STATISTICAL ANALYSIS PLAN

Early Bactericidal Activity, Demographics and Safety Analyses

PROTOCOL NUMBER: RESP30X-EBA

Phase 2 Study to Evaluate the Early Bactericidal Activity, Safety, and Tolerability of Nebulised RESP301 in Adults with Newly Diagnosed, Rifampicin Susceptible Pulmonary Tuberculosis

Version 1.2

Version and Date:	Version 1.2	22 Jul 2024

Statements

The statistical data analysis described in the present plan will be performed under Good Clinical Practice (GCP) principles and following the approved SOPs of TASK.

The information contained in this document is provided in confidence. It is understood that this information will not be disclosed to others without prior agreement of TASK, in accordance with the signed confidentiality

SIGNATURE PAGE

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Authors	Signature	Date
[redacted]	[redacted]	[redacted]

Table of Responsibilities

Table 1 shows the person(s) responsible for performing the respective statistical analysis:

[redacted]

Table 1: Table of Responsibilities

SAP Section	Analysis	Responsibility
10.1	Demography and Baseline Characteristics	[redacted]
10.2	Safety and Tolerability	[redacted]
10.3	Early Bactericidal Activity (EBA)	[redacted]

CONTENTS

List of Abbreviations and Definitions of Terms

Abbreviations	Stands for (Explanation)
%	Percentage
ADaM	Analysis Dataset Model
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
bd	Twice daily
BMI	Body Mass Index
CDISC	Clinical Data Interchange Standards Consortium
CRF	Case Report Form
CFU	Colony Forming Units
CS	Clinically Significant
eCRF	electronic Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DDT	Data Definition Table
DMP	Data management plan
EBA	Early Bactericidal Activity
EBEs	Empirical Bayes Estimates
EC	Ethics Committee
EWD	Early Withdrawal
FEV1	Forced Expiratory Volume in the first second
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HRZE	Isoniazid/Rifampicin/Pyrazinamide/Ethambutol
IMP	Investigational Medicinal Product
NCS	Not Clinically Significant
NEB	Nebulisation
PI	Principal Investigator
po	per os/orally
Q1	First quartile
Q3	Third quartile
qd	once daily
PTB	Pulmonary tuberculosis
SAP	Statistical Analysis Plan
SD	standard deviations
SDTM	Study data tabulation model
SOC	System Organ Class
TASK	TASK Applied Science (Pty) Ltd
TB	Tuberculosis
tds	three times a day
TEAEs	Treatment-Emergent Adverse Events
TSC	Trial Steering Committee
TTP	Time to Sputum Culture Positivity

ULN	Upper Limit of Normal

EBA Definitions

EBA	An agent's ability to kill mycobacteria expectorated in sputum during the first weeks of treatment.
EBA CFU	Determination of EBA by quantification of viable mycobacteria as colony forming units (CFU) on a solid media culture.
EBA TTP	Determination of EBA by quantification of viable mycobacteria as time to culture positivity (TTP) in an automated liquid culture system.

1 Document History

Version	Date	Reason for change
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]”

2 Introduction

This Statistical Analysis Plan (SAP) describes the statistical methods to be used during the analysis and reporting of demographics, baseline characterstics, safety and tolerability, and early bactericidal activity (EBA) on data collected from the Protocol RESP30X-EBA (Phase 2 Study to Evaluate the Early Bactericidal Activity, Safety and Tolerability of nebulised RESP301 in Adults with Newly Diagnosed, Rifampicin Susceptible Pulmonary Tuberculosis (PTB)). Investigational medicinal products to be used in this study include RESP301 and Isoniazid/ Rifampicin/ Pyrazinamide/ Ethambutol (HRZE). All study treatments will be given orally either via inhalation or ingestion.

This SAP should be read in conjunction with the Study Protocol. It has been developed using Protocol Version 1.0 dated 06 April 2023.

The purpose of this SAP includes the outline of the planned analysis to assess the demographics (including baseline), safety and tolerability, and EBA. This analysis will support the completion of the clinical study report (CSR) and may be included in regulatory submissions and/or future manuscripts. Any deviations from the planned analyses will be documented in the CSR.

3 Objectives of the Study

Primary Objective

Stage 1
To determine the EBA of three times a day (tds) dosing of inhaled RESP301 6 ml dosed via nebulisation (NEB) over 14 days of treatment.

Stage 2
To determine the EBA of inhaled RESP301 6 ml dosed via NEB administered qd and bd alone, or tds in combination with HRZE, over 14 days of treatment to optimise the dosing schedule.

Secondary Objectives
To determine the safety and tolerability of inhaled RESP301 6 ml via NEB administered qd, bd, and tds alone or tds in combination with HRZE, over 14 days of treatment.

Exploratory Objectives
To explore the EBA variability due to potential M.tb sterilisation in sputum samples as a result of NO contamination in sputum samples.

4 Design of the Trial

4.1 General

The study is a phase II, single-centre, open-label, parallel arm, randomised clinical trial in two sequential stages, with no stratification. The study endpoints are laboratory based and the microbiology staff will be blinded to treatment allocation. A total of up to 15 participants will be recruited per treatment arm (total of 75 participants in the study). Control arm participants will be split across stage 1 and 2.

Stage 1: Treatment arms and participant numbers are described in Table 2.

Table 2: Stage 1 Treatment Arms

Treatment Arm	Treatment	Number of participants
1 (Active):	Inhaled RESP301 6ml via NEB tds	15
5 (Control):	HRZE po qd	5

HRZE: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg
NEB: nebulisation, qd: once daily; po: per os/orally, tds: three times a day

On completion of Stage 1, recruitment will be paused and an interim analysis performed. Dependent on these results, a Trial Steering Committee (TSC) will determine whether the study should proceed to Stage 2.

Stage 2: Treatment arms and participant numbers are described in Table 3.

Table 3: Stage 2 Treatment Arms

Treatment Arm	Treatment	Number of participants
2 (Active):	Inhaled RESP301 6 ml via NEB qd	15
3 (Active):	Inhaled RESP301 6 ml via NEB bd	15
4 (Active)	Inhaled RESP301 6 ml via NEB tds plus HRZE* po qd	15
5 (Control):	HRZE po qd	10

HRZE: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg
NEB: nebulisation, qd: once daily; po: per os/orally, tds: three times a day

The per participant involvement in the study will consist of an up to 9-day screening period, followed by a 14-day treatment period and then a 15-day non-treatment day (for the purposes of exploratory samples). The participant will be admitted to the EBA unit from initial screening and discharged on day 16 of the study, with daily study "visits" where study assessments are performed, unless the participant is withdrawn early from the study. A follow-up visit will occur on day 28 following the start of treatment (Day 1) for all participants (completed treatment or early withdrawals).

4.2 Study population

The study is planned to enroll 75 participants male and female (5 groups of 15 participants

receiving arms/IMP) with sputum-positive newly diagnosed, rifampicin susceptible PTB that meet all inclusion/exclusion criteria. Twenty participants will be enrolled in Stage 1 with an additional 55 participants enrolled in Stage 2. Participants who do not complete treatment can be replaced. It is planned to enroll all participants at a single site in South Africa: TASK Clinical Research Centre, Bellville, South Africa

4.3 Sample Size Determination

This is a proof-of-concept study without hypothesis testing. The planned sample size of 15 participants per treatment group is in keeping with other phase 2 trials of this type and accounts for the possibility of up to 3 dropouts per arm which, based on previous studies of this type conducted at these sites, represents a conservative estimate of the expected drop-out rate. Previous EBA studies indicate that a sample size of 12 participants per arm allowed for the identification of proof-of-concept i.e., change in TTP/ CFU compared to no change in TTP/CFU for all evaluated arms.

