Statistical Analysis Plan

Addendum

A Phase 2a Randomized, Single-Center, Double-Blind, Placebo Controlled Study to Evaluate the Safety and Preliminary Efficacy of Oral iOWH032 Against Cholera Diarrhea in a Controlled Human Infection Model

Study Title	A Phase 2a Randomized, Single-Center, Double-Blind, Placebo Controlled Study to Evaluate the Safety and Preliminary Efficacy of Oral iOWH032 Against Cholera Diarrhea in a Controlled Human Infection Model
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1 INTRODUCTION

This SAP Addendum document is created to update secondary efficacy endpoints which were described in section 1.2.3 and section 4.4.2 of the final 1.1 statistical analysis plan (SAP), dated 23 March 2020.

2 CONTENT OF UPDATES

2.1. Section 1.2.3:

"Proportion of participants with moderate to severe diarrhea following cholera challenge, defined as >3 liters and >5 liters of loose stools, respectively, within 48 hours following challenge"

will be updated to

"Proportion of participants with moderate to severe diarrhea following cholera challenge, defined as >3 liters and >5 liters of loose stools, respectively, with onset within 48 hours following challenge."

2.2. Section 4.2.2:

"Secondary efficacy endpoints include a) the proportion of participants with moderate or severe diarrhea following cholera challenge defined as >3.0 L and >5.0 L of loose stools, as well as b) the attack rate of any diarrhea following cholera challenge defined as the number of participants with 2 or more loose stools (grade 3-5) totaling >200 mL within 48 hours or 1 loose (grade 3-5) stool > 300 mL."

will be updated to

"Secondary efficacy endpoints include a) the proportion of participants with moderate or severe diarrhea following cholera challenge defined as >3.0 L and >5.0 L of loose stools, as well as b) the attack rate of any diarrhea following cholera challenge defined as the number of participants with 2 or more loose stools (grade 3-5) totalling >200 mL with onset within 48 hours or 1 loose (grade 3-5) stool > 300 mL."

3 REPORTING OF ANALYSIS

The updates of secondary efficacy endpoints will be applied to all related analysis in Table, Figure and Listings (TFLs) and Clinical Study Report(CSR).

Statistical Analysis Plan

DRG-032-PO-2-01-USA

A Phase 2a Randomized, Single-Center, Double-Blind, Placebo Controlled Study to Evaluate the Safety and Preliminary Efficacy of Oral iOWH032 Against Cholera Diarrhea in a Controlled Human Infection Model

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Document Version History

Version Number	Reason for Update	Version Date
Final v1.1	• Add blood type group (O vs Non-O) in Time (hours, post-challenge) to the first formed stool, which will be summarized with Kaplan-Meier methods and compared with the log-rank test. • Adding timing of topline analyses. • Update the last unformed stool to the first formed stool.	23 Mar 2020
Final v1.0		08 Jan 2020

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ABBREVIATIONS

AE	Adverse Event
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AMS	Accelerator Mass Spectrometry
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Code
AUC	Area under the Concentration-time Curve
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
CBC	Complete Blood Count
CRU	Clinical Research Unit
CPK	Creatine phosphokinase
CSR	Clinical Study Report
CV	Coefficient of Variation
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ET	Early Termination
HBsAg	Hepatitis B Surface Antigen
HCV Ab	Hepatitis C Virus Antibody
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
ITT	Intent to Treat
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified ITT
MH	Medical History
ORS	Oral Rehydration Solution
PI	Principal Investigator
PK	Pharmacokinetic
PP	Per Protocol
PR	Time between the Start of the P Wave and the Start of the QRS Complex
PT	Preferred Term
QT	Time between the Start of the Q Wave and the End of the T Wave

QTcF	QT Interval Corrected by the Fridericia Formula
RR	R-R interval
SAE	Serious Adverse Events
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SRC	Safety Review Committee
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
WBC	White Blood Cell
WHO	World Health Organization

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide details and specifications for statistical analyses of the study DRG-032-PO-2-01-USA "A Phase 2a Randomized, Single-Center, Double-Blind, Placebo Controlled Study to Evaluate the Safety and Preliminary Efficacy of Oral iOWH032 Against Cholera Diarrhea in a Controlled Human Infection Model" as set forth in the clinical study protocol Version 4.0, dated 13 March 2020.

1.1 STUDY OBJECTIVES

1.1.1 PRIMARY EFFICACY OBJECTIVE

- To measure the rate and extent of diarrhea following cholera challenge, in participants treated with iOWH032 compared to placebo

1.1.2 PRIMARY SAFETY OBJECTIVE

- To evaluate safety of oral doses of iOWH032 compared to placebo

1.1.3 SECONDARY EFFICACY OBJECTIVES

- To evaluate additional measures of efficacy against cholera illness following cholera challenge, among those randomized to be treated with iOWH032 versus placebo

1.1.4 SECONDARY SAFETY OBJECTIVE

- To evaluate tolerability of oral doses of iOWH032 compared to placebo

1.1.5 EXPLORATORY OBJECTIVES

- To evaluate the pharmacokinetic (PK) properties of iOWH032
- To evaluate time of cessation of cholera organism in stool after challenge
- To evaluate a decrease for oral rehydration solution (ORS) and/or intravenous (IV) fluid replacement therapy need

1.2 STUDY ENDPOINTS

1.2.1 PRIMARY EFFICACY ENDPOINTS

- Diarrheal stool output rate, defined as the total volume of diarrheal stools (mL, Grade 3 and above) divided by the number of hours between initiation of study product dosing and initiation of antimicrobial therapy

