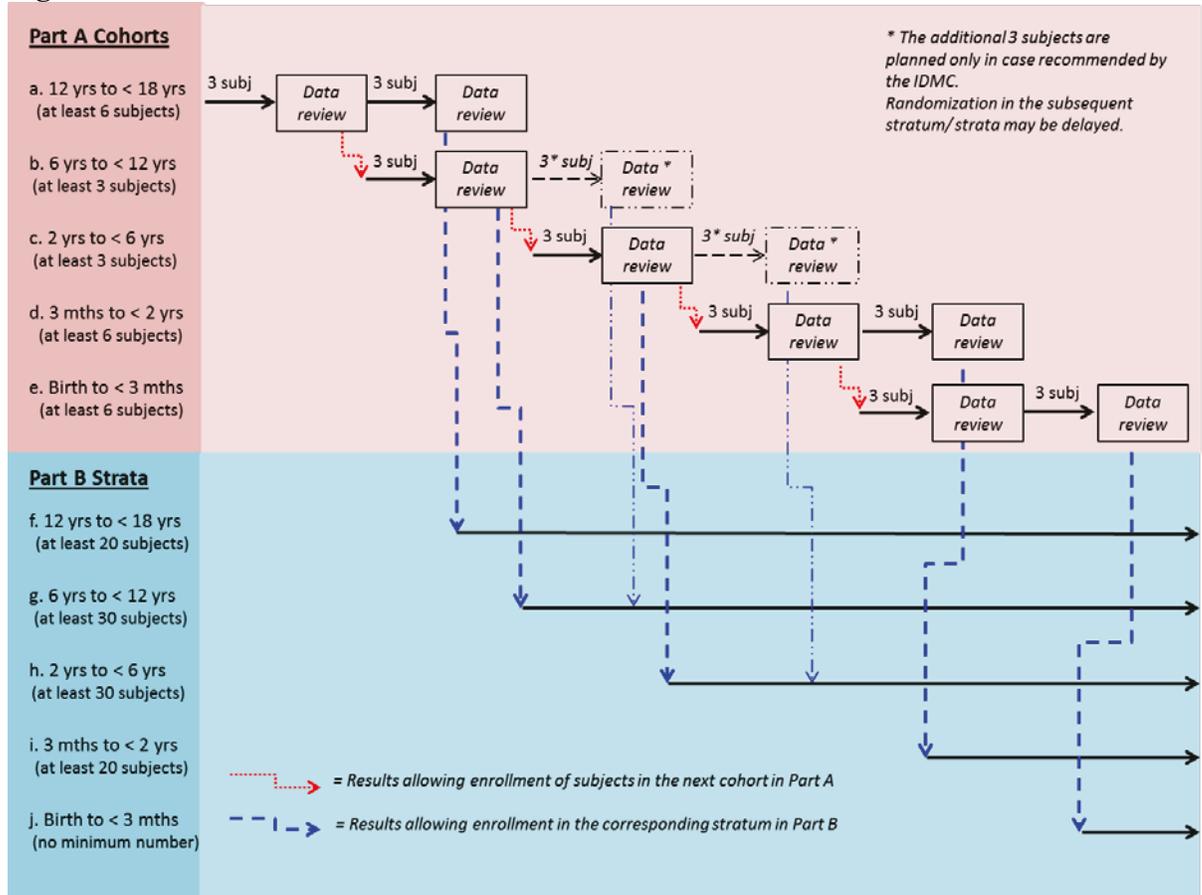


Figure 2 Study design of Part A

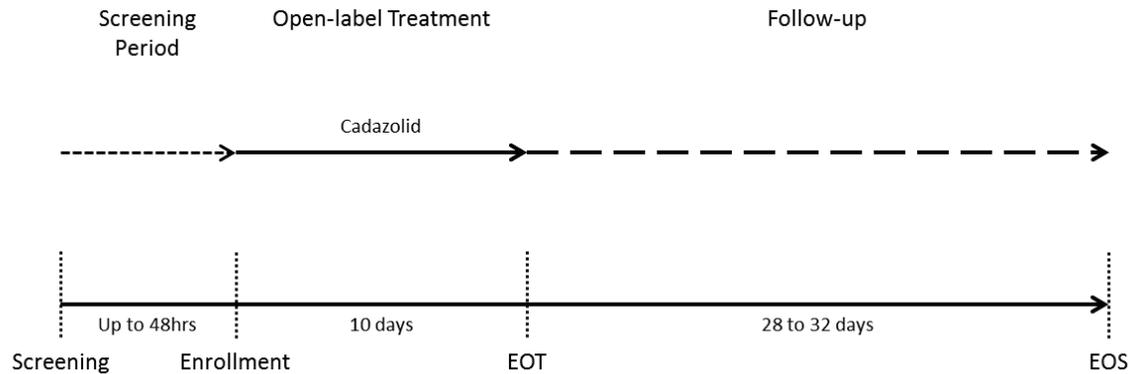
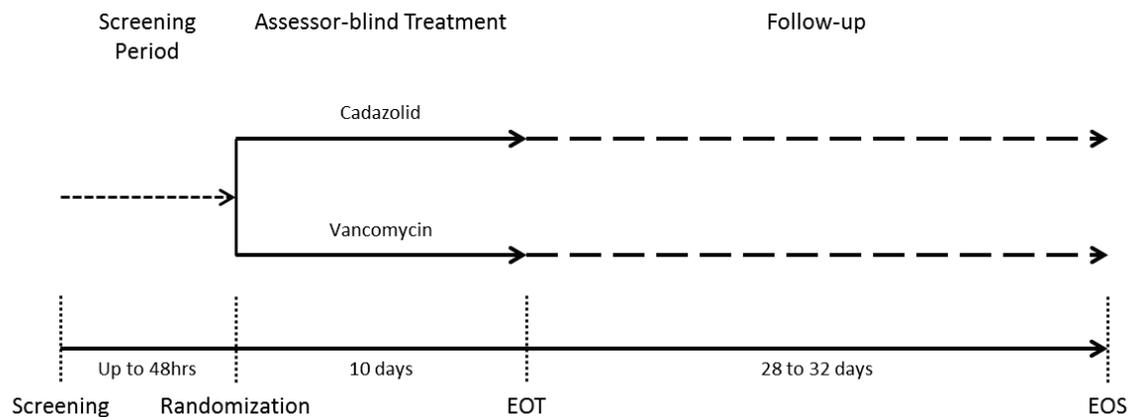


Figure 3 Study design of Part B



3.1.3 Study periods

There will be three study periods in Part A and Part B, respectively.

3.1.3.1 Screening period (up to 48 hours)

The Screening period starts with the signature of the Informed Consent Form (ICF) and ends before the subject's enrolment in Part A or randomization in Part B (within 48 h of the signature of the ICF) on Day 1.

3.1.3.2 Treatment period (10 days of treatment)

The Treatment period starts on Day 1 with the first dose of study treatment once the subject is enrolled in Part A or randomized in Part B and ends on the day of the last dose of study treatment (EOT).

The EOT visit will take place at Day 10 (Visit 3) or earlier in case of premature permanent discontinuation of study treatment. See Footnote 1 in Table 2 (Part A) or Footnote 1 in Table 3 (Part B), for special situations.

3.1.3.3 Follow-up period (28 to 32 days)

The Follow-up period starts after the last dose of study treatment and ends 28 - 32 days after the last dose of study treatment (Visit 5). In case of study withdrawal the EOS visit will be Visit 5.

Visits 4a, 4b, etc., to evaluate for Recurrence, occur when a subject experiences a New Episode of Diarrhea (NED) at any time between Day 13 (Visit 4) and Visit 5.

3.1.4 Study duration

The overall planned study duration is an estimated 48 months from First Subject First Visit (FSFV) in Part A to Last subject-Last visit (LSLV) in Part B.

Subject participation in the study (either Part A or Part B) will be up to 44 days.

3.2 Study design rationale

The study is a prospective, multicenter Phase 2/3 study in children with CDAD from birth to < 18 years of age.

Part A is a multicenter, open-label dose-finding part of at least 24 children from birth to < 18 years of age with confirmed CDAD to determine the cadazolid dose to be used in the second part of the study (Part B). Twice daily dosing of cadazolid for 10 days is an appropriate dosing and duration based on data from the adult clinical development program [see Section 1.2.1] and recommended duration of treatment from the CDI treatment guidelines [Cohen 2010] and the Committee on Infectious Diseases [Schutze 2013]. The design is considered appropriate and the sample size is sufficient to provide the necessary information including the systemic cadazolid exposure and fecal cadazolid concentrations needed to confirm the cadazolid doses to be used in Part B.

Part B is a multicenter, randomized, parallel-group, active-controlled part, randomizing at least 176 subjects (3:1 randomization ratio) stratified by age (with 5 different strata, corresponding to the age groups used for the cohorts of Part A). As specified in Section 1.1, the disease is the same in adults and children, and efficacy in the pediatric population can be partially extrapolated from adults, therefore a study not powered to demonstrate efficacy is acceptable. A placebo-controlled trial would not be ethical given the availability of effective treatments. Therefore, vancomycin will be utilized as the comparator. See also Section 1.2.2 for the justification for the choice of vancomycin as the comparator and for the dose selected.

Part B will utilize an assessor-blinded (single-blind) design for the following reasons:

1. The clinical efficacy endpoints are commonly accepted objective definitions of efficacy for CDAD. The introduction of a blinded efficacy assessor will further minimize the introduction of bias into the assessment.
2. Given that cadazolid and vancomycin are in different formulations, a double-blinding would require a double-dummy design that reduces the convenience for the subjects. In addition, palatability and acceptability of cadazolid need to be assessed in this study and a double-dummy design would complicate these assessments, especially in young children.

3.3 Site staff and their roles

Site personnel should have the appropriate medical and clinical expertise to perform the required study assessments.

It is recommended that the designated personnel remain unchanged throughout the entire course of the study and that an adequately trained back-up is designated in case of absence of any of the staff listed below.

3.3.1 Principal investigator

The principal investigator (PI) is responsible for the overall conduct of the study at the clinical site. It is her/his responsibility to assign appropriate personnel to the protocol-requested assessments (including safety, efficacy, and PK) and to define their roles. The PI oversees the accrual of appropriate subjects, the conduct of the study according to the trial protocol, and the collection of the required data.

3.3.2 Clinical coordinator/study nurse

Depending on the organization of the investigational site, a clinical coordinator / study nurse may be required to assist the PI in all aspects of subject management. She/he will be responsible for scheduling visits and assessments as planned in the study protocol, recording concomitant medications, maintaining source documentation and transcription of data into the electronic case report form (CRF). She/he will instruct the subjects (or their parents) on study treatment administration, and collect, process, and send all blood and fecal samples to the central laboratory (and to the analytical laboratory for PK samples).

3.3.3 Blinded efficacy assessor

Each site participating in Part B must have a designated physician who is fully blinded to a subject's treatment allocation (referred to as blinded efficacy assessor).

The blinded efficacy assessor must not have access to IRT (Interactive Response Technology), study treatment accountability logs, unblinding information in the CRF,

patient charts which indicate the actual treatment, or any other potentially unblinding information.

The blinded efficacy assessor must not be involved in any aspects of the subject's clinical care and management. She/he will perform all the efficacy assessments in Part B as detailed in Section 6.1.1 and Section 6.1.2 according to protocol schedule, in a blinded fashion.

A back-up blinded efficacy assessor may conduct those efficacy assessments as detailed above and must not be involved in the clinical care and management of the subject.

3.4 Study committees

An independent Data Monitoring Committee (IDMC) has overall responsibility for safeguarding the interests of subjects by monitoring safety, PK and efficacy data obtained in the study and making appropriate recommendations (including but not limited to dose recommendations) based on the reported data, thus ensuring that the study is being conducted with the highest scientific and ethical standards. The IDMC will be fully operational prior to enrolment of the first subject into the study. The composition and operation of the IDMC is described in the IDMC charter.

4 SUBJECT POPULATION

4.1 Subject population description

Study AC-061A303 will enroll children from birth to < 18 years with CDAD, which is defined by the presence of diarrhea and laboratory confirmation with a diagnostic *Clostridium difficile* test. Overall, at least 200 subjects will be enrolled in Part A and Part B.

In Part A, at least 24 subjects will be enrolled in five cohorts:

- Cohort A: From 12 to < 18 years (at least 6 subjects)
- Cohort B: From 6 to < 12 years (at least 3 subjects; 3 additional subjects may be added to this cohort if recommended by the IDMC)
- Cohort C: From 2 to < 6 years (at least 3 subjects; 3 additional subjects may be added to this cohort if recommended by the IDMC)
- Cohort D: From 3 months to < 2 years (at least 6 subjects)
- Cohort E: From birth to < 3 months (at least 6 subjects)

Subjects who discontinue the treatment before the PK sampling day will be replaced within the same cohort.

In Part B, at least 176 subjects will be randomized in a 3:1 ratio to cadazolid or vancomycin (approximately 132 cadazolid; 44 vancomycin), stratified by age.

- Stratum F: From 12 to < 18 years (at least 20 subjects)
- Stratum G: From 6 to < 12 years (at least 30 subjects)
- Stratum H: From 2 to < 6 years (at least 30 subjects)
- Stratum I: From 3 months to < 2 years (at least 20 subjects)
- Stratum J: From birth to < 3 months (no minimum number)

4.2 Rationale for the selection of the study population

The etiology, pathogenesis, evolution of infection, clinical manifestations, and treatment of CDI are similar in adults and children. Children with CDAD will be enrolled in the study based on the diarrhea criteria documented within a pre-specified period and a positive result from a *C. difficile* toxin assay, as suggested in the addendum to the guideline on the evaluation of medicinal products indicated for treatment of bacterial infections (EMA/CHMP/351889/2013) [EMA 2013]. Only subjects with first occurrence or first recurrence of CDAD will be eligible since treatment for subjects with multiple recurrences is not standardized by regimen, dose, and/or duration.

Risk factors for severe disease have been reported but there is no consensus on a specific CDI severity index in adults. Severe disease is often defined based on fever (temperature > 38.5 °C), leukocytosis (> 15,000 cells/mm³), or increase in serum creatinine (1.5 times above the premorbid level) [Bauer 2009, Cohen 2010].

In the pediatric population, children can experience severe disease and complications from disease. No adult severity index has been validated in children and use of adult criteria results in overestimation of disease severity [Pai 2012, Tschudin-Sutter 2014]. Complications such as the development of fulminant disease or death are uncommon [Crews 2015, Pai 2012, Tschudin-Sutter 2014, Kim 2012]. Fulminant CDAD is often unresponsive to medical therapy and a life-saving emergent colectomy is often required. The associated mortality rate in adults is up to 40% [Bhangu 2012]. Therefore, no severity criteria will be used in the trial; however, children with CDAD meeting the definition of fulminant (severe-complicated) CDAD within 72 h of enrolment will be excluded. Children who meet the criteria for fulminant (severe-complicated) CDAD on therapy will be discontinued from study treatment and treated as recommended per local guidelines and/or physician judgment.

In both Part A and Part B, the study population will be representative of children with laboratory confirmed CDAD. Subjects < 5 years of age with a positive Rotavirus test are excluded due to the high incidence of rotavirus infection in this age group. In Europe rotavirus is responsible for > 50% of hospitalizations in this age group [Ciccarelli 2013]. Children with inflammatory bowel diseases will be excluded.

The pathogenesis of CDAD is the same as in adults and children have similar risk factors for disease as adults. Similar to adults, risk factors for CDAD in the pediatric population include antibiotic therapy, healthcare exposure, immunosuppression, and acid-suppressive therapy. Other less traditional risk factors include rare intestinal conditions, such as Hirschsprung disease and primary immunosuppressive conditions. Subjects with immunosuppressive conditions or requiring immunosuppressive therapy and subjects with gastrointestinal abnormalities such as Hirschsprung disease will not be excluded from the pediatric study. Actelion will have investigators at tertiary care medical centres that potentially care for such at risk patient populations.