Fifteen individuals per treatment arm is a standardized sample size for EBA studies and is expected to show any potential effect of the studied drug on EBA. However, a difference in EBA between arms may not be detected if the effect difference is small. The suggested sample size of 15 individuals in conjunction with analysis using mixed effects models has previously been shown to be adequate for the assessment of EBA.

Using 12 participants per arm, a power of 80%, alpha of 0.05, and a between-patient variability in (time-to-positivity) TTP slope of 22% (coefficient of variation), will allow for the detection of EBA-TTP0-14days of 0.0483 hours, corresponding to TTP-EBA0-14days of 11 hours, using a nonlinear mixed effects model (Boeree, M. J., et al, 2015).

4.4 Treatment administration

Participants meeting eligibility criteria will be enrolled into five (5) cohorts/arms. Participants will participate in only one cohort. The control arm will enrol in stage 1 of the trial (with 5 participants) and stage 2 (with additional 10 participants).
Arm 2 to 4 will only enrol in stage 2 with all its participants.

Treatments include either RESP301 alone, RESP301 with HRZE, or HRZE alone (as a laboratory control). Doses of RESP301 will be administered NEB as displayed in Table 4.

Table 4. Summary of study treatment arms (dosing regimen of RESP301 and/or HRZE)

Stage	Arm	Abbreviation	# Per arm	Treatment/ Regimen components
1	1	RESP301tds	15	Inhaled RESP301 6 ml via NEB tds
	5	HRZE	5	HRZE po qd
2	2	RESP301qd	15	Inhaled RESP301 6 ml via NEB qd
	3	RESP301bd	15	Inhaled RESP301 6 ml via NEB bd
	4	RESP301tdsHRZE	15	Inhaled RESP301 6 ml via NEB tds plus HRZE* po qd
	5	HRZE	10	HRZE po qd

4.5 Randomisation

Eligible participants who have given written, informed consent will be screened on the trial during Visit 1 (Days -9 to -3) and will be identified by a study-generated participant identification code for anonymity (participant number).

Participants who meet all the inclusion criteria and none of the exclusion criteria will be randomised and assigned a treatment number. The randomization scheme will be generated by the study statistician. Randomisation will be conducted using permuted block randomisation. Randomisation will be done with 3:1 (arm 1, arm 5) in stage 1. Once enrolment into stage 2 is opened, randomisation into the remaining arms will be 2:3:3:3 (arm 5, arms 2-4) [Table 4].

Consecutively numbered, sealed, opaque envelopes will be prepared and provided to the site. The site research pharmacist will maintain the randomisation envelopes in a secured location. After being notified that a participant is eligible for the study, the research pharmacist will write the participant's number on the next numbered envelope and open it to determine the participant's treatment group. The participant number, participant initials, the date and time the envelope was opened, and the signature of the person opening the envelope will be recorded on the treatment group card in the envelope at the time it is opened. Opened envelopes containing treatment group cards will then be filed in the participants' source file.

Participants who withdraw from the study before they have received all days of IMP may be replaced per the discretion of the sponsor unless they are withdrawn for safety reasons.

4.6 Blinding

This is an open-label study and there is therefore no need for blinding or procedures to break the blind. The study endpoints are laboratory-based and the microbiology staff will be blinded to treatment allocation until the EBA database closure and release.

4.7 Sampling of Observations

Sampling of observation for fixed and random variables will be done over time per study procedure as shown in Table 5. Study Time and Study Treatment will be fixed factors (deterministic) in the study design while all other variables will be random (realised after sampling).

Table 5: Trial Flow Chart for Stage 1 and 2

	Screening			Treatment														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	(-9 to -3)	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B status	X																	
onsent	X																	
	X																	
nt History	X																	
on [A]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
[C], participant ompliance Check				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ation [D]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ent [F]	X																	
	X [G]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
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Screen	X																	
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nancy Test [L]	X																	
	X																	

EBA: Early bactericidal activity, EWD: Early withdrawal, FEV1: Forced Expiratory Volume in the first second, FU: Follow-up; IMP: Investigational Medicinal Product

A: Physical examinations: Will be complete at screening and targeted (symptom-directed) thereafter.

B: Vital signs, safety laboratory: on days where there is Investigational Medicinal Product (IMP) dosing, to be performed pre-dose.

C: IMP administration: The HRZE is to be administered fasted (1 hour before, or 2 hours after food) with approximately 240 mL of water. For study purpose this food should be breakfast.

D: Concomitant Medication: collected from 14 days prior to first dose of IMP.

E: Adverse Events: Collected from first dose of IMP to Follow-Up visit.

F: Eligibility Assessment: Continuous from screening to just prior to first IMP dose.

G: EBA unit inpatient: admission, anytime from initial screening to day -2, whichever is in the participant's best interest.

H: Randomisation: May occur any time after eligibility has been confirmed to just prior to first IMP dose.

I: Overnight sputum: Collection will start in the afternoons at approximately 15h00 and

continue for 16 hours ± 1 hour overnight. The 16-hour sputum sampling for each of the sampling days must be finished prior to the administration of the next morning's IMP. Collection will be paused for 30 minutes after evening RES301 dose where applicable.

J: Spot or Overnight Sputum: For eligibility assessments.

K: Blood collection for Haematology, Chemistry, and urinalysis on day 1 must be done prior to first dose.

L: β-HCG Serum Pregnancy Test: All female participants.

M: Follow-up visit: a window of ±3 days allowed.

N: EBA unit inpatient: discharge

4.8 Data Collection and Integration

The sampled observational information from study procedures will be recorded in the study source documents. This information will then be captured electronically onto a Clinical Study Database that is developed based on the electronic Case Report Forms (eCRFs). The variables names, types, formats, domains/tables will be based on the annotated eCRFs. Annotation of the eCRFs will done according to the Clinical Data Interchange Standards Consortium (CDISC) study data tabulation model (SDTM) guidelines. These guidelines will ensure compliance with regulatory bodies and ensure that the FAIR principle applies to the clinical study data. The Clinical Study Database will be developed in a Clinical Data Management System (CDMS) compliant with the Protection of Personal Information Act, 2013, 21 Code of Federal Regulation (CFR) Part 11 and General Data Protection Regulation (GDPR). Laboratory results from the Safety, Mycobacteriology, and Pharmacokinetic laboratories will be imported into the Clinical Study Database and reconciled with blood and sputum sampling information. The study Data Management Plan describes the data collection and integration described in more elaborate detail.

4.9 Stopping Rules

If the Investigator, the Sponsor, or Sponsor's Designee, or appropriate regulatory officials discover conditions arising during the study that indicate that the study should be halted or that the study centre should be terminated, this action may be taken after appropriate consultation among the Sponsor and Investigator/s. Conditions that may warrant termination of the study or discontinuation of a study site include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to the participants enrolled in the study
- A decision on the part of the Sponsor to suspend or discontinue testing, evaluation, or development of the product
- Failure of the Investigator to enroll patients into the study at a rate commensurate with good clinical research practice and acceptable to the Sponsor
- Failure of the Investigator to comply with pertinent regulations of appropriate regulatory authorities
- Submission of knowingly false information from the research facility to the Sponsor, Study Monitor, or appropriate regulatory authority
- Insufficient adherence to protocol requirements

The Regulatory Authorities and Ethics Committees/Institutional Review Boards (EC/IRB) will be informed should the study be terminated. All study materials (except documentation that must remain stored at the site) will be returned to the Sponsor. The Investigator will retain all other documents until notification is given by the Sponsor for destruction.

