TB Alliance

MRC Clinical Trials Unit Smarter Studies Global Impact Better Health

UCL

BPaMZ/SEM

Protocol Title

An Open-Label Phase 2 Trial to Evaluate the Male Reproductive Safety of a 6-month Combination Treatment for Pulmonary Tuberculosis of Bedaquiline plus Pretomanid plus Moxifloxacin plus Pyrazinamide (BPaMZ) in Adult Male Participants with Drug Resistant (DR-TB) Pulmonary Tuberculosis

Statistical Analysis Plan

Version 2.0 Dated 19 April 2023

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Author name:	Lindsay Thompson
Author position:	Statistician, UCL
Author signature and date:	DocuSigned by: L Thompson Signer Name: Lindsay Thompson Signing Reason: I am the author of this document Signing Time: April 19, 2023 | 11:03 AM EDT D57F305FCC3C498FA7C2CEF99BD708DF
Approval name:	Paul Bruinenberg
Approval position:	Study Physician, TB Alliance
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Contents Page

Version History:

Version Number/Date	Change
1.0 / 15 Feb 2021	Initial version
1.1 / 20 Dec 2022	Updates made to outcome analysis and circulated for comment
1.2 / 30 Jan 2023	Updates made to Statistical analysis and circulated for further comments
1.3/ 06 Feb 2023	Updates made to outcome sections to align more with the protocol Additional analysis details added
1.4/ 02 Mar 2023	Updates made post expert review
1.5/ 14 Mar 2023	Further updates made after expert review
1.6/ 17 April 2023	Updates made to section on pharmacokinetics

1. Introduction

This document outlines the statistical analysis for the BPaMZ/SEM trial, Protocol Version 4.0, dated 07 April 2022.

Pretomanid is being used in an antimicrobial combination regimen(s) to treat patients with pulmonary tuberculosis. The primary purpose of this trial "BPaMZ/SEM" is to evaluate the potential effect of pretomanid on human testicular function whilst being used in a 26-week antimicrobial combination regimen consisting of bedaquiline (B) plus pretomanid (Pa) plus moxifloxacin (M) and pyrazinamide (Z) (BPaMZ).

All participants will receive the same regimen of BPaMZ once daily for 26 weeks.

The participant population will be patients with drug resistant TB (DR-TB) defined as Mycobacterium tuberculosis (MTB) that is sensitive to fluoroquinolones but

1. Mono-resistant to rifampicin or isoniazid, OR
2. Resistant to both rifampicin and isoniazid (MDR-TB)

2. Objectives

The primary objective is to assess the male reproductive safety of pretomanid in the regimen (BPaMZ) of bedaquiline 200mg (200mg daily for 8 weeks then 100 mg daily for 18 weeks), together with pretomanid 200 mg (1x daily) + moxifloxacin 400 mg (1x daily) + pyrazinamide 1500 mg (1 x daily) for 26 weeks in participants with Drug-resistant (DR) pulmonary TB.

The secondary objective is to evaluate the efficacy, safety and tolerability after 26 weeks of active treatment for TB and follow up for 52 weeks after start of the above described treatment regimen in participants with Drug-resistant (DR) pulmonary TB.

3. Sample Size

A total of 20 evaluable male participants diagnosed with DR-TB who completed the trial will be required.

Twenty-five participants (25) will be enrolled in the trial assuming a 20% participant dropout of the trial. If the dropout rate is higher than 20% before finishing 26 weeks of treatment, the enrolment of additional participants may be considered to assure that 20 complete.

The sample size is not based on formal statistical considerations. It is based on practicality of enrolment and to rule out any severe effect of the regimen on sperm production.

4. Primary Outcome

The primary outcome (endpoint) will be change from baseline in total sperm count at 26 weeks of therapy.

5. Secondary male reproductive toxicity outcomes

1. Change from baseline in all semen parameters (semen count and semen concentration) at 13,

26 and 44 weeks.

2. The proportion of subjects experiencing an increase or decrease in sperm count and sperm concentration from baseline to the timepoint of interest (26 weeks and 44 weeks) will be calculated.

3. The proportion of subjects experiencing at least a 50 percent increase or decrease in sperm count and sperm concentration from baseline to the timepoint of interest (26 weeks and 44 weeks) will be calculated.

