CLINICAL TRIAL PROTOCOL NCT #: NCT06536465

Study Title:	A Phase 2 Clinical Study of REC-3964 in Adults for the Reduction of Recurrent *Clostridioides Difficile* Infection
Brief Study Title:	The *Clostridioides Difficile* Trial of REC-3964
Study Number:	REC-3964-201
Investigational Product:	REC-3964
IND:	169641
EU CT:	2024-520223-81-00
Sponsor:	Recursion Pharmaceuticals, Inc. 41 South Rio Grande St. Salt Lake City, UT 84101
Responsible Medical Officer	[redacted] [redacted] Recursion Pharmaceuticals, Inc.
Protocol Version	Version 7.0
Date	27 March 2025

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1. SIGNATURE PAGE

Sponsor's Approval

The protocol has been approved by:

{See appended electronic signature page}

[redacted]

Date

{See appended electronic signature page}

[redacted]

Date

INVESTIGATOR'S AGREEMENT

I have read the REC-3964-201 protocol and agree to conduct the study as outlined and in accordance with GCP. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Printed Name of Investigator

Signature of Investigator

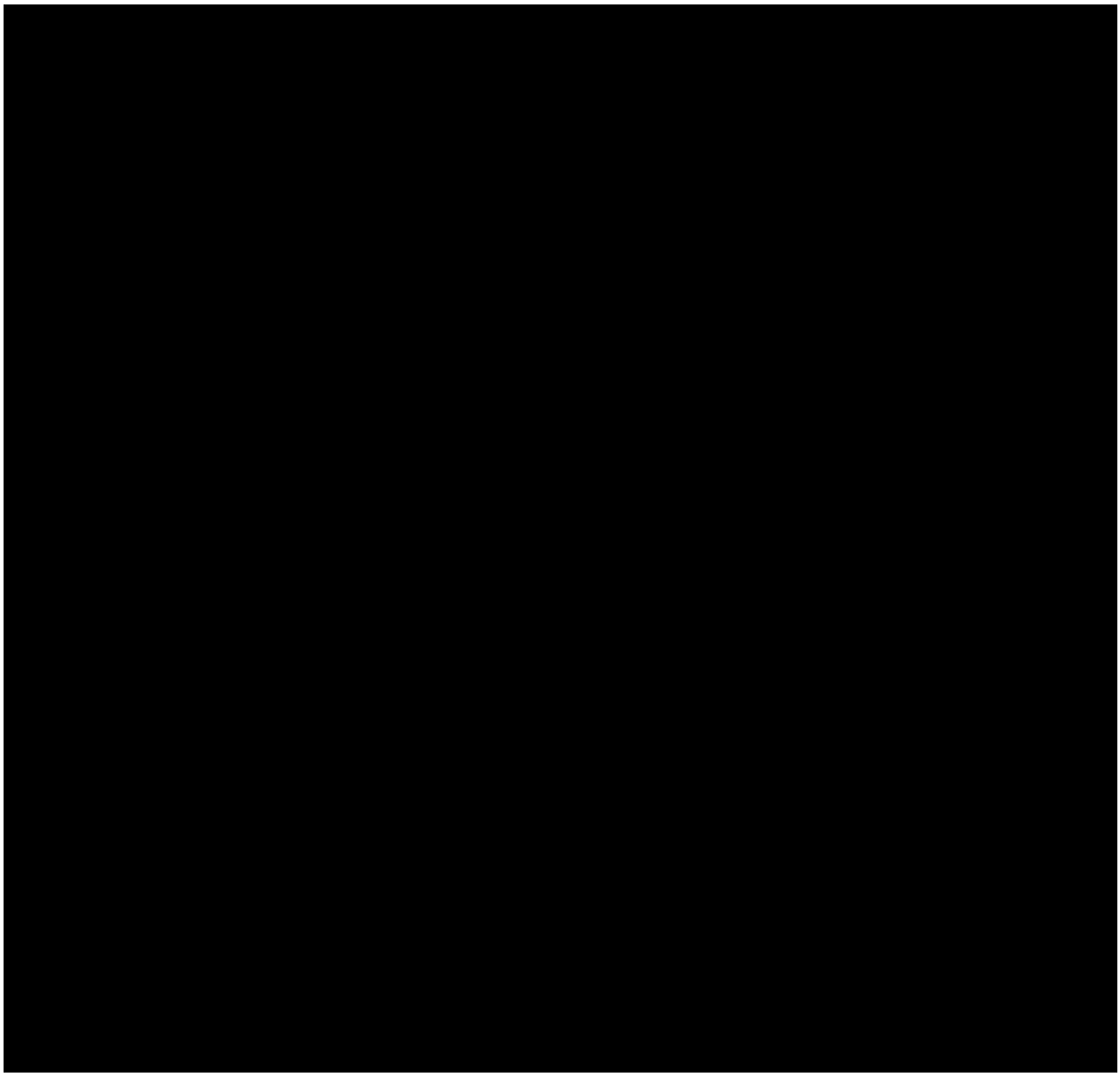
Date

EMERGENCY CONTACT INFORMATION

Name	Contact Number	Email Address
[redacted]	[redacted]	[redacted]

PROTOCOL AMENDMENT HISTORY

The following amendments have been made to this protocol since the date of preparation:



2. SYNOPSIS

Name of Sponsor / Company: Recursion Pharmaceuticals, Inc		
Name of Investigational Product: REC-3964		
Name of Active Ingredient: REC-3964		
Protocol Number: REC-3964-201	**Phase:** 2	**Countries:** Multi-site, multi-regional
Title of Study	A Phase 2 Clinical Study of REC-3964 in Adults for the Reduction of Recurrent *Clostridioides Difficile* Infection	
Study center(s):	Approximately 40-50 centers	

Objectives:

Primary

- To evaluate the efficacy of REC-3964 as determined by survival without recurrence or requirement for additional CDI treatment for at least eight weeks following cure of preceding CDI with vancomycin or fidaxomicin
- To evaluate the safety and tolerability of REC-3964

Secondary

- To assess the efficacy of REC-3964 as measured by other efficacy endpoints
- To characterize the pharmacokinetics (PK) of REC-3964

Methodology: This Phase 2, open-labeled, multi-center study is designed to characterize the safety, tolerability, PK, and efficacy of REC-3964 at doses of either [redacted] for the reduction of recurrent *Clostridioides difficile* infection (rCDI) after cure of preceding CDI with vancomycin or fidaxomicin. The study will enroll male and female participants who are 18 years of age or older.

Study Schema

Figure 1: [redacted]

[redacted]

Number of Participants (Planned): Approximately 80 participants [redacted] in the Observation arm) will be randomized.

Enrollment of participants initially treated with fidaxomicin will be capped at no more than 33% of the total study population.

Diagnosis and Main Criteria for Eligibility:

Inclusion Criteria

To be enrolled, the participant must provide informed consent and meet ALL of the following criteria at the time of enrollment:

- Be 18 years of age or older
- Have CDI diarrhea, defined as 3 or more unformed stools per day (types 5-7 on the Bristol stool scale) for at least 2 consecutive days AND
- Confirmed lab diagnosis through positive stool test for C. difficile toxin[s] prior to the preceding curative treatment
- The CDI episode, severe or otherwise, must have resolved (two or less unformed stools for at least 24 hours) after receiving a standard duration of vancomycin (or fidaxomicin) treatment for 10 – 14 days (+2 days). The standard initial curative treatment is vancomycin. Fidaxomicin is an optional alternative and may be chosen if deemed appropriate by the investigator, taking into account the participant's condition, treatment history, and other relevant clinical factors. The participant must be randomized within 2 days of completing the preceding curative treatment.
- Have high risk for rCDI, defined as any of the following:
  - having had at least one prior CDI episode within the last 6 months
  - age ≥65 years
  - immunocompromised state
  - severe CDI with resolution prior to enrollment into the study
    - **NOTE:** Severe CDI is defined as CDI with white blood cell count >15,000 cells/mL and/or serum creatinine ≥1.5 mg/dL. Severe CDI must have resolved prior to enrollment into the study.

Exclusion Criteria

To participate, the participant must meet NONE of the following at the time of enrollment:

- Have an active, symptomatic, chronic diarrheal illness from other causes such as ulcerative colitis or Crohn's disease.
- Have a life expectancy of less than 90 days.
- Have surgery planned to manage severe CDI colitis during the study period.
- Have fulminant CDI.
- [redacted].
- Chronic or recent use of laxatives, or anti-motility agents, such as loperamide.

Investigational Product, Dosage, and Mode of Action

REC-3964 [redacted]

Duration of Treatment

The duration of participation for each participant is up to [redacted], as follows:

- Screening [redacted]

- Treatment Period [redacted]
- Follow-up Period [redacted]

Statistical Methods:

Sample Size

Approximately 80 participants will be randomized to this study. No hypothesis testing will be conducted. This sample size is deemed appropriate to provide safety profile and preliminary efficacy information on the effect of treatment with REC-3964 in C. difficile participants.

