4.0 Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

This study is a phase II, randomized, placebo controlled, double blind efficacy and safety trial. The study is designed for the assessment of a novel fixed dose combination (FDC) drug VR-AD-1005 for the treatment of acute watery diarrhea in adult cholera patients. Adult (18-65 years) cholera patients admitted to icddr,b Dhaka Hospital will be randomized to intervention and placebo groups with 75 patients per group.

Group A (intervention product arm): Patients will receive VR-AD-1005 treatment for three days, or less if recovered earlier, in addition to the general cholera protocol treatment.

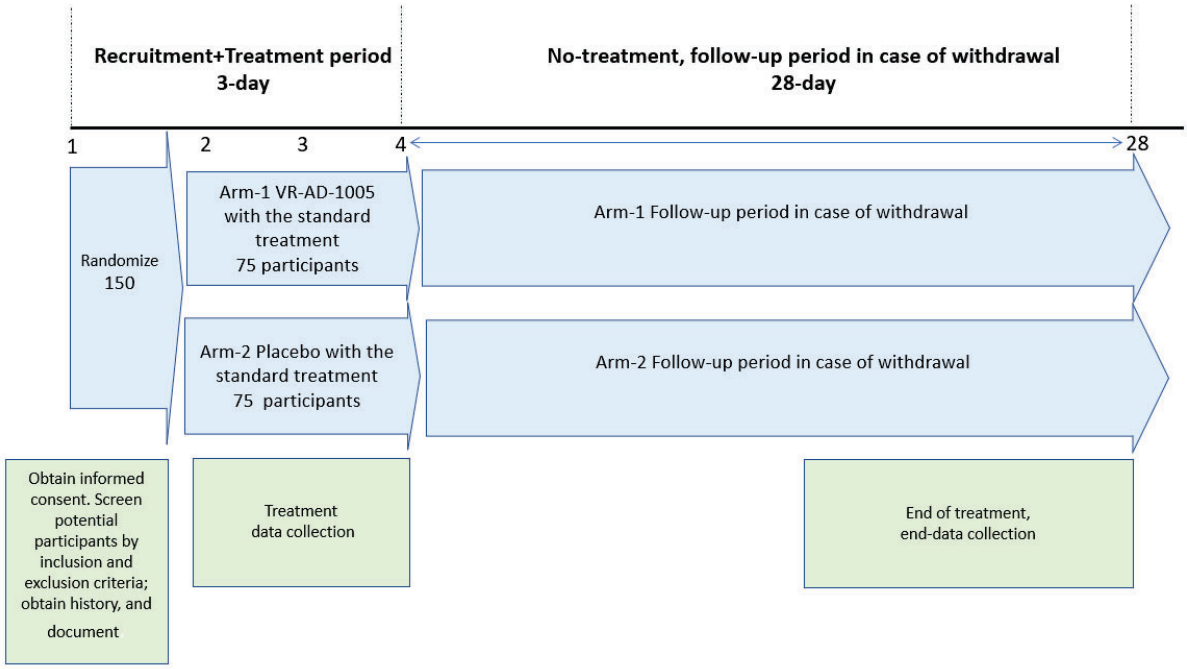
Group B (placebo arm): Patients will receive placebo for three days, or less if recovered earlier, in addition to the general cholera protocol treatment.

Key aspects of the trial design and Trial Schema are given below in table 2 and figure 2.

Table 2: Key aspects of the trial design

Intervention Model: Parallel arm intervention model with 2 Arms: Group A (intervention product arm) and Group B (placebo arm)		**Population Type:**	[Adult patient]
Control: **Intervention:**	[placebo] [VR-AD-1005]	**Population Diagnosis or Condition:**	[*Vibrio cholerae* infection]
Active Comparator:	[not applicable]	**Population Age:**	Minimum: [18y.o.] – Maximum: [65y.o.]
Trial Intervention Assignment Method:	Double-blind randomization	**Site Distribution:**	Single site

Figure 2-Trial Schema



4.1. Rationale for Trial Design

The double-blind randomized controlled trial (RCT) is accepted by the authorities as objective scientific methodology that, when ideally performed, produces knowledge untainted by bias. The most important design techniques for avoiding bias in clinical trials are blinding and randomization, and these should be normal features of most controlled clinical trials intended to be included in a marketing application. Most such trials follow a double-blind approach, in which treatments are pre-packed in accordance with a suitable randomization schedule and supplied to the trial center(s) labelled only with the subject number and the treatment period so that no one involved in the conduct of the trial is aware of the specific treatment allocate to any particular subject, not even as a code letter. The baseline characteristics will be summarized using descriptive statistics to facilitate evaluation of possible differences between treatment groups, which might bias the comparison of treatments. Study medication compliance, protocol violations and reasons for dropouts will be examined both as indicators of treatment differences and to evaluate any biases in the proposed treatment comparisons.

4.2. Trial Population

4.2.1. Selection of Trial Population

Adults, both sexes, aged 18-65 years of age.

4.2.2. Rationale for Trial Population

Cholera predominantly affects children and young adults[20]. The paediatric population is outside of the scope of the current protocol, however, during the outbreak all age groups are at risk of infection. With this consideration, capping the age at 65 years will facilitate the recruitment, while allowing to reduce the burden and complications of co-managing chronic diseases associated with elderly populations.

4.2.3. Inclusion Criteria

- Signed informed consent.
- Adults, both genders aged 18-65 years.
- Acute watery diarrhea (defined as passage of three or more liquid stools within the 24 hours before admission) with severe dehydration on arrival.
- Detection of *V. cholerae* by rapid diagnostic assay (e.g. dark field microscopy).

4.2.4. Exclusion Criteria

- Known or suspected hypersensitivity to trial product(s) or related products.
- Subjects with passage of bloody stools or muco-purulent stools.
- Subjects with chronic diarrhea (*>4 weeks of Diarrhea)*.
- Clinically significant concomitant systemic disease (i.e. cardiovascular diseases including heart failure, acute kidney injury, sepsis or life-threatening malignant cancer).
- Mental incapacity, unwillingness, or language barriers, precluding adequate understanding or cooperation.
- History of receiving antimicrobial or antidiarrheal drugs within 6 hours prior to admission.
- Positive urine pregnancy test for all female patients
- Failure to obtain informed consent.
- Failure to definitively diagnose cholera via culture or RT-PCR

4.2.5. Screen Failures

Patients with acute diarrhea, who go through the screening but do not fit the inclusion criteria or present with criteria excluding them from the clinical trial as per sections 4.2.3 and 4.2.4 above would be labelled as screen failures and will not receive the study treatment and will receive standard treatment in the hospital. Screen failures are not expected to contribute to the analysis of efficacy and safety. Screen failures who have medical conditions will be referred to the appropriate level of care.

4.3. Sequence of events

- All prospective patients visiting the facilities aged 18-65 years experiencing acute watery diarrhea (duration 24 hours or less) and severe dehydration will be assessed. Assessment will be done primarily by the study nurses (at least three nurses will rotate for the study duration). Study doctors will also assist with the assessment.
- Consent will be sought from suspected patients to enroll cases. Confirmatory microbiology for cholera will be done. Eligibility of the participants will be identified using the screening tool. This study will not include vulnerable participants (refer to inclusion and exclusion criteria in sections 4.2.3 and 4.2.4).
- Trained nurses/study doctors will take the patients (who fulfil all inclusion criteria and do not meet any of the exclusion criteria) or guardian for critically ill patients through the consenting process. This will involve explaining in plain language what the study is about and the risk/ benefits of participating. Participants will be informed that participation in the trial is voluntary and that they can withdraw at any time during the 3 days of inpatient care. They will be required to sign applicable consent forms, one form will be kept by investigators and another will be given to participants.
- Upon consenting, randomization will be done by the independent researcher and treatment started by the study nurses in consultation and supervision by study doctors, in addition to standard management for *V. Cholerae* for patients.
- All patients will be admitted in the dedicated wards of the study site for all the 3 days of treatment. Early discharges will not be permitted for enrolled study participants due to safety and endpoint measurement reasons. To facilitate data analysis, and in particular safety analysis (development of possible adverse events, while undergoing treatment with VR-AD-1005), all

participants with different level of severity of the disease must follow the same treatment protocol. Having variations in the treatment strategy, such as early discharge, may complicate the conduct of the trial. An example of such complication is the development of unrelated AEs which stem from changes in the lifestyle of participants after discharge, for example, change in diet or a community-acquired disease, which will be prevented and avoided by having all participants as in-patients.

