Statistical Analysis Plan

Bedaquiline Enhanced Post ExpOsure Prophylaxis for Leprosy (BE-PEOPLE) – PHASE 2

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Table of Contents

1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main, pre-planned analyses for the phase 2 study " Bedaquiline Enhanced Post ExpOsure Prophylaxis for Leprosy (BE-PEOPLE)". The purpose of this phase 2 study is to determine the safety of the combination of the trial regimen i.e. bedaquiline and rifampicin (BE-PEP) in healthy volunteers in Comoros. The study conduct is described in the study protocol (version 2.1 finalized on 17th March 2022) and in clinicaltrials.gov (registration to be completed).

These planned analyses will be performed by the statistician(s) at the Clinical Trials Unit of the Institute of Tropical Medicine (Antwerp) in collaboration with the research consortium. The analysis results will be described in a statistical analysis report, to be used as the basis of the main research publications according to the study publication plan. This document describes statistical methods for the primary and secondary safety outcomes of the phase 2 study as defined by the protocol. Additional analyses may be performed but are not covered by the current analysis plan. Statistical methods for these additional analyses will be described together with their respective results.

At the end of the study, all data points will be locked, analyzed and results may be submitted for publication. This analysis plan will be finalized and approved before the database lock for the phase 2 analysis. Major changes in statistical methodology used for the main and pre-planned analyses from this SAP, will require detailed description and justification in the statistical analysis report. The final analysis datasets, programs, and outputs are archived following good clinical practice guidelines (ICH E9).

2. Study design and objectives

2.1. Study design

This is a single-site, randomized, non-inferiority, phase 2 clinical trial, comparing the safety between a single dose rifampicin (SDR-PEP) and single dose bedaquiline plus rifampicin (BE-PEP) among contacts of recent leprosy cases. Safety will be closely monitored and evaluated by an independent data and safety monitoring board (DSMB). The study setting is the village of Gégé, Anjouan, that is part of arm 1 of the PEOPLE trial in which 8 new cases have been diagnosed since 2019 but no PEP has been provided.

During the final survey round of the PEOPLE study, all individuals in the village aged 5 years or above, weighing 20 kg or above and not currently suffering from leprosy will be enumerated and screened for the study inclusion and exclusion criteria (see 5Description of analysis populations Description of analysis populations for more details).
Three hundred of the eligible individuals from the village will be stratified proportionally over three age-groups (18 years and above, 13-17 years, and 5-12 years) and will be randomized (1:1) into one of the two study arms:

- Arm 1: single dose of bedaquiline plus rifampicin (BE-PEP) (n=150)
- Arm 2: single dose of rifampicin (SDR-PEP) (n = 150)

Randomization will occur sequentially, first enrolling persons above 18 years of age. Provided no concerning adverse events are observed, the randomization and enrollment will continue for the 13-17 years old age category. Again if no concerning adverse events are observed, the enrollment will proceed for children aged 5-12 years old. The remainder of the population of this village aged 2 years and above will be offered single dose rifampicin as per WHO recommendations.

All randomized participants will be followed up closely with active monitoring for adverse events, including measurement of the corrected QT interval and liver function, before and after administration, as well as any other adverse events, including but not limited to gastro-intestinal (nausea, vomiting), nervous system-related (headache, dizziness) and cutaneous reactions.

2.2. Study objectives

2.2.1. Primary objectives

To confirm overall safety of the study regimens, single dose bedaquiline plus rifampicin and single dose rifampicin, in a field setting in Comoros.

2.2.2. Secondary objectives

- To determine baseline liver function (frequency of ALT and AST elevations) and cardiac abnormalities (QTc prolongations) in the population.
- To document for each of the two regimens post administration QTc levels, ALT and AST results.
- To document for each of the two regimens any potentially frequent adverse events, such as gastro-intestinal (nausea, vomiting), nervous system-related (headache, dizziness) and cutaneous reactions.

2.3. Study hypothesis

It is hypothesized that BE-PEP is not inferior the SDR-PEP in terms of safety.

3. Study outcomes and variables of interest

3.1. Study outcomes

3.1.1. Primary outcomes

- Mean difference in QTc interval between the two arms 24 hours after treatment administration.