1.2.2 PRIMARY SAFETY ENDPOINT

- Frequency and incidence of serious adverse events (SAEs) throughout the study

1.2.3 SECONDARY EFFICACY ENDPOINTS

- Proportion of participants with moderate to severe diarrhea following cholera challenge, defined as >3 liters and >5 liters of loose stools, respectively, within 48 hours following challenge

- Attack rate of any diarrhea following cholera challenge, defined as the number of participants with either 2 or more loose stools (grades 3-5) totaling > 200 mL or 1 loose (grade 3-5) stool > 300 mL, respectively, within 48 hours following challenge
- Area under the curve (AUC) of diarrheal stool volume between challenge dose and initiation of antibiotics
- Density of cholera organisms in stool samples measured in stool samples via quantitative stool culture
- To evaluate cholera illness in each participant after cholera challenge, using the following objective parameters:
    1. Duration of diarrheal episode as defined by time to first formed stool
    2. Total number of loose (Grades 3-5) stools
    3. Occurrence of fever
    4. Occurrence of vomiting

1.2.4 SECONDARY SAFETY ENDPOINTS

- To measure the occurrence of solicited adverse effects during the three days of oral dosing, through 8 hours following the last dose, which are attributed to iOWH032 and placebo, to include:
    1. Nausea
    2. Abdominal discomfort and pain
    3. Occurrence of abdominal cramps
    4. Occurrence of headache
    5. Occurrence of malaise
    6. Anorexia
    7. Pollakiuria
    8. Micturition urgency
    9. Sinus tachycardia
    10. Increased alertness
- To measure the frequency and incidence of unsolicited adverse effects up to 28 days after the last dose.

1.2.5 EXPLORATORY ENDPOINTS

- To determine plasma levels of iOWH032 7±1 hours after the first and last dosing in all participants
- Time (hours) to cessation of detectable cholera in stool, defined as the time of the first sample negative via quantitative stool culture, after which all following samples are also negative for cholera.
- ORS and/or intravenous fluid replacement measured in liters

1.3 STUDY DESIGN

1.3.1 GENERAL STUDY DESIGN AND PLAN

This clinical trial is designed to be a Phase 2a, which will document the preliminary efficacy

of oral doses of iOWH032 in diminishing the severity of illness of cholera, using a well-established human challenge model.

In Cohort 1, 24 eligible healthy male and female participants will be randomized 1:1 to be assigned to the iOWH032 (active treatment) or placebo. Following a favorable assessment of iOWH032 in an interim analysis, Cohort 2 will include an additional 24 healthy participants randomized 1:1 to iOWH032 or placebo. All participants will be challenged with 10^6 cfu of V. cholerae El Tor Inaba strain N16961. Blinded treatment will begin at the onset of (any) diarrhea symptoms or 48 hours after challenge, whichever is sooner, and consists of three oral doses per day, over three days. The dosage of iOWH032 is 500 mg (two 250 mg tablets) per dose.

A 3-day course of antimicrobial therapy will be initiated at approximately 4 days post challenge or sooner if indicated. The Principal Investigator will be able to initiate antibiotics upon severe cholera definition being satisfied. The blinded study treatment will continue during antimicrobial therapy. For participants that remain asymptomatic, the last dose of iOWH032 and first dose of antibiotic will be separated by 1-2 hours. Discharge from the Clinical Research Unit will be contingent upon each participant meeting all the following criteria:

- 3 consecutive stool cultures that are negative for growth of vibrios (with each culture separated by at least 12 hours)
- The absence of moderate or higher grade objective reactogenicity (diarrhea, fever, and vomiting) for at least 12 hours prior to discharge
- The completion of a 3-day course of antimicrobial (preferred agent, ciprofloxacin, 500 mg twice daily for 3 days) therapy

The schedule of events is provided in Table 1.

Error! Reference source not found.: Schedule of study visits and evaluations

Study visit	**Visit 1 (screen)**	*Inpatient Containment Period*											**Visit 2**	**Visit 3**	**Visit 4**
Study Day	D-85 to-	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D15±1	D29±2	D180±1
Informed Consent	√														
Medical history, medications	√														
Comprehension Assessment Tool	√														
Physical examination	√		√h	√h	√h	√h	√h	√h	√h	√h	√h	√h	√h	√h	
Pregnancy test[a]	√	√													
Vital signs	√		√g	√ g	√ g	√ g	√ g	√ g	√ g	√ g	√g	√ g			
Screening laboratory[b]	18 mL														
12-lead ECG	√														
Pre-challenge Stool		√													
Safety laboratory[c]		10 mL											10 mL		
Eligibility confirmation	√	√	√												
Challenge			√												
Blinded Study Drug Administration[d]			(√)	(√)	(√)										
3-day course of antibiotics[e]							(√)	(√)	(√)						
Observe & manage illness[f]			√	√	√	√	√	√	√	(√)	(√)	(√)			
Stool grading and cultures[f]			√	√	√	√	√	√	√	(√)	(√)	(√)			
pK analysis[i]			√		√										
Cumulative blood volume	18 mL	10 mL	2 mL		2 mL								10 mL		
Solicited AEs 7 days post-challenge			√	√	√	√	√	√	√	√					
Unsolicited AEs			√	√	√	√	√	√	√	√	√	√	√	√	
SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant medications			√	√	√	√	√	√	√	√	√	√	√	√	√
Interval medical history			√	√	√	√	√	√	√	√	√	√	√	√	√
Unanticipated problems (UPs)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