- Patients will receive their first dose administered by the study nurse immediately after the consenting process and randomization is completed. The treatment is in a capsule form and participants will be instructed by the study nurse to swallow the treatment whole without chewing with a glass of water or oral rehydration solution. The volume of liquid used for ingesting the treatment will be recorded in patient water intake charts for monitoring and analysis purposes. Subsequent doses will be timed based on the first dose (i.e. second dose is 3 hours after first dose). The study nurses will record the timing for each patient's medication and will keep strictly to the timing between shifts by handing over study patient folder records after each shift. Study nurses will additionally monitor for vomiting or accidental loss of treatment dose (for example, dropped dose) and to re-administer, if vomiting occurs within 30 minutes of treatment administration or if the pill is clearly visible in the vomit.
- Stool samples will be taken to the laboratory for culture and identification of *V. cholerae* and blood samples taken for study laboratory assessments. Laboratory assessments will be conducted at the icddr,b Immunobiology, Nutrition and Toxicology Laboratory and/or diagnostics laboratories. Any other necessary study laboratory test may be performed at any of these laboratories depending on capacity and efficiency of study procedures.
- During the three days of admission, participants will be monitored daily by study doctors and nurses. Treatment will be given as per scheduled doses each day, monitoring and measurement of water intake, weighing of vomiting and stool output using calibrated scales (scales will be calibrated on daily basis) and all other study procedures. For recruited patients whose culture comes back negative for cholera, treatment will be stopped (culture results expected by day three) and they will not be included in the final analysis.
- In the event of SAE's during treatment or after discharge, participants will be referred to the appropriate clinic for comprehensive management.
- Participants will only be discharged when the study doctors deem so after the three days of treatment. Participants who are not discharged after 3 days will not receive further treatment but will be monitored by study staff.
- After discharge, participants will be followed up with weekly phone calls to check on their health and wellbeing and also ascertain, if they are experiencing any side effects of the treatment. For participants without phone, the phone number of their guardian or neighbor will be taken for the follow up. Where participant cannot be reached via phone, their personal address will be followed to conduct the follow up. After three attempts of phone calls and one home visit, participants that cannot be reached will be declared lost to follow up.

[redacted]

[redacted]

[redacted]

[redacted]

4.4.3. Route of Trial Intervention

Intervention is taken orally. Cholera is a gastrointestinal disease; the oral route is by far the most common route of drug administration in gastrointestinal diseases.

4.4.4. Dosing and Administration

Cholera typically presents with voluminous liquid stool due to hypersecretion of fluids by the intestine [21]. VR-AD-1005 is not absorbed and acts at the level of intestinal mucosa. Thus, first dose of the drug is dissolved in much larger than normal volume of fluid presented in the intestine of a patient recently admitted for cholera. In addition, due to the diarrhea, first dose of the drug is evacuated with stool faster than subsequent doses. In order to maintain working concentration and counteract dilution, first dose of the drug is tripled, representing the "loading dose" drug administration tactics. The remaining doses are single doses for the three days which is in line with average duration of the cholera episode and thus gives sufficient time for the drug to exert it's effect. The treatment will be administered to participants under the supervision of trained study personnel, i.e. study nurse. Nurses will be instructed to monitor for vomiting or accidental loss of treatment dose (for example, dropped dose). The participants will be instructed to swallow the treatment whole without chewing with a glass of water or oral rehydration solution. The volume of the liquid will be registered in the water intake charts.

Administration begins when the patient is able to swallow the tablet. If the patient vomits less than 30 minutes after administration of dose, the dose will have to be re-administered after about 15 minutes. If the patient vomits after 40 minutes, the clinician will check the vomitus to see if the medication is in there, if the medication is not visible then the dose is not re-administered. If the medication is visible, then the dose is re-administered after 15 minutes. Both arms will receive extra dosses of the medication for these purposes. In the event of lost dose, the pharmacy will have a backup of the medication doses for emergency purposes.

VR-AD-1005 oral dose will be administered orally 4 times per day (approximately every 4-5 hours with overnight break to allow for participants rest and recovery) with first administration of 3 capsules as loading dose. Dosing is indicated in the table below **(Table 3)**, with reasonable timing of administration at 7:00, 12:00, 17:00 and 21:00 hours ± 1 hour.

Table 3: VR-AD-1005 doses and dosing schedule

Time	Day 1	Day 2	Day 3
Time 0 hr	3 doses	1 dose	1 dose
Time +5:00 hr	1 dose	1 dose	1 dose
Time +10:00 hr	1 dose	1 dose	1 dose
Time +15:00 hr	1 dose	1 dose	1 dose

4.4.5. Access to Trial Intervention After End of Trial

After the end of trial, IP will no longer be accessible to the participants. Cholera is an acute infectious disease, and after resolution of cholera episode treatment is no longer warranted.

4.4.6 Participant Assignment, Randomization and Blinding

4.4.6.1. Participant Assignment

During recruitment, in addition to rehydration/antibiotics, each eligible participant will receive a numbered individual package of treatment, with package number being unique to the particular participant. After the end of the study for the purpose of data analysis the randomization tables will be supplied to the biostatisticians and data from all participants who have completed the trials will be assigned to the Group A (VR-AD-1005 intervention) or Group B (placebo) for analysis.

4.4.6.2. Participant Randomization

This trial is a double-blind, placebo-controlled trial. IP and placebo randomization will be performed by the IP manufacturer using uneven-block randomization strategy with the following considerations:

- A computer-generated randomization list that provides an associated unique study identification number is produced by a researcher unrelated to the study/trial
- The randomization list is saved as a document in a format that assigns appropriate identifiers to the study subjects. This document is safely and securely kept by the Sponsor along with a log of the code used to generate the list.
- The randomization list is sent to the assigned person to preserve any and all blinding and concealment. The blinded investigators/research team should not see the randomization list during the running of the trial (see 6.6.3 for blinding).
- Randomization is double-blind, and the clinical trial site is not aware of the type of intervention (IP or placebo) that a particular patient will receive.

A procedure for emergency breaking the code is implemented by the sponsor and the icddr,b Data Safety Monitoring Board (DSMB) in case there is a need for emergency access for the benefit of the participant.

4.4.6.3. Blinding and Unblinding

The blinding/randomization of the medication is determined when the individual patient study supplies are packaged by the IP manufacturer. Standard randomization methods are used, i.e. uneven block randomization. The study center is required to dispense the patient medication in chronological order to each of the patients entering the study. A sealed envelope containing the randomization code breaks will be given to the Investigator for safe keeping enabling easy access in the event of an emergency, requiring the code to be broken.

Management of Emergency Code Break:

The emergency code breaks will be placed by Hunazine Biotech in a sealed envelope and kept at the study center. The code must only be broken in the case of medical events which the investigator/physician in charge of the patient feels cannot be adequately treated without knowing the identity of the study medication.

Sponsor should be contacted before breaking the code. If it is not possible, and the situation is an emergency, the investigator may break the code and contact the Sponsor as soon as possible. The following information needs to be recorded in the event of an Emergency Code Break:

- Date of Code Break
- Identification of person(s) requesting the Code Break
- Reason for breaking the code
- Investigator Signature.

The person who broke the code must sign and date the envelope which, if opened at study site, will be returned to Safety Board (SB) by the SB site monitor. The patient will be withdrawn from the study.

4.4.6.4. Trial Intervention Compliance

During the study, the field monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the Case Report Form (CRF), the adherence to the protocol and to Good Clinical Practice (GCP), the progress of enrollment, and to ensure that study investigational product is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. The investigator must give the monitor access to all relevant follow-up source documents for safety monitoring purposes and safety database updates.