Statistical Analysis

The primary efficacy endpoint is survival without recurrence or requirement for additional CDI treatment for at least eight weeks following cure of preceding CDI with initial curative treatment defined in Inclusion Criteria 6.1.4. It will be analyzed as the rate of recurrent *C. difficile* infection after treatment with REC-3964 or observation at 8 weeks, upon resolution of the preceding CDI diarrheal episode with initial curative treatment. For secondary efficacy endpoints, rates/proportions will be calculated and summarized with 2-sided 95% CIs per treatment group using exact test while time to event endpoints will be analyzed using the KM method. Additional efficacy analyses will be conducted within stratum of prior antibiotic use (vancomycin versus fidaxomicin).

Safety and tolerability will be assessed by the following endpoints: incidence, severity, and relatedness of AEs and SAEs; and incidence of treatment-emergent adverse events (TEAEs) leading to study drug discontinuation. Safety and tolerability will be summarized by treatment arm in terms of the frequency and percentage of AEs. All TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The frequency and severity of AEs will be summarized by treatment arm in particular, all TEAEs, SAEs and deaths, and AEs leading to discontinuation of study drug will be presented. AEs by relationship to study treatment will also be summarized by the MedDRA preferred term (PT) and system organ class (SOC). Laboratory data, physical examination, vital signs, and 12-lead ECG data will be summarized descriptively by treatment arm over time, including displays of change from baseline. Values outside normal ranges will be identified and summarized by treatment arms. Incidence of abnormal physical examination findings will also be summarized. Plasma concentrations of REC-3964 and PK parameters derived from plasma concentration will be summarized and analyzed on PK Analysis Set.

Analyses of other endpoints and details will be provided in the Statistical Analysis Plan (SAP).

Table 1: [redacted]

[redacted]

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION	EXPLANATION
AE	Adverse event
AUC	Area under the curve
AUC_{inf}	Area under the curve to infinity
AUC_{last}	Area under the curve to the last timepoint measured
BCRP	Breast Cancer Resistance Protein
BID	twice a day
C. difficile	Clostridioides difficile
C_{max}	Maximum Observed Plasma Concentration
C_{trough}	Trough Plasma Concentration
CA	Competent authority
CDI	C. difficile infection
CI	Confidence Interval
CRO	Contract Research Organization
CSR	Clinical Study Report
EC	Ethics committee
ECG	Electrocardiogram
ECIS	Electric Cell-substrate Impedance Sensing
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOT	End of Treatment
ET	Early Termination
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
GCP	Good Clinical Practice
GMR	Geometric Mean Ratio
IC	Inclusion Criteria
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee

ABBREVIATION	EXPLANATION
IL	Interleukin
IRB	Institutional Review Board
IUD	Intrauterine Device
K-M	Kaplan Meier
ITT	Intent-to-treat population
MedDRA	Medical Dictionary for Regulatory Activities
[redacted]	[redacted]
$P\text{-}_{gp}$	P-glycoprotein
PBPK	Physiologically-based Pharmacokinetic
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic(s)
[redacted]	[redacted]
PT	Preferred Term
QTcF	Fridericia's correction formula
rCDI	Recurrent Clostridioides Difficile Infection
SUSAR	Suspected Unexpected Serious Adverse Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOA	Schedule of Assessments
SOC	System Organ Class
TCdiffB	C. difficile toxin B
T_{max}	Time to Maximum Plasma Concentration
TEAE	Treatment-Emergent Adverse Event
WBC	White Blood Cell
WOCBP	Women of Childbearing Potential

3. INTRODUCTION

Recursion Pharmaceuticals, Inc is proposing REC-3964 in this Investigational New Drug application for reduction of recurrent *Clostridioides difficile* (*C. difficile*) infections.

3.1. Clostridioides Difficile Background

C. difficile infection (CDI) is a worldwide disease affecting approximately half a million people in the United States and has been the leading cause of nosocomial infection and was responsible for 29,000 deaths in the United States in 2011 (Lessa, 2015). In North America, it is estimated that a cost of up to 4.8 billion dollars is associated with excess health care for CDI treatment (Lee, 2017; CDC, 2015) with endemic disease and outbreaks reported from all world regions including Asia, Western Pacific, Latin America, and Africa (Acuña-Amador, 2022; Rajabally, 2013; Chan, 2011; Ferguson, 2011; Camacho-Ortiz, 2009).

C. difficile is a Gram-positive, anaerobic, bacillus in which pathogenic strains produce protein exotoxins. These cytotoxic, enterotoxin, and pro-inflammatory toxins induce colonic mucosal injury, diarrhea, and in severe cases, pseudomembranous colitis, colonic perforation, and/or toxic megacolon (Cole, 2015; Viswanathan, 2010; Pothoulakis, 2000). Although clinical overt infection from *C. difficile* requires both acquisition of the bacterium and disruption of the normal bacterial flora in the colon (Bagdasarian, 2015), most clinical and pathophysiological characteristics of CDI are related to *C. difficile* toxins.

Often, CDI is precipitated by the use of antibiotics or other contributing factors (e.g., immunocompromised conditions), which affect normal gut flora and facilitate colonization by *C. difficile* spores in the gastrointestinal tract. The spores germinate in the gut after which *C. difficile bacteria* produce toxins that disrupt the normal architecture and function of the intestinal tract (Viswanathan, 2010). The main toxins produced by *C. difficile* are toxin A and toxin B which affect epithelial integrity via disruption of microtubules and cell-cell tight junctions, resulting in cytokine release such as interleukin (IL)-8 and other inflammatory factors (Bagdasarian, 2015; Pothoulakis, 2000). These actions promote pathogenic infiltration in the colonic mucosa, fluid shifts leading to diarrhea, and epithelial necrosis. Antibiotics alter normal microbiota, increasing CDI risk. Additional risk factors associated with the development of CDI include advanced age (>65 years), recent hospitalization, longer hospital duration, receipt of multiple antibiotics, longer antibiotic use duration, gastric acid suppression (e.g., with proton pump inhibitors), chemotherapy, chronic kidney disease, and the use of feeding-tubes (Cole, 2015; Viswanathan, 2010; Garey, 2008; Asha, 2006).

3.2. Clostridioides Difficile Pathogenesis

Rho family of guanosine triphosphatases has a pivotal role in cytoskeleton organization and rearrangements of intestinal cells. *C. difficile* mediate its pathogenicity via toxin-mediated glycosylation of the active form of the intracellular Rho GTPase. This leads to depolymerization of the actin cytoskeleton, followed by disruption of tight cellular junctions and ultimately results in apoptosis in colonic epithelial cells.

REC-3964 is a novel chemical entity identified from Recursion's unique artificial-intelligence platform. REC-3964 belongs to the diazepinedione chemical class and by inhibiting the *C. difficile* toxin effects on the glycosylation of the active form of the intracellular Rho GTPase,

prevents the disruption of cytoskeletal effects on the intestinal cells. REC-3964 has been formulated as an [redacted] active compound and has demonstrated antitoxin effects. REC-3964 is expected to be effective in several forms of CDI including recurrent CDI (rCDI).

3.3. Treatment of *Clostridioides Difficile*

Several agents can be used for the treatment of CDI, including antibiotics such as vancomycin and fidaxomicin, while monoclonal antibodies such as bezlotoxumab, or fecal microbiota transplants can be used to prevent recurrent CDI (Lee, 2017; Mada, 2022). While current antibiotic treatments, such as vancomycin and fidaxomicin, provide symptomatic relief by killing *C. difficile* bacteria, *C. difficile* spores are not affected by such antibiotics and can quickly propagate into virulent toxin-producing bacteria after treatment discontinuation. A significant number of patients have frequent recurrence or reinfection, or inability to prevent the disease from advancing to a more debilitating condition (Smits, 2016). Indeed, primary recurrence of CDI (recurrence after initial treatment of CDI) is seen in 20% to 30% of patients, and up to 45%--65% of these patients experience multiple recurrent episodes despite current available treatment (Kelly, 2010; Viswananthan, 2010; Kelly, 2012; Barbut, 2000). Recurrent CDI is associated with a 2.5-fold higher hospital readmission rate and a 33% higher mortality rate at 180 days compared with primary CDI, thereby highlighting the critical need for additional effective treatments (Olsen, 2015A; Olsen, 2015B).

Bezlotoxumab, a monoclonal antibody inhibitor to toxin B, reduced the rate of recurrence by 14.2%, 14.2%, and 24.8% in individuals who have between 1, 2, and 3 risk factors for recurrence, respectively, such as patients who are 65 years or older, those who have severe CDI on presentation, those who are immunosuppressed, those who have a history of recurrent CDI within 6 months, and those with certain virulent ribotypes (ribotypes 027/078/244) (IDSA, 2021). Bezlotoxumab injection has been discontinued as of January 31, 2025, according to the Food and Drug Administration (FDA) drug shortages tracker. Per guidance of Infectious Diseases Society of America (IDSA), fecal microbiota transplantation (FMT) is administered after initial cure with antibiotics to reduce the rate of recurrent CDI for patients with multiple recurrences of CDI who have failed appropriate antibiotics treatments. However, there are limitations of currently approved FMT treatments, including potential discomfort or difficulty with administration, possible transmission of pathogenic *Escherichia coli* from donor to FMT recipients, limited accessibility and gastrointestinal side effects, etc.. Given some of these limitations and the morbidity and mortality of recurrent *Clostridioides difficile* infection (rCDI), there is a significant unmet medical need for a highly effective, oral, toxin inhibitor that can be conveniently administered after initial cure with antibiotics to reduce the rate of recurrent CDI.