4.3 Inclusion criteria

For inclusion in the study, all of the following inclusion criteria must be fulfilled. It is not permitted to waive any of the criteria for any subject:

Parts A and B:

1. Signed informed consent by parents or legally authorized representatives (LAR) and assent by the child according to local requirements prior to initiation of any study-mandated procedure.
2. Male or female from birth to < 18 years.
3. A female of childbearing potential [see definition in Section 4.5.1] is eligible only if the following applies:
 - a. Negative pregnancy test at Screening.
 - b. Agreement to undertake another pregnancy test 28 - 32 days after the last dose of study treatment.
 - c. Agreement to use an adequate method of birth control described in Section 4.5 from Screening up to at least 30 days after study treatment discontinuation.
4. Subject is diagnosed with CDAD. As a minimum there must be a positive detection, within 72 h prior to enrolment/randomization¹, of either:
 - a. Enzyme Immuno-Assay (EIA) for glutamate dehydrogenase (GDH) plus EIA toxin A/B in stool or
 - b. EIA for GDH plus Nucleic Acid Amplification Test (NAAT) / Polymerase Chain Reaction (PCR) for toxin A/B in stool or
 - c. Positive cell cytotoxicity assay or

¹ Subjects who have failed at least 72 h of treatment of the current episode with metronidazole, continue to meet the definition of diarrhea without significant clinical improvement in the judgment of the investigator, and remain toxin positive (with a positive stool test done on a sample collected no more than 48 h prior to enrolment) may be enrolled in the study. Metronidazole therapy must be stopped prior to first dose of study treatment.

- d. Toxigenic culture
- and:
- e. For subjects from birth to < 2 years: Watery diarrhea within the 48 h period prior to enrolment/randomization.
 - f. For subjects from 2 years to < 18 years: ≥ 3 unformed bowel movements (UBMs) in a 24 h period within the 48 h period prior to enrolment/randomization.

4.4 Exclusion criteria

Subjects must not fulfill any of the following exclusion criteria. It is not permitted to waive any of the criteria for any subject:

Parts A and B:

1. Positive Rotavirus test for subjects < 5 years.
2. Fulminant or life-threatening CDAD. If in the judgment of the investigator there is a suspicion of fulminant or life-threatening CDAD, the presence of any of the following criteria during the 72 h period prior to enrolment/randomization and related to the fulminant or life-threatening CDAD episode excludes the potential subject from the study:
 - a. Septic shock based on the investigator judgment
 - b. Peritonitis
 - c. Ileus
 - d. Toxic megacolon
 - e. Significant dehydration based on investigator judgment
 - f. White blood cells count $> 30.0 \times 10^9/L$
 - g. Core body temperature $> 40^\circ C$
3. More than one previous episode of CDAD in the 3-month period prior to enrolment/randomization.
4. Antimicrobial treatment active against CDAD administered within 24 h prior to Screening except for metronidazole treatment failures (MTF) [see Note 1 in Section 4.3].
5. Subjects with body weight < 3 kg.
6. Life expectancy less than 30 days from any cause.
7. Inflammatory bowel disease, chronic abdominal pain, or chronic diarrhea of any etiology.
8. Planned treatment with forbidden concomitant medications.
9. Fecal microbiota transplant (FMT), immunoglobulin therapy, or any investigational drug to prevent or treat CDAD within 1 month period (or 5 half-lives in case of investigational drug, whichever is longer) prior to enrolment/randomization.

10. Subject has received an investigational therapy within 1 month prior to enrolment/randomization except primary therapy for cancer with non-novel mechanisms of action and that do not affect the assessment of diarrhea.
11. Monoclonal antibodies against *C. difficile* within 6 months prior to enrolment/randomization.
12. Previous vaccination against *C. difficile*.
13. Known hypersensitivity or contraindication to cadazolid, oxazolidinones, quinolones or drugs of the same class, or any of their excipients.
14. Females who are breastfeeding, or pregnant, or planning to become pregnant during the study.
15. Any circumstances or conditions, which, in the opinion of the investigator, may affect the subject's full participation in the study, or compliance with the protocol.
16. Known mental disorders.

Part B:

All listed above exclusion criteria and the following additional exclusion criteria:

17. Known hypersensitivity or contraindication to vancomycin or any of its excipients.
18. Subject was screened for Part A.

4.5 Criteria for female subjects of childbearing potential

4.5.1 Definition of childbearing potential

A female subject is considered to be of childbearing potential unless she meets at least one of the following criteria:

1. Previous bilateral salpingectomy, bilateral salpingo-oophorectomy, or hysterectomy.
2. Premature ovarian failure (confirmed by a specialist), XY genotype, Turner syndrome, uterine agenesis,
3. Pre-pubescence.

4.5.2 Acceptable methods of contraception

Females of childbearing potential [for definition, see Section 4.5.1] and who are sexually active must use one of the following methods of birth control from Visit 1 up to at least 30 days after study treatment discontinuation:

1. Diaphragm, female condom or cervical cap, partner's use of a condom, any of which must be used in combination with a spermicide (if spermicides are commercially available in the subject's country of residence).
 - Double barrier methods are not considered as highly effective (failure rate < 1%) contraceptive measures, and the acceptance of double barrier methods varies between concerned countries.

2. Intra-uterine devices.
3. Oral or injectable contraceptive agents, implants, or transdermal contraceptive hormone patches. If a hormonal contraceptive is used, it must have been taken for at least one month prior to enrolment/randomization.
4. Sterilization method (tubal ligation/occlusion, or partner's vasectomy).
5. True abstinence from intercourse with a male partner only when this is in line with the preferred lifestyle of the subject.

Rhythm methods are not considered acceptable methods of contraception for this study.

The methods of birth control used (including non-pharmacological methods) must be recorded in the CRF.

5 TREATMENTS

5.1 Study treatment

Study treatments include the investigational drug cadazolid and the active comparator vancomycin.

5.1.1 Investigational treatment: description and rationale

Oral cadazolid is provided by Actelion as granules in five strengths (250, 200, 150, 100, and 50 mg) for oral suspension to be reconstituted prior to administration. Cadazolid is supplied in an aluminum sachet. The inactive ingredients of the formulation are listed in the IB [Cadazolid IB].

5.1.1.1 Dosing in Part A

Based on similarities of the disease in children and adults and the very low systemic absorption of cadazolid, it is considered appropriate to initiate treatment of subjects from ≥ 12 years to < 18 years (*Cohort A*) with the dose for cadazolid (250 mg b.i.d.) similar to that tested in the ongoing Phase 3 studies in adults.

The anticipated starting doses of cadazolid in subjects from birth to < 12 years (*Cohort B to Cohort E*) are based on weight categories. Using as a reference the dose received by the lowest anticipated body weight of children of 12 years based on available Center for Disease Control (CDC) and UK/WHO growth charts (500 mg per day for a body weight of approximately 25 kg), it is proposed to initiate therapy at approximately 20 mg/kg/day, given as two daily doses of 10 mg/kg.

This weight category based dosing schedule is supported by simulations performed with a pediatric Physiologically Based Pharmacokinetic (PBPK) model (Gastroplus) that predicted that the systemic exposure obtained with a b.i.d. dose of 10 mg/kg will remain below the systemic concentrations observed in adults for all children aged from birth to 12 years old.

The anticipated starting dosing regimen based on weight categories for subjects < 12 years is shown in Table 1.

Table 1 Anticipated starting dosing regimen based on weight categories for subjects < 12 years

Weight categories (kg)	Cadazolid dose per day (mg/day)	Cadazolid dose per dosing (mg/dose)	Total volume administered per dosing (mL)
3 to ≤ 5	100	50	5
> 5 to ≤ 10	200	100	10
> 10 to ≤ 15	300	150	15
> 15 to ≤ 20	400	200	20
> 20	500	250	25

The daily doses are rounded up to adjust for the highest weight value of each weight category in order to ensure adequate concentrations.

The dose in any cohort may be adjusted based on accrued PK and safety information.

5.1.1.2 Dosing in Part B

Cadazolid b.i.d. for 10 days; dose to be determined based on PK and safety results from Part A.

5.1.2 Comparator: description and rationale

Vancomycin will be provided by Actelion as capsules of 125 mg and vancomycin powder for injection.

Vancomycin will be administered as oral capsule of 125 mg given q.i.d. for 10 days or as oral liquid at a dose of 40 mg/kg/day (500 mg/day maximum) in 3 or 4 divided doses for 10 days depending on the age of the child and ability to swallow capsules.

See also Section 1.2.2 for the rationale for the comparator chosen and the dose given.

5.1.3 Study treatment preparation and administration

Preparation and administration of, at least, the first dose of cadazolid prepared by the subject or the subject's parent(s) or LAR(s) must be done under the guidance of the site personnel.

If the subject is an out-patient or if subject is discharged from the hospital between Visit 1 and Visit 3, the subject's parent(s) or LAR(s) receive the instructions and reminder regarding study treatment intake and handling of study treatment.

5.1.3.1 Oral cadazolid suspension

Cadazolid granules (single dose sachet) will be administered as an oral suspension in water, which must be reconstituted prior to administration, in accordance with the following instructions:

1. Open the sachet and pour its content into a glass (or cup or beaker or goblet).
2. Add water to the glass: add approximately 12.5 mL (1 tablespoon) for the 250 mg sachet, 10 mL (2 teaspoons) for the 200 mg sachet, 7.5 mL (1 and ½ teaspoon) for the 150 mg sachet, 5 mL (1 teaspoon) for the 100 mg sachet, and 2.5 mL (½ teaspoon) for the 50 mg sachet.
3. Swirl immediately by hand with the base of the glass resting on a flat horizontal surface until a uniform suspension is obtained.
4. a) Drink the suspension.
or
b) Tilt the glass and take off the entire volume with a syringe and administer the suspension to the subject.
5. Repeat procedure from Step 2 to Step 4 with the remaining granules in the glass.

The oral suspension should be drunk within 5 minutes of reconstitution. If the suspension is not taken within 5 minutes of reconstitution, then it can be administered within the next 2 hours after having resumed at Step 3. It is recommended not to store the oral suspension in the fridge.

5.1.3.2 Cadazolid suspension for nasogastric delivery

If the suspension is to be delivered via nasogastric tube, see the following instructions:

- a. Prepare a cadazolid suspension as described in [Section 5.1.3.1] and continue with Step 4b.
- b. Connect the syringe containing the cadazolid suspension to the nasogastric tube.
- c. Press the plunger to empty all syringe contents.
- d. Repeat steps a) and b) after repeating cadazolid suspension preparation steps 2 to 4b.
- e. To clean, flush the nasogastric tube with water.

5.1.3.3 Oral vancomycin solution

The vancomycin powder for injection must be reconstituted as per package insert.

5.1.4 Treatment assignment

At the beginning of Visit 1 after the subject has signed the ICF:

At Screening, the investigator/delegate contacts the Interactive Voice/Web Response System (IRT) and obtains a subject number from the IRT which will identify the subject throughout the study. Subjects are allowed to be re-screened once if they failed eligibility

and were not enrolled in the study. The subject number assigned during the first screening procedure will be retained in re-screened subjects.

At the end of Visit 1, after having ensured that the responsible personnel/pharmacist is available to dispense the study treatment and that the subject fulfills all eligibility criteria, the investigator/delegate contacts IRT a second time and subjects will be assigned a:

- Part A: Treatment kit and a Subject number (it will be the same number obtained at Screening).
- Part B: Treatment kit and a Randomization number.

The randomization list is generated by an independent contract research organization (CRO), [REDACTED]. The randomization code is generated using SAS version 9.3 and is stratified by age.

5.1.5 Blinding

Site staff and subjects will not be blinded to treatment. However, at each site participating in Part B, there must be a blinded efficacy assessor who is fully blinded to a subject's treatment allocation [see Section 3.3.3].

5.1.6 Study treatment supply

Actelion will supply all study treatments to the site according to local regulations.

Manufacture, labeling, packaging, and supply of study treatment will be conducted according to Good Manufacturing Practice, Good Clinical Practice (GCP), and any local or national regulatory requirements.

All drug supplies are to be used only in accordance with this protocol, and not for any other purpose.

5.1.6.1 Study treatment packaging and labeling

Cadazolid will be provided as granules in sachet packs within a treatment kit. Each kit contains 24 sachets (10 days of treatment plus 2 reserve days) of adequate strength.

The following kits will be provided:

- 20 + 4 sachets of cadazolid 250 mg, granules for suspension
- 20 + 4 sachets of cadazolid 200 mg, granules for suspension
- 20 + 4 sachets of cadazolid 150 mg, granules for suspension
- 20 + 4 sachets of cadazolid 100 mg, granules for suspension
- 20 + 4 sachets of cadazolid 50 mg, granules for suspension

Vancomycin will be provided as capsules for children or powder for injection for children:

- 5 Blister packs containing capsules as provided by the supplier.
- Vials of vancomycin powder for injection (500 mg to be suspended in Water For Injection to prepare an oral solution).

Study treatment is labeled to comply with the applicable laws and regulations of the countries in which the study sites are located.

5.1.6.2 Study treatment distribution and storage

The investigator is responsible for safe and proper handling and storage of the study treatment at the investigational site and for ensuring that the study treatment is administered only to subjects enrolled in the study and in accordance with the protocol.

The study treatment kits must be stored at a temperature between 2 °C and 25 °C / 35.6 °F and 77 °F; do not refrigerate. It must be kept in a secure location, e.g., a locked room or a locked cupboard in a restricted access room, which can be accessed only by the pharmacist, the investigator, or another duly designated person as specified on the delegation of authority form. A temperature log is to be kept and temperature control should occur at least on a weekly basis at each location where study treatment is stored prior to subject dispensation. The adequacy of storage conditions should be documented. In the event of a temperature excursion, it must be immediately reported to the site Clinical Research Associate (CRA), preferably in writing and with supporting documentation (e.g., copy of the temperature log showing data for all excursion days). The CRA should immediately contact Actelion for further advice. The affected study treatment will not be used (e.g., it will be segregated physically at the study center) until confirmation from Actelion is obtained that its use is safe. In case the temperature deviation is outside of the acceptable limit, the study treatment is kept segregated at the study center and returned to Actelion following internal study treatment return processes. New study treatment supplies will be provided to the study center.

Temperature deviation correspondence must be kept in the Investigator Site File (ISF).

Subjects should store the medication at room temperature, less than 25 °C / 77 °F. The subject must be educated on the proper study treatment storage conditions at home.

Any temperature recording system routinely used at site is acceptable as long as all required information is included and certification of calibration is provided. If the temperature is captured electronically, a print-out should be made available to the CRA during each on-site visit.

The study treatment label is detached from the kit and placed on a Study treatment Label Dispensing Log. The time of study treatment administration and, in the event of the suspension, the time of preparation if the subject is an in-patient are recorded in the

source documents. The time of study treatment administration at Visit 3 is recorded in the CRF.

5.1.6.3 Study treatment dispensing

The subjects will receive sufficient study treatment to cover the 10-day treatment period and 2 additional days of treatment, if needed. All unused study treatment must be returned upon study treatment completion or discontinuation. An accurate record of the date and amount of study treatment dispensed to each subject must be available for inspection at any time.

If vancomycin is administered as oral solution, reconstitution and dispensing of the solution will be required every day.

Subjects are asked to return the treatment kit at Visit 3 [see Footnote 6 in Table 2 (Part A) or Footnote 7 in Table 3 (Part B), for special situations], including empty sachets, vials and blister packs, and all unused study treatment at each site visit. An accurate record of the date and amount of study treatment dispensed to each subject must be available for inspection at any time.

During the entire treatment period, a Study treatment Journal recording study treatment intake is completed by the study personnel based on the subject's parent(s) or LAR(s) interviews.

5.1.6.4 Study treatment return and destruction

On an ongoing basis (if required) or upon study completion or termination of the study at site level, the CRA will collect used and unused treatment kits, which will be sent to the warehouse, where Actelion personnel or a deputy will check treatment reconciliation. In certain circumstances, used and unused study treatment containers may be destroyed at the site once study treatment accountability is finalized and has been checked by Actelion personnel or the deputy, and written permission for destruction has been obtained from Actelion.

5.1.7 Study treatment accountability and compliance with study treatment

The inventory of each study treatment dispensed and returned (i.e., study treatment accountability) is performed by the study staff on the day of the subject visit. It is recorded on the IMP dispensing and accountability log and in the CRF and checked by the monitor during site visits and once each individual subject has completed the study. The study treatment accountability log in the CRF will include at least the following information for each study treatment (sachets/capsules/vials) dispensed to the subject:

- Kit number
- Date dispensed / number of sachets or capsules/vials dispensed

- Date returned / number of sachets or capsules/vials returned

All study treatment supplies, including partially used or empty, must be retained at the site for review by the monitor.