- Predetermined study stopping criteria, which will trigger an immediate pause on enrollment:

1. Death of a participant considered related to study drug
2. One or more participants experience an SAE or Gr 4 AE or a persistent (upon repeat testing) Gr 4 laboratory abnormality that is determined to be related to study drug
3. Three or more participants experience a Gr 3 or greater AE of the same type (as per medical judgement) that is determined to be related to study drug
4. Three or more participants experience a persistent (upon repeat testing) Gr 3 laboratory abnormality related to the same laboratory parameter and considered to be related to study drug
5. Two or more participants experience QTc > 500 ms
6. One or more participants has AST or ALT > 8x ULN, in absence of causative explanation

3.1.2. Secondary outcomes

- Baseline ALT and AST results
- Baseline QTc prolongations
- Post administration QTc prolongation, ALT and AST results
- Frequency of potentially common adverse events such as gastro-intestinal (nausea, vomiting), nervous system-related (headache, dizziness) and cutaneous reactions
- Occurrence of any (serious)AEs

3.2. Definitions

QT interval: The measured QT will be corrected by the Fridericia formula through a dedicated calculator. At baseline, QTc prolongations are defined as QTc >450ms. Participants with a QTc value above the cut-off point will be excluded from the study. Their QTc measurement will be entered in the database and will be used in the analysis to describe the baseline QTc distribution in the population.

Liver function measurements: ALT and AST (U/L) will be measured at baseline (day 0) and on day14. In both time points, participants with ALT and/or AST >3x upper limit normal (ULN) will be considered to have abnormal (elevated) values. If a participant has an elevated ALT/AST result at recruitment, they will be excluded from the study. The ULN values for ALT and AST used for this study are 55 U/L and 48 U/L respectively (https://www.mayoclinic.org/tests-procedures/liver-function-tests/about/pac-20394595#:~:text=Normal%20blood%20test%20results%20for,8%20to%2048%20U%2FL). Potassium and magnesium levels will only be recorded in case of QTc prolongation, ≥ 500 ms at baseline or > 60 ms increase as compared with baseline. Analysis of this data (potassium and magnesium results) will be purely descriptive.

Adverse events' categories and how they will be summarized are further described in section 6.3.

4. Description of study population

4.1. Participant accounting

The number of participants screened, those who meet the study inclusion criteria and enrolled (randomized) or excluded will be summarized according to reason for exclusion. Of the enrollees, the number of participants discontinued or lost to follow-up will be recorded by reason and time of discontinuation. Additionally, the number and proportion of participants who were excluded at baseline because of a QTc > 450 ms, as well as the number of exclusions due to elevated AST or ALT will be described by treatment arm and age stratum. These figures will be summarized in a CONSORT flow diagram. A table summarizing the enrolment process by treatment arm will also be produced.

4.2. Description of study population

Participants in each treatment group and overall will be described with respect to selected baseline characteristics, both by age strata and by allocation arm. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical

characteristics. Differences between the two arms will be noted, but no formal statistical testing will be performed.

5. Description of analysis populations

5.1. Analysis populations

For the safety analysis, both an intention-to-treat (ITT) and a per-protocol (PP) approach will be adopted. In both ITT and PP populations, participants will be analyzed according to their administered treatment. The PP population will be the primary analysis population as indicated for non-inferiority studies by the ICH-E9 guidelines.

5.1.1. Intention to treat (ITT) analysis

In the Intention-to-Treat analysis, all participants will be analyzed according to their administered allocation, even in case they show protocol violations prior to or during the study.

5.1.2. Per protocol (PP) analysis

An analysis based on a "per protocol" approach will be conducted to assess the non-inferiority of treatments, as recommended for non-inferiority studies. Thus, analyses based on the subjects following the study protocol will be the main strategy of analysis adopted for the primary and secondary endpoints. In the per-protocol analysis only participants who received PEP as planned, have completed follow-up, and follow the protocol as planned are included.

Table 1: The protocol deviations classified as minor or major deviation

Protocol Violation	Major/Minor Deviation	Comments
Inclusion criteria		
1. Being a permanent resident of the study village, in good state of health	Major	
2. Able and willing to provide informed consent	Major	
3. Age 5 years or above and weight of 20 kg or above.	Major	
Exclusion criteria		
1. Signs of active leprosy	Major	
2. Signs of active pulmonary tuberculosis (cough ≥2 weeks duration)	Major	
3. Signs of active extra-pulmonary tuberculosis (bluish-red nodules that cover the lymph nodes, bones or joints, or cervical glands with discharge)	Major	
4. History of liver- or kidney disease	Major	
5. Allergy to rifampicin or bedaquiline	Major	
6. Having received rifampicin or bedaquiline (if applicable) in the last 2-year period	Major	
7. Not able to swallow bedaquiline 100 mg tablets	Major	

8. Self-reported (suspected) pregnancy or breastfeeding	Major	
9. Concurrent (within the last three-week period before D0) use of medications not included in the safe list (for bedaquiline only)	Major	
10 .QT-prolongation of ≥450 msec in baseline ECG within the last week.	Major	
11. Jaundice or self-reported liver function abnormalities or hepatitis	Major	
12. Value of baseline ALT or AST >3x ULN within the last week. In case only ALT is available, this would suffice for enrollment.	Major	
Treatment deviations		
1. Vomiting of allocated treatment	Major	
2. Dosage miscalculation	Minor/Major	Minor if less dose is given, major if larger dose is given
Follow-up deviations		
1. Missing visit or samples of QTc at 24h or ALT/AST at day 14	Minor/Major	Minor if a participant gives ALT/AST until the day 30 visit

6. Statistical Methods

6.1. Primary safety analysis

The main objective about the difference in QTc between the two arms 24 hours after treatment administration will be assessed by calculating the 95% confidence interval for the difference.

Non-inferiority is established if the upper limit of a one-sided 95% confidence interval around the difference in means QTc 24 hours after treatment administration (BE-PEP - SDR-PEP) is below the non-inferiority limit of +10 ms.

Analysis of covariance (ANCOVA) or an equivalent regression model correcting for the baseline values of QTc will also be used to assess the primary objective. Alternatively, the individual difference scores will be calculated by subtracting the QTc values at baseline from the ones at day 1. The difference scores will be compared using a two-sample t-test.

6.2. Secondary safety analysis

Baseline measurements
Data from all screened patients with QTc, ALT or AST results will be used to determine the baseline distribution of the underlying population. Data will be described both visually and by using standard summary statistics for the whole population and by demographic characteristics, such as age (same age categories as the ones used for stratified randomization) and sex. The proportion of study participants with ALT/AST values above the normal range (> 3 ULN) will be calculated together with 95% Wilson confidence intervals. The proportion of study participants with QTc values above the normal range (> 450m) will be calculated together with 95% Wilson confidence intervals.

Post PEP measurements

ALT/AST results after study drug administration (day 14 and 30) will be described both numerically and as proportions above the normal range (> 3 ULN) using 95% Wilson confidence intervals pooled and by study arm. Chi-square or Fisher's exact test will be used to compare the proportions between the two study arms. The difference of the days 14 and 30 ALT/AST result with the results at baseline will be tabulated broken into categories (<0, 0-9, 10-19, etc).

The individual differences between baseline QTc and ALT/AST results and results from day 1 (QTc) or day 14 (ALT/AST) will be calculated. The difference will be described in 20ms categories (<0, 0-19, 20-39, 40-59 etc.) for QTc and in 10 U/L categories (<0, 0-9, 10-19, etc) for ALT and AST. Analysis of covariance (ANCOVA) or an equivalent regression model correcting for the baseline values of ALT/AST will be used similar to the methodology described for the primary objective. Alternatively, the individual difference scores will be calculated by subtracting the ALT/AST values at baseline from the ones at day 14 or 30. The difference scores will be compared using a two-sample t-test.

6.3. General safety aspects

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MEDDRA) and will be reported based on MEDDRA preferred terms and body systems. All AEs will be analyzed based on counts of subjects with a specific category and not on counts of individual adverse events. The relationship between AEs and treatment is determined by the investigator and categorized as "drug-related" if possibly, probably or definitely related to treatment. A general summary of safety events will be presented by treatment arm. Potentially frequent adverse events such as gastro-intestinal (nausea, vomiting), nervous system-related (headache, dizziness) and cutaneous reactions will be tabulated and presented using standard descriptive statistics (counts, percentages and 95% Wilson confidence intervals). AEs related to liver function, such as hepatitis, liver function elevation, transaminitis, liver toxicity, etc, will be grouped into one category. Fisher's exact test will be used to compare the association between the observed frequencies between the two treatment arms. A second table containing each reported AE by preferred terms and body system will be included in the analysis. All safety events will be presented both pooled and by time point (day 0, day 1, day 14 and day 30).

6.4. Interim analysis

An interim analysis will take place after the completion of recruitment of each age stratum. Data will be cleaned and locked before each interim analysis. The data will include any results until day 30.

6.5. Other aspects

6.5.1. Subgroup analyses

Since this is a stratified randomized trial, all analyses will be done by age-group.

6.5.2. Multiplicity adjustment

No adjustments for multiplicity are foreseen.

6.5.3. Missing data and sensitivity analysis

Missing data will be reported, though all specified analyses are complete case analyses. If a significant proportion of missing values is observed, then imputation methodology will be considered in a sensitivity analysis, after communication with the research consortium.

6.5.4. Exploratory analysis

The QTc prolongation pre- and post-PEP among participants who report diarrhea as an AE will be examined as an exploratory objective. Any surplus remaining serum may be used for exploratory analyses of leprosy related biomarkers at the LUMC. These analyses are not covered in the current SAP and are not a part of the main clinical trial.