4. Change from baseline (at screening) in male reproductive hormones at Week 2, 4, 8, 12, 16, 26, 35, 44, 52, 65, 78 and at early withdrawal. Reproductive hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH), Inhibin B, testosterone, and total testosterone

5. Plasma concentration of pretomanid from sparse sampling will be summarised as descriptive statistics.

6. Other secondary outcomes

6.1. TB Efficacy Outcomes

1. Clinical outcome (incidence of bacteriologic failure or relapse or clinical failure) at 78 weeks (52 weeks after end of therapy). Participants will be classified as having a favourable, unfavourable or unassessable outcome status. Patients achieving and maintaining culture negative status without any additional treatment will be considered favourable; participants who fail on treatment (or do not complete treatment for any reason) or relapse in follow-up will be considered unfavourable. Patients completing treatment and clinically well when last seen but lost to follow-up before week 78 will be considered unassessable.

2. Proportion of participants with sputum culture conversion to negative status in liquid culture (MGIT) at Week 4, 8, 12, 16, 22, 26, 35, 44, 52, 65 and 78.

6.2. Safety and tolerability outcomes

1. Change in weight from baseline
2. The incidence of all-cause mortality will be summarised.
3. All adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be presented by Preferred Term within each MedDRA System Organ Class (SOC).
4. Treatment emergent adverse events (TEAEs) are adverse events (AEs) which started or worsened on or after the first administration of BPaMZ up to and including 14 days after the last study drug administration. In the case where it is not possible to define an AE as treatment-emergent or not, the AE will be classified as treatment-emergent.
    a. The incidence of the following events will be summarised for further medical analysis:
        i. Incidence of TEAEs;
        ii. Incidence of TEAEs by Severity;

iii. Incidence of TEAEs by DMID toxicity grade;

iv. Incidence of Drug-Related TEAEs;

v. Incidence of Serious TEAEs;

vi. Incidence of TEAEs Leading to Early Withdrawal;

vii. Incidence of TEAEs leading to Death.

5. Quantitative and qualitative clinical safety laboratory measurements (newly notable grade 3 or 4 laboratory parameters according to DMID grading), including observed and change from baseline.
6. Electrocardiogram (ECG) results (heart rate, RR interval, PR interval, QRS interval, QT interval and QTc interval, including observed QT/QTc intervals and change from baseline) will be categorised.
7. Results of any paternity outcome during or after treatment with pretomanid at early withdrawal and week 78 follow-up will be tabulated.

7. Definitions and Data Handling Issues

7.1. Semen Samples

The semen parameters (semen count and semen concentration) at baseline and during treatment will represent the mean of two semen samples collected a few days apart at each time point.

In the case of one missing sample at a time point the only sample available will represent the value for that timepoint.

Any sample that was retaken within 2 weeks of the planned sample, due to technical/practical issues, will be included.

7.2. Positive and Negative TB Status

7.2.1. Positive culture

Positive culture refers to the culture being positive for *Mycobacterium tuberculosis* (MTB). The MGIT culture results that are positive with contamination will be considered positive. The MGIT culture results that are contaminated or with no result will be treated as missing. Two sputum samples per visit are collected at each visit throughout treatment and follow-up. The culture result for a given visit is established using all samples obtained for that visit. A positive culture takes precedence over a negative or contaminated culture at the same visit and a negative culture takes precedence over a contaminated culture at the same visit.

7.2.2. Isolated Positive Cultures

It is known that occasionally participants produce sputum samples that are “isolated positives,” that is, a positive culture preceded by a series of negative cultures and followed thereafter by at least two negative cultures without an intervening positive result. This phenomenon may be the result of a sealed cavity breaking down or laboratory contamination and does not in itself signify that the participant is relapsing. In the event of a single positive culture result occurring in a participant who has previously been classified as having culture negative status (in the absence of any retreatment), the participant will not be classified as a recurrence unless a second positive culture result is obtained at a separate visit (at least 7 days apart), without an intervening negative culture or unless the

participant is lost to follow up or completes the study (and is unable to be brought back) before two negative cultures are obtained. As there is a higher incidence of isolated positives with liquid culture and sometimes even serial "isolated positives", the clinical condition of the participant will also be considered in deciding whether the participant has an unfavourable outcome and re-treatment is indicated.

For example, if a participant after being culture negative has two positive cultures in a row, but is deemed to be doing well clinically, the investigator may choose to leave the participant untreated on clinical grounds. In such a case, so long as two consecutive negative cultures are eventually obtained in the absence of treatment, the participant will not be classified as an unfavourable outcome (defined in §6.1 above).

7.2.3. Culture negative status

Culture negative status is achieved when a participant produces at least two negative culture results at different visits (at least 7 days apart) without an intervening positive culture result for MTB. The date of the first negative culture (date of collection of culture) of these two is the date at which culture negative status was obtained. Once obtained, culture negative status continues until there are two positive cultures at different visits (at least 7 days apart), without an intervening negative culture, or until there is a single positive culture not followed by two negative cultures. Culture negative status can be achieved at any time during treatment or follow-up but before any re-treatment. Culture negative status can be re-established.