Participants currently on IMP will receive an appropriate TB regimen and all participants will be referred as soon as possible to the national TB programme.

5 Endpoints

5.1 Primary Endpoints

The following are the primary endpoints:

The EBATTP$_{0\text{-}14}$ in the Mycobacteria Growth Indicator Tube (BactecTM MGITTM 960) system as determined by the predicted rate of change in the TTP in sputum over the period day 0 to 14 will be analysed [redacted]

[redacted]

[redacted]

5.2 Secondary Endpoints

EBA

The EBACFU (0-14) as determined by the predicted rate of change in log10CFU per ml sputum over the period day 0 to day 14 will be described [redacted]

[redacted]

Safety and Tolerability

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). Treatment-emergent adverse events (TEAEs) are defined as AEs which started at or after the first administration of IMP and includes those events which started prior to the first administration of IMP but which worsened after the first intake. Adverse events starting after

the first administration of IMP until the last scheduled visit/ assessment/ measurement will be regarded as treatment-emergent.
The incidence of the following events will be summarized by treatment:

- Incidence of treatment-emergent adverse events (TEAEs);
- Incidence of TEAEs by Severity;
- Incidence of drug-related TEAEs;
- Incidence of Serious TEAEs;
- Incidence of TEAEs leading to early withdrawal;
- Incidence of TEAEs leading to death.

Descriptive summary statistics will be presented for other safety variables: laboratory parameters, physical examination, vital signs, concomitant medication. Information on all adverse events will be collected and captured in the eCRF.

Please refer to the Section 10.1 and 10.2 for additional information regarding analyses of these endpoints.

5.3 Exploratory endpoints

To explore the EBA variability due to potential M.tb 15terilization in sputum samples as a result of nitric oxide (NO) contamination in sputum samples. [redacted]

[redacted]

6 Analysis Populations

The Safety analysis population will consist of all randomised participants who took at least one dose of study treatment.

The EBA population will consist of all participants who have baseline and at least one post baseline TTP or CFU observation. There will be one EBA population for TTP and one for CFU.

7 General Principles

The SAP will be finalised and signed-off prior to database lock. Post-hoc analysis (analyses performed that are different from those described in the final version of the SAP) will be documented in the final CSR as changes to planned analysis. For the Demography, Safety and Tolerability, and EBA analyses, there will be two analysis periods, an interim analysis and an analysis at the end of stage 2 of the study.

All data collected will be listed, and sorted by treatment, participant number, visit, and time.

Descriptive summaries will be presented using summary statistics (e.g. number (n), mean, standard deviations (SD), median, first quartile (Q1), third quartile (Q3), minimum (min), maximum (max)) for continuous variables or number and percentage n (%) for categorical/discrete variables. Continuous variables will be expressed as median with inter-quartile range for skewed data and mean with SD for normal data. The percentages will not be provided for a zero count. The mean, median, SD, Q1, and Q3 will be presented to 1 more decimal place (dp) than the raw data, and min and max to the same number of dps as the raw data. Percentages will not be provided for a zero count and 100% will be provided to zero dps.

All output will be generated using SAS version 9.4 for safety analysis, and R software Version 4.3.1 or above for EBA analysis (including figures) and safety figures.

Handling of Dropouts or Missing Data: No missing data will be replaced, and it will be shown in any summaries as missing for all analyses.

Treatment of Outliers: TTP and CFU observations which are considered to be outliers by a team of microbiologist and clinical trialist, might be removed from the EBA analysis. Outliers and reason for removal will be documented.

If the start date of an Adverse Event (AE) (or concomitant medication) is incomplete, the most conservative approach will be taken and the AE treated as treatment emergent (or concomitant, in the case of medication) to the last study treatment given.

7.1 Analysis Datasets

For the Demography, Safety and Tolerability analysis, the study data tabulation model (SDTM) datasets after soft-lock of the clinical database will be used. The soft-lock of the clinical database is described by the study data management plan (DMP). Analysis datasets will be generated during the analysis according to the ICH E3, E9, and Clinical Data Interchange Standards Consortium (CDISC) Analysis Dataset Model (ADaM) guidelines.

For the EBA analysis, an EBA analysis dataset will be generated from the SDTM datasets according to the RESP30X-EBA Data Definition Table (DDT). The RESP30X-EBA DDT is a separate document that details the data specifications of the EBA analysis dataset and will be finalised prior to the first EBA analysis. In general, the EBA DDT will compromise at least the following variable:

CDSIC DOMAIN	Variable	Label	Type/Format	Length
ALL	#REF	Consecutive record number.	Numeric	LONG
ALL	STUD	Protocol number. Insert protocol number.	Character	200
DS	UID	SUBJECT (RANDOMIZATION NUMBER).	Character	200
ALL	ID	ID for NONMEM dataset	Numeric	LONG
ALL	TIME	Nominal sample production day	Numeric	LONG
LB MICRO	DVCFU	Dependent variable	Demical/Float	5.2
LB MICRO	DVTTP	Dependent variable	Numeric	LONG
LB MICRO	SSCFU	Sample status for CFU counts	Numeric	LONG
LB MICRO	SSTTP	Sample status for TTP counts	Numeric	LONG
LB MICRO	REP	Replicate number	Numeric	LONG
DS	GROUP	Group	Numeric	LONG
DS	TRT	Treatment	Numeric	LONG
DS	REGI	Regimen	Character	200
VS	BMI	BMI	Demical/Float	5.1
VS	HT	Height	Numeric	LONG
DM	ETHNICITY	Ethnicity	Numeric	LONG
VS	WT	Weight	Demical/Float	5.1
DM	AGE	Age	Numeric	LONG
DM	SEX	Sex	Numeric	LONG
LB MICRO	BBLCFU	Baseline bacterial load. (average log10 CFU on days -2 and -1)	Numeric	LONG
LB MICRO	BBLTTP	Baseline bacterial load. (average TTP on days -2 and -1)	Numeric	LONG
LB SAFETY	HIV	HIV	Numeric	LONG
CX	EXTENT	Severity or extent of disease	Character	200
CX	CAV	Presence of cavitation	Numeric	LONG
LB MICRO	SQUAL	Sputum quality	Numeric	LONG
LB MICRO	SVOL	Sputum volume	Numeric	LONG
LB MICRO	COMMENT	Comments from the Microbiology Laboratory associated with sample observation	Character	200
SU	SMOKING	Smoking	Character	200
LB MICRO	MBGENMTBULTRA	Genexpert MTB/RIF ULTRA Detection.	Numeric	LONG
LB MICRO	MBGENMTBULTRAPos	Genexpert MTB/RIF ULTRA Detection Positivity.	Numeric	LONG
LB MICRO	MBGENMTBULTRACyc	Genexpert MTB/RIF ULTRA Detection Cycle Threshold.	Numeric	LONG
RE SPIROMETRY	REFVC	Spirometry Forced Vital Capacity Actual value.	Numeric	LONG

RE SPIROMETRY	REFVC1	Forced Expiratory Volume in 1 Second Actual value.	Numeric	LONG
RE SPIROMETRY	REFEV1_FVC	Modified Tiffeneau-Pinelli index Actual value.	Numeric	LONG
CV SPMET	CVSPMET	SpMet Measurement Highest value.	Numeric	LONG
LB SAFETY	LBHGB	Haemoglobin in Blood Plasma.	Numeric	LONG
LB SAFETY	LBBASOA	Basophils Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBEOSA	Eosinophils Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBIGA	Imm Granulocyte Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBLYMPHA	Lymphocytes Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBMONOA	Monocytes Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBNEUTA	Neutrophils Absolute (ABS) in Blood Plasma.	Numeric	LONG
LB SAFETY	LBWBC	White cell count in Blood Plasma.	Numeric	LONG

7.2 Analysis Software/Statistical Packages

All study analysis datasets will be generated using SAS version 9.4. SAS version 9.4 will also be used for the Demography, Safety and Tolerability analysis. The R software version 4.3.1 or above will be used for EBA analysis and for safety figures and statistical models. SAS version 9.4 will be used to validate models to ensure consistent estimates from R software version. If updated versions of the above software become available these may be used and will be detailed in the Clinical Study Report.

