Uniformed Services University | Infectious Disease Clinical Research Program

IDCRP-115

CORE STATISTICAL ANALYSIS PLAN

IDCRP-115: A Randomized controlled trial evaluating single-dose rifaximin versus azithromycin with loperamide adjunct for treatment of acute watery diarrhea (TrEAT TD 2.0)

NCT05677282

Signatures

The signatures below indicate collaborative team preparation of the Statistical Analysis Plan and approval of the document.

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This Statistical Analysis Plan (SAP) outlines plans for the IDCRP-115 (TrEAT TD 2.0) primary analysis. Plans for additional analyses will be documented in standalone SAPs.

1. Population Characterization

Descriptive statistics will be used to summarize the study population overall, by treatment group, separately for the Adverse Event (AE)

An AE is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the research. An AE is NOT the symptoms and signs related to the target disease of interest, acute watery diarrhea, for which the trial is assessing treatment except for incidents of nausea or vomiting that occur during the 30-minute observation period following antibiotic administration.

Clinical Cure

Clinical cure at each follow-up time point will be defined according to the protocol-specific criteria combining diarrhea resolution and symptom resolution. The exact derivation rules will be documented here in final SAP language. TLUS recovery will be considered achieved if it occurs within the respective 24-hour assessment window and is accompanied by resolution of all TD-associated symptoms that were reported as moderate or greater and with no impact on activities for last 24-hour period.

Composite Pathogen

Composite pathogen positivity will be defined as a positive result from either BioFire or TAC. For STEC, confirmation will require a positive STEC EIA result.

Considerations for Rescue Therapy

It is anticipated that a minority of participants will fail treatment during the 72-hour clinical monitoring period when the primary endpoint for efficacy is being evaluated. In the event this occurs, the study physicians will first determine if the participant's illness continues to require antibiotic treatment. Traveler's diarrhea is a self-limited illness and, as emphasized in the eligibility determination, it is important to determine if antibiotics are still required. Oftentimes, the illness may not have resolved fully but additional antibiotics are not warranted because the illness now falls into the mild severity category and is not impacting the participant's functional status. In contrast, if the participant's illness is worsening or continues to impact functional capacity (as described in the eligibility determination) than antibiotic treatment is warranted. In these cases, the participant's treatment assignment needs to be known (unblinding for only this specific participant) so the study physician can appropriately prescribe rescue therapy with a different antibiotic.

Feeling Well

Feeling well is defined by participant self-report of wellness status at the given time point.

Intent-to-Treat (ITT) and Per-Protocol (PP) populations. Categorical variables will be summarized using frequencies and percentages. Continuous variables will be summarized using median and interquartile

range (Q1–Q3). Group comparisons will be exploratory and will use the chi-square test or Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables, as appropriate.

We will describe baseline comparability of treatment groups, characterize the enrolled population clinically, and context for interpretation of efficacy and safety outcomes will be provided.

1.1 Demographic Characteristics

Demographic characteristics will include study site, age, sex, race, ethnicity, military affiliation, enlisted rank, military service branch and Doxycline malaria prophylaixis use, where available. These characteristics will be summarized overall and by treatment group.

1.2 History of Acute Illness

History of acute illness (Day -4 to Day -1) will include symptom duration, diarrhea status, symptom burden and effect on activity level.

1.3 Symptoms at Clinical Presentation (Day 0 before randomization)

Symptoms at clinical presentation captures symptoms on Day 0 prior to study treatment and will include diarrheal stool burden, symptom timing relative to enrollment, impact on daily activity, and illness severity as defined in APPENDIX 2. TD Disease Severity Score.

1.4 Pre-Randomization Antidiarrheal Use

Anti-diarrheal medication used prior to study drug administration for Day -4 to Day -1 and the among of dose will be summarized overall and by treatment group.

2. Primary Clinical Endpoint (Treatment Efficacy)

The primary efficacy objective is to assess whether rifaximin plus loperamide is non-inferior to azithromycin plus loperamide in the treatment of moderate to severe acute watery diarrhea among ambulatory participants.