3.4. [redacted]

3.4.1. [redacted]

[redacted]

[redacted]

3.4.2. [redacted]

[redacted]

Table 2: [redacted]

[redacted]

3.4.3. Benefit / Risk Assessment

Both *in vitro* and *in vivo* nonclinical studies have been conducted to document the efficacy of REC-3964 to inhibit the effects of TCdiffB. Primary *in vitro* pharmacology studies conducted with REC-3964 demonstrate that it is a potent, effective inhibitor of the uridine diphosphate-glucose hydrolysis activity of TCdiffB in cell-free assays. [redacted]

[redacted]

[redacted]

Overall, it is considered that the potential benefit to patients, knowledge, and safety information derived from further investigation outweighs the potential risks derived from REC-3964 administration and provides ample support for a positive benefit-risk assessment.

3.5. REC-3964 Dose Justification

[redacted]

Participants in this study will receive REC-3964 for [redacted] after the end of initial curative treatment defined in Inclusion Criteria (IC) 6.1.4. REC-3964 is intended as a treatment to reduce the CDI recurrence rate by protecting the endothelial barrier from TCdiffB during recovery following antimicrobial treatment for CDI. REC-3964 was safe and well tolerated in healthy volunteers for multiple doses [redacted] and is not expected to impair the recolonization of the healthy enteric microbiota. A [redacted] regimen is expected to safely maximize the ability of REC-3964 to inhibit TCdiffB during the Follow-up Period. Additionally, since the risk of CDI recurrence is highest in the month following curative treatment (Feuerstadt, 2020), a [redacted] regimen provides adequate coverage to help mitigate the heightened risk of recurrence during this critical period.

4. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Efficacy of REC-3964	• Survival without recurrence or requirement for additional CDI treatment for at least eight weeks following cure of preceding CDI with initial curative treatment defined in IC 6.1.4. Recurrent CDI is defined as a new episode of CDI associated with a new positive C. difficile stool toxin or requirement for additional CDI treatment during the 8-week Follow-up Period after cure of preceding CDI with initial curative treatment.
Safety and Tolerability	• Number, severity, and relatedness of adverse events (AEs), serious adverse events (SAEs), AEs leading to study drug discontinuation.
Secondary	
Efficacy of REC-3964	• Rate of rCDI • Time to recurrence • Proportion of participants with severe rCDI (severe CDI is defined as CDI with white blood cell count >15,000 cells/mL and/or serum creatinine ≥1.5 mg/dL.) • rCDI-associated hospital admissions • Evaluation of diarrheal episodes (e.g., number of unformed bowel movements per day)
Pharmacokinetics	• Plasma concentrations of REC-3964 • Plasma PK parameters including, but not limited to, maximum observed plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), and trough plasma concentration (C_{trough})
Exploratory	
[redacted]	[redacted]

5. INVESTIGATIONAL PLAN

5.1. Overall Study Plan

This Phase 2, open-label, multi-center study is designed to characterize the safety, tolerability, PK, and efficacy of REC-3964 doses of [redacted] for the reduction of rCDI following cure of preceding CDI with treatment defined in IC 6.1.4. The study will enroll male and female participants who are 18 years of age or older.

The study will consist of three periods:

- Screening [redacted]
- Treatment / Observation Period [redacted]
- Follow-up Period [redacted]

Screening Period

During the Screening Period [redacted] participants will undergo assessments as described in the Schedule of Assessments (Table 1) to ensure they meet the enrollment criteria of the study. Medical history will be reviewed, including a detailed review of the participant's *C. difficile* history.

Treatment / Observation Period

Approximately 80 participants who have met the enrollment criteria will be randomized in the study and dosed [redacted].

Follow-up Period

Participants will be [redacted] after cure of preceding CDI with initial treatment.

An overview of the study design is presented in Figure 1.

5.1.1. Screening Period

During the Screening Period [redacted] participants will undergo assessments as described in the Schedule of Assessments (Table 1) to ensure they meet the Inclusion and Exclusion Criteria of the study. Medical history will be reviewed, including a detailed review of the participant's *C. difficile* history. In addition, a pre-vancomycin (or pre-fidaxomicin treatment fecal sample will be collected, if possible, and analyzed for *C. difficile* toxins, gut bile acid composition, microbiome, and an antibiotic resistance gene profile. Review of stool sample reports, including analysis of C. difficile toxin, will also be performed if available.

Individual assessments can be repeated once within the designated Screening Period window, and re-screening is possible with Sponsor approval in a circumstance for example, when a subject's Screening Period has elapsed.

5.1.2. Treatment / Observation Period

Approximately 80 participants who have met the enrollment criteria will be randomized in the study.

Participants will be stratified on prior antibiotic use (fidaxomicin versus vancomycin). Within each stratum participants are randomized 1:2:1 to receive REC-3964 [redacted] or REC-3964 [redacted] or observation, respectively, during the [redacted] Treatment Period of the study. Assessments to be performed during the Treatment Period are presented Table 1.

The duration of REC-3964 dose in treatment period is planned for [redacted]. However, the treatment period may vary if participants discontinue the study drug due to recurrence or other reasons. In such cases, the treatment period will be shorter than [redacted]. Should two or more participants experience treatment-related ≥G3 AEs in the same cohort, further enrollment in the cohort will be discontinued.

5.1.2.1. Early Discontinuation Visit

The Early Discontinuation Visit is required for all participants assigned to REC-3964 who discontinue study drug for reasons other than recurrence prior to the end of their [redacted] treatment cycle (Table 1). Participants in REC-3964 cohorts discontinued during the Treatment Period will be followed for [redacted] additional days for AEs, concomitant medications, and diarrheal symptoms (via stool diaries).

Should a participant assigned to REC-3964 discontinue during their [redacted] treatment cycle due to recurrence, the Recurrence Visit should be performed in lieu of the Early Discontinuation Visit.

5.1.3. Follow-up Period

After completing the [redacted] Treatment / Observation Period, all participants who have not experienced recurrence will be followed for an additional [redacted] of observation to assess safety and tolerability, and recurrence. Participants who have recurrence shall participate in a recurrence visit.

5.1.3.1. Recurrence Visit

The Recurrence Visit is required for all participants who experience a recurrence of CDI during the study (Table 1). While the requirement for hospitalization for rCDI will be made per the discretion of the Investigator, all visit specific procedures will need to be performed. Participants in REC-3-964 cohorts who recur during the Treatment Period will be discontinued from treatment with REC-3964 and will be followed for [redacted] additional days for AEs and concomitant medications. For participants in the Observation cohort, the Recurrence Visit will be the last study visit.

5.1.3.2. Safety Follow-Up Visit

The Safety Follow-Up Visit is required for all participants assigned to REC-3964 who discontinue study drug prior to the completion of their [redacted] treatment cycle (Table 1). This Safety Follow-Up Visit should occur [redacted] after the cessation of study drug.

5.1.3.3. Final Assessment Visit

The Final Assessment Visit will be performed for all participants approximately 8 weeks following the cure of their preceding CDI with initial curative treatment defined in IC 6.1.4. (Table 1). The purpose of this visit is to assess safety for participants assigned to treatment with REC-3964 who complete their full [redacted] treatment course and to assess recurrence of all trial participants who do not experience recurrence prior to and including this visit.

5.2. Number of Participants

Approximately 80 participants [redacted] will be enrolled.

5.3. Treatment Assignments

Participants will be randomized 1:2:1 to receive [redacted]: REC-3964 [redacted] (N=20) or REC-3964 [redacted] (N=40) or observation (N=20) within strata of prior antibiotic use (vancomycin or fidaxomicin), during the [redacted] Treatment Period of the study.

