NOVARTIS

Clinical Development

ABO809

CABO809A02101

An Open Label Cryptosporidium Controlled Human Infection Model (CHIM) to assess the efficacy and safety of ABO809 in healthy participants

Statistical Analysis Plan (SAP)

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Commercially Confidential Information (CCI)

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List of abbreviations

ATC	Anatomical Therapeutic Chemical
AE	Adverse Event
AESI	Adverse Event of Special Interest
BMI	Body mass index
bpm	beats per minute
CHIM	Controlled Human Infection Model
CE	Cell Equivalents
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
CSR	Clinical Study Report
DBL	Data Base Lock
DFA	Direct Fluorescent Antibody
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
EIA	Enzyme Immunoassay
EOS	End of study
FAS	Full Analysis Set
FDA	U.S. Food and Drug Administration
GP60	Gene Encoding Glycoprotein 60 kDa Protein
HR	Heart Rate
IA	Interim Analysis
IgG	Immunoglobulin G
Kg	Kilogram(s)
LLQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Drug Regulatory Affairs
mL	Milliliter(s)
mmHg	Millimeter(s) of mercury
MMRM	Mixed model for repeated measures
PD	Pharmacodynamics
PT	Preferred Term
qPCR	Quantitative Polymerase Chain Reaction
QTcB	QT interval corrected for heart rate according to Bazett
QTcF	QT interval corrected for heart rate according to Fredericia
RAP	Reporting & Analysis Planning
ROC	Receiver Operating Characteristic Curve
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SI	International System of Units
SOC	System Organ Class
ULQ	Upper Limit of Quantification
WHO	World Health Organization

1 Introduction

The Reporting and Analysis Planning (RAP) documents contain detailed information to aid the production of Statistics & Programming input into the Clinical Study Report (CSR) for trial ***"CABO809A02101".***

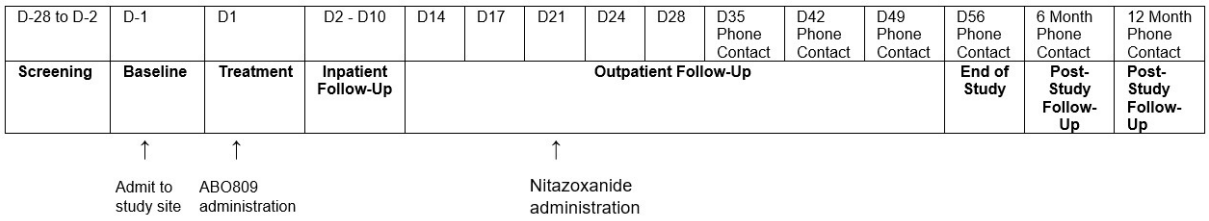
The statistical analysis plan (SAP) describes the implementation of the statistical analysis planned in the protocol to be reported in the CSR as well as any planned Interim Analysis (IA) as outlined in Section 2.12 below.

This SAP is based on the protocol v02 for study CABO809A02101 dated 02 December 2021

1.1 Study design

CABO809A02101 is a Phase 1, single-center, open-label, *Cryptosporidium* Controlled Human Infection Model (CHIM) study to characterize the incidence of infection and associated symptoms following the administration of single doses of *Cryptosporidium parvum* oocysts (ABO809) to healthy adult volunteers.

Figure 1-1 Study design



The study will consist of up to a 28-day Screening/Baseline period, a single ABO809 dose administration on Day 1, an inpatient monitoring period, an outpatient follow-up period, and a study completion evaluation on Day 56. A CHIM diary will be provided to each participant to collect information on the participant's clinical symptoms. To minimize the risk of long-term asymptomatic shedding and recurrence, participants may receive nitazoxanide per the U.S. Prescribing Information on Day 21, however, it may be administered earlier based on clinical findings. If a participant experiences diarrhea after Day 10 (after the participant has been discharged from the study site), the participant may be readmitted, at the discretion of the Investigator, to remain domiciled until the cessation of symptoms or Day 21, whichever is earlier. Participants may also come back to the study site for an unscheduled visit until end of study (EOS). The EOS visit will occur on Day 56. Additional Post-Study Follow-Up phone calls will be conducted after EOS at 6 and 12 months after ABO809 administration (Figure 1-1).

Participants will be monitored daily for infection (parasitological evaluation) and diarrheal illness (clinical evaluation) during the domicile period. The monitoring of symptoms will continue in the outpatient setting with the collection of information via the CHIM diary and Safety Follow-Up Calls. Participants will provide a health and stool update daily from Day 1 through Day 28 in the CHIM diary. Safety Follow-Up Calls will occur on Days 35, 42, 49, and 56. Finally, information on secondary transmission to household contacts (demographics (gender, age and relationship to participant), occurrence of diarrhea, abdominal cramps and pain,

or vomiting, and diagnosis of cryptosporidiosis) and potential recurrence of gastrointestinal symptoms in participants will be collected on Days 14, 17, 21, 24, 28 on site and on Days 35, 42, 49, and 56 via Safety Follow-Up calls.

The study will be comprised of three (to a maximum of six) sequential cohorts, each of approximately 10 healthy participants aged 18 to 50 years. This is an adaptive study, and for a particular cohort, safety, incidence of infection and incidence of clinical illness will be evaluated up to Day 14 to inform whether the ABO809 dose needs to be changed for dosing of the next cohort. If there are no safety issues, and the expected incidence of infection and illness are observed in one cohort, the next cohort will be dosed at the same dose level. If the dose of ABO809 is changed from one cohort to the next, additional cohorts may be added to further evaluate *Cryptosporidium* infection and clinical illness following dosing with ABO809.