Participants with two contaminated or missing samples at a given visit (from month 2 onwards) will be asked to return to produce two more sputum samples.

7.2.4. Inability to produce sputum

In general, inability to produce sputum is treated as being equivalent to having a negative culture result if no other sputum sample is produced at that visit. This includes the rare situation where a participant who never achieves culture negative status due to the inability to produce sputum, after TB has been confirmed on the applicable baseline sample, completes follow-up without clinical or microbiological evidence of relapse. Such participants will be considered to have a favourable outcome and an inability to produce sputum with no other sputum produced at that visit will be treated as a negative culture result.

7.3. Adequate treatment

The definition of adequate treatment sets a limit for the amount of treatment missed. Participants not taking the adequate amount of treatment by this definition will be excluded from the per protocol analyses (PP and TB-PP).

To meet the definition of adequate treatment participants must have taken at least 146 doses (80%) of their allocated 182 day (26 weeks) treatment regimen within 238 days of starting therapy (i.e. 26 weeks plus an allowable 56 day halt (including a maximum of 35 consecutive days) as per the protocol).

7.4. Major protocol deviations for analysis

A major protocol deviation for analysis is a protocol deviation likely to affect to a significant degree the scientific value of the trial and is CSR reportable. These include:

- participants who did not satisfy entry criteria but were entered anyway
- participants who developed withdrawal criteria during the study but were not withdrawn

- participants who received the wrong treatment or incorrect dose

These participants will be excluded from the PP and TB-PP analyses. A list of all major protocol deviations for analysis will be approved by a review committee prior to database lock.

Note: participants attending a long-term outcome visit outside the specified window will be evaluated and considered for potential major protocol violator. Visits within 2 weeks of the window opening will be considered *minor* protocol violators.

7.5. Trial Timings

Date of enrolment is Study Day 1 which is defined as the date on which a participant is administered the first dose of study medication. Other study days are defined relative to the Study Day 1 with Day 2 being the day after Study Day 1 and Day -1 being the day prior to Study Day 1.

For all safety endpoints, baseline is defined as the last non-missing measurement prior to first dose of study treatment unless otherwise stated.

In all analyses, visit date rather than day or week number will be used to define the timing of events.

Assessments should be collected as follows:

- Within ± 3 days of scheduled visit from Day 1 to Week 8
- Within ± 7 days of scheduled visit from Week 12 to Week 26
- Within ± 14 days of scheduled visit during the follow-up phase at or after Week 26 to Week 78

Unscheduled visits will be slotted into windows as appropriate. Scheduled visits falling outside of the defined protocol visit windows will be put into separate visits so that all data, both collected at scheduled and unscheduled time points, are used.

The ***treatment period*** is defined as a total of 26 weeks from start of therapy.

The ***follow-up period*** is defined as the period after the end of treatment to the end of follow-up.

7.6. General Statistical Considerations for Analysis

There are three planned analyses that will be performed. The first analysis will be done after all participants have completed the 26 weeks of treatment. The analysis will be on primary outcome data (semen data), secondary reproductive data, culture conversion data up to week 26 and safety data. The second analysis will be done at week 44 and will also be on the semen data available at 44 weeks, secondary reproductive data, culture conversion data to week 44 and safety data. The final analysis will take place after 78 weeks and will include analyses on all data including the TB efficacy analysis as outlined in §6.1.

There will be no specific strategy to deal with missing data i.e. it will not be imputed. A complete case analysis will be performed.

For the safety analysis, if there are multiple assessments in a visit, the highest grade non-missing value within a visit will be used in the summaries, however all will be shown in the listings. If numeric

data is beyond range of lab detectability and result is showed as "<XX" or ">XX" then the numeric XX value will be used for summary statistics.

Baseline is defined as last available measurement prior to dosing and post baseline abnormalities are included in the summary if the subject did not meet the abnormality criteria at baseline or toxicity grade at post baseline is higher than that on baseline.

7.7. Newly notable abnormalities

A newly notable laboratory abnormality is defined as an abnormality observed post baseline that meets the notable criteria shown in Table 1 below and that did not exist at baseline. Participants can still meet the criteria for newly notable laboratory event if the baseline value is missing.