2.1 Primary Endpoint Definition

The primary clinical endpoint is Time to Last Unformed Stool (TLUS), defined as the time interval in hours from study drug administration to the last unformed/diarrheal/loose/liquid stool meeting the Traveler's Diarrhea (TD) definition prior to recovery. A detailed derivation of TLUS is provided in APPENDIX 1. Key Definitions.

2.2 Primary Analysis

TLUS will be summarized using Kaplan–Meier methods by treatment group. Median time to event, survival estimates at selected time points, and associated confidence intervals will be presented. Treatment groups will be compared using the log-rank test.

The primary inferential analysis will use a one-sided Cox proportional hazards model with treatment group as the primary explanatory variable. Non-inferiority of rifaximin relative to azithromycin will be assessed using a prespecified hazard ratio margin of 1.53. Hazard ratios, confidence intervals, and p-values will be reported.

Because TLUS represents time to clinical recovery, a hazard ratio greater than 1 corresponds to faster recovery in the rifaximin group relative to azithromycin, while a hazard ratio less than 1 corresponds to slower recovery.

3. Safety

Safety analyses will summarize both immediate post-treatment tolerability and reported adverse events during follow-up.

3.1 Post Administration 30-Minute Observation

Participants will be observed for approximately 30 minutes following study treatment administration. Safety findings during this interval will focus on the occurrence of new onset nausea and new onset vomiting after treatment administration.

The proportion of participants experiencing nausea and/or vomiting during this interval will be summarized overall, by treatment group, and stratified by pre-existing versus new onset nausea and vomiting.

3.2 Adverse Events Outside of 30-Minute Post Administration Observation

All reported adverse events (AEs) and serious adverse events (SAEs) excluding nausea and vomiting during the 30-minute post administration observation, if any, will be summarized by treatment group. Frequency and percentage tables will be provided overall and by severity and relationship to study treatment when available.

3.3 Considerations for Rescue Therapy

It is expected that a minority of participants may experience treatment failure during the 72-hour clinical observation period for evaluation of the primary efficacy endpoint.

4. Microbiological Etiology

Pathogen etiology will be assessed using the BioFire (with fresh stool specimen), Enzyme Immunoassay (EIA) and TaqMan Array Card (TAC) (with OMNIgene GI stool collection tubes). The distribution of detected pathogens will be summarized by laboratory method and treatment group. The detection of positive pathogens will be evaluated at enrollment, day 7 and day 21 (TAC only).

4.1 Enrollment

Baseline pathogen detection results will be summarized by laboratory method, and treatment group. Detected pathogens will be described using frequencies and percentages.

Agreement between pathogen detection results obtained from BioFire and TAC will be evaluated using Cohen’s kappa coefficient.

4.2 Follow-Up

Pathogen detection at follow-up time points (e.g., day 7 and day 21, if available) will be summarized descriptively. Persistence, clearance, or recurrence of pathogen detection over time will be described by treatment group and laboratory method.

5. Secondary Clinical Endpoints

Secondary clinical endpoints are intended to complement the primary TLUS analysis by evaluating recovery at fixed assessment windows.

5.1 Clinical Cure

Clinical cure at 24, 48, and 72 hours will be summarized by treatment group and site. Clinical Cure is defined according to the protocol-specific rule set described in APPENDIX 1. Key Definitions. In general, clinical cure requires resolution of diarrhea within the respective time window together with resolution of associated symptoms meeting the predefined criteria.

For each time point, the proportion of subjects meeting the cure definition and corresponding two-sided 95% confidence intervals will be presented. Exploratory between-group comparisons will be conducted using chi-square or Fisher's exact test.

5.2 Return to Normal Activities

The proportion of participants reporting Return to Normal Activities at 24, 48, and 72 hours will be summarized by treatment group and site, with corresponding two-sided 95% confidence intervals. This endpoint is intended to reflect functional recovery.