7.3 Changes in the Conduct of the Study or Planned Analysis

Any deviations from the planned analysis will be provided and documented in the Statistical Reports and CSR (including Demographics, Safety and EBA). Information from these analyses will be combined into the Clinical Study Report.

9 Interim Analysis for Safety and EBA

The objective of stage 1 is to determine the EBA of inhaled RESP301, to inform ongoing development. When stage 1 is completed, recruitment will be paused, and an interim review will be conducted by a Trial Steering Committee (TSC) on the Safety and EBA. This will be detailed

in the Trial Steering Committee Charter.

In order to progress to Stage 2, the following minimum should be met:

- RESP301 tds active treatment arm EBATTP(0-14): the predicted rate of change in the TTP in sputum over the period day 0 to 14 must be statistically different from zero at the 5 % level (demonstrating acceptable EBA to continue to Stage 2).
- ≤50% of the participants experienced a grade 3 or higher AE.

A protocol amendment will be considered if the following safety and tolerability criteria are met:

- ≥25% of the participants experienced a grade 2 or higher AE of any description considered probably or definitely related to IP.
- ≥20% of the participants experienced a grade 3 or higher AE reflecting intolerability to IMP (gastrointestinal: diarrhoea, nausea, or dyspepsia; respiratory: bronchospasm).

The safety, tolerability, and EBA interim analysis will be conducted as described in Sections 10.1, 10.2, and 10.3 of this SAP respectively.

10 Analysis Methods

OVERVIEW OF THE ANALYSIS

The analyses to be conducted in this study will include Demography, Baseline Characteristics, Safety and Tolerability, and EBA.

10.1 Demography, Safety, and Baseline Characteristics

The disposition of participants, completion of the study, and reasons for withdrawal will be summarised by treatment and overall. The number of participants withdrawing and the reason for withdrawal will also be summarised by the last treatment taken.

In the event of more than a single data point (for a particular variable) prior to the administration of trial medication, "baseline" will be regarded as the first of these observations.

Demographic data will be summarised by treatment and overall. Summary statistics will be calculated for age, race and gender, height, weight, and body mass index (BMI) for the Safety Population.

Substance use of the safety population will also be summarised by treatment and overall.

The results of the medical history (refer to Adverse Events for an explanation regarding the coding of medical history), vital signs, safety laboratory values, and physical examination at baseline/screening will be tabulated by treatment for the Safety Population.

Prior medication is defined as any medication that ended prior to the first dose of trial medication. Incidence will be summarised by generic name and class by dose for the Safety Population.

Listings for the data used in the tabulations will also be given. Listings containing the following information will also be included:

1. Study Completion Status
2. Visit Dates (including hospital admission and discharge)
3. Eligibility and Inclusion and Exclusion Criteria
4. Subject Eligibility (including Informed Consent, Initial Diagnosis and Randomization)
5. Urine Drug Screen
6. Serum Pregnancy
7. Serology (HIV) and CD4 Count
8. Study Drug Administration (including compliance)
9. Adverse Events
10. Physical Examination
11. Vital Signs
12. Chest X-ray

The response to the eligibility assessments and inclusion/exclusion criteria will be listed.

For each treatment exposure (EX), the dose/number of tablets/capsules (group dependent) taken overall will be calculated along with the percent compliance out of the total number of

tablets/capsules expected and will be summarised by dose for the Safety Population.

10.2 Safety and Tolerability

10.2.1 Adverse Events

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).

An adverse event will be defined as Treatment Emergent (TEAE) if the onset date is after the first intake of study medication.

Where dates are incomplete such that a decision cannot be made, it will be assumed that the adverse event is TEAE.

The incidence of the following events will be summarised by treatment group:

13. TEAEs by SOC and preferred term.
14. TEAEs by maximum severity.
15. TEAEs by strongest relationship to IP
16. Related TEAEs.
17. Potentially IMP-related (Related and Possibly Related) TEAEs.
18. Potentially IMP- TEAEs by maximum severity.
19. TEAEs with a fatal outcome.
20. Serious TEAEs.
21. Discontinuations due to TEAEs.

The frequency of TEAEs, by severity and IMP relationship, will be presented by treatment. The sampled and recorded observations of AEs will be considered to be discrete random variables. AEs will be analysed based on absolute risk or crude incidence rates or exposure-adjusted incidence rates using frequency statistics. The AE variable will be modelled as a Bernoulli distribution with parameter (p) representing the probability of success/occurrence. The definition of absolute risk applied in this analysis will be the following:

- The number of individuals experiencing an AE divided by the total number of participants in that dose treatment group.

Applicable only in the AE summary table14.3.1.8, the following definition of absolute risk will be used for this table:

- The number of AEs divided by the total number of AEs in that dose treatment group

Furthermore, these rates do not adequately account for an individual participant's profile of adverse events over the study period when a participant may remain in the trial after experiencing one or more events (i.e., occurrence of multiple events of the same kind or different kinds). Moreover, the required statistical assumptions (e.g., constant hazard rate over time) for valid estimates of incidence rates are not likely to be met in practice by adverse events data of clinical trials. To overcome this, mean cumulative function (MCF) will also be performed for the safety data. MCF is a nonparametric approach that provides a valid statistical inference on recurrent adverse event profiles of drugs in randomized clinical trials (RCT). The

estimate involves no assumptions about the form of MCF. As compared to the crude or exposure-adjusted incidence rates of adverse events, the MCF estimates facilitate more understanding of the safety profiles of a drug in an RCT. Survival analysis models to understand the probability of occurrence over time will be applied using the Kaplan-Meier, Cox-Proportion Hazzard, and Binary Logistical Regression models. A special focus will be placed on Adverse Events that are considered related to study treatment.

10.2.2 Study Stopping and Amendment Criteria

The study stopping and amendment criteria will be summarised as described in Section 9 in the form of frequency statistics per treatment group and time point.

10.2.3 Other Safety Variables

Laboratory parameters
Laboratory safety tests are performed throughout the study. The data will be listed with values outside the normal range flagged and with the grade according to Common Terminology Criteria for Adverse Events (CTCAE) mentioned. The recorded values and the change from baseline will be summarized by treatment and time of collection. The assessment (normal/abnormal-NCS/ abnormal-CS as well as the grade according to CTCAE) will be summarized for haematology, blood chemistry, and urinalysis by treatment and time of collection.

Physical examination
The physical examination is repeated at each visit. The results (normal/abnormal-NCS/ abnormal-CS as well as the grade according to CTCAE, if applicable) will be summarized by body system by treatment and time of collection.

Vital signs
Vital signs are recorded at each visit. The change from the baseline will be calculated. The data will be summarized by treatment and time of collection.

Concomitant medication
Concomitant medication is defined as any medication taken within 14 days prior to IMP administration or during the trial until visit 19 (day 28). Where dates are incomplete such that a decision cannot be made, it will be assumed that a medication is concomitant. The incidence will be summarised by generic name, class, and treatment.