Study treatment compliance will be based on study treatment accountability.

Study treatment compliance will be calculated by site personnel when the IMP is returned, using the below formula, and entered in the CRF:

$$\text{Compliance} = \left[\frac{\text{(number of sachets/capsules/vials dispensed} - \text{number of sachets/capsules/vials returned)}}{\text{Total number of sachets/capsules/vials that should have been taken during the period}} \right] \times 100$$

The period is defined as current visit date – previous visit date + 1. The number of sachets/capsules/vials that should have been taken is derived from the number of treatment days.

Compliance is expected to be between 80% and 120%. Compliance values outside of this range will be considered as a protocol deviation, which will be reported in the CRF by the CRA.

The investigator must discuss the non-compliance with the subject to clarify the reasons and to take appropriate actions to avoid reoccurrence. This discussion and its outcome must be documented in the source documents. Permanent discontinuation of study treatment may be considered after consultation with Actelion.

5.1.8 Premature discontinuation of study treatment

The decision to prematurely discontinue study treatment may be made by the subject, the investigator, or Actelion. The main reason and whether discontinuation of study treatment is the decision of the subject, (e.g., tolerability- or efficacy-related), the investigator (e.g., due to pre-specified study treatment discontinuation criteria, an AE or lack of efficacy), or Actelion (e.g., study terminated) must be documented in the CRF.

A subject has the right to prematurely discontinue study treatment at any time by withdrawal from study treatment only or by withdrawal from any further participation in the study (i.e., premature withdrawal from the study; see Section 8.2).

At any time during the study, the study treatment must be discontinued if the subject experiences progressively worsening diarrhea, persistent fever > 38.3 °C, evidence of fulminant (severe-complicated) or life-threatening CDAD including the development of hypotension, septic shock, ileus, megacolon, peritoneal signs, or hypersensitivity to study treatment. The investigator must consider the subject as Clinical Failure.

In any case of Clinical Failure [Section 6.1.1.2] the investigator must discontinue study treatment (but follow up the subject until Visit 5 [EOS]) and an antimicrobial treatment active against CDAD (or FMT) should be initiated as recommended per local guidelines and/or physician judgment [Section 5.2.5].

In addition, the investigator should discontinue study treatment for a given subject if, on balance, he/she believes that continued administration would be contrary to the best interests of the subject. Such instances to warrant discontinuation of study treatment may include AEs, lack of efficacy (including disease progression), a protocol deviation (including eligibility failure, non-compliance with study requirements), a diagnostic or therapeutic procedure, a laboratory abnormality, or for administrative reasons.

In case of premature discontinuation of study treatment, Visit 3 is performed and the subject enters the Follow-up period. Every effort must be made to perform the Visit 3 assessments before initiation of alternative therapy for CDAD. Subjects prematurely discontinued from study treatment for any reason will not be replaced except in Part A if the PK samples were not collected.

A subject who prematurely discontinues study treatment is **NOT** considered as withdrawn from the study and will be followed up until Visit 5 (EOS), provided that the subject's consent for this limited participation in the study has not been withdrawn.

If a subject discontinues from study treatment but remains in the study, all subsequent visits (telephone or on site) will be performed according to protocol up to Visit 5 (EOS).

A subject who prematurely discontinues study treatment and withdraws consent to participate in any further study assessments is considered as withdrawn from the study. Subjects who die or are lost to follow-up are also considered as withdrawn from the study. Withdrawal from the study and follow-up medical care of subjects withdrawn from the study are described in Sections 8.2 and 8.4, respectively.

5.1.9 Study treatment dose adjustments and interruptions

Study treatment dose adjustments and/ or interruptions are not permitted.

5.2 Previous and concomitant therapy

5.2.1 Definitions

A previous medication is defined as a medication that was previously taken and was stopped prior to the first dose of study medication. A concomitant medication is defined as a medication that started, stopped, or was ongoing between first dose of study medication and Visit 5 (EOS).

5.2.2 Reporting of previous/concomitant therapy / auxiliary products in the CRF

The use of all study-concomitant therapy (including contraceptives and traditional and alternative medicines, e.g., plant-, animal-, or mineral-based medicines) will be recorded in the CRF.

Previous therapy must be recorded in the CRF if discontinued within 7 days or within 3 months (for antibiotics and acid suppressing medications) prior to signing of the informed consent.

The generic name, start/end dates of administration (as well as whether it was ongoing at start of treatment and/or EOS), route, dose, and indication will be recorded in the CRF.

5.2.3 Allowed concomitant therapy

Proton pump inhibitors and H2 blockers are allowed during the study.

5.2.4 Forbidden concomitant therapy

The following concomitant medications are prohibited during the study:

- Antimicrobial treatments active against CDAD or FMT up to Visit 5 (EOS) unless provided for Clinical Failure or Recurrence [Section 5.2.5]: Oral vancomycin (other than assigned comparator for subjects in Part B), metronidazole, bacitracin, fusidic acid, nitazoxanide, teicoplanin, tigecycline, fidaxomicin, rifampicin/rifampin or rifaximin.
- Other medication active against CDAD including probiotics, i.v. immunoglobulins, and binding agents (e.g., cholestyramine) up to Visit 5 (EOS).
- Initiation of treatment with opiates² after enrolment up to and including 2 days after the EOT.
- Anti-peristaltic medications (e.g., loperamide), kaolin or related products, pectin or charcoal-containing anti-diarrheals³ up to Visit 5 (EOS).
- Any investigational drug, antibody or vaccine to prevent or treat CDAD up to Visit 5 (EOS).

² In the event of ongoing opiate treatment at enrolment, a stable treatment or a decrease in the dose/regimen or discontinuation of treatment with opiates is allowed. For equivalence between opiates see APPENDICES Appendix 1.

³ The use of kaolin or related products, charcoal containing anti-diarrheals, or binding agents (e.g., cholestyramine) should be discontinued as soon as possible during the screening period for subjects likely to be enrolled because co-administration of kaolin, activated charcoal, and tolevamer with cadazolid resulted in a 4- to 8-fold increase in cadazolid MIC with kaolin to more than a 64-fold increase in cadazolid MIC with charcoal (Actelion data on file).

5.2.5 Auxiliary medicinal products / auxiliary therapy

At any time during the study treatment, if there is progressively worsening diarrhea, persistent fever > 38.3 °C, evidence of fulminant (severe-complicated) or life-threatening CDAD including the development of hypotension, septic shock, ileus, megacolon, or peritoneal signs, or hypersensitivity to study treatment, the investigator must consider the subject as Clinical Failure.

In case of Clinical Failure [Section 6.1.1.2] or Recurrence [Section 6.1.2.1], an antimicrobial treatment active against CDAD (or FMT) should be initiated as recommended per local guidelines and/or physician judgment. Subject considered as a treatment failure must discontinue study treatment (but be followed up to Visit 5 [EOS]).

6 STUDY ENDPOINTS

6.1 Efficacy endpoints

6.1.1 Primary efficacy endpoints

There is no primary efficacy endpoint in Part A.

The primary efficacy endpoint for Part B is Clinical Cure (at EOT + 2 days) assessed by the blinded efficacy assessor and recorded in the CRF.

6.1.1.1 Clinical Cure

Clinical Cure (at EOT + 2 days) is based on the following criteria:

- Less than 3 UBM (or no watery diarrhea if subject is < 2 years) per day for 2 consecutive days between first dose of study treatment up to EOT (inclusive),
AND
- Subject remains well up to EOT + 2 days (inclusive) based on investigator judgment,
AND
- No need for additional antimicrobial treatment active against CDAD between first dose of study treatment up to EOT + 2 days (inclusive).

6.1.1.2 Clinical Failure

Subjects who do not fulfill the requirements for Clinical Cure [Section 6.1.1.1], have a fatal serious adverse event (SAE), are lost to follow-up, or withdraw from the study prior to EOT + 2 days are considered Clinical Failures.

6.1.2 Key secondary efficacy endpoints

Key secondary efficacy endpoints in Part A are Clinical Cure [for definition see Section 6.1.1.1], Sustained Clinical Cure, and Recurrence assessed by the investigator.

Key secondary efficacy endpoints in Part B are Sustained Clinical Cure, Recurrence, time to Recurrence, and time to resolution of diarrhea assessed by the blinded efficacy assessor.

6.1.2.1 Recurrence

Recurrence (at EOT + 28 to 32 days) is based on the following criteria:

- Clinical Cure up to EOT + 2 days,
AND
- NED with ≥ 3 UBMs (or watery diarrhea for subjects < 2 years) on any day between EOT + 3 days and EOS,
AND
- Stool test showing positive *C. difficile* (as defined in Inclusion Criterion 4),
AND
- Antimicrobial treatment active against CDAD started between EOT + 3 days and EOS.

6.1.2.2 Sustained Clinical Cure

Sustained Clinical Cure (at EOT + 28 to 32 days) is based on the following criterion:

- Clinical Cure [Section 6.1.1.1] and no Recurrence [Section 6.1.2.1] until EOS.

Subjects with Clinical Failure, or with Clinical Cure and Recurrence, are considered to be without Sustained Cure.

6.1.2.3 Time to Recurrence

Time to Recurrence is defined as the time (in days) elapsed between the last dose of study treatment and the onset day of the NED component of the Recurrence endpoint.

6.1.2.4 Time to resolution of diarrhea

The date of Resolution of Diarrhea (ROD) is defined as the date of the first day of the 2 consecutive days on treatment with < 3 UBM (or no watery diarrhea for subjects < 2 years of age).

Time to ROD is the time (in days) elapsed between the first dose of study treatment and the ROD.

6.1.3 Other efficacy endpoints

For the other efficacy endpoints baseline is defined as the last assessment prior to study treatment start.

6.1.3.1 *C. difficile* culture and susceptibility

Susceptibility of *C. difficile* isolates to cadazolid, vancomycin, linezolid, moxifloxacin, metronidazole, and fidaxomicin will be evaluated at Screening, at EOT in the event of Clinical Failure, and at NED Visits in the event of Recurrence.

Change from baseline in susceptibility of *C. difficile* in case of Clinical Failure or Recurrence will be determined. Post-baseline MIC increases \geq 4-fold will be considered microbiologically relevant.

6.1.3.2 Vancomycin-Resistant Enterococci culture and susceptibility

Vancomycin-resistant *Enterococcus faecium* and vancomycin-resistant *Enterococcus faecalis* will be enumerated at Screening and EOT. Change in count from baseline up to EOT will be determined.

Susceptibility of VRE isolates to a panel of antibiotics including cadazolid and vancomycin will be assessed at Screening and EOT. Post-baseline MIC increases \geq 4-fold will be considered microbiologically relevant.

6.1.3.3 Change in Intestinal flora

Changes in intestinal flora composition including *Lactobacilli* and *Bifidobacterium* from baseline up to EOS will be determined. Fecal samples for intestinal flora composition analysis will be collected at Screening, EOT, NED, and EOS.

6.2 Safety endpoints

Safety will be evaluated during treatment and during the follow-up period of 28 to 32 days occurring after the last dose of study treatment. The following main secondary safety endpoints will be specifically evaluated:

- Deaths up to EOS.
- SAEs, AEs up to EOS.
- Treatment-emergent AEs and SAEs up to 7 days after EOT.
- AEs leading to premature discontinuation of study treatment.
- Change from baseline up to 7 days after EOT in vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], and heart rate [HR]) and body weight.
- Change from baseline up to 7 days after EOT in hematology, and blood chemistry parameters.
- Marked abnormalities in vital signs (SBP and DBP) up to 7 days after EOT (treatment-emergent).
- Marked abnormalities in hematology, and blood chemistry parameters up to 7 days after EOT (treatment-emergent).

Baseline is defined as the last assessment prior to study treatment start.

6.3 Pharmacokinetic endpoints

Systemic and fecal cadazolid concentrations will be analyzed to confirm that they are similar to the concentrations of adults in Phase 2.

Determination of plasma and fecal concentrations of cadazolid is the primary endpoint of Part A.

PK endpoints will be evaluated based on plasma (pre-dose, and 1 h, 2 h, 4 h, and 12 h post-dose) and fecal concentrations.

- The following endpoints will be derived by non-compartmental analysis of cadazolid plasma concentration-time profiles obtained at Visit 3.
 - C_{max} , time to C_{max} (t_{max}), and area under the concentration-time curve during a dose interval (AUC_T).
- Cadazolid fecal concentration will be measured at Visit 3.

In Part B, the cadazolid plasma concentration 2 h post-dose at Visit 3 (and fecal PK concentration if a stratum is administered a cadazolid dose that was not tested in subjects of the corresponding cohort in Part A) will be evaluated.

6.4 Other endpoints

6.4.1 Palatability and acceptability

- Palatability of cadazolid and vancomycin formulations at Day 1 and at Visit 3 using a 5-point facial hedonic scale.
- Acceptability of cadazolid and vancomycin formulations through a 3-point categorical scale at Day 1 and at Visit 3 whether the child swallowed the medication.

7 STUDY ASSESSMENTS

7.1 Study visits

The study visits are listed in Table 2 (Part A) and Table 3 (Part B). For all visits, the subjects must be seen or called on the designated day within the allowed visit window. A follow-up safety visit must be performed 28–32 days after intake of the last dose of study treatment.

In case of premature discontinuation of study treatment, the EOT visit must take place as soon as possible and no later than 7 days after the last dose of study treatment.

Subjects who prematurely discontinue study treatment for any reason will not be replaced except in Part A if the PK samples were not collected.

7.1.1 Screening/re-screening

Screening starts with the signature of the informed consent. The date on which the first screening assessment is performed corresponds to the date of the Screening Visit.

Screening Visit assessments can be performed up to 48 h prior to subject enrolment (Part A), or randomization (Part B).

Prior to performing any study-specific procedure or assessment, the subject must be consented. Pediatric subjects are legally unable to provide informed consent. Therefore, fully informed consent should be obtained from parent(s) or LAR(s) in accordance with national and/or local regulations. It is the responsibility of the investigator to obtain written informed consent from the subject's parent(s) or LAR(s) and assent by the child according to local requirements prior to initiation of any study-mandated procedure after adequate face-to-face explanation of the objectives, methods, and potential hazards of the study. The subjects who agree to participate in the study and the investigator/delegate must sign the ICF prior to any study-related assessment or procedure.

If a study-specific procedure or assessment has been performed as part of routine assessments and the results are available prior to the signing of the ICF, such procedure or assessment may be used to assess eligibility and does not have to be repeated. In such cases, it must be clear from the source document when and for which reason the assessment was done prior to the signing of the informed consent.

Subjects who are in screening when the enrolment target has been met may still be randomized.

It is permitted to re-screen subjects *once*, if the reason for non-eligibility was transient (e.g., abnormal laboratory test, insufficient wash-out period of a forbidden medication). All screening assessments should be repeated at the time of re-screening.

For subjects who failed screening, the reason for screen-failure will be recorded in the CRF if available:

7.1.2 Unscheduled visits

Unscheduled visits (Visits U1, U2, etc.) may be performed at any time during the study. Depending on the reason for the unscheduled visit (e.g., AE), appropriate assessments will be performed based on the judgment of the investigator and the results will be recorded in the CRF. After an unscheduled visit, the regular scheduled study visits must continue according to the planned visit and assessment schedule.