6. SELECTION AND WITHDRAWAL OF PARTICIPANTS

6.1. Inclusion Criteria

To be enrolled, the participant must provide informed consent and meet ALL of the following criteria at the time of enrollment:

1. Be 18 years of age or older
2. Have CDI diarrhea, defined as 3 or more unformed stools (types 5-7 on the Bristol stool scale) per day for at least 2 consecutive days AND
3. Confirmed lab diagnosis through positive stool test for *C. difficile* toxin[s] prior to the preceding curative treatment
4. The CDI episode, severe of otherwise, must have resolved (two or less unformed stools for at least 24 hours) after receiving a standard duration of vancomycin (or fidaxomicin) treatment for 10 – 14 days (+ 2 days). The standard initial curative treatment is vancomycin. Fidaxomicin is an optional alternative and may be chosen if deemed appropriate by the investigator, taking into account the participant's condition, treatment history, and other relevant clinical factors. The participant must be randomized within 2 days of completing the preceding curative treatment.
5. Have high risk for rCDI, defined as any of the following:
   - having had at least one prior CDI episode within the last 6 months
   - age ≥65 years
   - immunocompromised state
   - severe CDI with resolution prior to enrollment into the study

     NOTE: Severe CDI is defined as CDI with white blood cell count >15,000 cells/mL and/or serum creatinine ≥1.5 mg/dL. Severe CDI must have resolved prior to enrollment into the study.
6. Females must not be pregnant or lactating and either of non-childbearing potential (defined as having undergone surgical sterilization or being postmenopausal [i.e., greater than 40 years old with amenorrhea for at least 12 months prior to screening with follicle stimulating hormone (FSH) levels in the laboratory defined post-menopausal range])

   OR

   Using at least one of the following acceptable methods of contraception during the study and for up to 90 days after the last administration of study drug:

   a. Double-barrier method (e.g., condom plus spermicide, condom plus diaphragm with spermicide)
   b. Stable use of hormonal contraceptive treatment
   c. Intrauterine Device (IUD)
   d. Monogamous relationship with a vasectomized partner

e. Abstinence for 8 weeks prior to and throughout the study

7. Men who are sexually active must use at least one of the following forms of medically acceptable birth control during the study drug Treatment Period and for 90 days after the last administration of study drug:
   a. Vasectomy with medical assessment of surgical success
   b. Consistent use of a condom with partner also using either stable hormonal contraceptive or IUD.

   Sperm donation is prohibited during the study and for up to 90 days after the last administration of study drug.
8. Have NOT participated in a clinical study utilizing an investigational agent within 28 days or within 5 half-lives (if known) of the investigational drug (whichever is longer) prior to Day 1.
9. Acceptable laboratory parameters:
   - Hemoglobin ≥8.0g/dL
   - Platelet count ≥100,000/μL
   - Absolute neutrophil count ≥500 cells/mm^3

6.2. Exclusion Criteria

To participate, the participant must meet NONE of the following at the time of enrollment:

1. Have an active, symptomatic, chronic diarrheal illness from other causes, such as ulcerative colitis or Crohn's disease.
2. Have a life expectancy of less than 90 days.
3. Have surgery planned to manage severe CDI colitis during the study period.
4. Have liver dysfunction or active liver disease as defined by:
   - AST / ALT >2x ULN
   - Total bilirubin >2mg/dL
5. Have estimated glomerular filtration rate <60ml/min/1.73m^2, active renal disease or have previously received a kidney transplant.
6. Have a history of alcohol or substance abuse within 1 year prior to screening for study participation, or is currently using alcohol, drugs of abuse, or any prescribed or over -the -counter medication in a manner, which, in the opinion of the Investigator, indicates abuse.
7. Severe or uncontrolled intercurrent illness or clinically significant unrelated systemic illness that, in the opinion of the Investigator, could compromise participation in or the interpretation of the study or put the participant at risk.

8. Diarrhea that requires on-study treatment with agents that would confound the interpretation of the study results, including, but not limited to: bezlotoxumab, FMT, rifaximicin, nitazoxanide, loperamide, diphenoxylate/atropine, or cholestyramine.

   NOTE: bezlotoxumab and FMT use during the current episode is exclusionary whereas administration of rifaximicin, nitazoxanide, loperamide, diphenoxylate/atropine, or cholestyramine would be prohibited from Screening until the Safety Follow-up Visit.

9. Diarrhea attributable to other enteropathogens identified laboratory evaluation such as stool cultures or gastrointestinal panel, including but not limited to, Salmonella, Shigella, and Campylobacter at the point of enrollment.
10. Known stool studies positive for ova and/or parasites at the point of enrollment.
11. [redacted]
12. [redacted]
13. [redacted]
14. Chronic or recent use of laxatives, or gastric anti-motility agents, such as loperamide.
15. Have fulminant CDI.
16. Immunocompromised participants with:

    - Uncontrolled infection with human immunodeficiency virus (HIV). Participants on stable highly active antiretroviral therapy with undetectable viral load and normal CD4 counts for at least six months prior to study entry are eligible. Serological testing for HIV at screening is not required.
    - Known to be positive for hepatitis B virus (HBV) surface antigen, or any other positive test for hepatitis B indicating acute or chronic infection. Participants who are or have received anti-HBV therapy and have undetectable HBV DNA for at least six months prior to study entry are eligible. Serological testing for HBV at screening is not required.
    - Known active hepatitis C virus (HCV) as determined by positive serology and confirmed by polymerase chain reaction (PCR). Participants on or having received

antiviral therapy are eligible provided they are virus-free by PCR for at least six months prior to study entry. Serological testing for HCV at screening is not required.

- Known active or latent tuberculosis (testing at screening not required).

6.3. Removal of Therapy

Study medication may be discontinued at the discretion of the Investigator in consultation with the participant in the following instances:

- Unacceptable AEs or toxicity, as defined in Section 9 of the protocol, or toxicity that, in the judgment of the Investigator, compromises the ability to continue study-specific procedures or is considered to not be in the participant's best interest
- Participant's request to discontinue for any reason
- Participant's non-compliance
- Pregnancy during the study
- Dosing is interrupted for more than 7 days for any reason

The reason for study medication discontinuation must be documented. In the case of discontinuation due to an AE, SAE, or laboratory toxicity, the event that led to discontinuation must be specified and recorded on the AE electronic case report form (eCRF).

6.4. Discontinuation / Withdrawal from Study

Participants considering withdrawing from the study should be informed that they can discontinue treatment or withdraw from the study at any time without having to justify the reason for doing so and without jeopardizing their future medical care by the physician or institution. Participants must be discontinued from study treatment and withdrawn from the study under the following circumstances:

- Participant or participant's legally authorized representative requests discontinuation from the study
- Investigator advises that the participants safety and/or well-being could be compromised by further participation
- A treatment-related AE ≥Grade 3
- Sponsor request, for example, due to suspicion of fraud, multiple enrollments in clinical studies, lack of compliance

In all cases, prior to the Investigator discontinuing the participant from the study, the Investigator should confer with the Sponsor or Medical Monitor.

If a participant withdraws, or is withdrawn, from the study, the principal reason leading to the decision for withdrawal must be recorded in the appropriate section of the eCRF. The reasons for withdrawal from the study will be coded as:

- AE (serious or non-serious; related or unrelated to study procedures / medication, as assessed by the Investigator)

- Disease progression or lack of efficacy
- Non-compliance (participant fails to comply with protocol requirements)
- Withdrawal of consent by participant
- Lost to follow-up
- Discretion of Investigator (the Investigator determines that continuation in the study would be detrimental to a participant's well-being)
- Discretion of Sponsor
- Female participant becomes pregnant
- Other (specify)

For participants withdrawing due to AE(s), the Investigator should review the potential relationship of the AE to the study/study medication and record their assessment on the eCRF. If a participant withdraws due to an AE, the AE should be followed as described Section 9.

All participants who discontinue treatment early for reasons other than recurrence will attend the early discontinuation visit. Participants assigned to study drug will also need to complete the Post-treatment Follow-up Visit which will be [redacted] after the last dose of REC-3964 and the Final Assessment Visit (see Table 1).

6.5. Study Termination

The study may also be terminated at the discretion of the Sponsor for any reason at any time. In addition, for reasonable cause, either the Investigator or the IRB/IEC may terminate the study at their center.

Conditions that may lead to reasonable cause and warrant termination include, but are not limited to:

- Administrative decision by the Investigator (closure at a specific site)
- New toxicological or pharmacological findings or safety issues that alter the risk-benefit assessment
- Participant or Investigator noncompliance
- Unsatisfactory participant enrollment
- Lack of adherence to protocol procedures
- Lack of evaluable and/or complete data
- Decision by the Sponsor to discontinue development of REC-3964 or modify the plan for its development
- Decision by a regulatory authority
- Local discontinuation of the study within a territory or at a specific site at the request of an Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

6.6. End of Study Definition

The end of the study is defined as the date of the last participant's Final Assessment Visit, conducted approximately eight weeks following the cessation of initial curative treatment defined in IC 6.1.4. (unless superseded by a Recurrence Visit and subsequent Safety Follow-Up Visit). The study may be terminated at individual sites or in its entirety per the criteria listed in Section 6.5. The Investigator will be informed of additional procedures necessary to ensure the protection of participants' interests.

A participant is considered to have completed the study if he/she has completed all study visits per protocol as shown in the SoA (Table 1).

7. TREATMENT OF PARTICIPANTS

7.1. [redacted]

[redacted]

7.2. [redacted]

[redacted]

[redacted]

7.3. [redacted]

[redacted]

7.4. Randomization and Blinding

Participants will be stratified on prior antibiotic use (fidaxomicin versus vancomycin). Within each stratum participants are randomized 1:2:1 to receive REC-3964 [redacted] or REC-3964 [redacted] or observation, respectively. The specific arm assigned will be done by IRT. The study is open-label and is unblinded.

8.

8.1.

8.2.

8.3.

8.4.