a. For females of child bearing potential, a serum pregnancy test will be performed at screening and one day before challenge
b. Screening laboratory to include: Complete Blood Count (CBC) for WBC, ANC, Hemoglobin, and Platelets; Chemistry panel for Na, K, ALT, AST, Creatinine, Albumin, ALP, CPK, Glucose and Total Bilirubin; Serology for HbSAg, anti-HCV, and HIV; Blood Typing; and Urinalysis

c. Safety laboratory to include: Complete blood count (CBC) for WBC, ANC, Hemoglobin, and Platelets; and Chemistry panel for Na, K, ALT,AST,ALP, Creatinine, Albumin, and Total
Bilirubin, CPK, Glucose
d. Blinded therapeutic dosing will start at the onset of symptoms or by 48 hours after ingesting the cholera challenge inoculum.
e. A 3-day course of antibiotics will be initiated upon satisfying the criteria for severe cholera diarrhea or approximately 4 day post-challenge, whichever is earlier
f. The observation and management of cholera illness (includes measurement of vomiting) and stool grading and stool cultures are intended to be performed throughout the post-challenge inpatient period, until discharge criteria are satisfied. For the duration of diarrhea, the participant will be requested to provide a urine specimen from every void that they experience; the specimen will be tested for urine specific gravity. In case of IV fluid replacement, serum electrolytes, BUN, and creatinine will be measured.
g. Vital signs will be measured at least 3 times daily (approximately every 8 ±1 hours). Vital signs will also be measured every 4 hours when a participant has a fever ≥ 39°C (102.1°F), until the participant has 2 consecutive temperatures of ≤ 38°C (100.4°F). On Day 1, including the pre-challenge vitals assessment, there will be 4 vital signs assessments (1x pre-challenge and 3x post-challenge).
h. Focused physical examination at the discretion of the physician
i. pK plasma samples for the levels of iOWH032 will be collected 7±1 hours after the first and last dosing in all participants

1.3.2 POWER ESTIMATE

Because of the multiplicity of testing and accompanying efficacy and futility thresholds at the interim analysis, a group-sequential framework is employed. Traditional methods for trial design are intractable in this case, due to the anticipated distribution of the endpoint and the use of stratified nonparametric testing for its evaluation. For this reason, a simulation-based framework was used to simulate data under both the null and alternative hypotheses. Power is computed as the proportion of simulations under the alternative hypothesis which yield a conclusion of superiority at either interim or, given that futility is not declared at interim, at the final analysis. Type I error is computed as the proportion of simulations under the null hypothesis which yield the same conclusion, erroneously. The simulation procedure was submitted to a constrained optimization routine that selected boundary values which ensure overall ≥90% power and type I error ≤0.025, simultaneously. Further simulations confirmed the adequacy of these boundaries to provide high power while maintaining nominal type I error rate. The boundary values that will be used at the interim analysis are:

- If the one-sided Van Elteren test of the superiority of the diarrheal stool output rate (iOWH032 vs placebo) yields a p-value less than 0.0051, then the study product will be deemed superior to placebo, and the second cohort will not be enrolled
- If the one-sided Van Elteren test of the superiority of the diarrheal stool output rate (iOWH032 vs placebo) yields a p-value greater than 0.4585, this will be considered evidence of futility (lack of demonstrated efficacy), and the second cohort will not be enrolled
- In all other cases, the 2nd cohort will be enrolled

Due to the nonzero probability of concluding efficacy at the interim analysis, the alpha level (type I error rate) for the final analysis needs to be adjusted to maintain overall one-sided level alpha = 0.025. The threshold to define success on the primary efficacy endpoint at the final analysis is one-sided alpha = 0.0238. This hypothesis test will be supplemented by a two-sided 95% confidence interval for difference in median diarrheal stool output rate, using the percentile bootstrap method, with n=10,000 replicates.

With the abovementioned boundary values and sample size (24 participants per group), the power decreases to 70% for a 40% reduction in daily diarrheal stool rate and to 30% for a reduction of 25% in daily diarrheal stool rate. However, an effect size of 50% reduction is expected to represent a feasible effect size for an impactful therapy.

For the secondary endpoint of proportion of participants with moderate or severe diarrhea, with 24 participants per group, and assuming 40% of participants are Type O, adequate power

is available to detect odds ratios of moderate or severe diarrhea in iOWH032-treated participants vs placebo which are significantly less than 1, using a Cochran-Mantel-Haenszel test stratified by blood type group, with two-sided level alpha = 0.05. For example, if 67% (47%) of the type O (non-type O) control participants experience moderate or severe diarrhea, and only 20% (10%) of type O (non-type O) iOWH032-treated participants do (common odds ratio 0.123), then 80% power is available to detect a common odds ratio < 1. Only a single analysis will be conducted for efficacy endpoints other than the primary efficacy endpoint, upon collection of data from all participants included in the experiment.

1.3.3 RANDOMIZATION AND BLINDING

There will be 24 study participants challenged in the first cohort and an additional 24 study participants challenged in the second cohort, pending favorable results of an interim analysis.

Subject treatment assignment will be based on a computer-generated randomization scheme using SAS V 9.4 or higher with a ratio of 1:1 stratified by blood type status (O vs. Non-O) to receive either iOWH032 500 mg every 8 hours for three days or matching placebo.

The administration of iOWH032 and iOWH032 placebo will be double-blinded.

Subjects, the Investigator and the Sponsor will be blinded to the treatment administered until enrollment is complete and the study database is locked. Only the unblinded Pharmaron CPC pharmacists will be provided with the randomized treatment sequences.