Methemoglobin
The assessment of Methemoglobin using Spmet will be summarized by single SpMet measurement and Highest SpMet measurement per treatment and time of assessment using descriptive statistics.

Lung Function
The assessment of lung function using Spirometry will be summarized by Forced Vital Capacity, Forced Expiratory Volume in 1 Second and Modified Tiffeneau-Pinelli index per treatment and time of assessment using descriptive statistics.

10.3 Early Bactericidal Activity (EBA)

The below provides a summary of the general planned EBA analysis, however, more elaborate model definitions and methodology will be provided in the EBA statistical report as EBA modelling is subject to the EBA data.

Exploratory graphical analysis of CFU and TTP data versus time following drug treatment. Covariate distributions will be inspected for the identification of potential outliers and to inform the analysis.

Development of population EBA models for CFU and TTP

a) Evaluation of structural and stochastic models to describe CFU and TTP versus time profiles
b) Investigation and characterization of covariate relationships that influence initial bacterial load and bacterial killing.

10.3.1 Structural Model (Fixed Effects)

A separate EBA model will be developed for each of the two endpoints TTP and CFU. For each model, mono- and bi-linear (for CFU only) and mono- and bi-exponential models will be fitted to the data as defined below for TTP and CFU:

Functions for TTP and CFU should be explored, if needed, and indicated by the EBA data:

Mono-Linear and Mono-Exponential Model For TTP

1) **Logarithmic function:**

$$TTP = e^{\alpha_{log}} \cdot \log10(t) + e^{A_{log}}$$

The model includes one intercept defined by A and one slope defined by α.

2) **Exponential decay upwards function:**

$$TTP = A_{exp} + B_{exp} \cdot (1 - e^{\alpha_{exp} \cdot t})$$

The model includes one intercept defined by A, one asymptote defined as B, and one slope defined by α.

Mono-Linear, Mono-Exponential and Bi-Exponential Model For CFU

1) **Mono-linear model:**

$$Log10CFU = A_{mono} - \alpha_{mono} t$$

2) **Bi-linear (Step function) model:**

$$Log10CFU = A_{bi} - \alpha_{bi}\, t \quad \text{where } t \le k$$

$$Log10CFU = A_{bi} - (\alpha_{bi} - \beta_{bi})k - \beta_{bi}\, t \quad \text{where } t > k$$

The models will include one intercept defined by A and one or two slopes defined by α and β and k the node parameter.

3) Mono-exponential model:

$$Log10CFU = Log10(e^{A_{mono}} \cdot e^{-\alpha_{mono} \cdot t})$$

4) Bi-exponential model:

$$Log10CFU = Log10(e^{A_{bi}} \cdot e^{-\alpha_{bi} \cdot t} + e^{B_{bi}} \cdot e^{-\beta_{bi} \cdot t})$$

The models will include one or two intercepts defined by A and B and one or two slopes defined by α and β.

10.3.2 Stochastic model (Random Effects)

1. Inter-individual variability

During the course of the model development, the Inter-individual (IIV) parameters will be tested on the structural parameters of the EBA models. Inter-individual parameters will be included in an exponential manner to ensure that the individual EBA parameters have a lower bound of zero:

$P\_i = TVP.e^{\eta}$

where TVP is the typical (Expected) value of the parameter P, P_i is the individual value of the parameter and ŋ is a normally distributed random variable with mean 0 and standard deviation ω. Not all parameters need to be associated with IIV.

If the distributions of the ŋs are markedly non-normal, transformations of ŋs may be considered. Terms to describe correlations between ŋs will also be considered if indicated by the data. Initially, IIV should be added to the baseline only. IIV in other parameters should be explored after the treatment effect evaluation.

10.3.3 Residual unexplained variability (Random Error)

The aim of the residual unexplained variability (RUV)/random error model is to ensure that the individual weighted residuals (IWRES) are approximately homoscedastically distributed across all predictors (e.g., time, occasion, and study). The RUV describes the differences between the observations and the individual predictions (IPRED)/estimates of the model. The RUV model can be either additive (homoscedastic), log-additive (exponential), proportional (heteroscedastic) or combined additive and proportional error model. The different RUV models will be evaluated for the models.

10.3.4 Covariate model (Mixed Effects)

After confirmation of the structural and stochastic EBA models a covariate analysis will be done. The median of baseline values of the covariate will be used for time-independent covariates. For time-dependent covariates, the actual value of the covariate at the time of the observation will be used (or closest in time to the observation). The inclusion of time-dependent covariates in the covariate analysis will be informed by the exploratory data analysis. For time-varying covariates to be included in a model there must be a clear rational for assuming that the time variation is independent of the drug treatment and not vice versa. Time-varying covariates

for which the causality is unclear may be investigated after the assessment of other covariates or drug effects.

10.3.5 Model evaluation

Model evaluation will include graphical analysis of goodness of fit (GOF) plots, relative standard errors (RSEs), visual predictive checks (VPCs), and possibly other simulation-based diagnostics. Stratifications will be used when appropriate to ensure that the models perform adequately across important subgroups of the data.

10.3.6 Model discrimination

The discrimination between models will mainly be made based on the inspection of graphical diagnostics and changes in the objective function value (OFV) and Akaike information criterion (AIC). The differences in OFVs are nominally X2 distributed and a difference of -3.64 corresponding to approximately a p-value of < 0.05 for one degree of freedom, provided that the models are hierarchical. Preferably this should also demonstrate improvements in the graphical diagnostics and not lead to a high (>1000) conditional number.

10.3.7 Evaluation of shrinkage

Shrinkage in Empirical Bayes Estimates (EBEs) of model parameters used for diagnostic purposes will be evaluated. The shrinkage increases from 0 towards 100% as the EBEs becomes less informative, and EBE based graphs and diagnostics are more reliable when shrinkage is small. A shrinkage of <20% is generally considered as a low shrinkage. Shrinkage will be taken into account when EBE based graphs and diagnostics are evaluated.

10.3.8 Final model

A final model should provide a good description of the observed data, not have any important trends in the relevant GOF diagnostics, have a successful termination of the covariance step and preferably have a condition number less than 1000. Should a model be accepted as final without these criteria being met, it will be discussed and justified in the report.
There will be one final model for each of the endpoints.

Based on the final EBA models for TTP and CFU, mean bactericidal activity per treatment arm will be expressed as the daily change in TTP and CFU from day 0 to day 14. If required, periods from 0 to 2 days, 0 to 7 days, 7-14 days, or any other period will be predicted or calculated as well as the 95% Bayesian credibility interval using only the patient data included in the study. Clinical trial simulations (n= 1000) will be used to predict mean (95% prediction interval) bactericidal activity for different treatment arms and different characteristics depending on the covariates included in the final models.

Early bactericidal activity data definition table (DDT): A separate data specification table will be created for EBA. The dataset should be in excel csv format. A readme file describing the dataset column names should be created with an example for 1 participant.

11 Reporting

The Demography, Baseline Characteristics, Safety and Tolerability, and EBA analysis results will be reported. The CSR will incorporate these in one report. The statistical reports are independent and will be reported in parallel. The interim analysis report will be focused on safety and EBA.