8.5. Administration

Randomized participants will receive their first dose of study drug at the investigative site on Day 1. Participants will maintain dosing with study drug throughout the Treatment Period, taking the drug at approximately the same time in the morning and the evening.

[redacted]

8.6. Study Drug Accountability

The Investigator is responsible for the control of the study drug under investigation. Adequate records of the receipt and disposition of the study drug must be maintained as directed by the Sponsor. All records and study drug supplies must be available for inspection by the monitor at every monitoring visit.

8.7. Study Drug Handling and Disposal

Unused study drug will be returned to the clinical depot or will be locally destroyed according to the study site procedures and as per the study Pharmacy Manual. The completed Drug Dispensing Log and Drug Return Record(s) will be returned to the Sponsor. The Investigator's copy of the Drug Return Record(s) must accurately document the return of all study drug supplies to the Sponsor, if applicable.

9. ASSESSMENT OF SAFETY

9.1. Adverse and Serious Adverse Events

9.1.1. Adverse Event

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or safety Follow-up Periods, even if no study treatment has been administered.

All AEs that occur after any participant has signed the full-study informed consent, before treatment, during treatment, or within [redacted] following the cessation of treatment, whether or not they are related to the study, must be recorded on forms provided by the Sponsor. AEs occurring in patients randomized to observation will be recorded up to [redacted] after the patient's last day in the observation arm.

This includes events that occur after obtaining consent and throughout the duration of the study, including the follow-up off-study medication period. For example,

- Pre- or post-treatment complications that occur as a result of protocol mandated procedures (e.g., invasive procedures such as venipuncture) during or after screening (before the administration of study drug)
- Any pre-existing condition that increases in severity, or changes in nature during or as a consequence of the study drug phase of a human clinical study, will also be considered an AE.

The following are not considered AEs:

- Any medical condition or clinically significant laboratory abnormality with an onset date before the Screening Visit is not an AE; it is considered to be pre-existing and should be documented on the medical history eCRF
- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion) performed; the condition that leads to the procedure or the complication that occurs as a result of the procedure is an AE
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for planned elective surgery, social and / or convenience admissions for example, a pre-existing condition that is deemed clinically stable.)
- An overdose without clinical sequelae

For this protocol, an overdose is administration of study drug in an amount in excess of that intended for the participant according to this protocol. There is no information regarding overdose with REC-3964. Any overdose, with or without associated AEs, must be promptly communicated to the Medical Monitor. The overdose event does not need to be recorded as an AE in the eCRF. If an AE is associated with the overdose, the AE should be recorded on the relevant AE/SAE sections in the eCRF.

- Pre-treatment emergent AEs will be those recorded after the full-study informed consent is signed up to the time of the first administration of study drug.
- Treatment-emergent adverse events (TEAEs) will be those recorded from the time of the first administration of study drug to the last dose of study drug taken by the participant during this study.
- Post-treatment emergent AEs will be those recorded from the last dose of study drug taken by the participant through the last study-associated visit, end of the study, or early termination.

All participants who receive at least one dose of study drug will be included in the analyses of safety.

9.1.2. Serious Adverse Event

A SAE is an AE occurring during any study phase (i.e., Screening, Treatment, or Follow-up Periods), and at any dose of the study drug that fulfills one or more of the following:

- Results in death. The cause of death is the AE; death is an outcome, not the AE.
- It is immediately life-threatening. It does not refer to an event, which hypothetically might have caused death, if it were more severe
- It requires in-participant hospitalization or prolongation of existing hospitalization. Hospitalization refers to an admission to the facility, not simply the amount of time that the participant may have been in the emergency department (rehospitalizations for the same AE within one week of discharge will not be considered as a new admission)
- Results in persistent or significant disability or incapacity in conducting activities of daily living for at least [redacted]
- Results in a congenital abnormality or birth defect
- It is an important medical event that may jeopardize the participant or may require medical intervention to prevent one of the outcomes listed above

All SAEs that occur after any participant signed consent, before treatment, during treatment, or within [redacted] following the cessation of treatment, whether or not they are related to the study must be recorded on forms provided by the Sponsor.

If clinical site personnel become aware of an event fulfilling the criteria of an SAE, the Investigator or clinical site personnel must notify Recursion Pharmacovigilance within 24 hours, regardless of assessment of relationship to the study drug. The site should report SAEs via the AE eCRF in the electronic data capture system (EDC) AND email a completed SAE report form to Recursion Pharmacovigilance at safety@recursionpharma.com within 24 hours of awareness.

In addition, notification must be provided by the Investigator to the Institutional Review Board (IRB).

Recursion is responsible for notifying the relevant regulatory authorities of certain events. It is the Investigator's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site.

Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical study. Each site is responsible for notifying its IRB or Independent Ethics Committee (IEC) of these SAEs.

9.1.3. Clinically Significant Laboratory Findings

Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE. In addition, an abnormal test finding will be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluations or medical / surgical intervention, including significant additional concomitant drug treatment or other drug. Note: Simply repeating a test finding, in the absence of any of the other listed criteria does not constitute an AE
- The test finding leads to a change in study drug dosing or discontinuation of the participant in the clinical research study
- The test finding is considered clinically significant by the Investigator based on his / her medical judgment. If an abnormal laboratory result is recorded as an AE, then the corresponding laboratory value must be marked clinically significant in the database. Similarly, if an abnormal laboratory value is marked clinically significant in the database, there should be a corresponding AE entered.

In case of unexplained or unexpected clinical laboratory test values, the tests will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the Investigator will indicate which of these deviations after treatment are clinically significant. These clinically significant deviating laboratory results will then be recorded as AEs and the relationship to the treatment will be indicated.

9.2. Relationship to Study Drug

An Investigator who is qualified in medicine must make the determination of relationship to the study drug for each AE (unrelated or related). The Investigator should decide whether, in his or her medical judgment, there is a reasonable biological possibility that the event may have been caused by the study drug. If no valid reason exists for suggesting a relationship, then the AE should be classified as “not related.” If there is a valid biological basis for asserting that a causal relationship is present, then the AE should be considered “related.”

9.3. Recording Adverse Events

All AEs spontaneously reported by the participant and / or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded on the Adverse Event eCRF for that visit. All participants will be queried, using non-leading questions, about the occurrence of AEs at each study visit and throughout the study.

When possible, a constellation of signs and/or symptoms should be identified as 1 overall event or diagnosis. All AEs for randomized participants will be recorded in the eCRF and the participant's source documents. The following data should be documented for each AE:

- Description of the event
- Classification of "serious" or "not serious"
- Date of first occurrence and date of resolution (if applicable)
- Severity
- Causal relationship to study drug
- Action taken
- Outcome
- Concomitant medication or other treatment given.

Abnormal laboratory values that constitute an AE or lead to discontinuation of administration of study drug must be reported and recorded as an AE. Information about AEs and SAEs will be collected from signing of consent form until [redacted] after the participant has received their last dose of study drug. The AE term should be reported using standard medical terminology when possible.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate AE. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (e.g., diabetes mellitus rather than hyperglycemia).

For each AE, the Investigator will evaluate and report the onset (date), resolution (date), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the participant to discontinue the study or study treatment.

Table 3: Grading of Adverse Event Severity

Classification	Definition
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
Grade 4	Life-threatening consequences: urgent intervention required
Grade 5	Death related to an adverse event

General rules for the grading of AEs are found in Table 3. Investigators seeking further guidance on the assessment of intensity may consult the CTCAE, v 5.0 (information, including a searchable spreadsheet) can be found at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 9.1.2. An AE of severe intensity may not be considered serious, and a mild AE that resulted in a hospitalization, for instance, is always considered serious as it meets at least one of the criteria given in Section 9.1.2 as constituting an SAE.

9.4. Reporting Serious Adverse Events

All SAEs (and AESIs, if specified) that occur after any participant signed consent, before treatment, during treatment, or within [redacted] following the cessation of treatment, whether or not they are related to the study must be recorded. Any SAEs or AESIs (if specified) considered related to the investigational product and discovered by the Investigator at any time after the study should also be reported to Recursion.

All SAEs/AESIs must be reported to Recursion within 24 hours of the first awareness of the event by entering all known information into the electronic clinical database. If not entered into the database within the first 24 hours of awareness, the Investigator must complete, sign and date an SAE Report Form, verify the accuracy of the information recorded and email Recursion at safety@recursionpharma.com. The information must then be entered into the electronic clinical database as soon as possible. Should the SAE be entered into the database within the first 24 hours of awareness, the SAE will be automatically reported, and a paper SAE form will not be required.

Additional follow-up information, if required or available, must be sent to the Sponsor by entering all follow-up information into the electronic clinical database. If not entered into the database within the first 24 hours of awareness, the Investigator must complete, sign and date a follow-up SAE Report Form, verify the accuracy of the information recorded and email Recursion at safety@recursionpharma.com. The information must then be entered into the electronic clinical database as soon as possible. Should the follow-up information be entered into the database within the first 24 hours of awareness, the SAE will be automatically reported, and a paper SAE form will not be required. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

The original documentation must be maintained by the study site with the participant's study records.