The first dose level group will receive ABO809 at a dose of $1x10^4$ oocysts. If the desired incidences of infection and diarrheal illness are not observed, a new dose level group, receiving ABO809 at a dose of $1x10^6$ oocysts, may be initiated. If needed to optimize the model, intermediate ABO809 doses may be evaluated. The clinical dose is prepared using the most recent available % viability stability data per clinical batch.

The primary endpoint is *Cryptosporidium* infection as indicated by detection of oocysts in fecal samples by Enzyme Immunoassay (EIA) (and confirmed by direct fluorescent antibody (DFA) if positive) ≥72 hours following an oral administration of ABO809 (or sooner if associated with symptoms suggestive of diarrheal illness). Secondary endpoints include characterization of diarrheal illness, safety and tolerability.

1.2 Study objectives, endpoints and estimands

Table 1-1 Objectives and related endpoints

Objective(s)	Endpoint(s)
Primary objective(s)	**Endpoint(s) for primary objective(s)**
• To evaluate the incidence of *Cryptosporidium* infection following an oral administration of ABO809	• *Cryptosporidium* infection as indicated by *Cryptosporidium* oocysts in fecal samples measured by EIA (and confirmed by DFA if positive) at ≥72 hours post-administration (or sooner if associated with symptoms of diarrheal illness) up to Day 10 (inclusive).
Secondary objective(s)	**Endpoint(s) for secondary objective(s)**
• To evaluate the incidence and characteristics of diarrheal illness following an oral administration of ABO809	• Presence of clinical diarrheal illness by Day 10 (inclusive) and Day 28 • Number of diarrhea episodes • Stool weight • Grading of stool consistency • Time to onset and time to resolution of diarrheal illness • Characteristics of clinical signs and symptoms associated with clinical diarrheal illness such as (abdominal pain, abdominal cramping, nausea, vomiting, fever, electrolyte imbalance, dehydration) up to Day 28 • *Cryptosporidium* infection as indicated by *Cryptosporidium* oocysts in fecal samples measured by EIA (and confirmed by DFA if positive) at ≥72 hours

Objective(s)	Endpoint(s)
	post-administration (or sooner if associated with symptoms of diarrheal illness up to Day 28)
• To evaluate the time to onset and resolution of *Cryptosporidium* infection in participants who developed an infection following an oral administration of ABO809	• Detection of fecal shedding of *C. parvum* oocysts by EIA (and confirmed by DFA if positive) up to 28 days after administration of ABO809 • Time to onset and time to resolution of *Cryptosporidium* infection following an oral administration of ABO809
• To assess the safety and tolerability of ABO809	• Adverse events, vital signs, ECG findings, safety laboratory assessments including chemistry, hematology, and urinalysis results up to and including the Day 28 visit • Adverse events of special interest by telephone follow-up after Day 28 through 12 months after ABO809 administration
Exploratory objective(s)	**Endpoint(s) for exploratory objective(s)**

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1.2.1 Primary estimand(s)

Not Applicable

1.2.2 Secondary estimand(s)

Not Applicable

2 Statistical methods

2.1 Data analysis general information

Data will be analyzed by Novartis for the interim assessments and after final Data Base Lock (DBL) using SAS version 9.4 according to the data analysis presented in Section 12 of the study protocol which is also available in Appendix 16.1.1 of the CSR.

For key outcomes for the interim assessments and after final DBL, pooled analysis will be performed by pooling all cohorts up to the time of the interim/final DBL.

For statistical analyses and clinical study reporting, treatment group name (see Section 2.1.1) in addition to cohort number will be used. Information on imputation rules and the methods of efficacy and safety analyses is given in the sections to follow and details will be provided, as applicable, in Appendix 16.1.9 of the CSR. This SAP covers the methods for the planned interim analysis and the final analysis.

Unless otherwise stated, summary tables/figures/listings will include data for all participants in the population under consideration.

Categorical data will be presented as frequencies and percentages. For continuous data, number of participants (n), mean, standard deviation, median, 25th and 75th percentiles, minimum, and maximum will be presented.

2.1.1 General definitions

Study treatment: ABO809, 3x10^6 *C. parvum* oocysts(CE)/3 mL as concentration for oral suspension. There will be no control drug used in this study. Additional study treatment is nitazoxanide 500 mg as tablet for oral use, which will be used as rescue medication on Day 21 and may be administered earlier based on clinical findings. Both are referred to as study treatment and rescue medication in the document, respectively.

The treatment group name alone (pooled analyses) and in addition to cohort number will be used in statistical analyses and outputs. The following abbreviated treatment groups will be used as the headers in the CSR outputs:

- ABO809 1x10^4 CE cohort X
- ABO809 1x10^6 CE cohort Y, if applicable
- ABO809 1x10^x CE cohort Z, if applicable (e.g. alternative dose)

Baseline: The last measurement made prior to administration of the first dose of study treatment. Note this may include measurements taken on the day before single dose administration (e.g. lab tests, ECG, vital signs, etc.).

Study day: Study day will be calculated with respect to the first dose of study treatment. The first day of administration of study treatment (first dose) is defined as Day 1. Day -1 will be the day before Day 1. There is no Day 0 designated in this study.

For assessments collected on or after Day 1, study day = date of assessment ‑ date of first dose of study treatment + 1.