11.1 Demography and Baseline

The results of the demographic and safety analysis will be reported as per the tables listed below:

Table Number	Population	Table Title	Shell/ Reference to Shell
14.1 Demographic Data			
14.1.1	All Participants	Disposition of Participants	S
14.1.2	Safety	Demographics	S
14.1.3	Safety	Medical History	S
14.1.4	Safety	Vital Signs at Baseline	14.1.2
14.1.6.1	Safety	Physical Examination at Baseline	S
14.1.7	Safety	Prior Medications	S
14.1.8.1	Safety	Clinical Safety Laboratory Tests at Baseline – Haematology	14.1.2
14.1.8.2	Safety	Clinical Safety Laboratory Tests at Baseline – Serum Chemistry	14.1.2
14.1.8.3	Safety	Clinical Safety Laboratory Tests at Baseline – Urinalysis	14.1.2
14.1.9	Safety	Mycobacteriological Characteristics at Baseline	14.1.2
14.1.10	Safety	Exposure and Compliance	14.1.2
14.1.11	Safety	Substance Use	14.1.2
14.1.12	Safety	Methemoglobin Assessment	14.1.2
14.1.13	Safety	Lung Function Assessment	14.1.2

11.2 Safety and Tolerability

The results of the safety and tolerability analysis will be reported as per the tables, listings and figures listed below:

Listing Number	Listing Title / Summary
16.2 Safety Listings	
16.2.1.1	Study Completion Status
16.2.1.2	Visit Dates (including hospital admission and discharge)
16.2.2	Eligibility and Inclusion and Exclusion Criteria
16.2.3	Subject Eligibility (including Informed Consent, Initial Diagnosis and Randomization)
16.2.4.1	Demographic Data
16.2.4.2	Medical History
16.2.4.3	Urine Drug Screen
16.2.4.4	Serum Pregnancy
16.2.4.5	Serology (HIV) and CD4 Count
16.2.4.6	Prior and Concomitant Medication
16.2.5	Study Drug Administration (including compliance)
16.2.6.1	Adverse Events
16.2.6.2	Physical Examination
16.2.6.3	Vital Signs
16.2.6.4	Chest X-ray
16.2.7	Clinical Safety Laboratory Tests
16.2.8	Study Stopping and Amendment Criteria (for interim analysis)
16.2.9	Substance Use

Listing Number	Listing Title / Summary
16.2.10	Methemoglobin Assessment
16.2.11	Lung Function Assessment

14.3 Safety Data			
Table Number	Population	Table Title	Shell/ Reference to Shell
14.3.1.1	Safety	Summary of Incidence of Treatment Emergent Adverse Events	S
14.3.1.2	Safety	Incidence of Treatment Emergent Adverse Events by System Organ Class and Preferred Term	S
14.3.1.3.1	Safety	Incidence of IMP Related Treatment Emergent Adverse Events by System Organ Class and Preferred Term	14.3.1.2
14.3.1.3.2	Safety	Incidence of Potentially IMP Related Treatment Emergent Adverse Events by System Organ Class and Preferred Term	14.3.1.2
14.3.1.4	Safety	Incidence of Fatal Treatment Emergent Adverse Events by System Organ Class and Preferred Term	14.3.1.2
14.3.1.5	Safety	Incidence of Serious Treatment Emergent Adverse Events by System Organ Class and Preferred Term	14.3.1.2
14.3.1.6	Safety	Incidence of Treatment Emergent Adverse Events leading to discontinuation by System Organ Class and Preferred Term	14.3.1.2
14.3.1.7.1	Safety	Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Maximum Severity	S
14.3.1.7.2	Safety	Incidence of Potentially IMP-related Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Strongest Relationship to IMP	14.3.1.7.1
14.3.1.7.3	Safety	Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Strongest Relationship to IMP	14.3.1.7.1
14.3.1.7.4	Safety	Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term, Maximum Severity and Strongest Relationship to IMP	S
14.3.1.8	Safety	Summary of Frequency of Treatment Emergent Adverse Events	S
14.3.2.1.1	Safety	Clinical Safety Laboratory Tests – Haematology over time	S
14.3.2.1.2	Safety	Clinical Safety Laboratory Tests – Haematology Change from Baseline over time	S

14.3.2.1.3	Safety	Clinical Safety Laboratory Tests – Haematology Assessment over time	S
14.3.2.2.1	Safety	Clinical Safety Laboratory Tests – Serum Chemistry over time	14.3.2.1.1
14.3.2.2.2	Safety	Clinical Safety Laboratory Tests – Serum Chemistry Change from Baseline over time	14.3.2.1.2
14.3.2.2.3	Safety	Clinical Safety Laboratory Tests – Serum Chemistry Assessment over time	14.3.2.1.3
14.3.2.3	Safety	Clinical Safety Laboratory Tests – Urinalysis	14.3.2.1.1
14.3.4	Safety	Physical Examination	14.1.6
14.3.5	Safety	Vital Signs	14.3.2.1
14.3.6	Safety	Concomitant Medication	14.1.7
14.3.7	Safety	Summary of Assessment of Study Stopping and Amendment Criteria. (for interim analysis)	S
14.3.8	Safety	Methemoglobin Assessment Over Time	14.3.2.1.1

Figures Number	Population	Figure Title	Shell/ Reference to Shell
14.3.1	Safety	Summary of Incidence of Treatment Emergent Adverse Events	S
14.3.2	Safety	Clinically Significant Laboratory Tests by Treatment-Over Time	S
14.3.3	Safety	Clinically Significant Laboratory Tests by Treatment-Change from Baseline over Time	S

11.3 EBA

An EBA report will be written which will include

- Figures for identification of outliers and data points to be omitted
- Tables summarizing omitted data
- Figures showing observed EBA
- Figures showing predicted EBA

Addition tables and figures will be generated during the modelling process. The general results of the EBA analysis will be reported as per the tables, listings, and figures listed below:

14.4 EBA General Tables				
Table number	**Population**	**Objective**	**Table Title**	**Shell References to Shell**
14.4.1	EBA	Missing values	Positive and non-positive data in the dataset for CFU status at the different timepoints	S
14.4.2	EBA	Missing values	Positive and non-positive data in the dataset for TTP status at the different timepoints	14.4.1
14.4.3	EBA	Outliers	Listing of potential outliers and suspect values for CFU and TTP	S
14.4.4	EBA	Describing population	Summary statistics of baseline discrete covariates for the treatment arms	S
14.4.5	EBA	Describing population	Summary statistics of continuous covariates for the treatment arms at baseline	S
14.4.6	EBA	Describing population	Summary statistics of continuous covariates for the treatment arms over time	14.4.5
14.4.7	EBA	Describing population	Summary statistics of log 10 CFU over time	14.4.5
14.4.8	EBA	Describing population	Summary statistics of log 10 TTP over time	14.4.5

14.4.9	EBA	Model	Median and interquartile range of observed EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 days in CFU and TTP	S
14.4.10	EBA	Model	Parameter estimates for the final CFU model	S
14.4.11	EBA	Model	Expected model predicted EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 in CFU	14.4.9
14.4.12	EBA	Model	Expected model predicted daily EBA in CFU	S
14.4.13	EBA	Model	Individual model predicted EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 in CFU	14.4.9
14.4.14	EBA	Model	Individual model predicted daily EBA in CFU	14.4.12
14.4.15	EBA	Model	Parameter estimates for the final TTP model	14.4.10
14.4.16	EBA	Model	Expected model predicted EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 in TTP	14.4.9
14.4.17	EBA	Model	Expected model predicted daily EBA in TTP	14.4.12
14.4.18	EBA	Model	Individual model predicted EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 in TTP	14.4.9
14.4.19	EBA	Model	Individual model predicted daily EBA in TTP	14.4.12
14.4.20	EBA	Model	Overview of the analysis dataset per treatment arm	S