5.3 Feeling Well

The proportion of participants reporting Feeling Well at 24, 48, and 72 hours will be summarized similarly, with corresponding two-sided 95% confidence intervals. This endpoint reflects the participant's self-perceived wellness status and may differ from formal clinical cure or return to normal activities.

6. Multivariate Analysis

Multivariable analyses will be conducted to evaluate factors associated with the primary and secondary clinical outcomes as well as to assess whether treatment effects remain consistent after adjustment for baseline prognostic factors.

6.1 Stratified Analysis: Pre-Randomization Anti-Diarrheal Use

Anti-diarrheal medication used prior to randomization may alter baseline symptom burden and the subsequent TLUS recovery trajectory, analyses will be stratified by pre-randomization anti-diarrheal use (yes/no). Within each stratum, treatment effects on TLUS will be summarized.

6.2 Univariate Analysis of Selected Factors

A series of univariate analyses will be conducted to assess the association of selected baseline factors with TLUS and the secondary outcomes.

Candidate factors may include demographic characteristics, history of acute illness, baseline symptoms, composite pathogen detection, the dose of post-randomization anti-diarrheal use, baseline activity level, and baseline illness severity as measured by the TD disease severity score. Anti-diarrheal medication used prior to randomization will also be evaluated using an interaction model to provide a more robust analysis.

6.3 Cox Proportional Hazards Models for TLUS

The primary endpoint TLUS will be further analyzed using Cox proportional hazards regression models with treatment group as the primary explanatory variable and selected baseline factors identified from univariate analyses included as covariates.

The baseline TD disease severity score will be considered a prognostic predictor and may be included as an adjustment variable. Hazard ratios, 95% confidence intervals, and p-values will be reported. Proportional hazards assumptions will be assessed using standard diagnostic procedures where appropriate.

6.4 Logistic Regression Models for Secondary Outcomes

Binary secondary outcomes, including clinical cure and/or return to normal activities, feeling well at 24, 48, and 72 hours, will be analyzed using logistic regression models with treatment group as the primary explanatory variable and baseline covariates based on the univariate analysis selected may include demographic characteristics, history of acute illness, pre-randomization anti-diarrheal use, pre-randomization anti-diarrheal use interaction, baseline symptoms, composite pathogen detection, the dose of post-randomization anti-diarrheal use, baseline activity level, and baseline illness severity as measured by the TD disease severity score as adjustment factors.

7. Overall Assessment

This section will summarize integrated and exploratory analyses intended to help describe subject-level recovery patterns and factors associated with overall improvement.

7.1 Heat Map of Treatment Outcomes

A heatmap will be developed as a comprehensive visual summary of subject-level clinical response patterns. This figure will integrate selected baseline and follow-up endpoints, including baseline severity, TLUS, cure, wellness, return to normal activities, site, and activity level at enrollment.

For visualization purposes only, selected continuous variables may be normalized to a relative min–max percentage scale within site. Binary endpoints may be displayed on the same scale using 0 and 1 coding to support integrated visual comparison. These transformations are intended solely for visualization and do not replace the original-scale analyses described elsewhere in the SAP.

Rows (subjects) may be reordered using hierarchical clustering so that subjects with similar response patterns appear near one another. This reordering is intended to improve visualization of site-specific and subject-level patterns and does not alter the underlying data or analysis results.

The heatmap will be used descriptively to explore patterns across sites, relationships between baseline severity and outcomes, consistency of recovery profiles, and identification of subjects with atypical or discordant response trajectories.

7.2 Factors Impacting Ability to Return to Work / Normal Activities

Exploratory analyses will be conducted to evaluate which signs and symptoms may most strongly affect a subject's ability to return to normal activities. These analyses are intended to distinguish functional recovery from other forms of clinical recovery such as feeling well or formal cure.