4.5. Trial Assessments and Procedures

4.5.1. Screening/Baseline Assessments and Procedures

- Medical history: All patients admitted with secretory diarrhea with severe dehydration will be offered to participate in the study.
- Informed consent. Severely ill patients unable to sign the consent form will be resuscitated according to the proper SOPs, until they are able to make an informed decision regarding the enrollment.
- Randomization and assignment to treatment Group. IP assignment.
- Concomitant medication review.
- Physical examination: height, body weight, temperature, thirst, HEENT (note and record signs of dehydration, such as sunken eyes), skin (note and record signs of dehydration, such loss of turgor), level of consciousness using AVPU (active, response on voice or pain, unconscious) criteria, vomiting.
- Hydration status should be assessed in each participant including assessment of IV fluids, oral ORS intake and oral water intake.
- Vital signs: body temperature, heart rate, respiratory rate, blood pressure (including orthostatic blood pressure measurement when possible), urine output.
- Blood collection (5 ml) for Complete Blood Count (CBC), Basic Metabolic Panel (BMP) including serum electrolytes and creatinine.
- Stool collection for *V. cholerae* RDT (dark field microscopy) and culture.
- Initiate Re-hydration and antibiotic treatment in accordance with the SOPs for cholera patients until recovery.
- Initiate IP administration as soon as participants can tolerate oral intake.

4.5.2. Efficacy Assessments and Procedures

- Medications review.
- Physical examination: Body weight, thirst, HEENT (note and record signs of dehydration, such as sunken eyes), skin (note and record signs of dehydration, such loss of turgor), level of consciousness, vomiting.
- Hydration status should be assessed in each participant including assessment of IV fluids, oral ORS intake and oral water intake. The volume of rehydration solutions received by the participant is recorded continuously for both enteral and parenteral rehydration for the entire duration of the treatment. Access to unaccounted water (i.e. tap water) should be restricted, and measured volume of ORS or water should be provided for free access instead, with the entire volume of consumed liquid measured and recorded in the charts.

- Vital signs: body temperature, heart rate, respiratory rate, blood pressure (including orthostatic blood pressure measurement when possible), and urine output.
- Blood collection (5 ml) for Complete Blood Count (CBC), Basic Metabolic Panel (BMP) including serum electrolytes and creatinine at treatment dose #5.
- Stool collections for efficacy analysis starts immediately after participant receives the first dose of IP and continues for the entire duration of the treatment. Stool samples are collected continuously, and weighed at 12-hour intervals. Number of stool passages and stool weight is recorded in CRFs.
- At the end of the treatment the last physical exam is performed as per the schedule of events and stool sample will be sent for *V. cholerae* culture to confirm elimination of the pathogen. During the pre-treatment and treatment periods the data will be recorded into provided CRFs by the PI or his designee.

4.5.3 Safety Assessments and Procedures

- Physical examination: height, body weight, thirst, HEENT (note and record signs of dehydration, such as sunken eyes), skin (note and record signs of dehydration, such loss of turgor), level of consciousness, vomiting.
- Vital signs: body temperature, heart rate, respiratory rate, blood pressure (including orthostatic blood pressure measurement when possible), and urine output.
- Clinical Laboratory assessments: Complete Blood Count (CBC), Basic Metabolic Panel (BMP)

Procedures and assessments may be performed as part of the subject's standard medical care; however, data for these assessments should remain in the subject's medical record and should not be provided to HB, unless specifically requested by the sponsor. Laboratory assessments will be done at the on-site laboratories.

Schedule of activities during screening and three days of treatment period is given in appendix 1

4.6. Start of Trial and End of Trial

Trial starts once the participant has signed the informed consent form. The end of trial is defined as database lock after completion of the study by the last patient. A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in **Appendix I**.

4.7. Adverse Events and Serious Adverse Events

4.7.1. Definitions of AE, SAE and ADR

Terminology according to ICH S2A:

Adverse event: An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
Adverse Drug Reaction (ADR): In the *pre-approval clinical experience* with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established:
Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).
Serious Adverse Event or Adverse Drug Reaction: During clinical investigations, adverse events may occur which, if suspected to be medicinal product-related (adverse drug reactions), might be significant enough to lead to important changes in the way the medicinal product is developed (e.g.,

change in dose, population, needed monitoring, consent forms). This is particularly true for reactions which, in their most severe forms, threaten life or function. Such reactions should be reported promptly to regulators.

Serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

** results in death,*

** is life-threatening,*

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Requires inpatient hospitalisation or prolongation of existing hospitalisation,*

** results in persistent or significant disability/incapacity, or*

** is a congenital anomaly/birth defect.*

4.7.2. Time Period and Frequency for Collecting AE, SAE and ADRs Information

- **Single Cases of Serious, Unexpected ADRs**

All adverse drug reactions (ADRs) that are both serious and unexpected are subject to expedited reporting. This applies to reports from spontaneous sources and from any type of clinical or epidemiological investigation, independent of design or purpose. It also applies to cases not reported directly to a sponsor or manufacturer (for example, those found in regulatory authority-generated ADR registries or in publications). The source of a report (investigation, spontaneous, other) should always be specified.

Expedited reporting of reactions which are serious but expected will ordinarily be inappropriate. Expedited reporting is also inappropriate for serious events from clinical investigations that are considered not related to study product, whether the event is expected or not. Similarly, non-serious adverse reactions, whether expected or not, will ordinarily not be subject to *expedited* reporting.

Information obtained by a sponsor or manufacturer on serious, unexpected reports from any source should be submitted on an expedited basis to appropriate regulatory authorities if the minimum criteria for expedited reporting can be met.

Causality assessment is required for clinical investigation cases. All cases judged by either the reporting health care professional or the sponsor as having a reasonable suspected causal relationship to the medicinal product qualify as ADRs. For purposes of reporting, adverse event reports associated with marketed drugs (spontaneous reports) usually imply causality. Many terms and scales are in use to describe the degree of causality (attributability) between a medicinal product and an event, such as certainly, definitely, probably, possibly or likely related or not related. Phrases such as "plausible relationship," "suspected causality," or "causal relationship cannot be ruled out" are also invoked to describe cause and effect. However, there is currently no standard international nomenclature. The expression "reasonable causal relationship" is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

- **Other Observations**

There are situations in addition to single case reports of "serious" adverse events or reactions that may necessitate rapid communication to regulatory authorities; appropriate medical and scientific judgement should be applied for each situation. In general, information that might materially influence the benefit-risk assessment of a medicinal product or that would be sufficient to consider changes in medicinal product administration or in the overall conduct of a clinical investigation represents such situations. Examples include:

a. For an "expected," serious ADR, an increase in the rate of occurrence which is judged to be clinically important.

b. A significant hazard to the patient population, such as lack of efficacy with a medicinal product used in treating life-threatening disease.

c. A major safety finding from a newly completed animal study (such as carcinogenicity).

4.7.3. Identifying AE, SAE and ADRs

Principal investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. Time period and frequency for event assessment and follow-up:

The occurrence of an adverse event (AE) or serious adverse event (SAE) or adverse drug reaction (ADR) may come to the attention of study personnel during study weeks and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

4.7.4. Recording AE, SAE and ADRs

All AEs, including local and systemic reactions not meeting the criteria for AE/SAEs/ADRs, will be captured on the appropriate Adverse Event Form. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event.

All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

4.7.5. Reporting of Fatal or Life Threatening or Serious Unexpected ADRS

- **Fatal or Life-Threatening Unexpected ADRs**

All SAEs should be reported to local DSMB within 24 hours of known information by the PI/investigators.

Certain ADRs may be sufficiently alarming so as to require very rapid notification to regulators in countries where the medicinal product or indication, formulation, or population for the medicinal product are still not approved for marketing, because such reports may lead to consideration of suspension of, or other limitations to, a clinical investigations program. Fatal or life-threatening, unexpected ADRs occurring in *clinical investigations* qualify for very rapid reporting. Regulatory agencies should be notified (e.g., by telephone, facsimile transmission, or in writing) as soon as possible but no later than *7 calendar days after first knowledge by the sponsor that a case qualifies, followed by as complete a report as possible within 8 additional*

calendar days. This report must include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar medicinal products.

- **All Other Serious, Unexpected ADRs**

Serious, unexpected reactions (ADRs) that are not fatal or life-threatening must be filed as soon as possible but *no later than 15 calendar days* after first knowledge by the sponsor that the case meets the minimum criteria for expedited reporting.

- **Minimum criteria for reporting**

Information for final description and evaluation of a case report may not be available within the required time frames for reporting outlined above. Nevertheless, for regulatory purposes, initial reports should be submitted within the prescribed time as long as the following minimum criteria are met: an identifiable patient; a suspect medicinal product; an identifiable reporting source; and an event or outcome that can be identified as serious and unexpected, and for which, in clinical investigation cases, there is a reasonable suspected causal relationship. Follow-up information should be actively sought and submitted as it becomes available.