14.4 EBA General Figures					
Figure number	**Population**	**Biomarker**	**Objective**	**Table Title**	**Shell References to Shell**
14.4.1	EBA	CFU	Outlier	Log 10 CFU Cleveland dotplots	S
14.4.2	EBA	CFU	Outlier	Log 10 CFU Repetition 1 vs Repetition 2	S
14.4.3	EBA	CFU	Outlier	Individual Mean CFU versus mean TTP	S
14.4.4	EBA	CFU and TTP	Outlier	Individual Log 10 CFU and DVTTP over Time per subject(to be added)	S
14.4.5	EBA	CFU	Distribution	Distribution of log10CFU for HRZE and RESP301 over 14 days	S
14.4.6	EBA	CFU	Distribution	Distribution of log10CFU for HRZE over time	14.4.5
14.4.7	EBA	CFU	Distribution	Distribution of log10CFU for RESP301 over time	14.4.5
14.4.8	EBA	CFU	Trend	Log 10 CFU versus time stratified for different regimens	S
14.4.9	EBA	CFU	Trend	Individual Log 10 CFU and CFU versus time for different regimens	14.4.4
14.4.10	EBA	CFU	Multiple Colinearity(baseline)	Correlation Matrix of Continuous Variables at baseline	S
14.4.11	EBA	CFU	Multiple Colinearity(overtime)	Correlation Matrix of Continuous Variables over TIME for HRZE	14.4.10
14.4.12	EBA	CFU	Multiple Colinearity(overtime)	Correlation Matrix of Continuous Variables over TIME for RESP301	14.4.10
14.4.13	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline stratified for Sex	S

14.4.14	EBA	CFU	Multiple Colinearity(overtime)	Log 10 CFU versus time for different regimens stratified by Sex	S
14.4.15	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline versus AGE for different age groups	14.4.13
14.4.16	EBA	CFU	Multiple Colinearity(overtime)	Log 10 CFU versus time stratified for different age groups for all regimens	S
14.4.17	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline versus BMI for different BMI categories	14.4.13
14.4.18	EBA	CFU	Multiple Colinearity(overtime)	Log 10 CFU overtime versus BMI for different BMI categories and regimen	14.4.16
14.4.19	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline stratified for CAVITY	14.4.13
14.4.20	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline stratified for EXTENT	14.4.13
14.4.21	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline stratified for Sputum quality	14.4.13
14.4.22	EBA	CFU	Multiple Colinearity(overtime)	Log 10 CFU over TIME, stratified for TRT and Sputum quality	14.4.16
14.4.23	EBA	CFU	Multiple Colinearity(baseline)	Log 10 CFU at baseline vs MBGENMTBULTRAPos	14.4.13
14.4.24	EBA	TTP	Outlier	TTP Cleveland dotplots	14.4.1
14.4.25	EBA	TTP	Outlier	TTP Repetition 1 vs Repetition 2	14.4.2
14.4.26	EBA	TTP	Distribution	Distribution of TTP for HRZE and RESP301 over 14 days	14.4.5

14.4.27	EBA	TTP	Distribution	Distribution of TTP for HRZE over time	14.4.5
14.4.28	EBA	TTP	Distribution	Distribution of TTP for RESP301 over time	14.4.5
14.4.29	EBA	TTP	Trend	TTP versus time stratified for different regimens	14.4.8
14.4.30	EBA	TTP	Trend	Individual TTP versus time for different regimens	14.4.26
14.4.31	EBA	TTP	Multiple Colinearity(baseline)	Correlation Matrix of Continuous Variables at baseline	14.4.10
14.4.32	EBA	TTP	Multiple Colinearity(overtime)	Correlation Matrix of Continuous Variables over TIME for HRZE	14.4.10
14.4.33	EBA	TTP	Multiple Colinearity(overtime)	Correlation Matrix of Continuous Variables over TIME for RESP301	14.4.10
14.4.34	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline stratified for Sex	14.4.13
14.4.35	EBA	TTP	Multiple Colinearity(overtime)	TTP versus time for different regimens stratified by Sex	14.4.14
14.4.36	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline versus AGE for different age groups	14.4.13
14.4.37	EBA	TTP	Multiple Colinearity(overtime)	TTP versus time stratified for different age groups for all regimens	14.4.16
14.4.38	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline versus BMI for different BMI categoriBMIes	14.4.13
14.4.39	EBA	TTP	Multiple Colinearity(overtime)	TTP overtime versus BMI for different BMI categories and regimen	14.4.16

14.4.40	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline stratified for CAVITY	14.4.13
14.4.41	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline stratified for EXTENT	14.4.13
14.4.42	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline stratified for Sputum quality	14.4.13
14.4.43	EBA	TTP	Multiple Colinearity(overtime)	TTP over TIME, stratified for TRT and Sputum quality	14.4.16
14.4.44	EBA	TTP	Multiple Colinearity(baseline)	TTP at baseline vs MBGENMTBULTRAPos	14.4.13

12 Appendix

12.1 Table Shells

Table 14.1.1: Disposition of Participants

	All participants	RESP301tds	RESP301qd	RESP301bd
Screened				
Completed Screening Period				
Withdrawn during Screening Period				
Reason for withdrawal during screening:				
Screening Failure				
Patient withdrew consent				
: : :				
Safety Population				
Completed Study				
Withdrawn				
Reason for Withdrawal				
Patient withdrew consent				
Loss to follow-up				
: : :				

Key: RESP301tds: Inhaled RESP301 6 ml via nebulisation (NEB) three times a day (tds); RESP301qd: Inhaled RESP301 6 ml via NEB once daily (qd); RESP301bd: Inhaled RESP301 6 ml via NEB twice daily (bd); RESP301tdsHRZE: Inhaled RESP301 6 ml via NEB tds plus HRZE po qd; HRZE po qd: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg per os/orally (po) once daily (qd);

Table 14.1.2: Demographic Data
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301tdsHRZE n (%)
Gender					
Male					
Female					
Total					
Race					
Caucasian					
Black					
Coloured					
Other					
Total					

Table 14.1.2: Demographic Data
Safety Population

	All participants	RESP301tds	RESP301qd	RESP301bd	RESP301tdsHRZE
Age (yr)					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					
Weight (kg)					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					
Height (m)					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					
BMI (kg/m^2)					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					

Table 14.1.3: Medical History
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301tdsHR n (%)
Subjects With Any History					
SOC1					
Preferred term 1					
Preferred term 2					
SOC2					
Preferred term 1					
Preferred term 2					
< repeat for all SOC and Preferred Terms >					

Table 14.1.5.2: 12 Lead ECG at Baseline in Categories
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)
QT (msec)				
<= 450 msec				
> 450 msec and <= 480 msec				
> 480 msec and <= 500 msec				
> 500 msec				
Total				
< repeat for all QTcB, QTcF>				
QT (msec)				

Table 14.1.5.3: 12 Lead ECG at Baseline – Overall Interpretation
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301t n (
Normal					
Abnormal, not clinically significant					
Abnormal, clinically significant					
Total					

Table 14.1.6.1: Physical Examination at Baseline
Safety Population

Body System	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301td ZE n (%)
Body System 1					
Not Done					
Normal					
Abnormal					
Body System 2					
Not Done					
Normal					
Abnormal					
< repeat for all body systems >					

Table 14.1.7: Prior Medications
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301td n (%
Subjects With Any Prior Medication					
Class 1					
Generic term 1					
Generic term 1					
Class 2					
Generic term 1					
Generic term 1					
< repeat for all classes and Generic Terms >					