Symptom prevalence at 24, 48, and 72 hours will be summarized within cohorts defined by feeling well and return to normal activities. Comparative visual summaries, including symptom-profile plots, may be used to identify symptoms that appear to distinguish subjects who feel well from those who have resumed normal activities. Particular attention will be given to persistent symptoms such as fatigue, cramping, diarrhea, gas, urgency, and related functional limitations.

To support interpretation of symptom patterns associated with functional recovery, exploratory visualizations will be developed comparing the prevalence of selected symptoms among subjects who report feeling well versus those who report return to normal activities at 24, 48, and 72 hours. For each time point, the percentage of subjects reporting each symptom within each cohort will be calculated and displayed using symptom-profile plots (e.g., radar/spider plots) and other comparative visual summaries as appropriate. These figures are intended to highlight which residual symptoms may distinguish subjects who feel well from those who have resumed normal activities.

7.3 Forest Plots

Forest plots may be used to summarize selected treatment effect estimates across sites, baseline severity strata, dose of post-randomization anti-diarrheal use, composite pathogen groups, and other prespecified subgroups. These displays are exploratory and are intended to support interpretation of consistency and heterogeneity of effect estimates.

APPENDIX 1. Key Definitions

Adverse Event (AE)

An AE is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the research. An AE is NOT the symptoms and signs related to the target disease of interest, acute watery diarrhea, for which the trial is assessing treatment except for incidents of nausea or vomiting that occur during the 30-minute observation period following antibiotic administration.

Clinical Cure

Clinical cure at each follow-up time point will be defined according to the protocol-specific criteria combining diarrhea resolution and symptom resolution. The exact derivation rules will be documented here in final SAP language. TLUS recovery will be considered achieved if it occurs within the respective 24-hour assessment window and is accompanied by resolution of all TD-associated symptoms that were reported as moderate or greater and with no impact on activities for last 24-hour period.

Composite Pathogen

Composite pathogen positivity will be defined as a positive result from either BioFire or TAC. For STEC, confirmation will require a positive STEC EIA result.

Considerations for Rescue Therapy

It is anticipated that a minority of participants will fail treatment during the 72-hour clinical monitoring period when the primary endpoint for efficacy is being evaluated. In the event this occurs, the study physicians will first determine if the participant's illness continues to require antibiotic treatment. Traveler's diarrhea is a self-limited illness and, as emphasized in the eligibility determination, it is important to determine if antibiotics are still required. Oftentimes, the illness may not have resolved fully but additional antibiotics are not warranted because the illness now falls into the mild severity category and is not impacting the participant's functional status. In contrast, if the participant's illness is worsening or continues to impact functional capacity (as described in the eligibility determination) than antibiotic treatment is warranted. In these cases, the participant's treatment assignment needs to be known (unblinding for only this specific participant) so the study physician can appropriately prescribe rescue therapy with a different antibiotic.

Feeling Well

Feeling well is defined by participant self-report of wellness status at the given time point.

Intent-to-Treat (ITT)

The ITT population will include all participants who received study treatment.

Per-Protocol (PP)

The PP population will include participants who complete the 72-hour follow-up and do not have major protocol deviations affecting evaluability.

Return to Normal Activities

Return to normal activities is defined by participant report of no ongoing impact on usual activity level at the given time point.

Time to Last Unformed Stool (TLUS)

TLUS is defined as the time interval in hours beginning with study treatment administration and ending with the last unformed/diarrheal/loose/liquid stool meeting the TD definition prior to recovery.

Traveler's Diarrhea (TD)

Traveler's diarrhea is defined as three or more grade 3–5 stools within a 24-hour period (±30 minutes), or two grade 3–5 stools within a 24-hour period plus one or more TD-associated symptoms in the same 24-hour period, including abdominal pain/cramps, nausea, vomiting, fever, tenesmus, or gross blood in stool.

APPENDIX 2. TD Disease Severity Score

Overview

A modified TD disease severity score will be used to summarize illness burden at baseline and at 24, 48, and 72 hours. The score combines three domains: objective signs, subjective symptoms, and loose stool output.