It is the Investigator's responsibility to obtain, and redact of personal identifying information, any hospital discharge summaries, death certificates, autopsy reports, or birth certificates associated with any SAEs developing in a participant (or, in the case of pregnancy, a participant's partner) in this study. Such documents should be submitted to the Sponsor as soon as possible when the Investigator has access to a copy of the document.

The Sponsor is responsible for notifying the relevant regulatory authorities of certain events (SUSARs, etc.). It is the Principal Investigator's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7 / 15 Day Safety Reports) that occur during this clinical study and in other clinical studies using the same IMP (REC-3964). Each site is responsible for notifying its IRB or IEC of these additional SAEs.

9.4.1. Reporting of SUSARs

Suspected unexpected serious adverse reactions (SUSARs) will be reported to the Competent authority (CA), EC and the European Medicines Agency EudraVigilance database as required. Also, the investigators must be informed. The following timelines should be followed:

- The Sponsor will ensure that all relevant information about SUSARs that are fatal or life-threatening is recorded and reported as soon as possible to the CA concerned, the EC concerned and the EudraVigilance database but no later than seven days after first knowledge by the Sponsor. The sponsor shall submit a completed report within an additional eight days.
- All other SUSARs will be reported to the CA concerned, the EC concerned and the EudraVigilance database as soon as possible but no later than 15 days of first knowledge by the Sponsor and updated as soon as possible.

9.5. Pregnancies

Investigators must record and report pregnancies occurring in either a study participant or the partner of a study participant within 24 hours of becoming aware of the pregnancy. A Pregnancy Reporting Form should be completed by the Investigator / Designee, dated, signed, and emailed to Recursion. Pregnancy in itself is not regarded as an AE unless there is a suspicion that a study drug may have interfered with the effectiveness of a contraceptive medication.

Participants who become pregnant while on study should immediately cease using study drug. The participant should be monitored throughout the pregnancy up to its conclusion. If the pregnancy results in a live birth, the Investigator should report the results of the pregnancy on the Pregnancy Reporting Form. The infant should be followed (telephone follow-up is sufficient) for two months to ascertain any congenital anomalies. Any congenital anomalies and pregnancy conclusion other than a live infant should be reported using the SAE Report Form within 24 hours of the Investigator's learning of the information. Any delivery that is other than an unassisted (by a health care professional) vaginal one should also be reported as an SAE within 24 hours of the Investigator learnings of the information with the event being whatever condition necessitated either a cesarian section or other operative (e.g., forceps) delivery.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the participant was discontinued from the study unless and until the participant has withdrawn informed consent.

All reports of congenital abnormalities / birth defects are SAEs. Spontaneous miscarriages are also SAEs and should also be reported as such.

The Investigator should obtain a copy of any vital statistics document filed at the conclusion of the pregnancy (e.g., birth certificate) and, if circumstances warranted, an autopsy report of the stillbirth or the deceased infant, redacted of personal identifying details.

If during the pregnancy, the pregnant woman was hospitalized, unless the hospitalization had been planned prior to enrollment in the study, the Investigator should obtain and send to Recursion a copy of the redacted hospital discharge summary. At the conclusion of the pregnancy, the Investigator should obtain a copy of the discharge summary from the hospital or birthing center at which the delivery took place and send a redacted copy of it to Recursion.

Male participants with partners who become pregnant.

The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study.

The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. The investigator should follow-up with the participant regarding any congenital anomalies diagnosed at 2 months following the birth of the child. Any termination of the pregnancy will be reported including fetal status (presence or absence of anomalies) and indication for the procedure.

9.6. Overdose

An overdose is defined for this study as any administration of study drug in an amount in excess of that intended according to this protocol. In-and-of-itself, an overdose is not an adverse event (an exception being an intentional overdose); any clinical development resulting from an overdose, however, is an adverse event and should be reported by the Investigator accordingly. Should an overdose occur, the Investigator should contact the Medical Monitor as soon as possible using the contact information provided on the cover sheet of this protocol.

9.7. Additional Safety Assessments

9.7.1. Vital Signs

Vital signs including temperature, respiratory rate, heart rate, and blood pressure will be collected as outlined in the SOA (Table 1). Where applicable, vital signs will be collected pre-dose.

9.7.2. Physical Examination

A full physical examination is required [redacted]. For all other time points, an abbreviated PE (limited to organ systems based on presenting clinical signs and symptoms) is sufficient.

9.7.3. Electrocardiogram (ECG)

All standard digital single 12-lead ECGs will be performed during Screening. Heart rate, PR duration, QRS duration, QT duration, QTcF (Fridericia's correction), and the investigator's overall interpretation will be recorded in the eCRF.

9.7.4. Clinical Laboratory Assessments

Local laboratory testing will be used in this study. Screening Period labs will be used for eligibility assessments according to the inclusion and exclusion criteria. Further assessments will be made as specified in Table 1. The full testing panel to be carried out by sites is presented in Table 4. The Sponsor must be notified of any exceptions due to local testing restrictions to ensure safety assessment is not jeopardized.

Table 4: Laboratory Assessments

Hematology	Serum Chemistry	Urinalysis (dipstick)
• hemoglobin • hematocrit • reticulocytes • platelets • erythrocytes • leukocytes • neutrophils • lymphocytes • monocytes • eosinophils • basophils	• albumin • amylase • alkaline phosphatase • ALT • AST • bicarbonate • BUN / Urea • calcium • chloride • CPK • creatinine • GGT • glucose (random) • magnesium • phosphorous • potassium • sodium • total bilirubin • total protein	• appearance • color • ketones • leukocytes • protein • glucose • bilirubin • urobilinogen • occult blood (microscopic examination of sediment will be performed per the discretion of the Investigator)
Coagulation		
• INR • APTT		

10. ASSESSMENT OF EFFICACY

Efficacy assessments will be conducted as specified in the SOAs (Table 1). Efficacy endpoints of the study are listed in Section 4.

10.1. Efficacy Assessments

10.1.1. Survival without Recurrence or Additional Treatment

The primary efficacy endpoint is survival without recurrence or requirement for additional CDI treatment for at least eight weeks following the cure of preceding CDI with initial curative treatment defined in IC 6.1.4. (see also Section 12.3.1). Recurrent CDI is defined as a new episode of CDI associated with a new positive *C. difficile* stool toxin or requirement for additional CDI treatment within 8 weeks of the cessation of initial curative treatment defined in IC 6.1.4. All new episodes of diarrhea will be tested for toxigenic *C. difficile* to confirm CDI recurrence. The primary efficacy endpoint will be analyzed as the rate of recurrent *C. difficile* infection or requirement of additional CDI treatment for at least 8 weeks, upon resolution of the initial preceding CDI diarrheal episode with vancomycin or fidaxomicin.

10.1.2. Rate of Recurrence

The rate of recurrence of CDI associated with a new positive *C. difficile* stool toxin after treatment with REC-3964 or observation will be evaluated for participants who have rCDI during the study.

10.1.3. Time to Recurrence

The time to recurrence of rCDI after treatment with REC-3964 or after Day 1 for participants in the Observation arm will be evaluated.

10.1.4. Proportion of Participants with Severe rCDI

The proportion of participants with severe rCDI after treatment with REC-3964 or after Day 1 for participants in the Observation arm will be defined as CDI with white blood cell count >15,000 cells/mL and/or serum creatinine ≥1.5 mg/dL.

10.1.5. rCDI-associated hospital admissions

The incidence of rCDI associated hospital admissions will be evaluated for participants who have rCDI across the study arms during the study.

10.1.6. Evaluation of Diarrheal Episodes

The number of unformed [types 5-7 on the Bristol stool scale] bowel movements per day will be assessed as recorded by the participant in the stool diary, will be monitored through Day 57 in order to identify a new episode of diarrhea.

10.1.7. [redacted]

[redacted]

10.1.8. [redacted]

[redacted]

11. PHARMACOKINETIC ASSESSMENTS

Pharmacokinetic parameters of REC-3964 following [redacted] will be measured in plasma of the study as described below.

- Plasma concentrations of REC-3964 will be assessed within 15 minutes pre-dose, 1 hour, 3 hours, 6 hours at each clinic visit (Days 1 and 15. All post-dose PK time points have a window of +/- 30 minutes), and once (any time) during the Recurrence and Early Discontinuation visits.
- Pharmacokinetic parameters to be assessed include, but are not limited to:
  - C_{max}
  - T_{max}
  - C_{trough}

REC-3974 is the S-enantiomer of REC-3964. Remaining PK samples may be used to measure REC-3974 concentrations.

12. STATISTICAL METHODS

The statistical analysis plan (SAP) will be finalized prior to final database lock, and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints. The SAP will supersede the protocol should any differences arise. Any changes to the methods described in the final SAP will be described and justified in the clinical study report (CSR).