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Table 2 Visit and assessment schedule (PART A)

PERIODS	Name	SCREENING	TREATMENT			FOLLOW-UP			-
	Duration	Up to 48 h	10 days			28 to 32 days			-
VISITS	Number	1		2	3	4	4a, 4b...	5	U1, U2, ...
	Name	Screening	Enrolment	EOT ¹		NED		End of Study	Unscheduled
	Time	Day -2 to Day 1 (within 48 h)	Day 1	Day 5 (± 1 day) <i>On site or by telephone</i>	Day 10 (- 2 days) <i>incl. Premature Disc</i>	Day 13 (± 1 day) ² <i>On site or by telephone</i>	<i>If applicable, in case of Recurrence</i>	Day 40 (± 2 days) ² <i>or Study Withdrawal</i>	<i>If applicable</i>
Informed consent		X							
Eligibility		X	X						
Dispense kits and guidelines for fecal sampling		X			X		X		
Fecal sampling for microbiology		X			X		X	X	
Fecal sampling for PK					X				
<i>C. difficile</i> test		X ³					X		
Rotavirus test for children < 5 years		X							
Demographics, medical history		X							
CDAD disease characteristics		X	X						
Previous and concomitant medications		X ⁴	X	X	X	X	X	X	X
Physical examination		X			X		X	X ⁵	X
Height		X							
Body weight		X			X		X	X ⁵	X
Vital signs, body temperature		X			X		X	X ⁵	X
Palatability of study treatment			X		X				
Acceptability of study treatment			X		X				
Hematology and blood chemistry		X			X		X	X ⁵	X
Pregnancy test & contraception methods (females of childbearing potential only)		X						X	
Enrolment			X						
Study treatment dispensation			X						

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PERIODS	Name	SCREENING	TREATMENT			FOLLOW-UP			-
	Duration	Up to 48 h	10 days			28 to 32 days			-
VISITS	Number	1	2	3	4	4a, 4b...	5	U1, U2, ...	
	Name	Screening	Enrolment	EOT ¹		NED		End of Study	Unscheduled
	Time	Day -2 to Day 1 (within 48 h)	Day 1	Day 5 (± 1 day) <i>On site or by telephone</i>	Day 10 (- 2 days) <i>incl. Premature Disc</i>	Day 13 (± 1 day) ² <i>On site or by telephone</i>	<i>If applicable, in case of Recurrence</i>	Day 40 (± 2 days) ² <i>or Study Withdrawal</i>	<i>If applicable</i>
Study treatment intake			Daily						
Record time of study treatment intake					X				
Study treatment return					X ⁶				
Interview (face to face or by telephone) incl. site stool diary completion			Daily			X	Twice weekly		
Investigator assessment of Clinical cure						X			
Investigator assessment of Recurrence							X		
Investigator assessment of Sustained cure								X	
PK plasma sample					X				
AEs/SAEs		X	X	X	X	X	X	X	X
CDAD-related procedures (FMT, colectomy, hemi-colectomy)								X	

1. The treatment period may end on Day 10, or on Day 11 depending on the time of the day when the subject takes the first dose. In case Visit 3 is performed before Day 10, treatment must be continued until the subject has completed 10 days of treatment.
2. If treatment period ends on Day 11, Visit 4 is to be scheduled on Day 14 (± 1 day) and Visit 5 is to be scheduled on Day 41 (± 2 days).
3. To be performed within 72 h (or 48 h in case of MTF) prior to enrolment/randomization.
4. Previous and concomitant medications are only collected in the CRF if the subject is enrolled.
5. Assessments are not required unless indicated as follow-up for an AE/SAE.
6. Study treatment is to be returned at Visit 4 if Visit 3 is performed before Day 10.

AE = adverse event, BP = blood pressure, *C-difficile* = *Clostridium difficile*, EOT = End-of-Treatment, HR = heart rate, MTF = metronidazole treatment failure, NED = new episode of diarrhea, PK = pharmacokinetics, SAE = serious adverse event.

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Table 3 Visit and assessment schedule (PART B)

PERIODS	Name	SCREENING	TREATMENT			FOLLOW-UP			-
	Duration	Up to 48 h	10 days			28 to 32 days			-
VISITS	Number	1		2	3	4	4a, 4b...	5	U1, U2, ...
	Name	Screening	Randomization		EOT ¹		NED	End of Study	Unscheduled
	Time	Day -2 to Day 1 (within 48 h)	Day 1	Day 5 (± 1 day) <i>On site or by telephone</i>	Day 10 (- 2 days) <i>incl. Premature Disc</i>	Day 13 (± 1 day) ² <i>On site or by telephone</i>	<i>If applicable, in case of Recurrence</i>	Day 40 (± 2 days) ² <i>or Study Withdrawal</i>	<i>If applicable</i>
	Informed consent	X							
	Fecal sampling for microbiology	X			X		X	X	
	Eligibility	X	X						
	Fecal sampling for PK ³				X				
	Dispense kits and guidelines for fecal sampling	X			X		X		
	<i>C. difficile</i> test	X ⁴					X		
	Rotavirus test for children < 5 years	X							
	Demographics, medical history	X							
	CDAD disease characteristics	X	X						
	Previous and concomitant medications	X ⁵	X	X	X	X	X	X	X
	Physical examination	X			X		X	X ⁶	X
	Height	X							
	Body weight	X			X		X	X ⁶	X
	Vital signs, body temperature	X			X		X	X ⁶	X
	Palatability of study treatment		X		X				
	Acceptability of study treatment		X		X				
	Hematology and blood chemistry	X			X		X	X ⁶	X
	Pregnancy test & contraception methods (females of childbearing potential only)	X						X	
	Randomization		X						
	Study drug dispensation		X						

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PERIODS	Name	SCREENING	TREATMENT			FOLLOW-UP		-	
	Duration	Up to 48 h	10 days			28 to 32 days		-	
VISITS	Number	1		2	3	4	4a, 4b...	5	U1, U2, ...
	Name	Screening	Randomization	EOT ¹		NED		End of Study	Unscheduled
	Time	Day -2 to Day 1 (within 48 h)	Day 1	Day 5 (± 1 day) <i>On site or by telephone</i>	Day 10 (- 2 days) <i>incl. Premature Disc</i>	Day 13 (± 1 day) ² <i>On site or by telephone</i>	<i>If applicable, in case of Recurrence</i>	Day 40 (± 2 days) ² <i>or Study Withdrawal</i>	<i>If applicable</i>
Study treatment intake			Daily						
Record time of study treatment intake					X				
Study treatment return					X ⁷				
Interview (face to face or by telephone) incl. site stool diary completion			Daily			X	Twice weekly		
Blinded assessment of Clinical cure						X	X	X	
Blinded assessment of Recurrence							X		
Blinded assessment of Sustained cure								X	
Recording of CDAD-related procedures								X	
PK plasma sample⁸					X				
AEs/SAEs		X	X	X	X	X	X	X	X
CDAD related procedures (FMT, colectomy, hemi-colectomy)								X	

1. The treatment period may end on Day 10, or on Day 11 depending on the time of the day when the subject takes the first dose. In case Visit 3 is performed before Day 10, treatment must be continued until the subject has completed 10 days of treatment.
2. If treatment period ends on Day 11, Visit 4 is to be scheduled on Day 14 (± 1 day) and Visit 5 is to be scheduled on Day 41 (± 2 days)
3. Only to be collected if a stratum is administered a cadazolid dose that was not tested in subjects of the corresponding cohort in Part A.
4. To be performed within 72 h (or 48 h in case of MTF) prior to enrolment/randomization
5. Previous and concomitant medications are only collected in the CRF if the subject is randomized.
6. Assessments are not required unless indicated as follow-up for an AE/SAE.
7. Study treatment is to be returned at Visit 4 if Visit 3 is performed before Day 10.
8. Blood collection for plasma PK in part B required for patients in cadazolid arm only

AE = adverse event, BP = blood pressure, *C-difficile* = *Clostridium difficile*, EOT = End-of-Treatment, HR = heart rate, MTF = metronidazole treatment failure, NED = new episode of diarrhea, PK = pharmacokinetic SAE = serious adverse event.

7.2 Study assessments

7.2.1 Demographics / Baseline parameters

Demographic and baseline characteristic data to be collected on all subjects include: age, sex, race and ethnicity. Relevant medical history/current medical conditions (e.g., chronic and ongoing acute conditions, serious past conditions) present before signing informed consent will be recorded on the medical history page. Where possible, diagnoses and not symptoms will be recorded.

7.2.1.1 *Medical history of special interest*

Medical history of special interest will be captured on the specific Medical History CRF page and includes:

- Chronic medical conditions and new acute medical conditions in the past 6 months.
- Exposure to healthcare settings including outpatient office visits in the past 3 months.
- Antibiotic exposure in the past 3 months.
- Use of acid-suppressing medications in the past 3 months.
- History of chemotherapy and immunosuppression.
- History of gastrointestinal surgery including appendectomy.
- Number of daily bowel movements reported as usual by the subject or the parent, prior to the episode of CDAD.
- Serum creatinine, prior to the episode of CDAD, if available.

7.2.1.2 *Baseline CDAD disease characteristics*

Baseline CDAD disease characteristics will be captured on the specific CDAD Disease Characteristics CRF page and include:

- First CDAD occurrence or first Recurrence.
- MTF.
- Presence of pseudomembranes or histopathology classified as pseudomembranous colitis (for subjects having had endoscopy).
- Presence of distension of large intestine, colonic wall thickening, including low attenuation mural thickening, pericolonic fat stranding, or ascites not explained by other causes (for subjects having had imaging).
- In-patient or out-patient status at enrolment (Part A) or randomization (Part B).
- Number of bowel movements, including UBM, within 48 h during the screening period that qualified the subject for enrolment (Part A) or randomization (Part B).
- Abdominal physical examination (normal/abnormal and details) at enrolment (Part A) or randomization (Part B).

- *C. difficile* strain based on molecular testing at enrolment (Part A) or randomization (Part B).

7.2.1.3 *C. difficile* test to confirm subject eligibility and in case of Recurrence

At Visit 1, the result of a positive detection of *C. difficile* (EIA for GDH plus EIA toxin A/B in stool or EIA for GDH plus NAAT/PCR for toxin A/B genes in stool or positive cell cytotoxicity assay or toxigenic culture) performed locally is needed to document CDAD diagnosis and confirm subject eligibility.

If a test satisfying the inclusion criteria has been performed within 72 h of enrolment/randomization prior to Screening and returned a positive result, the test should not be repeated as this result will document eligibility.

If CDAD has been diagnosed prior to Screening using a test not satisfying the inclusion criteria, the test must be repeated after the subject has signed the ICF. The new test must satisfy the inclusion criteria and must show a positive result to confirm eligibility.

The assay for eligibility must be performed on a sample of stool (only on UBM or watery diarrhea) collected within the 72 h (48 h in case of MTF) prior to enrolment/randomization.

In case of suspected Recurrence during a NED, the same test used for eligibility is performed locally (only on UBM or watery diarrhea) to confirm Recurrence. If this is not possible or impractical then the test must be one that would satisfy the inclusion criteria.

The result of the test will be recorded in the CRF.

7.2.2 Efficacy assessments

7.2.2.1 Subject's parent(s) or LAR(s) interviews

After Visit 1, the investigator or site personnel (for Part A) or the blinded efficacy assessor (for Part B) perform an interview (face-to-face or by telephone) with the subject's parent(s) or LAR(s) every day up to Visit 4 and then twice weekly no more than 4 days apart until Visit 5. Interviews are performed to document the CDAD status of the subject and to collect stool information to be recorded in the site stool diary.

An interview log will be provided to investigators/study personnel to document site communication with the subject's parent(s) or LAR(s).

During the interviews the following information is discussed:

- Date and consistency of each bowel movement since last interview.
- Change in symptoms of CDAD since last interview.
- New concomitant medications or treatments since last interview.

- Compliance with study treatment.
- New AEs since last interview.

7.2.2.2 Site Stool Diary

During the subject's parent(s) or LAR(s) interviews (face-to-face or by telephone), the investigator or site personnel (for Part A) or the blinded efficacy assessor (for Part B) will collect and document stool information to be recorded in the site stool diary.

The investigator or site personnel (for Part A) or the blinded efficacy assessor (for Part B) records each bowel movement (or the presence or absence of < 3 UBMs if exact number is unknown), evaluates whether the bowel movement meets the definition of a UBM (i.e., taking the shape of the container in which it is produced), and determines the total number of stools in a site stool diary. For subjects from birth to 2 years of age, the site personnel will record the presence or the absence of watery diarrhea.

The full information from the site stool diary is entered in the Stool Log section of the CRF. The investigator is responsible for the accuracy of stool data entered into the CRF. The monitors performing source data verification ensure that the CRF entries match the site stool diary and the documentation of the daily subject's parent(s) or LAR(s) interviews.

The subject's parent(s) or LAR(s) and the study staff can determine when a NED happens and trigger a NED (Visit 4a, 4b, etc.) visit for assessment of Recurrence. A visit should be scheduled within 48 h.

7.2.2.3 Investigator or blinded efficacy assessor assessments

Investigator (for Part A) or blinded efficacy assessor (for Part B) assessments must be recorded in the CRF.

Based on the information gathered during daily subject's parent(s) or LAR(s) interviews [as defined in Section 7.2.2.1], and on clinical judgment, the investigator (for Part A) or blinded efficacy assessor (for Part B) will evaluate if the subject meets the definition of Clinical Cure [as defined in Section 6.1.1.1] at Visit 4. The information collected during the interviews will also allow the investigator or blinded efficacy assessor to assess ROD which is needed for the determination of Time to ROD [as defined in Section 6.1.2.4].

Based on the information gathered during subject's parent(s) or LAR(s) interviews, the investigator or blinded efficacy assessor will evaluate if the subject meets the definition of Recurrence [as defined in Section 6.1.2.1] at Visit 4a, 4b, etc., and Sustained Cure at Visit 5. The information collected during the interviews will also allow the investigator or blinded efficacy assessor to assess the Recurrence with its onset day.

7.2.3 Stool microbiology assessments

7.2.3.1 *Stool sampling for microbiology*

Fecal samples for microbiological analysis at the central laboratory will be taken at Screening, EOT, in case of NED (Visit 4a, 4b, etc.) and at EOS. If no stool is available on the day of the EOT visit, the NED visit, or the EOS visit, then a stool sample may be collected within 24 h after the visit and brought to the study site as described below.

The subject's parent(s) or LAR(s) will be instructed to collect the stool sample ideally within 24 h of the scheduled appointment or agreed upon drop-off time, and to store the sample in a refrigerator until departing for the study site. They should record the date and time at which the sample was produced. The samples should be delivered to the site using cooler bag and gel packs provided by the site.

At the study site, study staff will allocate stool that has been thoroughly mixed into two sample aliquots of 3–5 g or 3–5 mL for samples collected at Screening, EOT, and NED. For samples collected at EOS, the study staff will allocate stool that has been thoroughly mixed into one sample aliquot of 3–5 g or 3–5 mL. The investigator / site personnel will store the tubes containing stools from each sample at $-70\text{ }^{\circ}\text{C}/-94\text{ }^{\circ}\text{F}$ or below. In exceptional cases and after written confirmation by the sponsor, fecal samples for microbiology testing may be stored below $-20\text{ }^{\circ}\text{C} / -4\text{ }^{\circ}\text{F}$ in a frost-free freezer. Date and time at which the stool sample was produced are recorded on the tube labels and in the CRF.

In case of NED, only UBMs or watery diarrhea samples must be collected. If the stool sample is collected at the subject's place of residence, the subject's parent(s) or LAR(s) should call the study site immediately to make an appointment or arrangements to drop the sample at the study site as soon as possible.

The subject's parent(s) or LAR(s) will receive guidelines for fecal sampling and delivery to the study site.

The study sites will be instructed to ship the tubes at regular intervals, on dry ice, to the central microbiology laboratory. The tubes containing stools will be stored frozen at the central microbiology laboratory. Investigators will not receive results from the central microbiology laboratories but may be requested from the sponsor after the end of the trial.

7.2.3.2 *C. difficile strain identification and susceptibility*

This analysis will be performed on samples collected at Screening, EOT in the event of Clinical Failure, and NED in the event of Recurrence.

The central laboratory analyzes the feces received in the frozen tubes to isolate *C. difficile*. The *C. difficile* isolates are then tested for susceptibility against cadazolid, vancomycin, linezolid, moxifloxacin, metronidazole, and fidaxomicin in accordance with the Clinical and Laboratory Standards Institute (CLSI) testing standards.

The *C. difficile* isolates are shipped to specialty laboratories for typing of *C. difficile* strains including identification of epidemic hypervirulent strains currently defined as PCR ribotype 027, 078, or 244 [Freeman 2010, Lim 2014, De Almeida 2013]. New epidemic hypervirulent strains emerging during the course of the study may be added. Strain identification will also be used to differentiate relapse (*C. difficile* strain identical to the baseline strain) from re-infection (*C. difficile* strain different from the baseline strain) in subjects with Recurrence.

Investigators will not receive results from the central microbiology laboratory but results may be requested from the sponsor after the end of the trial.

7.2.3.3 VRE quantitative culture and susceptibility

This analysis will be performed on samples collected at Screening and EOT.