Table 14.3.1.1: Summary of Incidence of Treatment Emergent Adverse Events
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP30 n
Number of Subjects with TEAEs					
Number of Subjects with Serious TEAEs					
Number of Subjects with Fatal TEAEs					
Number of Subjects with Related TEAEs					
Number of Subjects with TEAEs Leading to Withdrawal					

Table 14.3.1.2: Incidence of Treatment Emergent Adverse Events by System Organ Class and Preferred Term
Safety Population

System Organ Class/ Preferred Term	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301 n
Subjects With Any Adverse Events					
SOC1					
Preferred term 1					
Preferred term 2					
SOC2					
Preferred term 3					
Preferred term 4					
< repeat for all SOC and Preferred Terms >					

Table 14.3.1.7.1: Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Maximum Severity
Safety Population

System Organ Class/ Preferred Term	Maximum Severity			
	Grade 1/ Mild n (%)	Grade 2/ Moderate n (%)	Grade 3/ Severe n (%)	Grade 4/Life-threatening n (%)
RESP301tds (N=xx)				
Subjects With Any Adverse Event				
SOC1				
Preferred term 1				
Preferred term 2				
SOC2				
Preferred term 3				
Preferred term 4				
< repeat for all SOC and Preferred Terms >				
< repeat for other groups>				

Table 14.3.1.7.4: Incidence of Treatment Emergent Adverse Events by System Organ Class, Preferred Term, Maximum Severity and Strongest Relationship to IMP
Safety Population

System Organ Class/ Preferred Term	Grade 1/ Mild		Grade 2/ Moderate		Grade 3/ Severe		Grade 4/Li
	Not Related n (%)	Related n (%)	Not Related n (%)	Related n (%)	Not Related n (%)	Related n (%)	Not Related n (%)
RESP301tds (N=xx)							
Subjects With Any Adverse Event							
SOC1							
Preferred term 1							
Preferred term 2							
SOC2							
Preferred term 3							
Preferred term 4							
< repeat for all SOC and Preferred Terms >							
< repeat for other groups>							

Table 14.3.1.8: Summary of Frequency of Treatment Emergent Adverse Events
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301tdsHRZ n (%)
Number of TEAEs					
Number of Serious TEAEs					
Number of Grade 1 (Mild) TEAEs					
Number of Grade 2 (Moderate) TEAEs					
Number of Grade 3 (Severe) TEAEs					
Number of Grade 4 (Life-threatening) TEAEs					
Number of Unrelated TEAEs					
Number of Possibly related TEAEs					
Number of Related TEAEs					
< repeat for other groups >					

Table 14.3.2.1.1: Clinical Safety Laboratory Tests – Haematology over time
Safety Population

	All participants	RESP301tds	RESP301qd	RESP301bd	RESP
Basophils (%)					
Baseline					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					
< repeat for all tests and days and parameters >					

Table 14.3.2.1.2: Clinical Safety Laboratory Tests – Haematology Change from Baseline over time
Safety Population

	All participants	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301tdsHR n (%)
Basophils (%)					
Day 1					
n					
Mean					
SD					
Median					
Q1-Q3					
Min- Max					
< repeat for all parameters >					

Table 14.3.2.1.3: Clinical Safety Laboratory Tests – Haematology Assessment over time
Safety Population

	All participants n (%)	RESP301tds n (%)	RESP301qd n (%)	RESP301bd n (%)	RESP301tdsHRZE n (%)
Basophils Abs (x10E9/L)					
Day 1					
High					
Normal					
Total					
< repeat for all parameters>					

Table 14.3.7: Summary of Assessment of Study Stopping and Amendment Criteria.
Safety Population

	All participants	RESP301tds	RESP301qd	RESP301bd	RESP
All Study Days					
AE Criteria					
Grade 3 or higher AEs					
Yes					
No					
Moderate (grade 2) or higher grade toxicity (CTCAE V5.0), that is probably or definitely related to IP					
Yes					
No					
Grade 3 or higher AEs (Gastrointestinal: diarrhoea, nausea, or dyspepsia; Respiratory: bronchospasm) that are at least possibly related to IP					
Yes					
No					

Table 14.4.1: Positive and non-positive data in the dataset for CFU status at the different timepoints
EBA

	All Participants		HRZE		RESP301tds		RESP301qd		
Sample status	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	R
Day -1									
Contaminated									
No Growth/Negative									
No Result									
No Done									
Positive									
Repeat for each Study Day									

Table 14.4.4: Summary statistics of baseline discrete covariates for the treatment arms
EBA

	All participants N=XX, n (%)	RESP301tds N=XX, n (%)	RESP301qd N=XX, n (%)	RESP301bd N=XX, n (%)
Discrete covariate				
Outcome 1				
Outcome 2				
Outcome 3				
< repeat for all days and covariates>				

Table 14.4.5: Summary statistics of continuous covariates for the treatment arms at baseline
EBA

	All participants	RESP301tds	RESP301qd	RESP301bd	RESP
Continuous covariate					
n					
Mean (SD)					
Median (IQR)					
Min-Max					
< repeat for all days and covariates>					

Table 14.4.9: Median and interquartile range of observed EBA for 0-2, 0-7, 0-14 days, 0-15 and 14-15 days in CFU and TTP
EBA

Treatment arm	All participants	RESP301tds	RESP301qd	RESP301bd
CFU log10 CFU/mL				
0-2				
Median (IQR)				
Repeat for study day differences and TTP (h)				

Table 14.4.10: Parameter estimates for the final CFU model
EBA

Parameter	Estimate	RSE(%)	Shrinkage

12.2 Graphical exploration of early bactericidal activity data

Examples of graphical analysis plots are shown below.

Figure 14.4.8: Log 10 CFU versus time stratified for different regimens. Solid black line is the median and red dashed lines from top to bottom are 5th and 95th percentiles of the observed data.

Figure 14.4.4: Individual log 10 CFU versus time for the HRZE treatment group. The lines connect the observations. The numbers represent the unique subject identification number in the analysis dataset.

Figure 14.4.26: Individual TTP versus time for the HRZE treatment group. The lines connect the observations. The numbers represent the unique subject identification number in the analysis dataset.

Figure 14.4.10: Correlation matrix for all continuous covariates. Scatterplot matrix with a loess smooth on the lower triangle. The diagonal illustrates the distribution for each covariate. Upper triangle displays the overall and per sex Pearson correlation coefficients, for all combinations of covariates.

Figure 14.4.13: Log 10 CFU at baseline stratified for sex.

Figure 14.4.14: Log 10 CFU versus time. Solid black and red lines are the median for males and females, respectively.

Figure 14.4.16: Log 10 CFU versus time stratified for different age groups.

Figure 6: Log 10 TTP at baseline versus BMI. Add info about what the different lines are etc.

13 References

1. ICH Topic E9, Step 5 Note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96). Final approval by CPMP: March 1998 / Date for coming into operation: September 1998

2. Protocol for RESP301-EBA Phase 2 RESP301-EBA_Protocol_V1.0 Dated 31 March 2023 Unapproved.

3. Boeree, M. J., Diacon, A. H., Dawson, R., Narunsky, K., du Bois, J., Venter, A., Phillips, P. P., Gillespie, S. H., McHugh, T. D., Hoelscher, M., Heinrich, N., Rehal, S., van Soolingen, D., van Ingen, J., Magis-Escurra, C., Burger, D., Plemper van Balen, G., Aarnoutse, R. E., & PanACEA Consortium (2015). A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. American journal of respiratory and critical care medicine, 191(9), 1058–1065. https://doi.org/10.1164/rccm.201407-1264OC