- **How to Report**

The CIOMS-I form has been a widely accepted standard for expedited adverse event reporting. However, no matter what the form or format used, it is important that certain basic information/data elements, when available, be included with any expedited report, whether in a tabular or narrative presentation. The listing in Attachment 1 addresses those data elements regarded as desirable; if all are not available at the time of expedited reporting, efforts should be made to obtain them.

All reports must be sent to those regulators or other official parties requiring them (as appropriate for the local situation) in countries where the drug is under development.

4.8. Safety Oversight - Data Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board (DSMB) will be formed by the Ethical Review Committee to evaluate and assess the safety of study participants. The DSMB will be composed of 3-5 members, three of which are nominated by the ERC chairperson. The PI also has the option of nominating 1 or 2 members with clinical expertise. The DSMB will convene two times, at the beginning before the trial is started, and at the end of the trial. Medical monitor will review the safety and have the option of convening a DSMB meeting if there is a safety issue that requires additional interpretation. The PI can also request a DSMB meeting depending upon the study complexity or in light of safety concerns. The DSMB will review all unanticipated problems involving risk to the participants or others, serious adverse events, and all participant deaths associated with the protocol. The DSMB will advise the PI of its findings and provide recommendations. The PI will inform the Sponsor in detail about the discussions of those meetings.

4.9. Case management

From screening to discharge, patients' safety will be ensured through following procedures, which are also indicated in **Table 4: schedule of activities.** Screening procedures will be started only after obtaining written informed consent. During screening, medical and medication history will be collected and patients' condition will be monitored through physical examination, obtaining vital signs, assessment of stool and urine output vomiting, dehydration status, water intake, ORS intake and IV fluid infused, and testing for complete blood count (CBC) and serum electrolytes and creatinine. For female of child bearing age pregnancy will also be evaluated as a part of the exclusion criteria. All the inclusion and exclusion criteria will be strictly maintained for enrollment of the patients in the study, and no vulnerable participants will be enrolled. After randomization and administration

of IP/placebo, physical examination will be performed and vital signs will be obtained for initial three hours followed by at least 8 hourly for the whole treatment period and also at the end of treatment. Assessment of hydration status, including dehydration, vomiting, urine output, water intake, ORS intake or IV fluid infusion will be performed throughout the treatment period. Concomitant medication review will also be performed at least once in a day. Blood testing for the re-evaluation of the patient will be done according to the clinical condition of the patient and once at the end of treatment. Beside giving study specific treatment, all patients will be provided standard care and treatments. Standard of care (SoC) for adult cholera patients having severe dehydration includes rehydration with IV cholera saline (100ml/kg over 3 hours) and Doxycycline 300 mg after initial hydration; SoC also includes replacement of ongoing purging with ORS (if no persistent vomiting or no high purging) or IV fluid (if there is persistent vomiting or high purging). Adverse events, including SAE will be closely monitored, and appropriate treatment will be provided; if needed, patients will be referred to specialized clinics or hospitals. Recovery from diarrhoea will be assessed continuously through stool output and frequency, and stool culture will be performed to confirm the absence of *V. cholerae.* Feeding with a normal diet will be continued /initiated based on clinical feasibility; feeding with a normal diet will be resumed when vomiting has stopped and patients are able to tolerate oral intake. Patients will be fed meals prepared and dispensed under hygienic conditions. Street food will be prohibited due to the high risk of bacterial contamination, which may hinder case management and also invalidate the results of this study. Patients will receive 2 to 3 cooked meals per day. Even after discharge, health and wellbeing of the participants will be followed up with weekly phone calls or home visits; it will be ascertained if they are experiencing any side effects of the treatment or any other AE or SAE.

4.10. Concomitant Therapy

4.10.1. Prohibited Concomitant Therapy

Medications that have shown a positive effect on any aspect of diarrhea will not be permitted during the trial, including:

- Cholestyramine
- EGF/urogastrone
- Loperamide
- Racecadotril
- Somatostatin
- Alosetron
- Mytesi (Crofelemer)
- Atropine/dyphenoxilate

4.10.2. Permitted Concomitant Therapy

Concomitant therapy should be administered to all patients in accordance with the icddr,b guidelines on the treatment of cholera patients with acute diarrhea. Important steps are listed below **(Table 5)**.

Table 5-Assessment of dehydration status [10]

Assess	Condition*	Normal	Irritable/Less active*	Lethargic / Comatose*
	Eyes	Normal	Sunken	Sunken
	Tongue	Normal	Dry	Dry
	Thirst*	Normal	Thirsty (drinks eagerly)	Unable to drink*
	Skin pulse*	Normal	Goes back slowly*	Goes back very slowly*
	Radial pulse*	Normal	Reduced*	Uncountable or absent*

Diagnosis		**No sign of dehydration**	If at least 2 signs including one (*) sign is present, diagnose. **Some Dehydration**	If some dehydration plus one of the (*) signs are present, diagnose **Severe Dehydration**
Management		**A**	**B**	**C**

A. No sign of dehydration – ORS

- 50 ml ORS per kg body weight over 4-6 hrs *plus* ongoing losses
- Send patient to home with 4 packets of ORS
- Continue feeding, including breast milk for infants and young children on

B. Some dehydration – ORS

- 80 ml ORS per kg body weight over 4 – 6 hours *plus* ongoing losses
- Observe patients for 6 - 12 hours.
- Continue feeding, including breast milk for infants and young children on breastfeeding
- Reassess patients and dehydration status frequently - hourly.

In case of frequent vomiting (>3 times in 1 hour) or some dehydration persists ≥6 hours even after oral rehydration therapy: Treat with I/V fluid

C. Severe dehydration – I/V solution containing sodium, potassium, chloride and bicarbonate (e.g. Ringer's Lactate/ cholera saline)

Start I/V fluid immediately:

- 100 ml / kg Adult and Children > 1 year
  30 ml / kg in first 1/2 hour
  70 ml / kg in next 2.5 hours
- Encourage the patient to take ORS solution as soon as he/she is able to drink without vomiting.
- Antibiotic, if needed, after full rehydration.
- Zinc-20 mg/day for 10 days in children 6 months-5 yr old.

4.10.3. Antibiotic Therapy

Stool samples for V. cholerae dark field microscopy and culture should be collected before the onset of antibiotic therapy for presumed cases of cholera (Profuse watery stools, typically looking like 'rice water', resulting dehydration; many individuals affected in the same locality).

- Adults : Azithromycin, 1 gram single dose orally
- Repeat dose if vomiting within 1/2 hour of taking the medicine

Do not use azithromycin when a patient is 60-65 years or older or at any age with an established heart disease to avoid adverse effects, specially conduction defects.

In such a case, Doxycycline 300 mg single dose is recommended.

Antibiotics should be administered no sooner than 2 hours after taking VR-AD-1005.

4.11. Discontinuation of Trial Intervention and Participant Withdrawal from Trial

Participants may withdraw from a research study for any reason, even without mentioning any reason. The participants will be able to receive standard treatment of the hospital without any penalty. For evaluation and reporting purposes, researchers may ask participants for their reasons for early withdrawal.

An investigator may discontinue trial intervention or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance.
- If any adverse event (AE), Serious adverse events (SAE), laboratory abnormality, or other medical condition or situation occurs, continued participation in the study would not be in the best interest of the participant.
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Participant unable to receive study intervention for 3 days.
- Reporting over-dose
- protect a participant from excessive risk or risk with a demonstrated lack of benefits (e.g., a participant experiences serious side-effect without the anticipated therapeutic effects), or
- maintain the integrity of the data (e.g., a participant is not following study procedures or may be deliberately providing false information).

Whenever an investigator terminates a subject's participation in research, the investigator must explain to the participant the reasons for the termination and, as appropriate, other treatment options available to the participant.
The data to be collected at the time of study intervention discontinuation will include the following:

- Physical examination
- Vital signs
- Complete blood count (CBC)
- Blood Basic metabolic panel, including Serum electrolytes and creatinine
- Assessment of vomiting
- Assessment of stool output
- Assessment of dehydration
- Assessment of IV and oral ORS and oral water intake
- Assessment of urine output
- Administration of questionnaires or other instruments helping participants to register symptoms, such as stool charts.
- Assessment of adverse events.