If adequate fecal sample is available, the central laboratory analyzes the feces received in the frozen tubes to culture and enumerate VRE isolates (*Enterococcus faecium* and *Enterococcus faecalis*). The VRE isolates are then tested for susceptibility against a panel of antibiotics including cadazolid and vancomycin, in accordance with the CLSI testing standards.

Investigators will not receive results from the central microbiology laboratories but results may be requested from the sponsor after the end of the trial.

7.2.3.4 Intestinal flora analysis

This analysis will be performed on samples collected at Screening, EOT, NED, and EOS.

If adequate fecal sample is available, the feces received in frozen tubes will be analyzed to assess changes in intestinal flora composition as compared to baseline, including changes in *Lactobacilli* and *Bifidobacterium*.

Investigators will not receive results from the central microbiology laboratories but results may be requested from the sponsor after the end of the trial.

7.2.4 Safety assessments

The definitions, reporting and follow-up of AEs, SAEs and pregnancies are described in Section 9.

7.2.4.1 Vital signs

Blood pressure (BP) and HR are to be measured at Screening, Visit 3, in case of a NED, at unscheduled visits (if applicable) and EOS (Visit 5)

SBP and DBP will be measured in a supine or sitting position using the same type of device throughout the study. It is recommended to allow the subject to rest for at least 5 minutes, and to use the same position (supine or sitting) throughout the study for an individual subject.

HR and BP are recorded in the CRF. Clinically relevant vital signs abnormalities which meet the definition of an AE [Section 9.1.1] must be recorded by the investigator on the AE page of the CRF.

Body temperature is measured or (if available in the subject medical chart) collected at Screening, Visit 3, in case of a NED, and at unscheduled visits (if applicable) and EOS (Visit 5) and must be recorded in the CRF (in case of multiple measurements available in a day the maximum temperature of the day must be entered in the CRF).

Recommended body temperature measurements are rectal, oral, ear, or axilla. Body temperature and site of measurement is recorded in the CRF. Conversion to core body temperature will be performed programmatically.

Calibration certificates for the sphygmomanometer and temperature measurement device must be available prior to the screening of the first subject.

7.2.4.2 Weight and height

Body weight will be measured in indoor clothing for subjects ≥ 2 years of age or in underclothes for subjects < 2 years of age but without shoes for all subjects at Screening, Visit 3, in case of a NED, at unscheduled Visits (if applicable) and EOS (Visit 5) and recorded in the CRF.

Height is to be measured at Screening and recorded in the CRF.

Body mass index (BMI) will be derived from height and weight.

Calibration certificates for the body weight scale must be available prior to the screening of the first subject.

7.2.4.3 Physical examination

A physical examination will be performed at Screening, Visit 3, in case of a NED, at unscheduled Visits (if applicable), and at EOS (Visit 5).

The observation should be reported according to body system in the CRF as either normal or abnormal. If an abnormality is found then it should be specified in the CRF page,

describing the signs related to the abnormality (e.g., systolic murmur) not the diagnosis (e.g., mitral valve insufficiency). Clinically relevant findings (other than those related to CDAD) that are present at Visit 1 must be recorded on the Medical History CRF page. Clinically relevant physical exam findings made after Visit 1 which meet the definition of an AE [Section 9.1.1], must be recorded by the investigator on the AE page of the CRF.

7.2.5 Laboratory assessments

7.2.5.1 Type of laboratory

A central laboratory (see central laboratory manual for contact details) will be used for all protocol-mandated laboratory tests, including re-tests due to laboratory abnormalities and laboratory tests performed at unscheduled visits. Central laboratory data will be automatically transferred from the central laboratory database to Actelion's clinical database.

At the time of Visit 1, local laboratory results are used for checking subject eligibility since central laboratory results would not be available within the 48 h screening period. If laboratory results have been performed as part of routine assessment and the results are available prior to the signing of the ICF, they can be used to assess eligibility and does not have to be repeated.

The local laboratory results (with the corresponding normal ranges) and pregnancy test will be recorded in the CRF.

Laboratory parameters required at Visit 1 are repeated at the central laboratory to be used as the baseline values for safety laboratory endpoints specified in the protocol.

Analysis of laboratory parameters for safety monitoring, including the routine monitoring of laboratory parameters and protocol mandated follow-up in case of abnormal values, is performed by the central laboratory.

In exceptional cases (e.g., subject is hospitalized in a different hospital from the study center due to a medical emergency, or missing central laboratory values) local laboratory results (with the corresponding normal ranges) must be entered into the clinical database via dedicated CRF pages.

In case a central laboratory sample is lost or cannot be analyzed for whatever reason, the investigator will collect an additional sample as soon as possible for repeat analysis, unless a local laboratory sample was collected within the same time-window and these test results are available.

Central laboratory reports, with the exception of microbiology results, will be sent to the investigator. In case of specific (pre-defined) laboratory abnormalities, the central laboratory will alert Actelion and the concerned site. All laboratory reports must be

signed and dated by the investigator or delegate within 10 working days of receipt and filed with the source documentation. The investigator or delegate must indicate on the laboratory report whether abnormal values are considered clinically relevant or not. Clinically relevant laboratory findings that are known at the time of signature of informed consent must be recorded on the medical history page of the CRF. Any clinically relevant laboratory abnormalities detected after signature of informed consent must be reported as an AE or SAE as appropriate [see Section 9], and must be followed until the value returns to within the normal range or is stable, or until the change is no longer clinically relevant. Further laboratory analyses should be performed as indicated and according to the judgment of the investigator.

Details about the collection, sampling, storage, shipment procedures, and reporting of results and abnormal findings can be found in the laboratory manual.

7.2.5.2 Laboratory tests

Hematology

- Hemoglobin (g/L)
- Hematocrit (L/L)
- Erythrocyte count ($10^{12}/L$)
- Leukocyte count with differential counts ($10^9/L$)
- Platelet count ($10^9/L$)

Clinical chemistry

- Alanine aminotransferase (ALT; U/L)
- Aspartate aminotransferase (AST; U/L)
- Alkaline phosphatase (U/L)
- Total and direct bilirubin ($\mu\text{mol}/L$)
- Creatinine ($\mu\text{mol}/L$)
- Blood urea nitrogen (mmol/L)
- Sodium, potassium, chloride, calcium (mmol/L)
- Protein (g/L)
- Albumin (g/L)

Pregnancy test

A urine or serum pregnancy test will be performed locally at Screening for females of childbearing potential to document eligibility at Screening prior to enrolment or randomization and at Visit 5 (EOS). Approximately 2.5 ml of blood will be collected to perform the serum pregnancy test.

If pregnancy is suspected during the study, a urine pregnancy test must be performed immediately.

7.2.5.3 Blood samples

Approximately 5 mL of blood[§] for the hematology and chemistry tests will be collected and sent to the central laboratory at each visit where laboratory parameters are assessed. The date and time of collection of samples for laboratory tests will be recorded in the CRF. Further details regarding blood sampling procedures, collection and shipment of samples and reporting of results are described in the central laboratory manual.

7.3 Pharmacokinetic assessments

7.3.1 Blood samples for PK assessments

7.3.1.1 Timing

The blood samples for PK assessment will be drawn at Visit 3 at the following time points:

- Part A: Morning pre-dose blood sample should be drawn immediately prior to morning study treatment administration, and then 1 h, 2 h, 4 h, and 12 h (prior to the evening administration) after cadazolid administration.
- Part B (only for subjects on cadazolid): 2 h post-dose blood sample (± 1 h).

The date and exact actual clock time of collection of each blood sample must be entered in the CRF.

The total blood volume to be taken for the PK assessments is $5 \times 1 \text{ mL} = 5 \text{ mL}$ in Part A and 1 mL in Part B.

7.3.1.2 Collection

Approximately 1 mL of blood^{**} will be collected in tubes containing EDTA via an i.v. catheter placed in an antecubital vein in the arm or via direct venipuncture. Anesthetic cream (e.g., EMLA) may be applied prior to placement of the catheter.

The indwelling catheter will be kept patent by insertion of a mandrin, which will be changed after each blood sampling or by flushing with saline. Immediately following collection of the required blood volume, the tubes will be slowly tilted backwards and forwards (no shaking) to bring the anti-coagulant into solution, and immediately cooled on ice. Within 30 min of collection, the tubes will be centrifuged at approximately 2000 g for 10 minutes at 2–8 °C. When a centrifuge that can be cooled is not available, the blood samples and the bucket of the centrifuge must be cooled on ice prior to centrifugation.

[§] The absolute minimum volume of blood required for complete hematology and chemistry tests is 3 mL (see central laboratory manual for details).

^{**} The absolute minimum volume of blood required for PK assessments is 0.5 mL.

The plasma will then be transferred into one labelled polypropylene tube, avoiding carry-over of erythrocytes. All samples will be stored in an upright position at -20°C or lower. The exact actual clock time of collection of the blood sample must be entered in the CRF.

7.3.1.3 Labeling

The tubes for both collecting blood for cadazolid determination and storage of plasma samples will be provided pre-labeled to each of the participating sites by the central laboratory before starting the study. They will provide fields to collect the following information:

Actelion Pharmaceuticals Ltd
AC-061A303 Plasma
Centre number / Subject number
Study day / scheduled time

7.3.1.4 Shipping procedures

The plasma samples for cadazolid determination must be shipped to the bioanalytical laboratory (either directly or via central lab) on a regular basis, as agreed by the sponsor. The samples must be packed securely with completed shipment forms, (detailing what the tubes contain, subject numbers, site number and date) in polystyrene insulated shipping containers, together with enough dry ice to keep the package frozen for at least 48 h. Arrangements must be made with the bioanalytical laboratory before the site sends the samples to the bioanalytical laboratory.

7.3.1.5 Bioanalysis

All samples from subjects on cadazolid will be analyzed by the bioanalytical laboratory. Cadazolid plasma concentrations will be determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. The foreseen limit of quantification (LOQ) is 0.25 ng/mL. Concentrations will be calculated by interpolation from a calibration curve. Quality control samples will be analyzed throughout the study, and their measured concentrations will be determined between-run and overall precision and accuracy of the analysis.

7.3.1.6 Determination of pharmacokinetic parameters

The PK parameters will be determined on the basis of actual blood sampling time points using a non-compartmental analysis.

Model-independent PK analysis will be performed under the responsibility of Actelion Pharmaceuticals Ltd using WinNonlin 6.3. or higher (Pharsight Corporation, Mountain View, CA, USA). The following PK parameters will be estimated from the individual plasma concentration versus time curves:

C_{\max}	is the maximum plasma concentration obtained directly from the measured plasma concentrations.
t_{\max}	is the time to reach C_{\max} .
AUC_{τ}	Area Under the concentration-time curve during one dosing interval τ . AUC_{τ} will be calculated according to the trapezoidal rule using the measured concentration-time values above the LOQ.

7.3.2 Fecal samples for PK testing

7.3.2.1 Timing

Part A: A fecal sample for PK testing will be collected at Visit 3 from each subject.

Part B: In case subjects in a stratum are administered a cadazolid dose that was not tested in subjects of the corresponding age in Part A, a fecal sample will be collected for confirmation of the expected PK profile of that stratum at Visit 3, and cadazolid stool concentration will be measured.

7.3.2.2 Collection

The subject's parent(s) or LAR(s) will be instructed to collect the stool sample for PK testing ideally within 24 h of Visit 3 or agreed upon drop-off time, and to store the sample in a refrigerator until departing for the study site. They should record the date and time at which the sample was produced. The samples should be delivered to the site using cooler bag and gel packs provided by the site.

At the study site, study staff will allocate stool that has been thoroughly mixed into a sample of 3–5 g or 3–5 mL. The investigator/ site personnel will store the tube containing the sample at -70°C / -94°F or below. Date and time at which the stool sample was produced are recorded on the tube labels and in the CRF.

The subject's parent(s) or LAR(s) will receive guidelines for fecal sampling and delivery to the study site.

7.3.2.3 Labeling

The tubes for fecal samples storage will be provided pre-labeled to each of the participating sites by the central laboratory before starting the study.

Actelion Pharmaceuticals Ltd
AC-061A303 stool
Centre number / Subject number
Study day

7.3.2.4 Shipping procedures

The fecal sample for PK testing of 3–5 g or 3–5 mL will be shipped on dry ice to the bioanalytical laboratory (either directly or via central lab)^{††}.

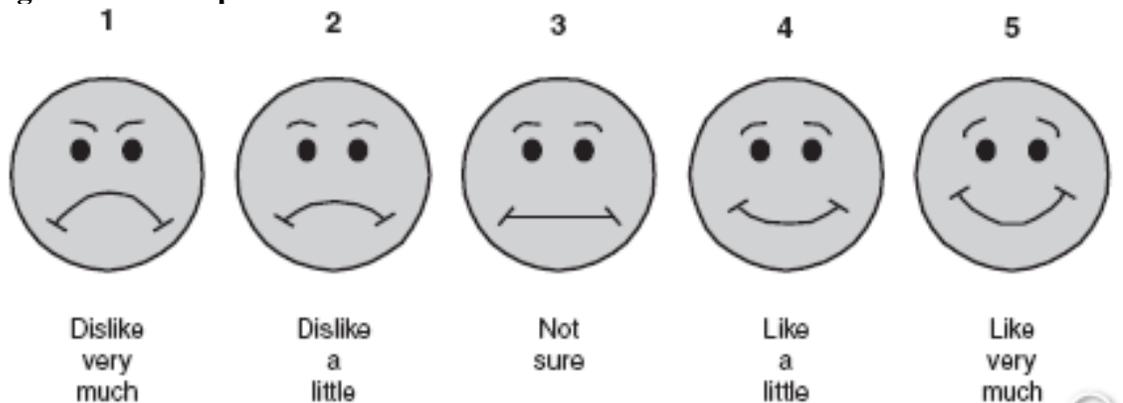
7.3.2.5 Bioanalysis

All samples from subjects on cadazolid will be analyzed by the bioanalytical laboratory. Cadazolid fecal concentrations will be determined using a validated LC-MS/MS assay. The foreseen LOQ is 5 ng/g.

7.4 Palatability and acceptability of study treatment

Palatability of cadazolid and vancomycin formulations will be assessed directly by children who are able to comply with the instruction of the test using a 5-point facial hedonic scale [see Figure 4].

Figure 4 5-point facial hedonic scale



[Davies 2008]

For children not able to comply with the instruction of the test, the palatability will be indirectly assessed by the subject's parent(s) or LAR(s) or study site personnel by answering the following question:

“On the basis of reaction / facial expression of the child, in your opinion, how much did the child like the taste of the medication?”:

- 1 dislike very much,
- 2 dislike a little,
- 3 not sure,

^{††} If in total only one fecal sample of 3–5 g or 3 5 mL could be collected at Visit 3 in Part A, i.e., there is not enough stool to prepare aliquots for both microbiology assessments and PK testing, the fecal sample *must* be used for PK testing.

- 4 like a little,
- 5 like very much.

The site study personnel will record the palatability score corresponding to the smiley face pointed by the subject or the response provided by subject's parent(s) or LAR(s) or study site personnel in the CRF.

Acceptability of cadazolid and vancomycin formulations will be assessed through a 3-point categorical scale. For all subjects, parent(s) or LAR(s) or study site personnel will be asked following first dose at Visit 1 and at Visit 3 whether the child swallowed the medication:

- a. fully,
- b. partially,
- c. not at all.

The site study personnel will record the acceptability score corresponding to the response provided by subject's parent(s) or LAR(s) or study site personnel in the CRF.

8 STUDY COMPLETION AND POST-STUDY TREATMENT / MEDICAL CARE

8.1 Study completion as per protocol

For an individual subject, study completion is reached when EOS (Visit 5) has been completed as per protocol schedule.

All enrolled or randomized subjects who received study treatment must be followed up to EOS (Visit 5), whether or not they are prematurely discontinued from study treatment. Enrolled or randomized subjects who do not receive any dose of study treatment will complete EOS (Visit 5) as soon as possible.

EOS on a study level occurs at the time all subjects have completed their EOS visits.

8.2 Premature withdrawal from study

Subject's parent(s) or LAR(s) may voluntarily withdraw the subject from the study without justification for any reason at any time. Subjects may voluntarily withdraw their assent for any reason at any time.

Subjects are considered withdrawn if, their parent(s) or LAR(s) state an intention to withdraw from further participation in all components of the study (i.e., withdrawal of consent), the subject withdraws their assent, the subject dies, or the subject is lost to follow-up. If a subject's parent(s) or LAR(s) withdraws consent or the subject withdraws assent, no further data will be collected in the CRF from the date of withdrawal onward. The investigator may withdraw a subject from the study (without regard to parent[s] or

LAR[s] consent or the subject's assent) if, on balance, he/she believes that continued participation in the study would be contrary to the best interests of the subject. Withdrawal from the study may also result from a decision by Actelion for any reason, including premature termination or suspension of the study.

Subjects are considered lost to follow-up if all reasonable attempts by the investigator to communicate with parent(s) or LAR(s) of the subject fail. The site must take preventive measures to avoid a subject being lost to follow-up (e.g., document different ways of contact such as telephone number of parent[s] or LAR[s], home address, email address, person to be contacted in case subject's parent[s] or LAR[s] cannot be reached). If the subject's parent(s) or LAR(s) cannot be reached, the site must make a reasonable effort to contact them, document all attempts, and enter the loss of follow-up information into the CRF. The following methods must be used: at least three telephone calls must be placed to the last available telephone number and one registered letter must be sent by post to the last available home address. Additional methods may be acceptable if they are compliant with local rules/regulations (e.g., site staff visit to the subject's home), respecting the subject's right to privacy. If the subject's parent(s) or LAR(s) is still unreachable after all contact attempts listed above, he/she will be considered to be lost to follow-up.

If premature withdrawal occurs for any reason, the reason (if known) for premature withdrawal from the study, along with who made the decision (subject's parent(s) or LAR(s), investigator, or Actelion) must be recorded in the CRF.

If for whatever reason (except death or loss to follow-up) a subject was withdrawn from the study, the investigator should make efforts to schedule a last appointment / telephone call to assess the safety and well-being of the subject, collect unused study treatment and discuss follow-up medical care. Data obtained during this last appointment / telephone call will be recorded in the subjects' medical records but it will not be collected in the CRF. The investigator must provide follow-up medical care for all subjects who are prematurely withdrawn from the study, or must refer them for appropriate ongoing care, as described in Section 8.4.

8.3 Premature termination or suspension of the study

Actelion reserves the right to terminate the study at any time globally or locally. Investigators can terminate the participation of their site in the study at any time.

If a study is prematurely suspended or terminated, Actelion will promptly inform the investigators, the IRBs/IECs, and health authorities, as appropriate, and provide the reasons for the suspension or termination.

If the study is suspended or prematurely terminated for any reason, the investigator in agreement with Actelion must promptly inform the parent(s) or LAR(s) of all enrolled

subjects, and ensure their appropriate treatment and follow-up, as described in Section 8.2. Actelion may inform the investigator of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the subjects' interests.

In addition, if the investigator suspends or terminates a study without prior agreement from Actelion, the investigator must promptly inform Actelion and the IRB/IEC, and provide both with a detailed written explanation of the termination or suspension.

If the IRB/IEC suspends or terminates its approval / favorable opinion of a study, the investigator must promptly notify Actelion and provide a detailed written explanation of the termination or suspension.

Any suspension or premature termination of the study must be discussed with the IDMC.

8.4 Medical care of subjects after study completion/withdrawal from study

After the subject's study completion or premature withdrawal from the study, whichever applies, the investigator/delegate will explain to the subjects and subject's parent(s) or LAR(s) what treatment(s)/medical care is necessary and available according to local regulations.

9 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

9.1 Adverse events

9.1.1 Definitions of adverse events

An AE is any adverse change, i.e., any unfavorable and unintended sign, including an abnormal laboratory finding, symptom, or disease that occurs in a subject during the course of the study, whether or not considered by the investigator as related to study treatment.

A treatment-emergent AE is any AE temporally associated with the use of study treatment (from study treatment initiation until 7 days after study treatment discontinuation) whether or not considered by the investigator as related to study treatment.

AEs include:

- Exacerbation of a pre-existing disease. Failure to achieve Clinical Cure or evidence of Recurrence are not to be reported as AEs unless in the investigator opinion the signs and symptoms or the disease are exacerbated or are of unusual type or intensity.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.

- Disease or medical condition detected or diagnosed during the course of the study even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at study start that worsen following the start of the study (i.e., signing of informed consent).
- Abnormal assessments, e.g., change on physical examination, if they represent a clinically significant finding that was not present at study start or worsened during the course of the study.
- Laboratory test abnormalities if they represent a clinically significant finding, symptomatic or not, which was not present at study start or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study treatment.

Overdose, misuse, and abuse of the study treatment will be reported as an AE and, in addition, study treatment errors must be documented in the study treatment log of the CRF.

9.1.2 Intensity of adverse events

The intensity of clinical AEs is graded on a three-point scale – mild, moderate, severe – and is reported on specific AE pages of the CRF.

If the intensity of an AE worsens during study treatment administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.

If the intensity of an AE with an onset date between informed consent signature and start of study treatment and which is ongoing at the start of treatment worsens after the start of study treatment, a new AE page must be completed. The onset date of this new AE corresponds to the date of worsening in intensity.

The three categories of intensity are defined as follows:

Mild

The event may be noticeable to the subject. It does not influence daily activities, and usually does not require intervention.

Moderate

The event may make the subject uncomfortable. Performance of daily activities may be influenced, and intervention may be needed.

Severe

The event may cause noticeable discomfort, and usually interferes with daily activities. The subject may not be able to continue in the study, and treatment or intervention is usually needed.

A mild, moderate, or severe AE may or may not be serious [see Section 9.3.1]. These terms are used to describe the intensity of a specific event. Medical judgment should be used on a case-by-case basis.

Seriousness, rather than severity assessment, determines the regulatory reporting obligations.

9.1.3 Relationship to study treatment

Each AE must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to the study treatment, and reported as either related or unrelated. The determination of the likelihood that the study treatment caused the AE will be provided by an investigator who is a qualified physician.

9.1.4 Reporting of adverse events

All AEs occurring after study start (i.e., signing of informed consent) and up to Visit 5 (EOS) (30 ± 2 days after study treatment discontinuation) must be recorded on specific AE pages of the CRF except events described in Section 9.1.1 that are an exacerbation of the pre-existing disease.

9.1.5 Follow-up of adverse events

AEs still ongoing at Visit 5 (EOS) must be followed up until they are no longer considered clinically relevant.

9.2 Serious adverse events

9.2.1 Definition of serious adverse events

An SAE is defined by the ICH guidelines as any AE fulfilling at least one of the following criteria:

- Fatal.
- Life-threatening: refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death had it been more severe.
- Requiring inpatient hospitalization, or prolongation of existing hospitalization.
- Resulting in persistent or significant disability or incapacity.
- Congenital anomaly or birth defect.

- Medically significant: refers to important medical events that may not immediately result in death, be life-threatening, or require hospitalization but may be considered to be SAEs when, based upon appropriate medical judgment, they may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions above.

The following reasons for hospitalization are exempted from being reported:

- Hospitalization for cosmetic elective surgery, or social and/or convenience reasons.
- Hospitalization for pre-planned (i.e., planned prior to signing informed consent) surgery or standard monitoring of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for coronary angiography in a subject with stable angina pectoris.

However, complications that occur during hospitalization are AEs or SAEs (for example if a complication prolongs hospitalization).

9.2.2 Reporting of serious adverse events

All SAEs occurring after signing of informed consent up to Visit 5 (EOS) must be reported on AE pages in the CRF and on an SAE form, regardless of the investigator-attributed causal relationship with study treatment or study-mandated procedures.

An SAE is defined as related to protocol-mandated procedures if it appears to have a reasonable possibility of a causal relationship to either the study design or to protocol-mandated procedures (e.g., discontinuation of a subject's previous treatment during a washout period, leading to exacerbation of underlying disease).

9.2.3 Follow-up of serious adverse events

SAEs still ongoing at Visit 5 (EOS) must be followed up until resolution or stabilization, or until the event outcome is provided. The follow-up information obtained after the subject's EOS visit / telephone call must be reported to Actelion Global Drug Safety, but it is not recorded in the CRF.

9.2.4 After the 28 to 32-day Follow-up period

New SAEs occurring after Visit 5 (EOS) must be reported to the Actelion drug safety department within 24 h of the investigator's knowledge of the event, **only** if considered by the investigator to be causally related to previous exposure to the study treatment.

9.2.5 Reporting procedures

All SAEs must be reported by the investigator to Actelion Global Drug Safety within 24 h of the investigator's first knowledge of the event.

All SAEs must be recorded on an SAE form, irrespective of the study treatment received by the subject, and whether or not this event is considered by the investigator to be related to study treatment.

The SAE forms must be sent to Actelion Global Drug Safety (contact details are provided on the SAE form). The investigator must complete the SAE form in English, and must assess the causal relationship of the event to study treatment.

Any relevant information from source documents regarding the SAE, e.g., hospital notes or discharge summaries, etc., must be summarized on the SAE form.

Follow-up information about a previously reported SAE must also be reported within 24 h of receiving it. Actelion Global Drug Safety personnel may contact the investigator to obtain further information.

If the subject is hospitalized in a hospital other than the study site, it is the investigator's responsibility to contact this hospital to obtain all SAE relevant information and documentation.

The expectedness of an adverse reaction is determined by Actelion in the reference safety information (RSI) section provided in the most recent version of the IB for cadazolid and for the comparator product, vancomycin, the RSI is its US Prescribing Information. Any SAE that is assessed as related and unexpected against the RSI is known as a SUSAR and must be reported by Actelion to concerned health authorities (including the EudraVigilance database if the study is conducted in Europe), IECs/IRBs and investigators.

9.3 Pregnancy

If a female subject becomes pregnant while on study treatment, study treatment must be discontinued. The investigator must counsel the subject and subject's parents or LAR(s) and discuss the risks of continuing with the pregnancy and the possible effects on the fetus.

9.3.1 Reporting of pregnancy

Irrespective of the treatment received by the subject, any pregnancy occurring after study start (i.e., signing of informed consent) up to 30 days following study treatment discontinuation must be reported within 24 h of the investigator's knowledge of the event.

Pregnancies must be reported on the Actelion Pregnancy form, which is faxed to Actelion Global Drug Safety (see contact details provided on the Actelion Pregnancy form), and on an AE page of the CRF.

9.3.2 Follow-up of pregnancy

Any pregnancies must be followed to their conclusion and the outcome must be reported to Actelion Global Drug Safety.

Any AE associated with the pregnancy occurring during the Follow-up period after study treatment discontinuation must be reported on separate AE pages in the CRF. Any SAE occurring during the pregnancy must be reported on an SAE form as described in Section 9.2.2.

9.4 Study safety monitoring

Clinical study safety information (AEs, SAEs, laboratory values, vital signs, and project-specific laboratories/examinations as required) is monitored and reviewed on a continuous basis by the sponsor (in charge of ensuring subjects' safety as well as data quality).

In addition, an IDMC is monitoring safety, PK and efficacy data on a regular basis [see Section 3.4].

10 STATISTICAL METHODS

All statistical analyses will be conducted by Actelion or by designated CRO supervised by Actelion.

A Statistical Analysis Plan (SAP) will provide full details of the analyses, data displays, and algorithms to be used for data derivations.

10.1 Analysis sets

10.1.1 Screened Analysis Set

The screened analysis set (SCRAS), includes all subjects who were screened and received a subject number.

10.1.2 Full Analysis Set

The Full Analysis Set (FAS) includes all enrolled subjects assigned to a study treatment, whether or not they have received study treatment. In order to adhere to the intention-to-treat principle as much as possible:

Subjects are evaluated according to the study treatment they have been assigned to, which may be different from the study treatment they have received.

All available data are included.

Summaries will be prepared separately for subjects in Part A (FAS_A), and for subjects in Part B (FAS_B).

10.1.3 Modified ITT Analysis Set

The mITT analysis set includes subjects in the FAS who have received at least one dose of study treatment and have a confirmed diagnosis of CDAD.

Two sets including subjects from Part A or Part B will be identified respectively by mITT_A and mITT_B.

Like for the FAS, subjects are evaluated according to the study treatment they have been assigned to, which may be different from the study treatment they have received.

10.1.4 Per-protocol Analysis Set

The Per-Protocol Analysis Set (PPS), pertaining to subjects in Part B alone, will be defined in the (SAP).

Major protocol deviations or conditions which result in the exclusion of subjects from the PPS will be fully defined and documented. Full details of the reasons for exclusion from the PPS will be documented in the SAP.

Subjects are evaluated according to the study treatment they have been assigned to.

10.1.5 Safety Set

The Safety Analysis Set (SS) includes all subjects enrolled in Part A and Part B who received at least one dose of study treatment.

10.1.6 PK Sets

Part A

The plasma PK Set A (PKS_A) comprises all subjects treated with cadazolid included in the FAS_A who were able to provide, at least, evaluable pre-dose, 2 h, and 12 h post-dose samples.

The stool PK Set A1 (PKS_A1) comprises all subjects treated with cadazolid included in the FAS_A who were able to provide an evaluable stool sample.

Part B

The plasma PK Set B (PKS_B) comprises all subjects treated with cadazolid included in the FAS_B who were able to provide an evaluable 2 h PK sample (± 1 h).

The stool PK Set B1 (PKS_B1) comprises all subjects treated with cadazolid included in the FAS_B who were required and able to provide an evaluable stool sample.

10.1.7 Usage of the analysis sets

Subject disposition and study completion/discontinuation, including reason for screening failure, will be summarized using the SCRAS.

Protocol deviations, baseline demographic and disease characteristics, and previous/concomitant medications, will be summarized in the FAS_A and FAS_B.

The statistical analysis of the efficacy variables CCR, SCR, and recurrence will be performed on the mITT_A, miTT_B and the PPS_B.

All remaining efficacy variables will be analyzed on the mITT_B analysis set only.

All analyses of PK data will be performed on the PKS_A or PKS_A1 in Part A and on PKS_B and PKS_B1 (if applicable) in Part B.

Summaries of safety parameters will be performed on the SS.

Listings will be prepared on the FAS_A and FAS_B, unless otherwise specified.

10.2 Variables

For efficacy and safety variables, unless otherwise specified, the baseline value is the last valid assessment obtained prior to start of study treatment intake.

10.2.1 Efficacy variable(s)

The following efficacy variables will be analyzed in Part A and in Part B:

10.2.1.1 Clinical Cure

Clinical Cure Rate (CCR; %) is the variable to be analyzed for the primary endpoint Clinical Cure, as defined in Section 6.1.1. CCR (%) is the percentage of subjects assessed as meeting the criteria for Clinical Cure as assessed by the investigator / blinded efficacy assessor up to EOT + 2 days.

CCR (%) is derived as:

$$\left[\frac{\text{Number of subjects experiencing clinical cure up to EOT + 2 days}}{\text{Number of subjects in the analysis set}} \right] \times 100$$

Subjects without investigator assessment of Clinical Cure are considered to have Clinical Failure as defined in Section 6.1.1.2.

10.2.1.2 Recurrence

RR (%) is the variable to be analyzed for the endpoint Recurrence as defined in Section 6.1.2.1. RR(%) is the percentage of subjects experiencing a recurrence, as assessed by the investigator / blinded efficacy assessor up to EOS. The denominator for this variable is the number of subjects meeting the criteria for Clinical Cure up to EOT + 2 days.

RR(%) is calculated as:

$$\left[\frac{\text{Number of subjects experiencing a recurrence up to EOS}}{\text{Number of subjects experiencing clinical cure up to EOT} + 2 \text{ days}} \right] \times 100$$

10.2.1.3 Sustained Clinical Cure

Sustained Clinical Cure Rate (SCR; %) is the variable to be analyzed for the endpoint Sustained Clinical Cure as defined in Section 6.1.2.2. SCR(%) is the percentage of subjects assessed as meeting the criteria for Sustained Clinical Cure as assessed by the investigator / blinded efficacy assessor up to EOS.

SCR% is calculated as:

$$\left[\frac{\text{Number of subjects experiencing sustained clinical cure up to EOS}}{\text{Number of subjects in the analysis set}} \right] \times 100$$

Subjects with Clinical Failure, or with Clinical Cure and Recurrence, are considered to be without Sustained Cure.

10.2.1.4 Time to Recurrence

Time to recurrence is the variable to be analysed for the endpoint time to recurrence and is defined for subjects meeting the criteria of recurrence as defined in Section 6.1.2.3.