12.1. Analysis Population

For analyses, the following analyses population are defined in Table 5:

Table 5: Analyses Population

Analysis Population	Description
All Randomized	All participants who finished the Screening Period, eligible to, and randomized to study Treatment Period.
Intent-to-treat Population	All randomized participants who received at least 1 dose of REC-3964 intervention or who are intended to be followed-up on in the Observational arm. Participants will be analyzed according to the treatment arm they were originally assigned to, regardless of the treatment received, or withdrawals. ITT will be the primary efficacy analyses population.
Per-protocol Population	All randomized participants who completed the study without major protocol deviations. Participants with protocol deviations that affect primary efficacy analysis may be included in the Per-protocol population depending on the actual impact of the protocol deviation. Per-protocol Population will be used for sensitivity analysis.
As-treated Population	All randomized participants who received at least 1 dose of REC-3964 intervention or who are intended to be followed-up on in the Observation arm. Participants will be analyzed according to the treatment received, regardless of assigned treatment. The as-treated Population Set will be used for safety analysis.
Pharmacokinetic (PK) Population	All participants in the As-treated Population Set who provide at least 1 measurable post-dose PK sample with no protocol deviation affecting PK evaluation.

12.2. Statistical Analyses

12.2.1. General Consideration

In general, participant characteristics, demographics data, and safety summary statistics will be presented by treatment arm and overall unless otherwise specified.

Descriptive analyses and summary statistics will be provided for all data collected during the study, where applicable. Continuous data will be summarized in terms of the number of participants, mean, standard deviation, median, minimum, maximum and number of observations, unless otherwise stated, e.g., additional lower and upper quartiles. Categorical data will be summarized in terms of the number of participants providing data at the relevant time point (n), number of participants with missing data, frequency counts and percentages. Changes from baseline in categorical data will be summarized using shift tables where appropriate.

In addition to the overall crude analyses, stratified analyses, as specified, will be performed on all randomized subjects on the following stratification factor (recorded at randomization as per IRT):

- prior antibiotic use (vancomycin versus fidaxomicin)

12.3. Efficacy Endpoints Analyses

12.3.1. Primary Endpoint Analyses

The primary endpoint is survival without recurrence or requirement for additional CDI treatment for at least eight weeks after the cure of preceding CDI with initial curative treatment defined in IC 6.1.4.

The primary endpoint will be analyzed as the proportion of participants with recurrent *C. difficile* infection (rCDI) or requirement for additional CDI treatment among randomized participants.

The proportion and 2-sided 95% confidence interval (CI) based on the Clopper-Pearson exact method will be calculated for each REC-3964 treatment group and the Observation group. The estimated difference and corresponding 2-sided 95% CI in recurrence rate between randomized groups will be provided using a binomial model. No hypothesis test will be conducted for comparison. In addition, analyses will be stratified on prior antibiotic use and stratum specific proportions and corresponding 95% CI's will be reported for each REC-3964 treatment group and the Observation arm.

12.3.2. Secondary Endpoints Analyses

- Rate of recurrence

The rate of recurrent *C. difficile* infection is defined as the proportion of participants who had the *C. difficile* recurrence among randomized participants.

The rate of recurrence and 2-sided 95% confidence interval (CI) based on the Clopper-Pearson exact method will be calculated for each REC-3964 treatment group and the Observation group. The estimated difference and corresponding 2-sided 95% CI in recurrence rate between

randomized groups will be provided using a binomial model. No hypothesis test will be conducted for comparison.

In addition, analyses will be stratified on prior antibiotic use and stratum specific proportions and corresponding 95% CI's will be reported for each REC-3964 treatment group and the Observation arm.

- Time to recurrence

Time to recurrence is defined as the time from the date of randomization until the occurrence of rCDI. The rCDI date is determined by the start of a new episode of symptoms in conjunction with a new positive *C. difficile* stool toxin result, or the need for additional CDI treatment. The KM method will be used to analyze the time to recurrence for each of the study arms. Participants who did not have recurrence by the time of analysis cutoff date will be censored. The estimated median time to rCDI along with the respective 95% confidence interval will be provided. In addition, analyses will be stratified on prior antibiotic use and stratum specific KM medians and corresponding 95% CI's will be reported for each REC-3964 treatment group and the Observation arm.

- Severity of rCDI

The proportion of participants with severe rCDI and 2-sided 95% CI based on the Clopper-Pearson exact method will be calculated for each REC-3964 treatment and the Observation arm. Analyses stratified on prior antibiotic use will also be performed and stratum specific proportions and corresponding 95% CI's will be reported for each REC-3964 treatment group and the Observation arm.

- The rCDI-associated hospital admissions

The incidence of rCDI associated hospital admissions will be summarized for participants who have rCDI during the study for each REC-3964 treatment and the Observation arm. Analyses stratified on prior antibiotic use will also be performed and stratum specific incidences and corresponding 95% CI's will be reported for each REC-3964 treatment group and the Observation arm.

12.3.3. Evaluation of Diarrheal Episodes

The number of unformed [types 5-7 on the Bristol stool scale] bowel movements per day will be assessed as recorded by the participant in the stool diary, will be monitored through Day 57 in order to identify a new episode of diarrhea. In addition, analyses will be stratified on prior antibiotic use.

12.3.4. [redacted]

12.3.4.1. [redacted]

[redacted]

12.3.4.2. [redacted]

[redacted]

12.4. Safety Endpoint Analyses

Safety and tolerability will be assessed through AEs, clinical laboratory values (hematology including reticulocyte count, biochemistry, coagulation, urinalysis, hepatic enzymes, bilirubin, and complete blood count), vital signs, ECGs, and physical examination findings.

Safety analysis will be conducted using data from the As-treated Population. No formal inferential analyses will be conducted for safety variables. Safety will be evaluated using descriptive statistics.

12.4.1. Adverse Events

Treatment-emergent adverse events (TEAEs) are defined as AEs that occur after the participant received first dose of study treatment or if a pre-existing condition worsens in severity or becomes serious after receiving the first dose of study treatment up to [redacted] after the last dose of study treatment.

AEs will be graded by the Investigator according to the National Cancer Institute (NCI) CTCAE v5.0 or higher.

The frequency and percentage of participants with TEAEs will be displayed by System Organ Class (SOC) and Preferred Term (PT). Summary of TEAEs by severity and relationship to study medication will also be provided. Serious AEs and TEAEs resulting in discontinuation of study medication will be summarized separately in a similar manner.

12.4.2. Clinical Laboratory, Vital Signs, Electrocardiograms, and Physical Examinations

Vital signs, ECG parameters, and clinical laboratory data (hematology including reticulocyte count, biochemistry, coagulation, urinalysis, hepatic enzymes, bilirubin, and complete blood count) will be described using raw data and changes from baseline (Day 1), where both baseline assessments are available. For clinical laboratory values, vital signs, ECG readings, and physical examination results, the values that are below, within, or above a defined normal range will be counted and tabulated by the number and percentage of participants with such values.

12.5. Statistical Analyses for Pharmacokinetic Data

The SAP for PK analysis will be a component of the clinical SAP and will be generated by Recursion PK group; the PK SAP will be finalized prior to database lock. Full details of the analyses to be performed will be included in the PK SAP. Any deviation from the SAP will be reported in the Section “Changes in Planned Analysis” in the CSR.

Population PK and/or exposure-response analysis may be conducted for all endpoints in this study. If this analysis is conducted, a separate report may be prepared.

12.5.1. Pharmacokinetic Evaluation

PK parameters will be derived from the plasma concentration-time data.

A noncompartmental PK method, as appropriate, will be used to analyze the plasma concentrations of REC-3964 and its metabolites, if applicable.

Individual and summary statistics of plasma concentrations at each sampling time point will be presented for each treatment and cohort. Individual and mean (±SD standard deviation where appropriate) plasmaconcentration-time profiles will be presented on linear and semi-logarithmic scales.

Individual and summary statistics of all PK parameters will be presented for each treatment and cohort. Scatterplots of mean and median values will be presented by treatment and cohort for PK parameters.

12.6. Sample Size Evaluation

Approximately, 80 participants will be randomized to this study. No formal hypothesis testing will be conducted. The sample size is deemed appropriate to provide preliminary safety and efficacy profiles of REC-3964 on *C. difficile* participants.

12.7. Interim Analyses

No interim analyses are planned.

13. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The clinical research site will be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study will be conducted according to the relevant regulatory requirements. The eCRF entries will be verified with the source documentation.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group may request access to all source documents, eCRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

An explanation will be given for all missing, unused, and spurious data in the relevant sections of the CSR.

13.1. Case Report Form

The Study Data Management Plan will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process. Furthermore, the Study Data Management Plan will describe the data flow and timelines within the study.

Data captured electronically will be immediately saved to a central database and changes tracked to provide an audit trail. When the principal investigator (PI) has signed the eCRF electronically as per eCRF instructions, then the participant's data will be locked.