Each domain contributes a score from 0 to 3, yielding a total score range of 0 to 9, where higher values indicate greater illness severity.

Calculation Formula

TD Disease Severity Score = Objective Signs Score + Subjective Symptoms Score + Loose Stool Output Score

Objective Signs Score (0–3)

The objective signs component is derived from vomiting and fever severity in the applicable time window. Where multiple objective signs are present, the maximum applicable score within the domain is assigned.

Subjective Symptoms Score (0–3)

The subjective symptom component is derived from symptoms including tenesmus, malaise, nausea, faecal incontinence, and abdominal cramps. Where multiple symptoms are present, the maximum applicable score within the domain is assigned.

Loose Stool Output Score (0–3)

Loose stool burden is scored according to the maximum stool output observed during the relevant time window: ≥8 stools = 3; 5–7 stools = 2; 2–4 stools = 1; 0–1 stools = 0.

Time Points

The score will be calculated at pre-randomization baseline, 24 hours, 48 hours, and 72 hours.

Baseline Pre-Randomization Severity Window

To standardize baseline illness severity at enrollment, each subject's pre-randomization illness burden will be summarized over a 24-hour observation window preceding randomization. This window is anchored to the exact enrollment time and uses 6-hour intervals to ensure that each subject contributes a complete and comparable 24-hour history immediately before treatment administration.

Because enrollment can occur at different times of day, the selected intervals may span two calendar dates. This approach is intended to preserve alignment with the acute clinical state at enrollment while supporting comparability across subjects.

Appendix Table. TD Disease Severity Score Framework

Modified		
Parameter	Outcome	score
Objective signs at Each Time Point	Severe vomiting	3
	Moderate vomiting	2
	Mild vomiting	1
	No objective symptoms	0
Subjective symptoms at Each Time Point	Moderate or severe fever	3
	Severe tenesmus, malaise, nausea, faecal incontinence	3
	Moderate tenesmus	3
	Severe abdominal cramps	2
	Moderate nausea	2
	Mild tenesmus	2
	Moderate abdominal cramps, faecal incontinence, malaise	1
	Mild abdominal cramps, nausea, malaise, faecal incontinence, fever	1
	No subjective symptoms	0
Each Time Point Loose stool output (max.24 hr)	≥8	3
	5-7	2
	2-4	1
	0-1	0

We refer to the original approach published in (ref. Maier, 2021)

APPENDIX 3. Heatmap Definition and Interpretation

The heatmap is an integrated, exploratory visual summary of subject-level clinical response patterns. Each row represents a participant and each column represents a selected endpoint or severity measure. Rows may be grouped or split by site, and row order may be defined using hierarchical clustering based on included outcome variables.

For visualization purposes, selected continuous variables may be normalized to a min–max percentage scale within site. This normalization reflects relative ranking within each site and is intended to improve pattern recognition across subjects and endpoints. Binary outcomes may be displayed using 0 and 1 coding on the same color scale for visual continuity.

The heatmap is intended to help identify site-specific response heterogeneity, associations between baseline severity and outcomes, consistency or inconsistency across endpoints, and atypical subject-level response profiles. This figure is exploratory and does not replace formal statistical inference.

APPENDIX 4. Exploratory Symptom Factors Affecting Return to Normal Activities

To explore symptom domains associated with functional recovery, symptom prevalence at 24, 48, and 72 hours will be summarized separately among subjects who report feeling well and return to normal activities.

These summaries will be presented using percentages of subjects reporting each symptom within each cohort at each time point. Comparative visual displays in terms of radar plot may be used to identify symptoms that appear to distinguish the two groups, with particular focus on fatigue, cramping, diarrhea, gas, urgency, and related symptoms that may continue to impair activity even when subjects otherwise report feeling well.

These analyses are exploratory and intended to improve clinical interpretation of functional recovery beyond formal cure definitions.