2 STATISTICAL CONSIDERATIONS

2.1 GENERAL CONSIDERATIONS

SAS version 9.4 or greater (SAS Institute Inc., Cary, NC, SAS System) will be used for analysis. For descriptive statistics, continuous variables will be summarized by the number of non-missing observations, mean, median, standard deviation, and minimum and maximum. Categorical variables will be summarized by presenting the frequency and percent. Unless otherwise specified, inference such as confidence interval construction will be conducted with two-tailed Type I error level $\alpha = 0.05$. All secondary endpoints will be considered as supportive evidence of efficacy and will be analyzed without any procedures to account for multiple comparisons.

In general, nonparametric analyses or exact methods (e.g., Fisher's exact test) will be preferred for efficacy analyses, with confidence intervals for binary variables computed via the Clopper-Pearson exact method, and confidence intervals for continuous variables computed via the percentile bootstrap method, using n=10,000 replicates each.

Primary efficacy analysis will be stratified by blood type status (O vs Non-O) as described below. All efficacy endpoints will be summarized, by group and by group and O vs Non-O status, using daily and overall summaries, where appropriate. Exploratory analyses will consider stratification by cohort to account for potential differences in challenge inoculum between cohorts.

Analysis of diarrheal stool output rate, diarrheal stool volume, cholera-containing diarrheal stool volume, and density of cholera organisms will be supplemented with reverse cumulative distribution curves.

Unscheduled results will not be included in the summary tables except for determining baseline, but will be presented in data listings.

All data listings will be sorted by group, subject and visit/timepoint where applicable. All dates will be displayed in DDMMMYYYY format. No algorithm for missing data imputation will be employed.

2.2 ANALYSIS SETS

Assignment of participants to analysis populations will be completed prior to unblinding, for each cohort.

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the informed consent form (ICF).
Intent to Treat (ITT)	All participants who are randomized to study treatment Participants will be analyzed according to their randomized treatment.
Modified ITT (mITT)	The subset of ITT who receive at least one dose of study drug. Any participant displaying no indication of cholera infection (no diarrheal stool output of grade 3 or higher) within 48 hours of challenge will be removed from the mITT population, prior to unblinding of data.
Per Protocol Population	The subset of the mITT consisting of those participants who had no major protocol deviations and received all doses of assigned study drug. All protocol deviations will be assessed and documented on a case-by-case basis before the database lock, and deviations considered having a serious impact on the efficacy results will lead to the relevant participant being excluded from the per-protocol population. Before database lock, potential participant exclusions from per-protocol population will be reviewed by the Sponsor and documented.
Safety	The Safety Analysis Set includes all participants who received any study treatment. Safety analyses and demographic/baseline characteristic summaries will be based on the safety population, according to treatment received.

3 DATA HANDLING CONVENTIONS

3.1 DERIVED AND TRANSFORMED DATA

3.1.1 BASELINE AND CHANGE FROM BASELINE DEFINITION

The baseline value of a variable is defined as the last value obtained on or before the date and time of the dose of iOWH032 and iOWH032 placebo, including unscheduled assessments.

Change from baseline will be derived for each subject as the post-baseline evaluation minus the baseline evaluation.

3.1.2 STUDY DAY

Study Day will be calculated from the reference start date (defined as the day of administration of study drug), and will be used to show start/stop day of assessments/events:

- Study Day = (date of assessments/events - reference start date) if event/assessment is prior to the reference start;
- Study Day = (date of assessments/events - reference start date + 1) if event/assessment is on or after the reference start.

There is no study day 0.

3.1.3 LAB PARAMETER RESULTS WITH SPECIAL CHARACTERS

If any lab parameter results contain special characters such as '<' or '>', the numerical portion of the results will be used for descriptive summary.

3.2 ANALYSIS VISIT WINDOWS

For analysis of data over time (wherever specified), the nominal study days/visits will be used as analysis days/visits. No windowing algorithm will be used.

3.3 PREMATURE WITHDRAWAL AND MISSING DATA

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Participants who sign the ICF and are randomized but do not receive the challenge may be replaced. Participants who sign the ICF, and are randomized and receive the challenge, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

All reasonable measures will be taken to ensure minimal missing data. Despite this, some data are likely to be missing at the end of the study. No imputation is planned for any endpoints in this study, and all data will be considered to be missing completely at random. Should observations indicate this is not likely to be true for a given endpoint, additional sensitivity analyses may be conducted, such as multiple imputation, to assess the impact of the missing data.

3.4 MULTIPLE COMPARISON/MULTIPLICITY

Adjustment for multiplicity for the group-sequential analysis of the primary efficacy endpoint is described in Section 5. All secondary endpoints will be considered as supportive evidence of efficacy and will be analyzed without any procedures to account for multiple comparisons.

Due to the nonzero probability of concluding efficacy (under alternative hypotheses) at the interim analysis, the alpha level (type I error rate) for the final analysis needs to be adjusted to maintain overall one-sided level alpha = 0.025. The threshold to define success on the primary efficacy endpoint at the final analysis is one-sided alpha = 0.0238.

4 STATISTICAL ANALYSES

4.1 SUBJECT INFORMATION

4.1.1 DISPOSITION OF SUBJECTS

Subject disposition will be summarized for all enrolled subjects by group. The number and percentage of subjects in all Populations, as well as number and percentage of subjects who completed the study and discontinued the study will be summarized along with the primary reason for discontinuation. The percentages will be based on the number of subjects in the Safety Population.

A listing will present whether the subject is in the Safety, ITT, mITT and PP Population, whether the subject completed the study, date of completion/withdrawal, and the primary reason for discontinuation of study, if applicable.