Table 1: Notable Criteria for Laboratory Data

Lab Test Type	Laboratory Variable	SI Units
Liver	AST	> 3 x ULN and ≤ 5 x ULN > 5 x ULN and ≤ 8 x ULN > 8 x ULN
	ALT	> 3 x ULN and ≤ 5 x ULN > 5 x ULN and ≤ 8 x ULN > 8 x ULN
	Total Bilirubin	> 2 x ULN
	Alkaline Phosphatase (ALP)	> 2 x ULN
Chemistry Labs	Other: ALT or AST > 3 x ULN and total bilirubin > 2 x ULN ALT or AST > 3 x ULN and total bilirubin > 2 x ULN and ALP <2 x ULN (potential Hy's law case)	
	Lipase	> 2 x ULN to ≤ 5 x ULN > 5 x ULN

8. Analysis Populations and Definitions

8.1. Primary Semen Analysis populations

8.1.1. The intent-to-treat (ITT) population

The primary and secondary semen analyses will be conducted on the ITT population, defined as all participants who at took at least one dose of the study medication. All data available will be considered for each time-point.

8.1.2. The per-protocol (PP) population

The primary and secondary semen analyses will also be conducted on the PP population, defined as all participants who were enrolled and started treatment, excluding:

1. Participants not meeting the definition of having received an adequate amount of treatment.

2. Participants classified as major protocol deviations for analysis (see §7.4).

8.2. TB efficacy analysis populations

The TB efficacy outcomes will be reported by ITT (§8.1.1) and the following populations:

8.2.1. The TB-specific modified intent-to-treat (TB-MITT) population

Defined as all participants who were enrolled and started treatment, excluding those listed below:

1. Any late exclusions
    a. Participants enrolled and later found to be ineligible because of a protocol violation at enrolment (based on data collected prior to enrolment).
    b. Participants without culture confirmation of TB at Day 1 (baseline) (or screening to week 4 if baseline is contaminated or negative)
2. Any participants who completed treatment and were later lost to follow-up or withdrawn from the study.
3. Participants who died during treatment from violent or accidental cause.
4. Participants who died during follow-up with no evidence of failure or relapse of TB.
5. Participants who are able to produce sputum at the endpoint visit, but whose endpoint visit sputum samples are all contaminated or missing, who cannot be brought back for repeat cultures, provided their last positive culture was followed by at least two negative cultures. N.B.: This does not apply to participants who are unable to produce sputum at the given endpoint, or to participants who are able to be brought back subsequently and produce negative cultures.

Participants already classified as having an unfavourable outcome will not be excluded.

8.2.2. The TB-specific per-protocol (TB-PP) population

In addition to those excluded from the TB-MITT population (§8.2.1), the following participants will be excluded:

1. Participants lost-to follow-up or withdrawn for reasons other than treatment failure before the end of treatment.
2. Participants whose treatment was modified or extended beyond what was permitted.
3. Participants not meeting the definition of having received an adequate amount of treatment.
4. Participants who classified as major protocol deviations for analysis (see §7.4).

Participants already classified as having an unfavourable outcome will not be excluded.

8.3. The Safety analysis population

The safety and tolerability outcomes will be conducted on the safety population, which in this trial is the same as the ITT population; defined as all participants enrolled who received at least 1 dose of trial treatment.

8.4. Pharmacokinetics (PK) analysis population

Defined as all participants who were enrolled and started treatment, excluding participants who do not have at least one valid PK measurement.

9. Statistical Analysis

All statistical analysis will be based on descriptive statistics for the whole population and by key subgroups.

All statistical analyses tables, listings and figures will be produced using STATA Version 17.0 or higher.

9.1. Primary semen outcome analysis

The change from baseline in total sperm count at 26 weeks will be summarised by mean, standard deviation, median, IQR and range. Both the baseline value and change from baseline will be summarised.

9.2. Secondary outcome analyses

The following applies to outcomes outlined in sections 5 and 6. Continuous outcomes will be summarised by mean, standard deviation, median, interquartile range and range. For change from baseline, both baseline value and change from baseline will be summarised.

Binary and categorical outcomes will be summarised with n's and percentages. Shift tables will be provided to summarise the status changes from baseline to week 13, week 26 and week 44. Each shift table will include shift analyses from within the reference range at baseline to above the reference range at 13, 26 or 44 weeks and from within the reference range at baseline to below the reference range at the same time points.

9.3. Pharmacokinetics (PK) analysis

The following analyses will be carried out on the PK population as outlined in §8.4:

1. For each analyte and each scheduled sampling time/window (week 2, 4, 8, 12, 16, 22, 26, 44 and early withdrawal), the plasma concentration will summarised using the mean, SD, coefficient of variation (CV), median, minimum, maximum, geometric mean and geometric CV (%).
2. The plasma concentration will be summarised by those who had a 50% increase or 50% decrease in total sperm count and overall.
3. Mean and/or median plasma concentration vs. time graphs will be provided, with error bars and/or scatter plots as appropriate.
4. Mean of the observed Pretomanid troughs from week 2 through to week 26 will be plotted against the percentage change from baseline in total sperm count at week 26.