Follow-up calls will be made with patients who completed the protocol with questionnaires focusing on the side effects. Calls well be made once a week during the 28-day follow-up period.

If any serious adverse events occur before treatment phase, study treatment may be postponed at the researcher's decision.

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

4.12. Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for three out of four scheduled phone calls or one missed in-person visit (for withdrawals) and is unable to be contacted by the study site staff. The following actions must be taken if a participant fails to return to the clinic for a required study visit: The site will attempt to contact the participant and reschedule the missed visit within 7 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and/or electronic messages, one home visit). These contact attempts should be documented in the participant's medical record or study file.
Should the participant continue to be unreachable, he or she will be considered to have been lost to follow-up and will be excluded from the long-term safety data analysis.
Since follow-up occurs after the completion of the treatment course, loss to follow-up is not critical for the primary and secondary endpoints and treatment data obtained during the course of the trial will remain valid.

4.13. Protocol Deviations

A deviation is any noncompliance with the clinical trial protocol, GCP, or manual of procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. Deviations from the protocol should be avoided. If deviations occur, The implications of the deviation must be reviewed and discussed. Investigator must document and explain protocol deviations by stating the reason, date, and the corrective action(s) taken. Deviations, for which no corrections are relevant, can be acknowledged and confirmed via edit checks in the CRF. The documentation for the protocol deviations must be kept in the Investigator's trial file and Hunazine Biotech trial master file. Major deviations from the protocol, as defined as deviations that impact participant safety or endpoints, will be promptly reported to the DSMB and Sponsor. Minor deviations from the protocol will be recorded on a source document and corresponding eCRF, and included in the annual report. Examples of minor deviations are events outside the control of the investigators (e.g., participant missed visit window or study parameters that are not part of the primary endpoint was not performed at a visit).

4.14. Study monitoring plan (monitoring visits, level of source data verification, etc.)

Trial monitoring activities will be provided by the CRO monitor(s), which will be conducted prior to beginning, at initiation, during, and at closeout of the trial by the monitor(s). At the beginning of the study, the monitor(s) will (1) verify the physical facilities of the study site. (2) ensure that the study team including the investigators and study staff are qualified and experienced. (3) provide training to study staff. During the course of the study, the monitor will visit the clinical site at regular intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations and requirements, including GCP on the conduct of clinical research. At a minimum the monitor(s) will do the following: (1) ensure that study staffs are performing their duties as per the delegation of authority log. (2) ensure that the study is being performed according to the protocol and applicable regulatory requirements. (3) review adherence to the protocol-specific SOPs. (4) verify source documents and eCRF are completed accurately and perform verification of eCRF against the available source documents. The monitor should have access to participant medical records and other study-related records needed to verify the entries on the eCRFs. (5) check that all AEs / SAEs and deviations have been appropriately reported. (6) ensure that each participant or parents/guardians have signed the approved ICF. (7) Ensure adequacy of study drugs and clinical trial supplies at the site. (8) review different study logs, e.g. 'delegation of authority', 'IP accountability log', 'temperature log' etc. (9) review visit findings with PI / investigator(s) and provide action items for the site personnel. (10) provide refresher training if necessary. The PI and the monitor must agree to cooperate to ensure that any problems detected in the course of these monitoring visits, including eCRF completion and query resolution, are resolved. Close-out monitoring visit includes (1) Final review of the source documents and eCRF, and resolution of missing and questionable data (2) Final 'IP accountability'; returning remaining IPss to the sponsor or destruction of remaining IPs at study site. (3) Final 'specimen accountability'. (5) Review and record site close-out report submitted to sponsor, IRB and regulatory authorities.

4.15. Regulatory Agency inspection

The PI must be aware that representatives from regulatory authorities (e.g. DGDA) or the IRB may wish to inspect the eCRFs and associated study records. The PI and study coordinator must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies.

4.16. General Considerations: Risk Management and Quality Assurance

4.16.1. Quality Tolerance Limits

A QTL is a trial-level parameter, not a patient-level parameter. Examples include inclusion/exclusion protocol violations, incomplete/missing endpoint data, and AEs/SAEs of special interest.

4.16.2. Data Quality Assurance

QA is a systematic and independent assessment of all the clinical trial activities and records. It checks for following aspects: Generation, recording, analysis, and reporting of the clinical data are in accordance with the protocol, Standard Operating Procedures (SOPs) and Good Clinical Practices (GCPs).

4.16.3. Source Data

The executive order on GCP defines source data as any information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

Source data may be both electronic and on paper. The following list includes examples of where source data may be stored:

- medical records
- laboratory reports
- diaries
- dispensing logs
- Any research records
- Case Report Forms (CRF)
- ECG printouts
- X-ray images
- radiological reports, etc.
- communication: verbal

5.0 Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

Planned screening is 500 participants. ITT (intention to treat) group is 150 patients. Enrollment will stop once 150 participants are recruited, or once 100% of per-protocol dataset (PP) is achieved. Sample size was calculated based on the mean volume of stool output in cholera patients. Mean stool output was assumed at 280±156 g/kg [22–24]. Assuming 33% reduction (alternative hypothesis) vs no reduction (null hypothesis), sample size was calculated using GraphPad Prizm software for two-tailed t-test with alpha 5% and power 0.8. Using recruitment of 50 participants per arm (100 total), power level of 0.82 can be achieved. Although, this is a sufficient level of power for the projected study, data points lost due to participant drop-out, cross-over to other study arms, withdrawal and missing data will affect the power of the study. We calculated that recruitment of up to 75 patients per study arm would allow power of 0.94.

Endpoints associated with primary and secondary objectives, and justification for endpoints are given below (Table 1).

Table 1-Primary and Secondary Objectives and Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
• To assess the efficacy of VR-AD-1005 for the treatment of acute diarrhea in cholera patients in combination with standard treatment (rehydration and antibiotics, please see attached SoP for more details) versus standard treatment with placebo. Efficacy is measured as reduction in stool output between the start of treatment until final diarrheal stool before clinical recovery (defined as disappearance of watery stool and two consecutive passages of soft stool) or end of study treatment (treatment duration 72 hours), which is earlier.	• Reduction in stool output volume (ml/hour/kg) over a treatment period measured as stool weight and recalculated to stool volume using 1:1 w/v ratio, and plotted as stool output quantity per each 12-hour period for the duration stool collection. 6 points are expected for a 72-hour stool collection. Measurement of stool output will be performed by collecting the stool from a participant into the pre-weighed vessel. The vessel with stool will be weighed on the calibrated scale, the weight of the vessel would be subtracted, and the resulting stool weight recorded into the stool diary form. During weighing of stool, Infection Prevention and control (IPC) standards will be upheld by implementing protection of personnel via using appropriate personal protective equipment. Hand washing before and after stool weighing will be strictly adhered to thereby preventing infection of study personnel. After weighing, stool will be disposed of as biohazardous waste at study sites and the weighing vessel appropriately disinfected before next measurement or discarded, if disposable.	• Primary endpoint allows to monitor and characterize the efficacy of VR-AD-1005 for reduction of stool output. Over-secretion of hypotonic fluid occurs predominantly in the small intestine, and fluid is lost with stool. The volume of fluid loss in cholera patients can be life-threatening due to rapid dehydration. Thus, the primary efficacy endpoint is designed to demonstrate potential of VR-AD-1005 treatment to reduce fluid loss with stool.
Secondary		
• Assessments of improvement of hydration status, fluid input and duration of	• Time until the stool volume output reduction to 200 ml/hour or less. Duration of stool output in excess of 200 ml/hour per patient will be recorded between the start and end of	• Secondary end-points are designed to supplement the primary end-point by providing a

diarrhea following VR-AD-1005 administration. • Evaluation of safety and tolerability of VR-AD-1005 in addition to standard of care (SoC) versus SoC with placebo in adults with symptomatic cholera via documentation of adverse events, physical examination, and vital signs.	the treatment for both treatment groups. • Time until clinical recovery (defined as disappearance of watery stool and two consecutive passages of soft stool). Duration of diarrhea until the passage of first non-watery stool (soft/loose) or no stool for 12 hours or the end of treatment (72 hours) will be derived from date and time records in daily stool charts. • Number of subjects with clinical recovery as above within treatment group. The number of subjects with clinical recovery will be recorded as total for the entire treatment duration, and as number of recoveries per each 24-hour period of treatment. • Number of unscheduled IV rehydration episodes per treatment. The number of unscheduled IV rehydration episodes per treatment will be recorded for the entire duration of the treatment. Unscheduled IV rehydration is defined as "receipt of IV fluid during the maintenance phase of hydration due to development of severe dehydration or some dehydration with high purging (>15 ml/kg/hour) or persistent vomiting (>3 episodes/hour), or failure to take ORS for any reason" • Duration of IV rehydration for the entire treatment period per treatment group and per each 24- hour period of treatment. • Safety will be measured by the proportion of patients that will present with AE/SAE as defined in Appendix II following administration of VR-AD- 1005.	measure of hydration status of the participants, as well as providing a measure of the duration of the illness. Dehydration is the primary cause of death in cholera; duration of illness is the primary measure of the burden on the healthcare system and quality of life/productivity loss for cholera patients. Assessment of reduction in the need for unscheduled intravenous rehydration and time until switch to oral rehydration therapy will allow to assess the benefit of VR-AD-1005 for in-patient management of cholera cases. Thus, secondary end-points allow to characterize the benefits related to potential improvements in the clinical course of the disease, case management and quality of life for cholera patients.