An eCRF casebook will be completed by an authorized study personnel member whose training for this function is completed in the EDC system unless otherwise directed. The electronic case report form casebook will only capture the data required per the protocol schedule of assessments and procedures, unless collected by a non-EDC vendor system (e.g., central laboratory). The Inclusion/Exclusion Criteria and Enrollment electronic case report forms should be completed only after all data related to eligibility are available. Data entry should be performed in accordance with the case report form Completion Guidelines provided by the Sponsor. Subsequent to data entry, a study monitor may perform source data verification. System-generated or manual queries will be issued in the EDC system as data discrepancies are identified by the study monitor or CRO Monitor who routinely review the data for completeness, correctness, and consistency. The site investigator, site coordinator, or other designee is responsible for responding to the queries in a timely manner, within the system, either by confirming the data as correct or updating the original entry, and providing the reason for the update (e.g., data entry error). Original entries as well as any changes to data fields will be stored in the audit trail of the system. Regular oversight by the PI of the data entered into the EDC system is expected to occur on an ongoing basis throughout the study to ensure quality and completeness. At a minimum, before any interim, final, or other time points (as instructed by Recursion), the Investigator applies their electronic signature to confirm that the forms have been reviewed and that the entries accurately reflect the information in the source documents.

13.2. Study Monitoring

Before an investigational site can enter a participant into the study, a representative of Recursion Pharmaceuticals, Inc. will visit the investigational study site to:

- Determine the adequacy of the facilities and
- Discuss with the Investigators and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of Recursion Pharmaceuticals, Inc. or its representatives. This will be documented in a Clinical Study Agreement between Recursion Pharmaceuticals, Inc. and the Investigator.

During the study, a monitor from Recursion Pharmaceuticals, Inc. or representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the Investigators,
- Confirm that facilities remain acceptable,
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that study drug accountability checks are being performed,
- Perform source data verification. This includes a comparison of the data in the eCRFs with the participant's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each participant (e.g., clinic charts),
- Record and report any protocol deviations not previously sent to Recursion Pharmaceuticals, Inc., and
- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to Recursion Pharmaceuticals, Inc. and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the Investigators or other staff needs information or advice.

13.3. Audits and Inspections

Authorized representatives of Recursion Pharmaceuticals, Inc., a regulatory authority, an IEC, or an IRB may visit the site to perform audits or inspections, including source data verification and drug accountability verification. The purpose of a Recursion Pharmaceuticals, Inc. audit or inspection is to systematically and independently examine all study-related activities and data to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the ICH, and any applicable regulatory requirements. The Investigator should contact Recursion Pharmaceuticals, Inc. immediately if contacted by a regulatory agency about an inspection.

13.4. Institutional Review Board (IRB)

The Investigator must obtain IRB approval for the investigation. Copies of the initial IRB approval and all materials approved by the IRB for this study, including the participant consent form and recruitment materials, must be maintained by the Investigator, and be made available for inspection.

13.5. Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

The Sponsor or its representative will not provide individual genotype results to participants, any insurance company, any employer, their family members, general physician, or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the participant. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a participant. For example, in the case of a medical emergency, the Sponsor or representative physician or an Investigator might know a participant's identity and also have access to his or her genetic data. Also, regulatory authorities may require access to the relevant files.

Any required notifications (e.g., serious breaches, other reports relevant for participant safety) will be completed, as per the applicable laws and regulations.

14. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, Recursion Pharmaceuticals, Inc. may conduct a quality assurance audit. See Section 13.3 for more details regarding the audit process.

15. ETHICS

15.1. Ethics Review

The final study protocol, including the final version of the full study Informed Consent Forms, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The Investigator must submit written approval to Recursion Pharmaceuticals, Inc. before he or she can enroll any participant into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit participants for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the study drug. Recursion Pharmaceuticals, Inc. will provide this information to the Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

15.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (World of Medical Association, 2013) and are consistent with ICH/GCP (ICH E6, 2016), applicable regulatory requirements and Recursion Pharmaceuticals, Inc.'s policy on bioethics.

15.3. Written Informed Consent

The Investigators at each center will ensure that the participant is given full and adequate oral and written information about the nature, purpose, possible risk, expectations, responsibilities, and benefit of the study. Participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the individual is otherwise entitled, and the participant may discontinue at any time without penalty or loss of benefits. The participant should be given the opportunity to ask questions and allowed time to consider the information provided.

The participant's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigators must maintain the original, signed informed consent forms. A copy of the signed informed consent forms must be given to the participant.

16. DATA HANDLING AND RECORDKEEPING

16.1. Inspection of Records

Recursion Pharmaceuticals, Inc. will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, participant charts and study source documents, and other records relative to study conduct.

16.2. Retention of Records

An Investigator shall retain all documentation relating to the study for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

If it becomes necessary for Recursion Pharmaceuticals, Inc., or the regulatory authority to review any documentation relating to the study, the Investigator must permit access to such records.

17. PUBLICATION POLICY

By signing this clinical study protocol, the Investigator reaffirms to the Sponsor that he or she will maintain in confidence all information furnished to him or her or resulting from this study. The Investigator and designees will only divulge such information as may be necessary to the IRB, the members of the staff, and the participants who are involved in this study.

18. LIST OF REFERENCES

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APPENDIX A. [redacted]

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Table 6: [redacted]

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Table 7: [redacted]

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APPENDIX B.

Table 8:

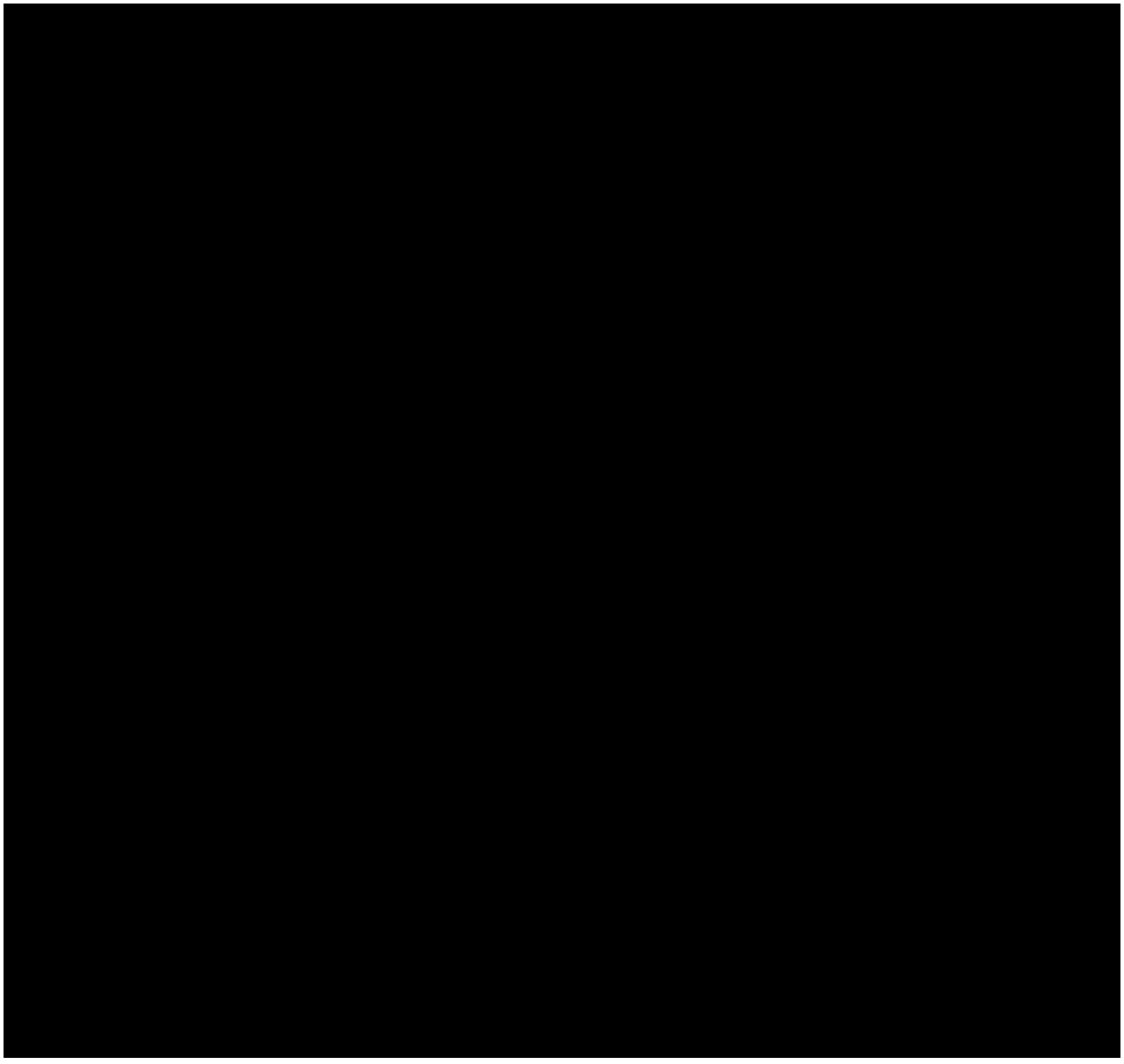

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Table 9: [redacted]

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Table 10: [redacted]

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Table 12: [redacted]

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Signature Page for: REC-3964-201 Protocol_Version 7_03252025
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Approval Task Verdict: Approved	[redacted] Medical 27-Mar-2025 19:27:12 GMT+0000

Approval Task Verdict: Approved	[redacted] Biostats 27-Mar-2025 21:42:36 GMT+0000

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