A subject eligibility listing will also be provided to describe if the subject meets all inclusion/exclusion criteria.

4.1.2 PROTOCOL DEVIATIONS

The study site will record all deviations that occurred during the conduct of the study. Protocol deviations will be classified as either minor or major by the Sponsor or designee prior to database lock.

The protocol deviation data with the verbatim description, the reason for the deviation and whether the deviation is classified as minor or major will be summarized by group and listed.

4.1.3 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics (blood type, age, gender, race, height (cm), weight (kg) and body mass index (BMI) (kg/m2)) will be summarized descriptively by treatment group for the Safety Population and mITT. Descriptive statistics will be presented for age, height, weight, and BMI. Frequency counts and percentage will be presented for blood type, sex, ethnicity origin, and race.

The demographic and baseline characteristics data will also be presented as data listings by group and subject.

4.1.4 SEROLOGY

The screening serology for hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV) and human immunodeficiency virus antibodies (anti-HIV) will be listed by group and subject.

4.1.5 PREGNANCY

A serum pregnancy test (Serum β-HCG) at screening and one day before challenge will be listed by group and subject for all women.

4.1.6 Prior and Concomitant Medications

Concomitant medications will be coded using the World Health Organization (WHO) Drug Global September 1 2019.

Prior medications will be those discontinued prior to the start of the study drug. Concomitant medications will be medications started prior to the start of the study drug and continued after administration of study drug or started during the study after administration of study drug.

Prior and concomitant medications will be summarized (separately) by anatomical-therapeutic-chemical (ATC) class and preferred term (PT) after coding with the WHO Drug dictionary.

Prior and concomitant medication information will be listed by group and subject with verbatim text given by the investigator, WHO Drug Dictionary PT, indication, whether it is related to AE/MH and associated AE/MH number, start and stop date/time, dosage, route, and frequency.

4.1.7 Medical History

A comprehensive medical history will be collected in screening by interview with the participant including participation in clinical trials, surgery, previous hospitalization, allergy to food/drugs, and history of any chronic or recurrent medical conditions. Interval medical history will inquiry regarding changes since the last medical history discussion including signs and symptoms.

All medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) 22.1. Medical history will be listed by group and subject.

4.1.8 Cholera Challenge

The cholera challenge information will be listed by group and subject including whether participant was administered challenge, whether subject fasted for 90 minutes prior to challenge, date and time of challenge, and comments.

4.1.9 Study Drug Administration

The study drug administration information will be listed by group and subject including date and time of dose, whether the drug was delayed and reason, AE number if AE, specify if other, and comments.

4.1.10 Antibiotics treatment

Antibiotics treatment information will be listed by group and subject including day of treatment, date and time of dose, antibiotic name, prescribed dose, dosing frequency and comments.

If the subject is terminated early from the study, antibiotic treatment information for early termination will be listed including day of treatment, date and time of dose, antibiotic name, prescribed dose, whether doses were provided for participant to take home, total dose provided for the participant to take home and comments.

4.1.11 Comprehension Assessment Tool and Venous Assessment

Whether the subject passed the quiz of Comprehension Assessment Tool (≥70% correct answers) and assessment for venous will be listed by group and subject.

4.2 EFFICACY ANALYSIS

4.2.1 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is "Diarrheal stool output rate", defined as the total volume of diarrheal stools (mL, Grade 3 and above) divided by the number of hours between initiation of study product dosing and initiation of antimicrobial therapy. Diarrheal stool output rate will be summarized, by group and by group and O vs Non-O status, using daily and overall summaries.

Statistical comparison of the primary endpoint between groups will be conducted with the stratified (by blood type group) version of the Wilcoxon rank-sum test, the Van Elteren test.

A sample of SAS implementation code for the Van Elteren test is shown below:

```
proc npar1way data=adeff;
    strata stratum; /* O vs Non-O status */
    class trt01p; /* iOWH032 vs Placebo */
    var effrate;   /* Diarrheal stool output rate */
run;
```

Due to the nonzero probability of concluding efficacy at the interim analysis, the alpha level (type I error rate) for the final analysis needs to be adjusted to maintain overall one-sided level alpha = 0.025. The threshold to define success on the primary efficacy endpoint at the final analysis is one-sided alpha = 0.0238. The hypothesis test will be supplemented by a two-sided confidence interval for the difference in median diarrheal stool output rate, using the percentile bootstrap method, with n=10,000 replicates.

The principal analysis will be performed using the mITT population. If greater than 20% of subjects are excluded from the mITT due to onset of symptoms after 48 hours, the primary endpoint will be calculated again including patients with symptom onset after 48 hours. For subjects with symptom onset after 48 hours, the time between onset of symptoms and administration of antimicrobials will define the denominator used to calculate the diarrheal stool output rate. Supportive analysis will be performed using the per-protocol population.

Analysis of diarrheal stool output rate will be supplemented with reverse cumulative distribution curves. The horizontal axis of the plot is the rate after logarithmic conversion, and the vertical axis is the percentage of individuals larger than this rate.

Diarrhea Collection information will be listed by group and subject including date and time of collection, volume of Diarrhea collection, Grade and comments.

4.2.2 ANALYSIS OF THE SECONDARY EFFICACY ENDPOINTS

Secondary efficacy endpoints include a) the proportion of participants with moderate or severe diarrhea following cholera challenge defined as >3.0 L and >5.0 L of loose stools, as well as b) the attack rate of any diarrhea following cholera challenge defined as the number of participants with 2 or more loose stools (grade 3-5) totaling >200 mL within 48 hours or 1 loose (grade 3-5) stool > 300 mL.

Both endpoints will be computed as the proportion within each group satisfying the respective

definitions, and will be accompanied by exact two-sided 95% confidence intervals. This will be supplemented with corresponding summaries within study arm and blood type group. Statistical inference for each endpoint will be conducted with the Cochran-Mantel-Haenszel test, stratified by blood type group.

If a two-sided statistical test with Type I error rate of 0.05 rejects H0 and the proportion of participants is less for the iOWH032 group than for the placebo group, the study will have demonstrated that iOWH032 is superior.

The AUC of diarrheal stool volume and cholera organisms will be computed via the trapezoidal rule. The secondary endpoints of AUC of cholera-containing diarrheal stool volume, AUC of cholera organisms, and peak shedding of cholera organisms (highest cfu counts for each participant) will be analyzed in quantitative stool culture. Statistical comparison of the three secondary endpoints between groups will be conducted with the stratified (by blood type group) version of the Wilcoxon rank-sum test, the Van Elteren test. If a one-sided statistical test rejects H0 and the median is less for the iOWH032 group than for the placebo group, the study will have demonstrated that iOWH032 is superior. The hypothesis test will be supplemented by a two-sided 95% confidence interval for the difference in median AUC of cholera-containing diarrheal stool volume, AUC of cholera organisms, and peak shedding of cholera organisms , using the percentile bootstrap method, with n=10,000 replicates.

Cholera illness during the interval immediately following challenge and prior to initiation of antibiotics will be summarized by group and by blood type group (O vs Non-O) within group.

- Time (hours, post-challenge) to the first formed stool (grade <2, overall, as well as by blood type group(O vs Non-O)), will be summarized with Kaplan-Meier methods and compared with the log-rank test by blood type group(O vs Non-O).

- The total count of loose stools will be summarized as a continuous variable and compared separately for each blood type group with the Wilcoxon rank-sum test and jointly with the Van Elteren test, stratified by blood type group (O vs Non-O).The hypothesis test will be supplemented by a two-sided 95% confidence intervals for the difference in median total count of loose stools, using the percentile bootstrap method, with n=10,000 replicates.

- The presence of fever or vomiting will each be summarized as categorical variables, with between-group inference conducted separately for each blood type group with Fisher's exact test, and jointly via the Cochran-Mantel-Haenszel test, stratified by blood type group (O vs Non-O).

The principal analyses will be performed using the mITT population. Supportive analysis will be performed using the per-protocol population.

Analysis of diarrheal stool volume, cholera-containing diarrheal stool volume, and density of cholera organisms will be supplemented with reverse cumulative distribution curves. The horizontal axis of the plot is the volume and density after logarithmic conversion, and the vertical axis is the percentage of individuals larger than this rate.

Stool collection information will be listed by group and subject including Not done, date and time of collection, type of Collection, weight of stool, Grade, result of quantitative culture and comments.

Vomitus collection information will be listed by group and subject including none, date and time of collection, volume of vomitus, grade and comments.

4.3 SAFETY ANALYSIS

4.3.1 ADVERSE EVENTS (AES)

AEs will be coded by system organ class (SOC) and preferred term（PT）according to MedDRA 22.1.

The summary of AEs will be based on Treatment-emergent AEs (TEAEs) are defined as AEs which start or worsen following the start of study medication and up until the follow-up visit. If the time of the AE is not available, then TEAEs will be those occurring on or after the first day of study medication.

The summary of AEs will also include an overall event count by treatment group. The treatment percentages (solicited/unsolicited AEs, etc.) will be supplemented with two-sided 95% confidence intervals.

4.3.1.1 Overview of AEs

A summary table will be presented, by treatment group, for the numbers and percentages of participants with any AE, any TEAEs, study drug related TEAEs, study related TEAEs, serious TEAEs, serious and study drug related TEAEs, serious and study related TEAE, any severe TEAEs, TEAEs leading to discontinuation from the study and TEAE leading to death.

4.3.1.2 Incidence of TEAEs

A summary of the frequency (number and percentage of subjects) of TEAEs will be presented by treatment group and by SOC and PT. Multiple occurrences of the same AE (SOC or PT) will be counted only once when calculating the number and percentage of subjects. This summary will be sorted in decreasing order of overall frequency of SOC, and then in decreasing order of overall frequency of PT within the SOC.

Number and percentage of subjects with TEAEs will also be presented by PT only. This summary will be sorted in decreasing order of overall frequency.

All information pertaining to AEs will be listed by subject and all the details collected on the eCRF, including verbatim term given by the investigator, PT, SOC, onset date/time, end date/time, course, severity, solicited AE category if solicited AEs, seriousness, serious AE definition (congenital abnormality or birth defect, significant disability, death, hospitalization or prolongation of hospitalization, life threatening, other medically important event), autopsy performed and date of death if died, causality, study product administration, treatment required, and outcome.

4.3.1.3 Incidence of TEAEs by Severity

The number and percentage of subjects reporting a TEAE will be tabulated by treatment group for SOC and PT and severity.

A subject experiencing the same AE multiple times within same SOC/PT will be counted only once for that SOC/PT at the worst severity.

4.3.1.4 Incidence of Study Drug Related TEAEs

The number and percentage of subjects reporting a study drug related TEAE will be tabulated by treatment group for SOC and PT.

4.3.1.5 Incidence of Study Related TEAEs

The number and percentage of subjects reporting as study related (defined as those related to study drug or related to cholera) TEAE will be tabulated by treatment group for SOC and PT.

4.3.1.6 Serious Adverse Events (SAEs)

SAEs will be summarized and listed in the same manner described above for TEAEs (Section 4.3.1.2).

4.3.1.7 TEAEs Leading to Discontinuation of Study Drug

TEAEs leading to discontinuation of study drug will be summarized and listed in the same manner described above for TEAEs (Section 4.3.1.2).

4.3.1.9 TEAEs Leading to Death

TEAEs leading to death will be listed in the same manner described above for TEAEs (Section 4.3.1.2).

4.3.1.10 Solicited Adverse Events

The occurrence of solicited adverse events during the three days of oral dosing, through 8 hours following the last dose, which are attributed to iOWH032 and placebo be summarized.

Solicited adverse events collected during the dosing phase (three days of oral dosing through 8 hours following the last dose) and considered to be related to study drug will be summarized by treatment group for causality and severity. For summary table by causality, subjects will be counted only once within each causality category, but can be counted in more than one causality category. For summary table by severity, subjects will only be counted once at the worst severity for an AE.

All information pertaining to solicited AEs will be listed by subject and all the details collected on the eCRF, including solicited AE category, PT, SOC, onset date/time, end date/time, whether AE occurs during the dosing period, course, severity, seriousness, serious AE definition (congenital abnormality or birth defect, significant disability, death, hospitalization or prolongation of hospitalization, life threatening, other medically important event), autopsy performed and date of death if died, causality, study product administration, treatment required, and outcome.

4.3.1.11 Unanticipated Problems (UPs)

Unanticipated problems (UPs), following the definition the Office of Human Research Protections as described in Protocol Section 10.1.3, are collected for this study. A listing including event description, date/time of occurrence, corrective action, ongoing or not, and end date/time of occurrence will be provided.

4.3.2 Safety Laboratory Tests

Clinical laboratory values will be graded per Appendix A.

The observed values and change from baseline of Complete blood count (CBC) for white blood cell (WBC), absolute neutrophil count (ANC), Hemoglobin, and Platelets; and Chemistry panel for Na, K, ALT, AST, ALP, Creatinine, Albumin, and Total Bilirubin, CPK, Glucose will be tabulated with descriptive statistics by visits if applicable.

Abnormalities for each lab will be summarized by maximum grade overall and per time point. A corresponding summary will be produced only for those considered to be clinically relevant which corresponds to the abnormal clinically significant.

The complete blood count (CBC) for WBC, ANC, Hemoglobin, and Platelets; and Chemistry panel for Na, K, ALT, AST, ALP, Creatinine, Albumin, and Total Bilirubin, CPK, Glucose will be listed by subject and visit, respectively.

4.3.3 Urinalysis

The screening urinalysis for Glucose, Protein, Occult blood, RBC and specific gravity will be listed by group and subject.

4.3.4 Vital Signs

Vital signs (blood pressure, pulse, and oral temperature) will be measured approximately every 8 hours, unless more frequent monitoring is needed. Once a participant has passed a diarrheal stool (Grade 3 or higher), vital signs will be measured every 4 hours until the participant passes a Grade 1 or 2 stool or 24 hours have passed since the last Grade 3 – 5 stool, whichever comes first.

Vital signs will also be measured every 4 hours when a participant has a fever ≥ 39°C (102.1°F), until the participant has 2 consecutive temperatures of ≤ 38°C (100.4°F).

Any participant that complains of dizziness or lightheadedness upon standing will have orthostatic blood pressures assessed—BP after supine for ~5 minutes and BP after standing ~2-3 minutes. Orthostatic hypotension is defined as a drop in systolic BP > 20 mmHg or in diastolic BP > 10 mmHg.

Vital signs and their change from baseline will be tabulated using descriptive statistics by visits and time point.

All vital signs including vital sign parameters and overall interpretations will be listed by subject.

4.3.5 ECGs

ECGs will be measured at screening. Subjects should rest in the supine position for at least 5 min before each 12-lead ECG recording. The results will include heart rate (HR), R-R interval (RR),

PR interval, QRS interval, QT interval, and QTcF interval. The corrected QT interval will be corrected for heart rate according to the following formula:

$$\text{Fridericia's formula: } QTcF = QT/RR^{0.33}$$

The listing will be provided for ECG including individual parameters, ECG interpretation and comments on findings if applicable.

4.3.6 Physical Examination and Physical Measurement

Full physical examination will be done at Screening, Post-Challenge Observation Period (Day 1 through discharge), visit2 and visit3. A symptom-directed physical examination may be performed at other times at the Investigator's or Sub-Investigator's discretion.

All subjects with any abnormal physical examination findings will be listed.

Physical Measurement for weight will be done at Post-Challenge Observation Period (Day 1 through discharge). All subjects with weight will be listed.

4.4 Exploratory Analyses

4.4.1 Pharmacokinetic (PK) Properties of iOWH032

PK plasma samples for the levels of iOWH032 will be collected 7±1 hours after the first and last dosing in all participants.

Concentration-time data will be summarized by treatment group using descriptive statistics (n, arithmetic mean, geometric mean, median, arithmetic standard deviation, minimum, maximum, and arithmetic coefficient of variation [%]) at each timepoint. At a particular timepoint, a concentration value reported as "<LLOQ" will be considered zero and a mean/median will not be calculated in the instance where >50% of the concentration values at a timepoint are reported as "<LLOQ", then only the minimum and maximum will be reported.

4.4.2 Evaluate Time of Cessation of Cholera Organism in Stool after Challenge

Time (hours) to cessation of detectable cholera in stool is defined as the time of the first sample negative via quantitative stool culture, after which all following samples are also negative for cholera.

Time to cessation of detectable cholera in stool (hours) will be analyzed using Kaplan-Meier methods.