6.0 Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

Data analysis must comply with ICH E9 Guideline and ICH E9(R1) Guideline. All relevant data collected in the trial should be considered in the statistical considerations section.
The analysis will be conducted on all participant data at the time the trial ends.

Statistics for primary end-point data and parametric secondary end-point data will be presented via means (alternatively, LSMEANS) with standard deviations; primarily two-tailed Student's t-test will be used for analysis with significance level of $p \leq 0.05$. For non-parametric analysis, such as lifetime data, Kaplan-Meier analysis will be used with significance level of $p \leq 0.05$. Some data might be expressed and analyzed as % values, such as the occurrence of unscheduled IV rehydration.

Analysis of secondary endpoints will be performed through the same method of the primary endpoint, which is found via a one- sided two sample unequal variance Student's t-test. The scale for each secondary endpoint will be nominal and will be determined at a single endpoint, which is at the end of intervention. The results will be presented by LSMEANS (Least-squares Means) with standard errors.

Primary Endpoint:
Reduction in stool output volume (ml/hour) over a treatment period measured and plotted as total stool output during 12-hour stool collection at every 12 hours for 72 hours.

Secondary Endpoints:

- Time until the stool volume output of 200mL/hour or less. Duration of stool output in excess of 200mL/hour per patient will be recorded between the start and end of the treatment for both treatment groups.
- Time until recovery. Duration of diarrhea until the passage of first non-watery stool or no stool for 12 hours or the end of treatment (72 hours) will be derived from date and time records in daily stool charts.
  Number of unscheduled IV rehydration episodes per treatment. The number of unscheduled IV rehydration episodes per treatment will be recorded for the entire duration of the treatment. Unscheduled IV rehydration is defined as "receipt of IV fluid during the maintenance phase of hydration due to development of severe dehydration or some dehydration with high purging (>15 ml/kg/hour) or persistent vomiting (>3 episodes/hour), or failure to take ORS for any reason"
- Duration of IV rehydration for the entire treatment period per treatment group and per each 24-hour period of treatment.
- Number of recovered subjects within treatment group. The number of recovered subjects will be recorded as total for the entire treatment duration, and as number of recoveries per each 24-hour period of treatment.
- Safety will be measured by the proportion of patients that will present with AE following administration of VR-AD-1005.

6.1. Analysis set

Data for VR-AD-1005 intervention vs. placebo groups will be analyzed in the following comparison data sets:
A. Stool output comparison
B. Comparison of the number of unscheduled hydration episodes (both IV and oral, as defined above).

C. Comparison of the total volume of the rehydration solutions used during the study.
D. Duration of diarrheal illness will be compared between the groups.
E. Duration of stool output in excess of 200ml/hour will be compared.
F. Comparison for the number of AE/SAE.
G. Comparisons for the base-line descriptive statistics.

6.2. Analysis Supporting Primary Objectives

6.2.1. Statistical Model, Hypothesis, and Method of Analysis

Primary Endpoint:
Reduction in stool output volume over a treatment period.
Primary Endpoint 1., Hypothesis 1. Treatment with VR-AD-1005 reduces stool output in patients with diarrhea due to V. cholerae infection. Null hypothesis is stated as no statistically significant difference in stool output volume as compared to placebo; alternative Hypothesis 1 is stated as statistically significant reduction in stool output volume in patients treated with VR-AD- 1005.
Two analytical approaches are proposed. Approach 1 will be based on the total quantity of stool purged by participant during the intervention administration period. Stool output will be expressed as g/kg body weight based on the stool charts, and the means will be compared between intervention and placebo groups using Student's unpaired t-test. In approach 2, stool purging is measured and plotted as total stool output in ml/kg/12 hours based on the individual stool collection charts. Weight of stool will be converted to volume using 1:1 weight/volume ratio. Diarrhea of cholera resolves rapidly, and majority of patients are expected to recover within 30-50 hours after randomization.12-hour analysis increments allow a better insight into the onset and timing of the effect of study intervention as compared to cumulative data over the duration of the treatment. Means will be compared for each 12-hour period after first intervention administration using unpaired Student's t-test. Normal distribution is assumed for both cases. Analysis will be conducted in the ITT, MITT and PP groups. Missing data and outliers will be handled using imputation technique. Non-adherence and lost to follow-up patients will be categorized into the appropriate group and handled accordingly. No subtractions will be made from the ITT group after randomization assignment.

6.2.2. Handling of Missing Data

Missing data other than protocol-planned baseline values will be classified as missing completely at random (MCAR) or missing at random (MAR). MCAR can be confirmed by partitioning the data into two parts: one set containing the missing values, and the other containing the non-missing values. After partitioning the data, the t-test of mean difference is carried out in order to check whether there exists any difference in the sample between the two data sets. If the number of missing values is small, i.e. if the number of the cases is less than 5% of the sample, then the researcher can drop them. Otherwise, when analyzing using ITT population, model based multiple imputation (MI) will be used for both primary and secondary outcomes. The number of imputations will be the largest value of:

- 10, or
- The proportion of missing values for the actual variable * 100.

200 burn in iterations will be used for all analyses. Trace plots and distribution plots will be created to check the accuracy of imputations.

6.3. Analysis Supporting Secondary Objectives

Analysis of secondary end-points is not dependent on the analysis of the primary end-point.

***Secondary Endpoint 1*, Hypothesis 2.** Treatment with VR-AD-1005 improves hydration control in patients with diarrhea due to V. cholerae infection. Null hypothesis is stated as no statistically

significant difference in the number of unscheduled hydration episodes as compared to placebo; alternative hypothesis 2 is stated as statistically significant improvement in hydration control, expressed as number of unscheduled rehydration episodes over the treatment period; in patients treated with VR-AD-1005. Unscheduled IV rehydration is defined as "
"receipt of IV fluid during the maintenance phase of hydration due to development of severe dehydration or some dehydration with high purging (>15 ml/kg/hour) or persistent vomiting (>3 episodes/hour), or failure to take ORS for any reason"

Secondary Endpoint 2, **Hypothesis 3.** Treatment with VR-AD-1005 shortens duration of diarrhea in patients with diarrhea due to V. cholerae infection. Null hypothesis is stated as no statistically significant difference in the duration of diarrhea as compared to placebo; alternative hypothesis 2 is stated as statistically significant reduction in the duration of diarrhea, expressed as number of days till normalization of stool, in patients treated with VR-AD-1005.

Secondary Endpoint 3, **Hypothesis 4.** Treatment with VR-AD-1005 shortens duration of stool output in excess of 200ml/hour in patients with diarrhea due to V. cholerae infection. Null hypothesis is stated as no statistically significant difference in the duration of stool output >200ml/hour as compared to placebo; alternative hypothesis 2 is stated as statistically significant reduction in the duration of diarrheal output >200ml/hour, expressed in hours, in patients treated with VR-AD-1005.