Time to first recurrence in days, is calculated as:

$$[\text{Onset date of recurrence} - \text{Date of last dose of study treatment} + 1]$$

or, for censored subjects, as:

$$[\text{Censoring date} - \text{Date of last dose of study treatment} + 1].$$

Subjects considered as sustained clinical cure without establishment of recurrence or death, are censored at their date of EOS. Subjects who died after EOT + 3 days are censored at the date of death. Subjects who are lost to follow-up without an EOS visit are censored at the date of last contact.

Subjects not clinically cured and therefore not at risk for a recurrence are not considered for this endpoint.

10.2.1.5 Time to Resolution of Diarrhea

Time to ROD is the variable to be analysed for the endpoint time to ROD and is defined for subjects meeting the criteria of ROD as defined in Section 6.1.2.4.

Time to ROD in days, is calculated as:

$$[\text{Date of ROD} - \text{Date of first dose of study treatment} + 1]$$

or, for censored subjects, as:

$[Censoring\ date - Date\ of\ first\ dose\ of\ study\ treatment + 1]$.

Subjects without ROD are censored at EOT + 2 days. Subjects who died prior to EOT + 2 days are censored at the date of death. Subjects who are lost to follow-up prior to EOT + 2 days are censored at the date of last contact.

10.2.1.6 Other efficacy variables

10.2.1.6.1 Susceptibility of *C. difficile* (MIC₅₀ and MIC₉₀, in µg/mL)

Susceptibility is defined as the minimum inhibitory concentration (MIC in µg/mL) of the test agent (cadazolid, vancomycin, linezolid, moxifloxacin, metronidazole and fidaxomicin) which inhibits bacterial growth:

- At baseline.
- In case of Clinical Failure, as the MIC of *C. difficile* from a fecal sample collected at EOT.
- In case of Recurrence as the maximum MIC of *C. difficile* in fecal samples collected at any NED visit.

10.2.1.6.2 Change from baseline in susceptibility of *C. difficile*

Fold change from baseline in susceptibility of *C. difficile* is the variable to be analyzed for the endpoint change from baseline in susceptibility of *C. difficile* isolates as defined in Section 6.1.3.1. This variable is derived only in subjects with Clinical Failure or Recurrence.

The fold change from baseline is derived as:

$$\left[\frac{\text{MIC at post} - \text{baseline}}{\text{MIC at baseline}} \right]$$

and is categorized as:

- ≥ 4 fold decrease,
- 2-fold decrease,
- no change,
- 2-fold increase, or
- ≥ 4 -fold increase.

10.2.1.6.3 Change from baseline in VRE count

Fold change from baseline in VRE count is the variable to be analyzed for the endpoint change from baseline in VRE count as defined in section 6.1.3.2. This variable is derived for all subjects up to EOT.

The fold change from baseline is derived as:

$$\left[\frac{\text{VRE count at EOT}}{\text{VRE at baseline}} \right]$$

10.2.1.7 Susceptibility of VRE

Fold change from baseline in susceptibility of VRE is the variable to be analyzed for the endpoint change from baseline in susceptibility of VRE as defined in section 6.1.3.3. This variable is derived for all subjects up to EOT.

The fold change from baseline is derived as:

$$\left[\frac{\text{MIC at EOT}}{\text{MIC at baseline}} \right]$$

The proportion of subjects experiencing a ≥ 4 -fold increase will be calculated.

10.2.1.8 Change in intestinal flora composition from baseline up to EOS

Change from baseline to EOS in intestinal flora composition will be derived for intestinal flora components including *Lactobacilli* and *Bifidobacterium* and is the variable for the analysis of the endpoint, change from baseline to EOS in intestinal flora.

10.2.2 Safety variables

The following safety variables will be analyzed during all study periods: AEs, SAEs deaths, vital signs, body temperature, laboratory variables, and concomitant medications.

A treatment-emergent AE is any AE temporally associated with the use of study treatment from study treatment initiation up to 7 days after study treatment discontinuation.

- Deaths, AEs and SAEs up to EOS.
- Treatment-emergent AEs and SAEs up to 7 days after EOT.
- AEs leading to premature discontinuation of study treatment.
- Change from baseline up to 7 days after EOT in vital signs (SBP, DBP, and HR), body temperature and body weight.

- Change from baseline up to 7 days after EOT in hematology, and blood chemistry laboratory parameters measured in Standardized International (SI) units.
- Marked abnormalities in vital signs (SBP and DBP) up to 7 days after EOT (treatment-emergent).
- Marked abnormalities in hematology, and blood chemistry up to 7 days after EOT as measured in SI units (treatment-emergent).

If laboratory values are received in a different unit, they will be converted to SI units.

10.2.3 PK variables

Part A

The following endpoints will be derived by non-compartmental analysis of plasma concentration-time profiles obtained at Visit 3: C_{max} , t_{max} , and AUC_{τ} .

Fecal drug concentration at Visit 3.

Part B

Cadazolid plasma concentration (ng/mL) 2 h post-dose and fecal concentration (if applicable) at Visit 3.

10.2.4 Other variables

10.2.4.1 Treatment exposure and compliance

- Treatment exposure for cadazolid and vancomycin will be described in terms of duration in days. The duration of exposure is defined as the time elapsing between study treatment initiation and discontinuation, inclusive.
- The mean daily dose per subject is defined as the ratio between the total study treatment dose taken and the total exposure time.
- Compliance, in percent, is defined as number of doses taken divided by expected number of doses during the treatment period.

10.2.4.2 Demographic variables

- Country
- Sex
- Age categories
- BMI
- Race and Ethnicity

10.2.4.3 Baseline disease characteristics

- In-patient or out-patient.
- First occurrence or first recurrence.

- MTF (yes/no).
- Number of bowel movements, including UBM within 24 h during the screening period that qualified the subject for enrolment.
- *C. difficile* strain based on molecular testing.

10.2.4.4 Palatability and acceptability

Palatability of cadazolid and vancomycin formulations at Day 1 and at Visit 3 assessed on a 5-point facial hedonic scale.

Acceptability of cadazolid and vancomycin formulations at Day 1 and at Visit 3 assessed on a 3-point categorical scale.

10.3 Description of statistical analyses

10.3.1 Overall testing strategy

This is a study to evaluate PK, safety, and efficacy. As no formal statistical testing is applied, there is no overall testing strategy. Descriptive statistics will be used to summarize the PK, safety, and efficacy study endpoint variables.

Analysis of safety variables will be performed on a pooling of subjects from Part A and Part B.

For the analyses conducted using data from Part A, the results will be presented by age cohort, unless otherwise specified.

Any statistical tests done on efficacy variables will be conducted for exploratory purposes. Differences between treatment arms in Part B will be estimated and presented with corresponding 95% CLs.

- SAS version 9.3 or higher will be used for all the statistical analyses.
- All analysis variables will be listed and presented in tables; variables to be presented in figures will be specified in the SAP.
- Data will be listed and summarized by appropriate descriptive statistics (tables or figures), typically including:
 - Number of non-missing observations, number of missing observations, mean, standard deviation (SD), minimum, Q1, median, Q3 and maximum for continuous variables.
 - Number of events, number of censored observations, number of subjects at risk, and Kaplan-Meier estimates of the survival function for time-to-event variables.
 - Number of non-missing observations, number of missing observations and frequency with percentage per category (percentages based on the sum of number

of non-missing observations and total number of observations) for categorical variables.

- For susceptibility data: number of non-missing observations, number of missing observations, geometric mean, median (MIC₅₀), 90% quantile (MIC₉₀), minimum, maximum.

Absolute changes from baseline are defined as: post-baseline value minus baseline value, such that a positive sign indicates an increase as compared to baseline.

All listings will be prepared on the FAS.

10.3.2 Analysis of efficacy variables

10.3.2.1 Hypotheses and statistical model

Not applicable.

10.3.2.2 Handling of missing data

This section describes the concepts to be used. Further details for handling of missing data and missing dates and times will be described in the SAP. Additional imputation methods may be specified in the SAP to assess sensitivity of analyses to the imputation methods used.

In general, summaries and analyses will be based upon observed data, without imputation of missing values; exceptions to this rule for the most important variables are described in Section 10.3.2.2.1 and Section 10.3.2.2.2.

10.3.2.2.1 Clinical Cure

In the event of death, study withdrawal, loss to follow-up, or missing investigator / blinded efficacy assessor assessment of Clinical Cure up to EOT + 2 days, the subject is considered to have experienced a Clinical Failure.

10.3.2.2.2 Sustained Clinical Cure

In the event of death, study withdrawal, loss to follow-up, or missing investigator / blinded efficacy assessor assessment of SCR during the period from EOT + 3 days up to EOS the subject is not considered to have experienced a SCR.

10.3.2.3 Main analysis

The statistical analysis of efficacy variables will be performed on the analysis datasets as described in section 10.1.7.

The CCR, Recurrence rate, and SCR variables will be summarized by treatment group, presenting the percentage of subjects experiencing the event along with the

corresponding 95% 2-sided confidence limits (CLs), estimated using the Wilson's Score method [Newcombe 1998].

10.3.2.3.1 Part B analyses:

The treatment difference between cadazolid and vancomycin for CCR, Recurrence, and SCR will be summarized, presenting the difference in proportions of subjects experiencing the event along with corresponding 2-sided 95% CLs, estimated using the Wilson's Score method [Newcombe 1998].

Time to Recurrence and Time to ROD will be estimated by treatment group on the mITT_B analysis set using the Kaplan-Meier method. Time to Recurrence and Time to ROD will be analyzed using Kaplan-Meier estimates along with 95% 2-sided CLs at relevant timepoints for each treatment group in both graphical and tabular form. In addition, the number of subjects with event, the number of subjects at risk, and the number of subjects censored will be computed at each timepoint for each group. The difference in treatments will be tested by means of the log-rank test comparing cadazolid versus vancomycin for each time to event endpoint.

Graphical displays of time-to-event variables using Kaplan-Meier survival curves will be produced.

10.3.2.4 Subgroup analyses

Consistency of results on CCR and in SCR will be explored over different subgroups defined by anthropometric and baseline characteristics: first occurrence and first recurrence, sex, race, age category, in-patient status, and initial strain of CDAD (hypervirulent or not).

10.3.2.5 Analysis of the other efficacy variables

Susceptibility of *C. difficile* isolates test results in (MIC in µg/mL) at baseline and post-baseline visits will be summarized by treatment group using descriptive statistics on MIC range, MIC₅₀, MIC₉₀, and the frequency of isolates at each MIC test concentration for each test agent.

Changes from baseline in susceptibility will be summarized by treatment group in subjects with Clinical Failure or Recurrence. Frequency and percent of subjects with ≥ 4-fold decrease, 2-fold decrease, no change, 2-fold increase or ≥ 4-fold increase will be presented.

VRE counts at baseline and up to EOT, as well as changes from baseline up to EOT in difference of VRE counts, will be summarized using descriptive statistics.

Susceptibility test results of VRE to a panel of antibiotics including cadazolid and vancomycin will be summarized by treatment group for each test agent with descriptive statistics including MIC range, MIC₅₀, MIC₉₀.

10.3.3 Analysis of the safety variable(s)

10.3.3.1 Adverse events

The SS will be used to summarize the safety variables on the pooled subject data from Part A and Part B.

All AEs and SAEs will be coded using the MedDRA dictionary version 18 or higher.

All AEs will be listed by treatment group, age cohort, and subject in chronological order (using onset date). Similarly, all SAEs, as well as all AEs leading to discontinuation of study treatment and those leading to death, will be listed separately. The listings will also include the date of starting study, the number of days from the start of study treatment to the onset date (also known as “relative onset day”; relative days in general are associated with an assessment or event date, expressed using “days” as a unit).

Treatment-emergent AEs and treatment-emergent SAEs up to 7 days after EOT will be tabulated by system organ class (SOC), and individual preferred terms within each SOC. The incidence of subjects who experienced AEs coded with the same preferred term will be tabulated by treatment group. AEs will also be tabulated by maximum intensity and by relationship to study treatment.

AEs leading to permanent discontinuation of study treatment will be summarized by SOC and individual preferred terms within each SOC.

AEs and SAEs from first dose up to Visit 5 (EOS) will be summarized in a similar manner to that used for treatment-emergent AEs.

10.3.3.2 Deaths

The primary reason for death as reported in the Death CRF will be listed and summarized in a frequency table, per treatment group.

10.3.3.3 Vital signs, body weight, and body temperature

Descriptive summary statistics by visit and treatment group will be provided for observed values and absolute changes from baseline at Visit 3 in SBP, DBP, HR, body temperature, and body weight. The number of subjects with at least one marked abnormality up to EOT + 7 days will be summarized.

10.3.3.4 Laboratory values

Statistical analysis of laboratory values will be performed on SI units. Observed values and absolute changes from baseline up to EOT + 7 days will be summarized by visit and

treatment group using descriptive statistics. The number of subjects with at least one treatment-emergent abnormality and the number of subjects with at least one marked abnormality up to EOT + 7 days will be summarized for each laboratory variable by treatment group. Treatment-emergent laboratory test abnormalities will be assessed according to the normal ranges.

10.3.3.5 Treatment exposure and compliance

All tabulations and listings of exposure to, and compliance with study medication will be generated by treatment group and type of study treatment (capsules or sachets). The duration of exposure will be summarized using descriptive statistics.

Compliance will be summarized by treatment group using descriptive statistics. Percent compliance and overall mean daily dose will be summarized both continuously and categorically by reporting the number and percent of subjects with < 80%, 80–120%, > 120%, and indeterminate compliance. Subjects whose compliance cannot be determined will be flagged in the listings.

10.3.4 Analysis of PK parameters

10.3.4.1 Part A

PK endpoints: the PKS_A and PKS_A1 will be used for plasma and fecal samples analysis, respectively.

Concentration-time data of cadazolid in plasma:

- Individual concentration-time data will be listed by age group, dose, subject, and time.
- Concentration-time data will be summarized by dose presenting the number of observations, arithmetic mean, median, minimum, maximum, SD, standard error (SE), % coefficient of variation (%CV), and 95% CI of the arithmetic mean.

Cadazolid fecal concentrations (when applicable):

- Individual fecal concentration will be listed by age group, dose and subject.
- Fecal concentrations will be summarized by dose presenting the number of observations, arithmetic mean, geometric mean, median, minimum, maximum, SD, SE, %CV, 95% CI of the arithmetic and the geometric means, and the fold over MIC90 for the geometric mean and for the observed minimum.

Derived plasma PK parameters of cadazolid.

- Individual PK parameters (C_{max} , t_{max} , and AUC_{τ}) will be listed by dose and by subject.

- C_{\max} , t_{\max}^* , and AUC_{τ} will be summarized by dose with arithmetic mean, geometric mean, minimum, median, maximum, SD, SE, %CV, and 95% CI of the arithmetic and geometric means.

(*For t_{\max} the geometric mean and its 95% CI will not be calculated).

10.3.4.2 Part B

PK endpoint: for cadazolid plasma concentration: The PKS_B will be used.

Cadazolid plasma concentrations:

- Individual concentration at 2 h will be listed by dose and by subject.
- Concentrations at 2 h will be summarized by dose presenting the number of observations, arithmetic mean, geometric mean, median, minimum, maximum, SD, SE, %CV, and 95% CI of the arithmetic and the geometric means.

Fecal concentrations will be listed and summarized as for Part A if applicable. The PKS_B1 will be used.

10.3.5 Analysis of other variable(s)

10.3.5.1 Subject disposition

A detailed description of subject disposition will be provided for all subjects. It will include a summary (number and percentage) of subjects screened and undergoing enrolment or randomization, with the latter, for Part B, divided into the cadazolid and vancomycin treatment groups to which they were randomized. The number of subjects by treatment group continuing and discontinuing treatment and study, along with reasons for discontinuations will be displayed along with the number of subjects completing the study in each treatment group.

The number and percent of patients included in and excluded from each analysis set, based on the set definitions in Section 10.1, will be summarized in tables and listings, and will be supported by a summary table of protocol deviations leading to exclusion from each analysis set based on the number of subjects in the FAS_B.

10.3.5.2 Demographic characteristics

Demographic and baseline characteristic variables: age, sex, race, ethnicity, height, weight, and region will be summarized per treatment group and overall.