4.4.3 Evaluate a decrease for oral rehydration solution and/or intravenous fluid replacement therapy need

Statistical comparison of this exploratory endpoints will be conducted with the stratified (by blood type) version of the Wilcoxon rank-sum test, the Van Elteren test. The hypothesis tests will be supplemented by a two-sided 95% confidence interval for the difference in median diarrheal stool output, using the percentile bootstrap method, with n=10,000 replicates.

4.4.4 EXPLORATORY ANALYSES OF DIARRHEAL STOOL OUTPUT

In addition to the primary and secondary analyses of diarrheal stool output rate, assessments of diarrheal stool output (volume) over 12- and 24-hour periods will aid interpretation of study results. The following will be summarized by group and by blood type (O vs non-O) within group.

- Diarrheal stool output in first 12 hours, after initiation of study product and prior to initiation of antibiotic
- Diarrheal stool output in first 24 hours, after initiation of study product and prior to initiation of antibiotic
- Diarrheal stool output during subject's individual 24-hour window of peak output, after initiation of study product and prior to initiation of antibiotic
- Diarrheal stool output during subject's individual 24-hour window of peak output, after initiation of study product and including time after initiation of antibiotic
- Diarrheal stool output in first 24 hours after initiation of antibiotic

Statistical comparison of these exploratory endpoints will be conducted with the stratified (by blood type) version of the Wilcoxon rank-sum test, the Van Elteren test. The hypothesis tests will be supplemented by a two-sided 95% confidence interval for the difference in median diarrheal stool output, using the percentile bootstrap method, with n=10,000 replicates.

5 INTERIM ANALYSIS

Once efficacy data are available from the completion of Cohort 1, an interim analysis of the primary efficacy endpoint and accompanying safety data will be conducted to guide the decision to move forward with Cohort 2. The details of the interim analysis are described separately in an interim SAP.

Additionally, topline results will be conducted after the efficacy data from Cohorts 1 and 2 have been collected (i.e. including data prior to Visit 2). These topline results will include analysis of the primary efficacy endpoint, as well as the analyses of the secondary endpoints of proportion of participants with MSD, attack rate of any diarrhea, AUC of stool volume, duration of episode (time to first formed stool), and total number of loose stools conducted on the mITT population. In addition, subject disposition and demographic and baseline characteristics tables will be provided. The topline results will be unblinded only to the treatment-level and not to the individual subject. To avoid identification of individual subjects, some summary statistics such as minimum and maximum will not be presented in the summary tables.

The final statistical analyses will be performed after database lock and final unblinding of the data.

6 SUMMARY OF MAJOR CHANGES IN THE PLANNED ANALYSES

The following updates from Protocol v4.0 were made in this SAP:

- TEAE definition has been revised to AEs which start or worsen following the start of study medication and up until the follow-up visit.
- The Clinical Safety Laboratory Toxicity Scales defined in the Appendix D of the protocol have been revised as shown in Appendix A of this SAP.

7 PROGRAMMING SPECIFICATIONS

Programming specifications will be provided in the SAP table, listing, and figures shells document.

8 TABLES, LISTINGS, AND FIGURES SHELLS

The Tables, Listing, and Figures Shells will be provided as a separate document.

APPENDIX A: CLINICAL SAFETY LABORATORY TOXICITY SCALES

Test	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Potentially Life Threatening Grade 4
WBC, increase	1.1 – 1.5 xULN	1.6 – 2.0 xULN	2.1 – 2.5 xULN	≥ 2.6 xULN
WBC, decrease*	LLN/1.5 – 1.1	LLN/2.0 – 1.6	LLN/2.5 – 2.1	≤ LLN/2.6
ANC decrease	LLN/1.5 – 1.1	LLN/2.0 – 1.6	LLN/2.5 – 2.1	≤ LLN/2.6
Hemoglobin decrease	LLN/1.5 – 1.1	LLN/2.0 – 1.6	LLN/3.0 – 2.1	< LLN/3
Platelet count	LLN/1.5 – 1.3	LLN/2.0 –1.6	LLN/3.0 – 2.1	< LLN/3
Glucose Nonfasting, High	116-160	161-250	251-500	> 500
Glucose, Low	55-64	40-54	30-39	< 30
Sodium, low	132 -134	130 - 132	125 - 129	< 125
Sodium, high	146 – 148	149 - 150	151 - 152	> 152
Potassium, high	5.6 – 5.8	5.9 – 6.1	6.2 – 6.5	> 6.5
Potassium, low	3.3 – 3.4	3.1 – 3.2	2.9 – 3.0	<2.9
Creatinine*	1.1 – 1.5 xULN	1.6 – 2.0 xULN	2.1 – 3.0 xULN	> 3.0 xULN
AST (SGOT)	1.6 -.2.5 xULN	2.6 – 4.0 xULN	4.1 – 10.0 xULN	>10 xULN
ALT (SGPT)	1.6 -.2.5 xULN	2.6 – 4.0 xULN	4.1 – 10.0 xULN	>10 xULN
Alkaline Phosphatase	1.6 -.2.0 xULN	2.1 – 3.0 xULN	3.1 – 10.0 xULN	>10 xULN
Total Bilirubin	1.2 -.1.5 xULN	1.6 – 2.0 xULN	2.0 - 4.0 xULN	>4 xULN

ULN = upper limit of normal range

LLN = lower limit of normal range

*if the baseline is outside of the reference range (e.g., African-American acceptable value), baseline value will replace LLN for WBC decrease, and replace ULN for Creatinine, to evaluate toxicity