Analysis:
Secondary Endpoint 1, assessment of hydration control. There are two measurable parameters to be assessed in the patient hydration. Parameter 1 is the number of unscheduled hydration episodes. The numbers will be derived from the individual rehydration records, and the number of patients receiving IV rehydration will be expressed in % and compared for control and study intervention group. Alternatively, total number of IV rehydration episodes cand be expressed as means for control and study intervention group and analyzed using Student's t-test. Parameter 2 is the volume of the oral rehydration solution received during the course of interventional treatment. Total volume of rehydration solution (oral and IV where applicable) will be derived from the individual rehydration records, expressed in ml/kg of patient weight, and analyzed as means using unpaired Student's t-test. Analysis subgroups will include 1) total hydration received over the course of study after first administration of interventional medication, and 2) hydration received during first 12 hours and 3) hydration received during first 24 hours.

Secondary Endpoint 2. Assessment of duration of diarrhea. Total duration of diarrheal illness is an important parameter in the assessment of antidiarrheal treatment efficacy. Study intervention is expected to reduce the overall duration of the diarrhea. Duration of diarrhea is assessed from first administration of study intervention until the termination of diarrhea, which is defined as first formed stool, or no stool for 12 consecutive hours. Duration of diarrhea will be derived from individual stool charts. Kaplan-Meier survival curves will be built to compare duration of diarrhea between control and intervention groups.

Secondary Endpoint 3. Duration of stool output in excess of 200ml/hour. As stated above, duration of diarrheal disease in cholera is limited, averaging 30-35 hours[24]. This could mask the beneficial effect of antidiarrheal treatment (as measured solely by stool quantification) if high initial stool output terminates abruptly. In order to establish the time course of the study intervention's effect, in addition to analyzing the total duration of diarrhea in patients we need to introduce a parameter that combines the measured quantity of stool output and the time factor, addressing the question weather interventional medication can reduce the severity of diarrhea in the early stage of administration of study intervention. For analysis, individual stool charts will be used to calculate stool output per patient per 12-hour period and express it as stool volume in ml/hour. Duration of time when stool

output is in excess of 200ml/hour will be calculated for each participant, expressed as means for control and study intervention group and analyzed using Student's t-test.

Normal distribution is assumed for Endpoints 1 and 3. Non-parametric statistics is used in the analysis of Endpoint 2. Analysis will be conducted in the ITT, MITT and PP groups. Missing data and outliers will be handled using imputation technique. Non-adherence and lost to follow-up patients will be categorized into the appropriate group and handled accordingly. No subtractions will be made from the ITT group after randomization assignment.

6.4. Safety Analyses

Safety analysis is not the primary or secondary objective of this study, thus safety will be analyzed as summary statistics during treatment. AEs will be coded per Medical Dictionary for Regulatory Activities (MedDRA), and calculated. Each AE will be counted once only for a given participant, and presented using standard available criteria for severity, frequency, and relationship of AEs to study intervention. Start date, stop date, severity, relationship, expectedness, outcome, and duration will be reported for each SAE. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be listed. Proportion of the patients presented with AE or SAE will be calculated for IP and Placebo arms as a fraction of the total number of participants per arm and expressed as percent. Percentage of AE and SAE occurrence will be compared between IP and placebo groups to provide information regarding the safety of the investigational product.

6.5. Other Analyses

In addition to the overall analysis of the intervention vs. placebo, it is important to elucidate the efficacy of the IP as a function of the severity of the diarrheal disease. Thus, participants will be segregated into three subgroups based on the volume of stool output. All subgroups will be analyzed using both ITT and PP populations. moderate subgroup (stool volume 3-8 liters per 24 hours) and severe subgroup (stool volume >8 liters per 24 hours). Subgroups are intended for analytical purposes and will not affect randomization. Within the subgroups the analysis will be performed as above.

6.6. Interim Analyses

No interim analysis is currently planned.

7.0. Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

7.1. Data Protection

All study data and records will be treated confidentially and used solely for research purposes; We will ensure that, only the researcher will have access to the collected data/information obtained from the participant's records. We will also make sure that the data that are gathered are saved and well encrypted under password to prevent unauthorized access. If the subject and parents or the subject's legal representative withdraws the previously given informed consent or refuses to consent to continue in the trial, or if the subject dies and no consent is available from a subject's legal representative, or if the subject is lost to follow up then the subject's data will be handled as follows:

- Data collected will be retained by Hunazine Biotech and entered into the database.
- Safety events will be reported to the department responsible for global product safety, Hunazine Biotech/regulatory authorities according to local/national requirements.
- If data is used, it will always be in accordance with local law and IRB/IEC procedures.

7.2. Reports and Publications

Information obtained during the conduct of this trial is considered confidential and can be used by Hunazine Biotech for regulatory purposes and for the general development of the trial product. All information supplied and obtained by Hunazine Biotech in connection with this trial shall remain the sole property of Hunazine Biotech and is to be considered confidential information. No confidential information shall be disclosed to others without prior written consent from Hunazine Biotech. Such information shall not be used except in the performance of this trial. The information obtained during this trial may be made available to other physicians who are conducting other clinical trials with the trial product, if deemed necessary by Hunazine Biotech. One principal Investigator will be appointed to review and sign the CTR (Signatory Investigator) on behalf of all participating Investigators. The signatory Investigator will be appointed based upon the criteria defined by the International Committee of Medical Journal Editors (ICMJE) for research publications. The protocol will be registered in a publicly accessible database such as clinicaltrials.gov, CTIS/EudraCT, WHO Pan African Clinical Trial Registry (PACTR), or other applicable registers. In addition, after study completion (i.e., LPLV) and finalization of the study report the results of this trial will be submitted for publication and posted in a publicly accessible database of clinical trial results.

Hunazine Biotech commits to communicating, and otherwise making available for public disclosure, results of trials regardless of outcome. Public disclosure includes publication of a paper in a scientific journal, abstract submission with a poster or oral presentation at a scientific meeting, or by other means.

Hunazine Biotech reserves the right to not release data until specified milestones, e.g. when the clinical trial report is available. This includes the right to not release interim results of clinical trials, because the release of such information can invalidate the results of the entire trial.

At the end of the trial, one or more manuscripts for publication will be prepared collaboratively between Investigator(s) and Hunazine Biotech. Hunazine Biotech reserves the right to postpone publication and/or communication for less than 60 days to protect intellectual property.

The results of this trial will be subject to public disclosure at external web sites according to international regulations, which is reflected in Hunazine Biotech Code of Conduct for Clinical Trial Disclosure.

In all cases, the trial results shall be reported in an objective, accurate, balanced, and complete manner, with a discussion of the strengths and limitations of the trial. Screen In the event of any disagreement about the content of any publication, both the Investigators' and Hunazine Biotech's opinions shall be fairly and sufficiently represented in the publication.

Hunazine Biotech maintains the right to be informed of any Investigator plans for publication and to review any scientific paper, presentation, communication or other information concerning the investigation described in this protocol. Any such communication must be submitted in writing to the Hunazine Biotech Trial Manager prior to submission for comments. Comments will be given within four weeks from receipt of the planned communication.

7.3. Authorship

Authorship of publications should be in accordance with guidelines from The International Committee of Medical Journal Editors' Uniform Requirements (sometimes referred to as the Vancouver Criteria). The Investigator(s) offered authorship will be asked to comment and approve the publication. No permission to publish will be granted to any clinical research organization involved in the trial described in this protocol.

8.0. Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

8.1. Regulatory and Ethical Considerations

The trial will be conducted in compliance with ICH GCP, applicable regulatory requirements, and in accordance with the Declaration of Helsinki. The sponsor and investigator will comply with all applicable regulations. In addition, the investigator will follow national and institutional requirements including, but not limited to Investigational Medicinal Product (IMP), clinical research, Informed Consent Forms (ICF) and Institutional Review Board (IRB) regulations. The approval of the trial protocol will be obtained from Ethical Review Committee (ERC) of icddr,b and Directorate General of Drug Administration (DGDA), which is functioning under the Ministry of Health & Family Welfare, Government of the People's Republic of Bangladesh. The investigator will be aware that representatives from DGDA or ERC may wish to inspect if the trial is in compliance to the protocol and adhered to GCP and local regulatory requirement. They may also look at eCRFs and associated study records to check the accuracy and consistency of the data.

All subjects included in the trial will be treated according to the icddr,b SoC for the treatment of cholera (attached) in addition to either VR-AD-1005 (75 subjects) or placebo (75 subjects). Subjects assigned to the trial will be transferred to a treatment regimen anticipated to be better than or equal to the treatment they receive at the time they enter the trial. However, they will have to spend some extra time, as additional visits to the clinic are required and some of these tests performed during the trial are outside normal practice.