For assessments collected prior to Day 1, study day = date of assessment - date of first dose of study treatment.

Date of administration of study treatment: the day of administration of study treatment (ABO809 dose), is defined as Day 1.

Inpatient Follow-Up period: All participants, whether or not they are symptomatic, will be domiciled at the study site until completion of Day 10 assessments and then discharged if diarrheal symptoms have been resolved for ≥24 hours. Participants who experience symptoms

will remain domiciled for the duration of the diarrheal illness up to Day 21. If a participant experiences diarrhea after Day 10 (after the participant has been discharged from the study site), the participant may be readmitted, at the discretion of the Investigator, to remain domiciled until the cessation of symptoms or Day 21, whichever is earlier.

Outpatient follow-up period: After the domicile period, participants will be followed on an outpatient basis ending on Day 56 following ABO809 administration. Participants will return for follow-up visits on Days 14, 17, 21, 24 and 28 for clinical assessments and to provide collected stool samples for analysis.

Safety Follow-Up Calls: Participants will be contacted by telephone on Days 35, 42, 49 and 56 to collect information on any adverse events that are considered related to administration with ABO809. Any participants who experience diarrheal symptoms between Day 28 and Day 56 may provide a stool sample for analysis and may return to the study site for an unscheduled visit to consult with the Investigator. Additional Post-Study Follow-Up phone calls will be conducted after EOS at 6 and 12 months after ABO809 administration.

2.1.1.1 Definition of clinical and parasitological response

Diarrhea: A participant will be considered as having diarrhea if they have at least one stool sample grading 3-5 on the Stool Grading System (Table 5-5). Grade 1-2 stools are considered normal and are formed or soft stool which do not easily take the shape of the container. Grade 3-5 stools are considered diarrheal and are thick liquid, opaque watery, rice water or clear watery stool which do take the shape of the container.

Number of days to diarrhea onset: is defined as the first diarrhea stool date minus the dosing date.

Number of days of diarrhea: is defined as all days with at least one stool sample grading 3-5.

Number of days to resolution of diarrhea: is defined as the last diarrhea stool date minus the dosing date +3

Duration of diarrhea (hours): is defined as the last diarrhea stool date/time minus the first diarrhea stool date/time.

Clinical diarrheal illness: A participant will be considered as having clinical diarrheal illness if diarrhea is observed with at least two diarrheal bowel events within 24 hours on at least two days during the observation period beginning after ABO809 administration (Day 1) and ending on Day 28.

Resolution of clinical diarrhea illness: A participant will be considered recovered from diarrheal illness when no diarrhea occurred on ≥2 consecutive days following the last diarrhea in participants with clinical diarrhea illness.

Number of days to clinical diarrhea onset: is defined as the first day of clinical diarrhea illness date minus the dosing date.

Number of days of clinical diarrhea illness: is defined as all days of diarrhea in participants with clinical diarrhea illness. It will be set to 0 for participants with no clinical diarrhea illness.

Number of days to resolution of clinical diarrhea illness: is defined as 48 hours after the last day of clinical diarrhea illness, i.e., last diarrhea date minus the dosing date +3 in participants with clinical diarrhea illness.

Duration of clinical diarrheal illness (hours): is defined as the last diarrhea date/time minus the first diarrhea date/time in participants with clinical diarrhea illness.

***Cryptosporidium* infection:** is defined as the presence of oocysts in at least 1 stool sample starting 72 hours following the administration of ABO809 (or sooner if associated with symptoms suggestive of diarrheal illness), up to Day 10 or Day 28 (separately). If clinical symptoms associated with cryptosporidiosis occur earlier than 72 hours post-administration, then stool samples after the onset of symptoms can be collected for parasitological evaluation. Oocysts will be detected by a commercially available diagnostic EIA test and confirmed by a DFA test if the EIA is positive. The tests will be performed at a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. Samples that test negative for *Cryptosporidium* by EIA will be reported as negative for *Cryptosporidium* without confirmation by DFA.

Resolution of *Cryptosporidium* infection: A *Cryptosporidium* infection will be considered resolved when no evidence of *Cryptosporidium* is detected in stool samples collected over ≥2 consecutive days. Please refer to the definition of "Number of days to resolution of Cryptosporidium infection" for further details.

Number of days to Cryptosporidium infection onset: is defined as the first occurrence of positive EIA test if it is at/after 72 hours post-challenge, otherwise as the first occurrence of positive EIA test with clinical diarrhea symptoms (if earlier than 72 hours), minus the date of dosing.

Number of days of Cryptosporidium infection: is defined as all days that have at least 1 positive EIA test.

Number of days to resolution of Cryptosporidium infection: is defined as:

- 48 hours after the last occurrence of positive EIA test during the inpatient period following the initial admission into the unit or,
- 48 hours after the last occurrence of positive EIA test during the inpatient period following the readmission into the unit or during the outpatient period up to Day 20 (without clinical diarrhea symptoms and before the start of NTZ) or,
- if there is a clinical relapse during the outpatient period, 48 hours after the last occurrence of positive EIA test with GP60 result showing recrudescence or,
- if clinical relapse during the outpatient period with GP60 result showing reinfection, 48 hours after the last occurrence of positive EIA test before onset of clinical symptoms,

i.e., the last positive EIA test date minus the dosing date +3.

Duration of *Cryptosporidium* infection: is defined as the last *Cryptosporidium* infection date/time minus the first *Cryptosporidium* infection date/time.

Recrudescence: A *Cryptosporidium* infection will be considered as recrudescent if *Cryptosporidium* infection occurs again after resolution of infection with a genotype identical

to that of *Cryptosporidium parvum* Iowa isolate used for this study. Recrudescence must be confirmed by Gene Encoding Glycoprotein 60 kDa Protein (GP60) genotyping analysis.

Reinfection: A participant will be considered as reinfected if *Cryptosporidium* infection occurs again after resolution of infection with a genotype different from the *Cryptosporidium parvum* Iowa isolate used for this study. Reinfection must be confirmed by GP60 genotyping analysis.

Secondary transmission: Person-to-person transmission of *Cryptosporidium* may occur, particularly among study participant's household members or sexual partners.

2.2 Analysis sets

For all analysis sets, participants will be analyzed according to the dose of ABO809 received (*C. parvum* oocysts, also referred to as "treatment").

The full analysis set (FAS) and safety analysis set (SAF) will be comprised of all participants who took one dose of ABO809 during the treatment period.

The Pharmacodynamic (PD) analysis set includes all participants with available parasitological and clinical data and with no protocol deviations with relevant impact on PD data. Fecal stool samples will also be assessed for other enteric pathogens which may cause diarrheal illness. Any participant with a positive test for these other enteric pathogens will be excluded.

The protocol deviation codes are summarised in Appendix Section 5.6.

2.2.1 Subgroup of interest

Exploratory subgroup analyses for primary and secondary endpoints may be performed by age, sex and fecal indole at baseline.

2.3 Participant disposition (All participants), demographics, other baseline characteristics, prior, concomitant and rescue medications (Safety analysis set)

2.3.1 Participant disposition

The number of participants screened and screening failures will be presented. Information collected on screening failures are the demographic information, informed consent, and Inclusion/Exclusion pages. In addition, the reasons for screen failures will be provided.

The following disposition status will be summarized as the number and percent of participants

- who completed each period (i.e. screening, treatment, inpatient and outpatient follow up) and who discontinued each period prematurely (including the reason for discontinuation) will be presented for each treatment group and overall.

For each protocol deviation, the number and percent of participants for whom the deviation applies may be tabulated.

2.3.2 Demographics and other baseline characteristics

All data for background and demographic variables will be listed by ABO809 treatment group and cohort number for all participants. Summary statistics will be provided by ABO809

treatment group and cohort number. Relevant medical histories and current medical conditions coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary will be listed and may be summarized by system organ class (SOC) and preferred term (PT) of the MedDRA dictionary and by treatment group.

Following demographic variables will be summarized:

- Age
- Sex (male, female)
- Race (Caucasian, Black, Asian, native American, Pacific Islander, unknown, other)
- Ethnicity
- Body weight
- Body height (cm)
- Body mass index (BMI)

Following disease characteristics at baseline will be summarized:

- Fecal indole concentration level
- Presence of anti-*Cryptosporidium* antibody in serum and saliva, if available

2.3.3 Study treatment / compliance

Each participant will be supervised while receiving their single dose of ABO809 and will be domiciled for a minimum of 10 days to support compliance with study requirements.

If vomiting occurs within 30 minutes of administration of ABO809, a second administration of ABO809, at the same dose, may be given. If a participant vomited following the original dose, but did not vomit following the replacement dose, the participant is considered fully dosed. If a participant vomited the original dose and did not receive a replacement dose, or vomited both the original dose and the replacement dose, the participant is considered not fully dosed. The number and percent of participants who vomited following ABO809 administration and were dosed a second time may be presented by ABO809 treatment group and cohort number.

2.3.4 Prior, concomitant and post therapies

Prior and concomitant medications will be coded according to the latest version of World Health Organization (WHO) Drug Reference List dictionary which employs the Anatomical Therapeutic Chemical (ATC). The number and percentage of participants taking prior and concomitant medications may be tabulated for each treatment by ATC class and PT. Concomitant rescue and other medications, procedures, and significant non-drug therapies may also be summarized by ABO809 treatment group and cohort number.

Prior medications are defined as drugs taken and stopped prior to first dose of study medication. Any medication given at least once between the day of first dose of study medication and the last day of study visit will be a concomitant medication, including those which were started before dosing and continued into the treatment period.

Rescue medication nitazoxanide

To minimize the risk of severe clinical illness, long-term asymptomatic shedding and recurrence of *Cryptosporidium* infection, nitazoxanide will be administered on Day 21 to all participants who receive ABO809 and develop a *Cryptosporidium* infection, whether or not clinical symptoms are observed.

Nitazoxanide will be administered at 500 mg by mouth every 12 hours with food for 3 days as per the U.S. Prescribing Information, under the clinical conditions outlined below. All participants who receive ABO809 and develop an infection (i.e. oocyst shedding after Day 3 or sooner if associated with symptoms of diarrheal illness), will receive nitazoxanide on Day 21 or as soon as results of EIA analysis (and confirmed by DFA if positive) of Day 17 sample becomes available whether or not they develop clinical symptoms. There are conditions under which participants may receive nitazoxanide earlier: (i) participants with diarrhea persisting until Day 10 will start nitazoxanide on Day 10, (ii) participants with severe diarrheal illness (≥ Grade 3 as per Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)) may receive nitazoxanide unless deemed contraindicated by the Investigator irrespective of timing. Whenever nitazoxanide treatment is administered to participants experiencing clinical symptoms, participants may be domiciled at the discretion of the Investigator until free of clinical symptoms for ≥24 hours. A second course of nitazoxanide may be considered for participants with severe diarrhea or treatment failure following first course of nitazoxanide at the discretion of the Investigator. The duration of treatment will depend upon the participant's response to therapy.

Participants will not receive nitazoxanide if:

- they did not shed oocysts at any time after Day 3 and
- they did not have diarrhea between Day 1 and Day 21 and
- there was no diagnosis of cryptosporidiosis in any of the participant's household contacts between Day 11 and Day 21

The summary of the following characteristics may be provided by ABO809 treatment group and cohort number:

- Tabulation of number of participants who required nitazoxanide on Day 21 with confirmation that all 3 doses of nitazoxanide are taken on consecutive days
- Tabulation of number of participants who required nitazoxanide earlier or if the participant requires a second course of nitazoxanide
- Tabulation of number of participants who had evidence of infection or clinical symptoms after last dose of nitazoxanide

Other rescue medications (other antiparasitic drug(s) e.g. paromomycin alone or in combination with azithromycin) will be captured under rescue medication category.

In participants with severe diarrheal illness, oral and/or intravenous hydration, and replacement of electrolytes as needed, is expected as part of the standard of care management of adverse events (dehydration and severe diarrhea). Fluid replacement therapy will be recorded as a concomitant medication.

2.4 Analysis supporting primary objective(s) (PD analysis set)

2.4.1 Primary endpoint(s)

The primary endpoint is *Cryptosporidium* infection up to Day 10 (inclusive). This is defined as detection of oocysts

- in at least one fecal sample at ≥72 hours after ABO809 administration
- or sooner than 72 hours if associated with symptoms suggestive of diarrheal illness, in at least one fecal sample after ABO809 administration.

Fecal stool samples will be assessed for *Cryptosporidium* infection using a commercially available diagnostic EIA test and confirmed by DFA test if the EIA is positive. Tests will be performed at a CLIA certified laboratory. Samples that test negative for *Cryptosporidium* by EIA will be reported as negative for *Cryptosporidium* without confirmation by DFA.

To overcome stool-to-stool and day-to-day oocyst shedding variability, up to 3 consecutive stool specimens, produced about 3-4 hours apart and on the same day will be tested. If at least one of the stool samples is *Cryptosporidium* positive, the participant will be considered infected. Otherwise, the participant will not be considered infected.

2.4.2 Statistical hypothesis, model, and method of analysis

The number and percentage of participants with *Cryptosporidium* infection will be tabulated by treatment. The 2-sided 80% exact confidence interval of the infection rate will also be reported.

In addition, summary tables will display the number and percentage of participants with positive EIA results (confirmed by DFA) over time (by visit) until Day 10 (inclusive) as well as until EOS. All EIA time-courses will be listed by ABO809 treatment group and cohort number and by participant.

2.4.3 Handling of intercurrent events

For participants receiving a rescue medication such as nitazoxanide during the 10 day comicling period the negative Cryptosoridium infection data collected after the start of the rescue medication will be set to missing when summarizing the time profiles as described in Section 2.4.2. The corresponding data collected during the whole study will be handled similarly. Handling of missing values not related to the intercurrent event. No missing data are expected for the primary efficacy analysis.

2.4.4 Sensitivity analyses

Not Applicable

2.4.5 Supplementary analyses

Not Applicable

2.5 Analysis supporting secondary objectives

The secondary objectives of this study are to (1) evaluate the incidence and characteristics of diarrheal illness (PD analysis set), (2) to evaluate the time to onset and resolution of *Cryptosporidium* infection (PD analysis set), and (3) to assess the safety and tolerability of ABO809 (Safety analysis set).

2.5.1 Secondary endpoint(s)

The secondary endpoints include the presence of clinical symptoms (diarrheal and associated symptoms) by Day 10 and Day 28, *Cryptosporidium* infection up to Day 28, time to infection and symptom onset, time to resolution of infection (number of days to resolution of Cryptosporidium infection) and diarrheal illness, and characteristics of clinical signs and symptoms associated with diarrheal illness, as well as safety and tolerability assessments up to Day 28 and the 12 month post-study follow-up.

Presence of clinical diarrheal illness will be determined by analysis of diarrhea data: number of days to diarrhea onset (first day with Grade 3 or higher stool), number of days of diarrhea (stool grade 3-5), number of days to resolution of diarrhea (stool grade 1-2), number of diarrheal episodes, total stool output (weight in grams) and the worst (highest number) stool grade each day will be recorded.

Data on other gastrointestinal symptoms associated with *Cryptosporidium* infection will be recorded as follows: presence (yes/no), onset, severity (mild, moderate, severe), therapeutic intervention (yes/no; if yes, what) and progression (ongoing, resolved). These gastrointestinal symptoms include:

- abdominal pain or discomfort
- abdominal tenderness
- abdominal rebound
- abdominal distension
- fecal urgency
- nausea
- vomiting
- flatulence
- tenesmus
- poor appetite
- presence of blood
- fever
- dehydration
- electrolye imbalance

The number of days to gastrointestinal symptoms onset will be defined as the date of the first symptom minus the dosing date.

2.5.2 Statistical hypothesis, model, and method of analysis

The number and percentage of participants with clinical symptoms (diarrheal and associated symptoms) (by Day 10 (inclusive) and Day 28) and with *Cryptosporidium* infection up to Day 28 will be tabulated by treatment group. The 2-sided 80% exact confidence interval of the clinical symptom rate will be reported by treatment group.

Frequency and percentage will be presented for categorical data such as grading of stool, evidence of occult blood in stool and number of diarrhea episodes. Mean, standard deviation, median, minimum, and maximum will be presented for continuous data such as stool weight. Overlaying individual stool weight-time profiles will be generated.

Time to onset and time to resolution of infection and diarrheal illness will be summarized using Kaplan-Meier graphs with separate lines by treatment group. Median time to event for each treatment group will be estimated along with 90% confidence intervals.

Duration of infection and diarrheal illness will be summarized by treatment group.

2.5.3 Handling of intercurrent events

Stool data with grade <3 will be set to missing after the participant took the rescue medication. For “Number of days of ...”, “Duration of ...”, “Stool output (weight)” variables, data will be right censored if the participant took the rescue medication. Proc Lifetest and Proc Lifereg will be used to calculate the median and mean (SE), respectively. If there are convergence issues with Proc Lifereg for some visits such as those starting on Day 21 when most measurements are censored then Proc Means will be used instead.

For all Kaplan-Meier graphs, participants will be censored under the following circumstances:

- If the study ended before the subject experienced the event.
- If the subject discontinued the study before experiencing the event.
- If the subject died before experiencing the event.
- If the subject took the rescue medication before experiencing the event.

2.5.4 Handling of missing values not related to intercurrent event

No imputation will be made.

2.5.5 Sensitivity analyses

Not Applicable

2.5.6 Supplementary analyses

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2.6 Safety analyses (Safety analysis set)

All safety analyses will be performed using the SAF. All listings and tables will be presented by treatment group.

2.6.1 Adverse events (AEs)

AEs and serious adverse events (SAEs) will be monitored, treated and appropriately recorded by the Investigator from Day 1 until EOS (Day 56).

The number (and percentage) of participants with adverse events will be tabulated in the following ways:

- by Primary system organ class and preferred term
- by Primary system organ class and preferred term and maximum severity

A participant with multiple AEs within a primary system organ class is only counted once towards the total of the primary system organ class. If a participant reported more than one adverse event with the same preferred term, the adverse event with the greatest severity will be presented.

Summaries will also be presented for AEs by severity (toxicity grade), for study treatment related AEs, for AEs duration (start and end dates) or if the event is ongoing, of not recovered/not resolved, for whether an AE constitutes an SAE and for its outcome (i.e. recovery status or whether it was fatal).

The most common adverse events reported (≥ z% in any group for each preferred term in the table by SOC and PT) will be presented in the clinical study report by descending frequency according to its incidence in overall group starting from the most common event. Here threshold value z is set to 5 (%) but it may be updated following review of the dry run outputs.

For the legal requirements of ClinicalTrials.gov, two required tables on treatment emergent adverse events which are not serious adverse events with an incidence greater than 5% and on treatment emergent serious adverse events and SAE suspected to be related to study treatment will also be provided by system organ class and preferred term on the safety set.

If for a same participant, several consecutive AEs (irrespective of study treatment causality, seriousness and severity) occurred with the same SOC and PT:

- a single occurrence will be counted if there is ≤1 day gap between the end date of the preceding AE and the start date of the consecutive AE
- more than one occurrence will be counted if there is >1 day gap between the end date of the preceding AE and the start date of the consecutive AE

For occurrence, the presence of at least one SAE / SAE suspected to be related to study treatment / non SAE must be checked in a block e.g., among AE's in a ≤1 day gap block, if at least one SAE is occurring, then one occurrence is calculated for that SAE.

Separate summaries will be provided for ABO809 related AEs, SAEs, adverse events of special interest **(**AESIs) and deaths.

No statistical analysis will be performed for safety and tolerability evaluation.

2.6.1.1 Adverse events of special interest / grouping of AEs

The number and percentage of participants with these special AEs will be tabulated. In addition, listings of related adverse events will be provided.

All clinical signs and symptoms associated with *Cryptosporidium* infection will be characterized as AEs. . The Investigator will report AEs through Day 56 (EOS) and from EOS through 12 months after ABO809 administration and follow-up accordingly; These include:

- **Gastroenteritis in the absence of *Cryptosporidium* infection**: In the event of gastroenteritis in the absence of *Cryptosporidium* infection, the event will be considered an AEs. The Investigator will investigate other potential causes of diarrhea, including infectious diarrhea and manage participant's care according to local standards.
- **Extraintestinal cryptosporidiosis:** *Cryptosporidium* infection involving the biliary tract, lungs, liver, pancreas, or other organs will be considered AEs.
- **Persistent or recurrent cryptosporidiosis:** Persistent clinical symptoms after administration of standard of care (e.g. Nitazoxanide, oral or IV hydration and electrolyte replacement), or after initial resolution of *Cryptosporidium* oocysts in the stool and/or diarrheal symptoms, will be considered an AEs.
- **Persistent *Cryptosporidium* shedding:** Persistent detection of *Cryptosporidium* oocysts in stools after treatment with nitazoxanide will be considered an AEs.
- **Dehydration:** Moderate or severe dehydration, as defined below will be considered an AESI.
  - **Mild:** dry mucous membranes; diminished skin turgor; increased oral fluids are indicated.
  - **Moderate:** IV fluids are indicated.
  - **Severe:** hospitalization is indicated, or it is considered to have life-threatening consequences; or an urgent intervention is indicated.
- **Non-intestinal sequelae:** Development of new onset non-intestinal symptoms, including eye pain or joint pain, will be considered AEs.

2.6.2 Deaths

The number of deaths resulting from SAEs suspected to be related to study treatment and number of deaths resulting from SAEs irrespective of causality will be provided by SOC and PT.

2.6.3 Laboratory data

Clinical laboratory data will include chemistry, hematology and urinalysis results up to EOS. All laboratory values will be converted into International System of Units (SI) units. Grading will be assessed using the U.S. Food and Drug Administration's (FDA) "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)". For AEs that are not contained in the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)", Mild/Moderate/Severe will be used as categorization. Additional information is provided in Section 5.3.

Descriptive statistics (means, medians, standard deviations, ranges) by time point will be generated for all clinical laboratory tests performed (actual values and changes from baseline) for three groups of laboratory tests by laboratory test and treatment group. Change from baseline

will only be summarized for participants with both baseline and post baseline values and will be calculated as:

change from baseline = post baseline value - baseline value

Box plot for lab parameters will also be provided by timepoint and treatment group as applicable.

2.6.4 Other safety data

Boxplots to visualize trends in longitudinal safety data (vitals, ECG) will be created.

2.6.4.1 ECG and cardiac imaging data

The following quantitative variables will be summarized: heart rate, RR interval, PR interval, QRS interval, QT interval, QTcF (QT interval corrected for heart rate according to Fredericia) and optionally QTcB (QT interval corrected for heart rate according to Bazett). Summary statistics for change from baseline will be presented for ECG variables by timepoint and treatment group.

ECG parameters will be summarized categorically by computing the number and percentage of participants at each time point and at the maximum post baseline value with following:

- QT, QTcF, or QTcB
  - >450 and ≤480 ms
  - >480 and ≤500 ms
  - >500 ms
  - Increase from Baseline of ≥30 ms to <60ms
  - Increase from Baseline of ≥60 ms
- HR
  - Increase from baseline >25% and to a value >100 bpm
  - Decrease from baseline >25% and to a value <50 bpm
- PR
  - Increase from baseline >25% and to a value >200 ms
  - New value of >200 ms
- QRS
  - Increase from baseline >25% and to a value >120 ms
  - >120 ms

All ECG data will be listed; notably all newly occurring or worsening abnormalities as compared with baseline will be provided, abnormal values will be flagged, and the overall interpretation of abnormality will be presented.

2.6.4.2 Vital signs

Vital signs will include the collection of weight (kg), body temperature (recorded in °C), supine systolic and diastolic blood pressure (mmHg) and pulse (beats/min) measurements with the possibility of repeated vital assessments.

All vital signs data will be listed by treatment group, participant, and visit/time and if ranges are available, abnormalities (and relevant orthostatic changes) will be flagged. Summary statistics will be provided by treatment and visit/time.

A listing of all newly occurring or worsening abnormalities as compared with baseline will be provided, as well as a by-participant listing of all vital signs.

2.7 Pharmacokinetic endpoints

Not Applicable

2.8 PD and PK/PD analyses

Exploratory longitudinal PD modeling may be performed to identify potential relationships for safety, clinical and parasitological endpoints.

2.9 Participant-reported outcomes

Not Applicable

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2.11 Other Exploratory analyses (PD analysis set)

Commercially Confidential Information

Commercially Confidential Information

Commercially Confidential Information

2.12 Interim analysis

Commercially Confidential Information

Commercially Confidential Information

3 Sample size calculation

3.1 Primary endpoint(s)

Approximately 10 participants in each cohort will be enrolled to receive ABO809. The sample size is driven by clinical trial site capacity.

Commercially Confidential Information

Commercially Confidential Information

The calculation is based on the 1-sided 80% exact confidence interval.

3.2 Secondary endpoint(s)

Commercially Confidential Information

The calculation is based on the 1-sided 80% exact confidence interval.

4 Change to protocol specified analyses

None

5 Appendix

5.1 Imputation rules

5.1.1 Study treatment

The study treatment administration date should be complete since it is taken at the clinic. In case missing or partial, the visit date will be used as the study treatment administration date.

5.1.2 AE date imputation

The following missing dates will not be imputed:

- Missing AE start dates
- AE start dates missing the year
- Partial/missing AE end dates

For other type of partial missing start dates, rules specified in Table 5-1 to Table 5-3 will be used.

Table 5-1 AE/Treatment Date Abbreviations

	Day	Month	Year
Partial Adverse Event Start Date	<not used>	AEM	AEY
Treatment Start Date (TRTSTD)	<not used>	TRTM	TRTY

Table 5-2 describes the possible combinations and their associated imputations. The upper text indicates the imputation (NC, A, B, C, etc.) and the lower text the relationship of the AE start date to the treatment start date (TRTSTD).

Table 5-2 Imputation algorithm

	AEM MISSING	AEM < TRTM	AEM = TRTM	AEM > TRTM
AEY MISSING	NC Uncertain	NC Uncertain	NC Uncertain	NC Uncertain
AEY < TRTY	(D) Before TRTSTD	(C) Before TRTSTD	(C) Before TRTSTD	(C) Before TRTSTD
AEY = TRTY	(B) Uncertain	(C) Before TRTSTD	(B) Uncertain	(A) After TRTSTD
AEY > TRTY	(E) After TRTSTD	(A) After TRTSTD	(A) After TRTSTD	(A) After TRTSTD

The legend to the above table is shown in Table 5-3.

Table 5-3 Imputation algorithm legends

Relationship		
	Before TRTSTD	Indicates AE start date prior to Treatment Start Date
	After TRTSTD	Indicates AE start date after Treatment Start Date
	Uncertain	Insufficient to determine the relationship of AE start date to Treatment Start Date
Imputation calculation		
	NC/Blank	No convention/imputation
	(A)	01MONYYYY
	(B)	TRTSTD+1
	(C)	15MONYYYY
	(D)	01JULYYYY
	(E)	01JANYYYY

5.1.3 Concomitant medication date imputation

Missing concomitant dates will be imputed similar as to AE dates.

5.1.3.1 Prior therapies date imputation

Start date: The same rule which is applied to the imputation of AE/concomitant medication start date will be used.

End date:

- Imputed date = min (reference end date, DEC 31 of the year), if month and day are missing.
- Imputed date = min (reference end date, last day of the Month), if day is missing. where reference end date will be study informed consent date.

If the end date is not missing and the imputed start date is after the end date, use the end date as the imputed start date. If both the start date and the end date are imputed and if the imputed start date is after the imputed end date, use the imputed end date as the imputation for the start date.

5.1.3.2 Post therapies date imputation

Start date:

- If Day is missing, then impute to the max (reference start date, first day of the month).
- Day and month are missing then impute to the max (reference start date, Jan 1)
- Reference start date will be last date of study treatment administration + 1.

End date: No imputation

5.1.3.3 Other imputations

N/A

5.2 Prohibited medications

Table 5-4 Table of prohibited medication with respective ATC codes

Prohibited medication	ATC code	ATC name
Antiperistaltic, antidiarrheal agents or stool softeners	A02BX22	Bismuth subsalicylate
	A03BA01	Atropine
	A07DA01	Diphenoxylate
	A07DA03	Loperamide
	H01CB02	Octreotide
	A07DA02	Opium tincture
	C10AC01	Cholestyramine
	C10AC02	Colestipol
	A06AA02	Docusate sodium
	A06AD11	Lactulose
Antithrombotic agents and other agents prolonging QTc interval	B01AA03	Warfarin
Antibacterial agents and other anti-parasitic therapies	A07AA06	Paromomycin alone or in combination with azithromycin
Yogurt, kefir, probiotics	N/A	N/A
vaccinations	N/A	N/A

5.3 AEs coding/grading

Grading will be assessed using the U.S. Food and Drug Administration's (FDA) "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)". For AE reporting based on laboratory values, local laboratory normal ranges may be used at the discretion of the Investigator, and if so, must be used consistently throughout the study. These may differ from the normal ranges given in the FDA grading scale. In case of such discrepancies, the Investigator will take into consideration the FDA recommendations as to the magnitude of deviations from normal provided in the FDA grading scale.

For AEs that are not contained in the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)" the following categorization will be used:

- Mild: usually transient in nature and generally not interfering with normal activities
- Moderate: sufficiently discomforting to interfere with normal activities
- Severe: prevents normal activities

Stools will be graded according to this Stool Grading System by the Investigator. Grades 1 and 2 are considered normal stool and Grades 3-5 are considered diarrheal stool.

Table 5-5 Stool Grading System

Clinical Stool Classification	Stool Grade	Stool Description
Normal stool	1	Formed stool which does not take the shape of the container
Normal stool	2	Soft stool which does not easily take the shape of the container
Diarrheal stool	3	Thick liquid stool which does take the shape of the container
Diarrheal stool	4	Opaque watery stool
Diarrheal stool	5	Rice water stool, clear watery stool

Levine et al 1988, Sack et al 1998, Tribble et al 2009

5.4 Laboratory parameters derivations

Grading will be assessed using the U.S. Food and Drug Administration's (FDA) "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007)", see Section 2.6.3.

5.5 Statistical models

5.5.1 Analysis supporting primary objective(s)

The primary analysis of *Cryptosporidium* infection at ≥72 hours post-administration (or sooner if associated with symptoms suggestive of diarrheal illness) up to Day 10 (inclusive is detailed in Section 2.4.1.

SAS procedure FREQ with EXACT statement for one-way tables will be used to estimate the proportion of responders (binary outcome = 1 or "Yes"), along with the associated 80% 2-sided Clopper- Pearson CI (Clopper and Pearson 1934).

5.5.2 Analysis supporting secondary objective(s)

Kaplan-Meier estimates

An estimate of the survival function in each treatment group will be constructed using Kaplan-Meier (product-limit) method as implemented in PROC LIFETEST with METHOD=KM option. The PROC LIFETEST statement will use the option CONFTYPE=LOGLOG.

Median survival for each treatment group will be obtained along with 90% confidence intervals calculated from PROC LIFETEST output. Kaplan-Meier estimates of the survivor function with 90% confidence intervals at specific time points will be summarized. The standard error of the Kaplan-Meier estimate will be calculated using Greenwood's formula (Collett 1994).

5.6 Rule of exclusion criteria of analysis sets

Table 5-6 Protocol deviations leading to exclusion

Analysis Set	***Criteria that cause* subjects to be excluded**
SAF	*Not treated with study treatment;*
PD	*Not having informed consent; Not treated with study medication;* *Not meeting entry criteria;* *No evaluable, parasitological and clinical data;* *Use of prohibited medication or vaccination during the study;*

6 Reference

Clopper, C. J. and Pearson, E. S. (1934), "The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial," *Biometrika*, 26, 404‑413.

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