10.3.5.3 Baseline disease characteristics

The baseline CDAD characteristics In-patient / out-patient, first recurrence/occurrence, initial strain hypervirulent or not, MTF (yes/no), number of bowel movements, and *C. difficile* strain based on molecular testing will be summarized by treatment group and overall.

10.3.5.4 Previous and concomitant medications

Previous and concomitant medications will be coded according to the WHO drug code and the Anatomical Therapeutic Chemical class code and will be summarized by treatment group, class code, and preferred term for previous and study-concomitant medications with the number and percentages of subjects having received each treatment. Listings of Concomitant medications will be presented including drug name, start of administration, end of administration, dosages administered, and route of administration.

10.3.5.5 Medical history

Medical history will be coded using MedDRA and summarized in a similar manner to AEs.

10.3.5.6 Protocol deviations

Protocol deviations will be listed and summarized per treatment in Part A and Part B.

10.3.5.7 Palatability and acceptability

Palatability of cadazolid and vancomycin formulations at Day 1 and at EOT assessed on a 5-point hedonic facial scale will be summarized by treatment with counts and percentages.

Acceptability of cadazolid and vancomycin formulations at Day 1 and at EOT assessed on a 3-point categorical scale will be summarized by treatment arms with counts and percentages.

10.4 Interim analyses

Not applicable.

10.5 Sample size

10.5.1 Sample size justification

Approximately 156 pediatric subjects will be treated with cadazolid at the proposed duration of treatment, 24 in Part A and 132 in Part B. Assuming that systemic absorption in children is as low as in adults, a database of approximately 156 pediatric subjects is adequate to characterize the safety profile of cadazolid, including gastrointestinal tolerability side effects that are the most likely with a non-absorbed medication and which occurred at a rate of approximately 4–5% in the adult Phase 2 study. Assuming that a treatment-emergent AE is not observed, there is 95% confidence that the upper bound of the true rate is at most 2%.

Part A will include at least 24 subjects treated with cadazolid, which is a number considered sufficient to evaluate PK and safety in a dose level cohort.

Part B will include approximately 132 subjects treated with cadazolid and 44 treated with vancomycin (randomization ratio 3:1).

11 DATA HANDLING

11.1 Data collection

The investigator/delegate is responsible for ensuring the accuracy and completeness, of the data reported and ensures that it is entered in a timely manner. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of the data. Data reported in the CRF derived from source documents must be consistent with the source documents.

Electronic CRF data will be captured via electronic data capture. The investigator and site staff will be trained to enter and edit the data via a secure network, with secure access features (username, password, and identification – an electronic password system). A complete electronic audit trail will be maintained. The investigator/delegate will approve the data (i.e., confirm the accuracy of the data recorded) using an electronic signature (ref. to 21 CFR Part 11).

For each subject screened, regardless of study treatment initiation, a CRF must be completed and signed by the investigator/delegate. This also applies to those subjects who fail to complete the study.

11.2 Maintenance of data confidentiality

The investigator/delegate must ensure that data confidentiality is maintained. On CRFs or other documents (e.g., documents attached to SAE reports) submitted to Actelion and any external service providers, subjects must be identified only by number, and never by name or initials, hospital numbers, or any other identifier. The investigator/delegate must keep a subject identification code list, at the site, showing the subject screening/randomization number, the subject's name, date of birth, and address or any other locally accepted identifiers. Documents identifying the subjects (e.g., signed ICFs) must not be sent to Actelion, and must be kept in strict confidence by the investigator/delegate.

11.3 Database management and quality control

The investigator will have access to the site CRF data until the database is locked. Thereafter, they will have read-only access. The CRF must be kept current to reflect subject status at any time point during the course of the study.

While entering the data, the investigator/delegate will be instantly alerted to data queries by validated programmed checks. Additional data review will be performed by Actelion on an ongoing basis including but not limited to looking for unexpected patterns in data

and study monitoring. If discrepant data are detected, a query specifying the problem and requesting clarification will be issued and visible to the investigator/delegate via the CRF. All electronic queries visible in the system either require a data correction (when applicable) or a response from the investigator/delegate to clarify the queried data. The investigator/delegate must, on request, supply Actelion with any required background data from the study documentation or clinical records. This is particularly important when errors in data transcription are suspected. In the event of health authority queries, it is also necessary to have access to the complete study records, provided that subject confidentiality is protected.

This process will continue until database closure.

Fecal and blood samples will be processed through a central laboratory and the results will be sent electronically to Actelion.

After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time may only be made as described in the appropriate SOP. After database lock, the investigator will receive the CRFs of the subjects of her/his site on electronic media or as a paper copy.

12 PROCEDURES AND GOOD CLINICAL PRACTICE

12.1 Ethics and Good Clinical Practice

Actelion and the investigators will ensure that the study is conducted in full compliance with ICH Good Clinical Practice (GCP) Guidelines, the principles of the “Declaration of Helsinki”, and with the laws and regulations of the country in which the research is conducted.

12.2 Independent Ethics Committee / Institutional Review Board

The investigator will submit this protocol and any related document provided to the subject (such as Subject Information Leaflet used to obtain informed consent) to an Institutional Review Board (IRB) or independent Ethics Committee (IEC). Approval from the Committee must be obtained before starting the study, and must be documented in a dated letter to the investigator, clearly identifying the study, the documents reviewed, and the date of approval.

Modifications made to the protocol or subject information leaflet after receipt of the approval must also be submitted as amendments by the investigator to the IRB/IEC in accordance with local procedures and regulations [see Section 12.6].

A list of members participating in the IRB/IEC meetings must be provided, including the names, qualifications, and functions of these members. If that is not possible, the attempts made to obtain this information along with an explanation as to why it cannot be obtained

or disclosed must be documented in the study documentation. If a study staff member was present during a meeting, it must be clear that this person did not vote.

12.3 Informed consent

It is the responsibility of the investigator to obtain informed consent and assent by the child according to local requirements according to GCP and local regulations from each individual participating in this study, after adequate explanation of the aims, methods, objectives and potential hazards of the study.

The investigator must also explain to the parent(s)/LAR(s) and the subjects that they are completely free to refuse to enter the study, or to withdraw from it at any time for any reason. Appropriate forms for documenting informed consent and assent will be provided to the sites prior to the study.

The ICF and Subject Information Leaflet will be provided in the local language.

Site staff authorized to participate to the consent process and/or to obtain consent from the subject's parent(s) or LAR(s) will be listed on Actelion Delegation of Authority form. A study physician must always be involved in the consent process.

The subject's parent(s) or LAR(s) must sign, personally date, and time (if appropriate) the ICF before any study-related procedures (i.e., any procedures required by the protocol) begin. The ICF must also be signed, personally dated, and timed (if the first study-mandated procedure was performed on the same day informed consent was obtained) by the authorized site staff listed on Actelion Delegation of Authority form.

A copy of the signed and dated assent and ICF is given to the subject and subject's parent(s) or LAR(s); the original is filed in the site documentation. The informed consent process must be fully documented in the subject's medical records. This must include the study reference, the subject number, the date and, if applicable, time when the subject was first introduced to the Actelion clinical study, the date and, if applicable, time of consent, who participated in the consent discussion, who consented the subject, and any additional person present during the consent process (e.g., subject family member), a copy of the signed assent and ICF given to the subject or parent(s)/LAR(s).

12.4 Compensation to subjects and investigators

Actelion provides insurance in order to indemnify (with both legal and financial coverage) the investigator/site against claims arising from the study, except for claims that arise from malpractice and/or negligence.

The compensation of the subject's parent(s) or LAR(s) in the event of study-related injuries will comply with applicable regulations.

12.5 Protocol adherence/compliance

The investigator must conduct the study in compliance with the approved version of the protocol and must not implement any deviation/change from the protocol, except when deviation is necessary to eliminate an immediate hazard to the subject.

If a protocol deviation occurs, the investigator/delegate will inform Actelion or its representative, in a timely manner. The investigator/delegate must document and explain any deviation from the approved protocol. Deviations considered to be a violation of GCP must be reported to the IRB/IEC and regulatory authorities according to Actelion or (overruling) local requirements.

12.6 Protocol amendments

Any change to the protocol can only be made through a written protocol amendment. A protocol amendment must be submitted to IRB/IEC and regulatory authorities, according to their requirements.

12.7 Essential documents and retention of documents

The investigator/delegate must maintain adequate records necessary for the reconstruction and evaluation of the study. A number of attributes are considered of universal importance to source data and the records that hold those data. These include that the data and records are accurate, legible, contemporaneous, original (or certified copy), attributable, complete, consistent, enduring, and available when needed.

These records are to be classified into two different categories of documents: investigator site file (ISF) and subject clinical source documents.

These records must be kept by the investigator for as long as is necessary to comply with Actelion's requirements (i.e., as specified in the clinical study agreement), and national and/or international regulations, whichever would be the longest period. If the investigator cannot guarantee this archiving requirement at the site for any or all of the documents, special arrangements, respecting the data confidentiality, must be made between the investigator and Actelion to store these documents outside the site, so that they can be retrieved in case of a regulatory inspection. No study document should be destroyed without prior written approval from Actelion. Should the investigator wish to assign the study records to another party, or move them to another location, Actelion must be notified in advance.

If the site is using an electronic/computerized system to store subject medical records, it can be used for the purpose of the clinical study if it is validated (as per 21 CFR Part 11 or equivalent standard) and if the monitor has been provided personal and restricted access to study subjects only, to verify consistency between electronic source data and the CRF during monitoring visits.

If the site is using an electronic/computerized system to store subject medical records but it could not be confirmed that the system is validated or if the monitor could not be provided access to the system, the site is requested to print the complete set of source data needed for verification by the monitor. The print-outs must be signed and dated by the investigator/delegate to confirm that these certified copies are exact copies with the same information as the original subject's data. The printouts will be considered as the official clinical study records and must be filed either with the subject medical records or with the subject's CRF.

In order to verify that the process the site uses to prepare certified copies is reliable, the monitor must be able to observe this process and confirm that the comparison of the source documents and the certified copy did not reveal inconsistencies. The monitor does not need to verify this process for all data of all subjects but at least for some of them (e.g., first subject; regular check during the study of critical data like inclusion/exclusion criteria, endpoints for some subjects) as per Actelion's instructions. If it were not possible for the monitor to observe this process, it would not be possible to rely on the site's certified copies and therefore the site cannot be selected for the clinical study.

12.8 Monitoring

Prior to study start, a site initiation visit (SIV) will be performed after the required essential study documents are approved by Actelion. The study treatment will be shipped to the site upon approval of the required essential documents.

The PI must ensure that all site personnel involved in the study are present during the SIV and will dedicate enough time to it. Site Information Technology support should also be available during the initiation visit.

The SIV must be completed before the site can start the screening of study subjects. Following the SIV, a copy of the completed initiation visit report and follow-up letter will be provided to the PI and filed in the ISF.

During the study, the monitor will contact and visit the site regularly and must be permitted, on request, to have access to study facilities and all source documents needed to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered in the CRFs and other protocol-related documents. Actelion monitoring standards require full verification that informed consent has been provided, verification of adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of the main efficacy, safety, and tolerability endpoints. Additional checks of the consistency of the source data with the CRFs will be performed according to the study-specific monitoring plan. The frequency of the monitoring visits will be based on subject recruitment rate and critical data collection times.

The PI must ensure that the CRF is completed after a subject's visit (site visit or telephone call), and that all requested subject files (e.g., ICFs, medical notes/charts, and other documentation verifying the activities conducted for the study) are available for review by the monitor. The required site personnel must be available during monitoring visits and allow adequate time to meet with the monitor to discuss study related issues.

The investigator agrees to cooperate with the monitor(s) to ensure that any issues detected in the course of these monitoring visits are resolved. If the subject is hospitalized or dies in a hospital other than the study site, the investigator is responsible for contacting that hospital in order to document the SAE, in accordance with local regulations.

A close-out visit will be performed for any initiated site when there are no more active subjects and all follow-up issues have been resolved. In case a site does not enroll any subjects, the close-out visit may be performed prior to study database closure at the discretion of Actelion.

12.9 Investigator site file

Each site will be provided with an ISF prior to the initiation visit. It will contain all the essential documents that are required to always be up-to-date and filed at site as per ICH E6 GCP section 8.

The ISF will include a table of content listing the essential documents. All study-related documentation must be maintained in the ISF.

In some cases, exceptions can be discussed with the monitor regarding the filing of the study documents outside the ISF. It should be clearly documented where each document is filed. This note to file should be present in the specific tab of the document in the ISF.

The ISF must be stored in a secure and access-restricted area during and after the study. It must be kept by the site for as long as needed to comply with any applicable rules and regulations, ICH-GCP, as well as instructions from Actelion. If the site needs to transfer the ISF to another location and/or if site facility can no longer store the ISF, the PI must inform Actelion immediately.

If the PI will change, or if the site will relocate, the monitor must be notified as soon as possible.

12.10 Audit

Actelion's Global Quality Management representatives may audit the investigator site (during the study or after its completion). The purpose of this visit will be to determine the investigator's adherence to ICH-GCP, the protocol, and applicable regulations; adherence to Actelion's requirements (e.g., SOPs) will also be verified. Prior to initiating this audit, the investigator will be contacted by Actelion to arrange a time for the audit.

The investigator and staff must cooperate with the auditor(s) and allow access to all study documentation (e.g., subject records) and facilities.

12.11 Inspections

Health authorities and/or IRB/IEC may also conduct an inspection of Actelion's clinical study (during the study or after its completion).

Should an inspection be announced by a health authority and/or IRB/IEC, the investigator must inform Actelion immediately (usually via the monitor) that such a request has been made.

The investigator and staff must cooperate with inspector(s) and allow access to all study documentation (e.g., subject records) and study facilities.

12.12 Reporting of study results and publication

Study results will be documented in a clinical study report that will be signed by Actelion representatives and the Coordinating Investigator (or PI for single-center studies).

The coordinating investigator and the Steering Committee, if any, will have the opportunity to review the analysis of the data and to discuss the interpretation of the study results with Actelion prior to publication.

Actelion will post results from the clinical study on Actelion's Clinical Trial Register and on external/national registries, as required by local law.

In accordance with the Good Publication Practices and ethical practice, the results of the study will be submitted for publication in a peer-reviewed journal. Study results can be submitted for presentation at a congress before publication in a peer-reviewed journal.

Authorship will be determined in accordance with the International Committee of Journal Editors (ICMJE) criteria, and be based on:

- substantial contributions to the conception or design of the study, or the acquisition, analysis, or interpretation of data; and
- drafting of the publication or critical review for important intellectual content; and
- providing final approval of the version to be published; and
- agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The list of authors of any publication of study results may include representatives of Actelion and will be determined by mutual agreement.

Any study-related publication written independently by investigators must be submitted to Actelion for review at least 30 days prior to submission for publication or presentation. Upon review, Actelion may provide comments, and may also request alterations and/or deletions for the sole purpose of protecting its confidential information and/or patent rights. Neither the institution nor the investigator should permit publication during such a review period.

Actelion's policies on Scientific Publications and Disclosure of Clinical Research information can be found at:



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14 APPENDICES

Appendix 1 Equivalence between opiates

Drug	Equianalgesic dose		Interval (h)
	Oral (mg)	IV (mg)	
Morphine	30	10	3–4 (PO, SL) 2–4 (IV, SQ)
Hydromorphone	6–8	1.5–2	3–4 (PO, SL) 2–4 (IV, SQ)
Oxycodone	15–20	N/A	3–4 (PO)
Fentanyl	N/A	0.1 (100 mcg)	0.5 (IV, SQ)
Methadone	See methadone conversion table		4 (first 2–3 doses) then 8–12 (PO, SQ, IV, SL)

IV = intravenously; N/A = not applicable; PO = orally; SL = sublingual; SQ = subcutaneous.

Equianalgesic Conversion to Methadone		
Oral morphine equivalent	Mg of oral methadone	Mg of oral morphine
	=	
	ratio	
< 100 mg/day	1	3–4
100–200 mg/day	1	5–8
301–600 mg/day	1	10
601–800 mg/day	1	12
801–1000 mg/day	1	15
> 1000 g/day	1	20

IV methadone is twice as potent as oral methadone

IV = intravenously.

[Dana Farber Cancer Institute 2011]

[Macintyre 2001]