There is no information available today indicating any risks in connection with the use of VR- AD-1005 FDC as compared to other products from the same pharmaceutical category. Data available for individual API indicate low risk which is far outweighed by the benefits.

All subjects participating in the trial will be monitored closely by the site with frequent visits and telephone contacts. When the subjects' participation in the trial ends, the subjects will consult their Investigator to decide on the best available marketed treatment.

8.2. Committees

Prior to commencement of the trial the protocol, any protocol amendments, subject information/informed consent form, any other written information to be provided to the subject and parents/subject's legal representative, subject recruitment procedures (incl. advertisement), if any, IB, available safety information, information about payments and compensation available to subjects if not mentioned in the subject information, the Investigator's current curriculum vitae (CV) and/or other documentation evidencing qualifications, and other documents as required by the local Institutional Review Board/ Independent Ethics Committee (IRB/IEC) should be submitted. The submission letter should clearly identify the trial identification number, version, European Union Drug Regulating Authorities Clinical Trials (EudraCT) number, title and/or the date of the documents that have been submitted to the IRB/IEC. Written approval favorable opinion must be obtained from IRB/IEC prior to commencement of the trial. During the trial, the Investigator or sponsor, as applicable, must promptly report the following to the IRB/IEC, in accordance with local requirements: updates to IB, unexpected SAEs where a causal relationship cannot be ruled out, substantial protocol amendments to the protocol, non- substantial protocol amendments according to local requirements, deviations to the protocol implemented to eliminate immediate hazards to the subjects, new information that may affect adversely the safety of the subjects or the conduct of the trial (including new risk/benefit analysis in case it will have an impact on the planned follow-up of the subjects),

annually written summaries of the trial status, and other documents as required by the local IRB/IEC and Ethics Review Committee.
Substantial protocol amendments must not be implemented before approval/favorable opinion, unless necessary to eliminate immediate hazards to the subjects.

8.3. Informed Consent Process

All potential study participants will be informed on the purpose of the study, procedures, risks and benefits of participating in the study, participant's expectations, and the compensation plan for their participation. It will be ensured that all information collected will be stored under lock and key or on the researchers' computer under password to prevent unauthorized access and used only for the purpose for which the data was collected. We will guarantee data privacy and confidentiality of all data obtained by ensuring that personal identifiers collected from participant folders would not be used during the analysis in the study. The participants will be encouraged to ask questions about the study, have the questions answered and then be given time to decide if they would participate in the study. It will also be emphasized that participation is voluntary, and that the participant has the right to withdraw themselves from the study at any time without prejudice. An impartial witness will be present during the informed consent discussions with an illiterate participant. A person who can only write his/her name and cannot read or write in Bangla will be considered as illiterate for the study purposes. The Investigator must obtain voluntary, personally signed and dated informed consent from participants before any study-related procedure. The original, signed ICF must be kept in the site study file; one copy of the ICF will be given to the participant / parents.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animals will be used in this study.

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

Hunazine Biotech will serve as the sponsor of the study and will provide the Clinical Trial Material for the phase II study. Hunazine Biotech will be responsible for cGMP manufacture of the investigational product.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

- The trial will be conducted at icddr,b Dhaka Hospital. It has adequate facilities for the management of acutely ill patients, particularly those are suffering from diarrheal illnesses. The patients are used to manage in short stay unit of the hospital. Those who require hospital stay for>48 hours with comorbidities require admission in longer stay unit. Additionally, the hospital has a dedicated intensive care unit for the management of seriously ill patients, like those with septic shock.

- The Clinical Haematology and Microscopy Lab, the Clinical Biochemistry Lab, and the Clinical Microbiology and Immunology Lab under the Clinical and Diagnostic Services, Office of Executive Director support clinical research activities beside performing routine diagnosis. We have the facility for sample processing and storage. We have refrigerator (4°C) and deep freezer ((-20°C and- 80°C) and backup generator for 24 hours supply.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

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List of Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
AS	Active Substance
AWD	Acute Watery Diarrhea
BMP	Basic Metabolic Panel
Caco-2	Heterogeneous human epithelial colorectal adenocarcinoma cell line
cAMP	Cylic adenosin monophosphate
Ca2+	Calcium ion
CBC	Complete Blood Count
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
cGMP	Cyclic Guanosine Monophosphate
CFR	Code of Federal Regulations
CICC	Calcium-induced chloride channel
Cl-	Chloride ion
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CTC	Cholera Treatment Centres
CTx	cholera toxin
CV	Cardiovascular
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EM	Electron Microscopy
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDC	Fixed-Dose Combination
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GRS	Generally Recognized as Safe
GI	Gastrointestinal
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICD	International Classification of Disease
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption

IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
Kg	Kilogram
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
ml	Milliliter
MOP	Manual of Procedures
MQAE	N-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide
MSDS	Material Safety Data Sheet
NDC	National Drug Code
Na+	Sodium ion
NCT	National Clinical Trial
NHE3	Sodium-hydrogen exchanger 3
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
ORS	Oral Rehydration Salt
ORT	Oral Rehydration Therapy
PBS	Phosphate buffered saline
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RCT	Randomized Controlled Trial
RDT	Rapid Detection Test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
WT	Wild type
WHO	World Health Organization

Appendix I

Schedule of activities

Procedures	Screening Hour -4	Day1 Hour 0:00	Day 1 Time +5:00 hr	Day 1 Time+1 0:00 hr	Day 1 Ho_Time +15:00 hr	Day 2 Hour 0:00	Day 2 Time +5:00 hr	Day 2 Time +10:00 hr	Day 2 Time +15:00 hr	Day 3 Hour 0:00	Day 3 Time +5:00 hr	Day 3 Time +10:00 hr	Day 3 Time +15:00 hr	End of treatment
Informed consent	X													
Demographics	X													
Medical history	X													
Pregnancy screening	X													
Randomization and dispensation of numbered IP	X													
Concomitant medication review	X	X				X				X				
Physical exam (including Height and weight)	X	X				X				X				X
Vital signs BP, pulse, temperature	X	X				X				X				X
Blood collection for CBC	X	X				X				X				X
Blood collection for BMP	X					X								X
Assessment of stool output	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of vomiting	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of rehydration	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of dehydration	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of IV fluid infused	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of water intake	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of ORS intake	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of urine output	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Collection of stool samples for V. Cholerae confirmation	X													X
~~Stool collection for electrolytes~~	~~X~~					~~X~~								~~X~~
Administer study intervention		X	X	X	X	X	X	X	X	X	X	X	X	
Initiate rehydration and/or antibiotics treatment		X	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X
Complete Case Report Forms (CRFs)	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Appendix II : ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS – DEFINITIONS, SEVERITY, AND CASUALTY

Definitions

Adverse event: An untoward medical occurrence after exposure to a medicine, which is not necessarily caused by that medicine.

Serious Adverse event: An adverse reaction that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a birth defect

Assessment of severity

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe, or according to a pre-defined grading criterion; the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each adverse event recorded on the CRF.

Serious adverse events are required to be reported by the Investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

The assessing adverse event severity table below will be used for assessing severity for adverse events.

Table -Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living[a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living[b,]
4	Life-threatening consequences or urgent intervention indicated[d]
5	Death related to adverse event[d]

Note:

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Examples of self-care activities of daily living include bathing, dressing and undressing, feeding oneself, using the toilet, and taking medications, as performed by patients who are not bedridden.

[c] If an event is assessed as a "significant medical event," it must be reported as a serious adverse event

[d] Grade 4 and 5 events must be reported as serious adverse events

Assessment of causality

Investigators should use their knowledge of the participant, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an adverse event is considered to be related to the study treatment,

indicating "yes" or "no" accordingly. The following guidance should be taken into consideration:

- Temporal relationship of event onset to the initiation of study treatment.
- Course of the event, considering especially the effects of dose reduction, discontinuation of study treatment, or reintroduction of study treatment
- Known association of the event with the study treatment or with similar treatments.
- Known association of the event with the disease under study.
- Presence of risk factors in the participant or use of concomitant medications known to increase the occurrence of the event.
- The presence of non-treatment-related factors that are known to be associated with the occurrence of the event.
- For participants receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy.