CONFIDENTIAL

Protocol Title:

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

Protocol Number: RESP30X-EBA
Protocol Version: 1.0
Protocol Date: 25 May 2023

Version History:

Version Number _ Date	Incorporates
v1.0 _ 06 April 2023	Initial
v1.0 _ 25 May 2023	Administrative change – PI details

Sponsor Protocol Signature Page

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Sponsor: Thirty Respiratory Limited (30 Respiratory), 1 Red Place W1K 6PL, London, United Kingdom

I agree to the terms of this study protocol.

Principal Investigator Protocol Signature Page

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Principal Investigator:

I hereby confirm that I have read the above protocol and agree to conduct this clinical study as outlined in the above protocol. I will work in accordance with the ethical principles that have their origin in the Declaration of Helsinki and with the principles of Good Clinical Practice and local regulations. I will provide copies of the protocol and access to all the information required to conduct the clinical study according to the above protocol to the site personnel under my supervision. I will discuss this material with them and ensure they are fully informed on all study requirements.

Development Phase:	2
Funding by:	Thirty Respiratory Limited (30 Respiratory)
Sponsor:	Thirty Respiratory Limited (30 Respiratory) 1 Red Place W1K 6PL London United Kingdom
Sponsor Representative(s):	[redacted] [redacted] Thirty Respiratory Limited 1 Red Place London, W1K 6PL
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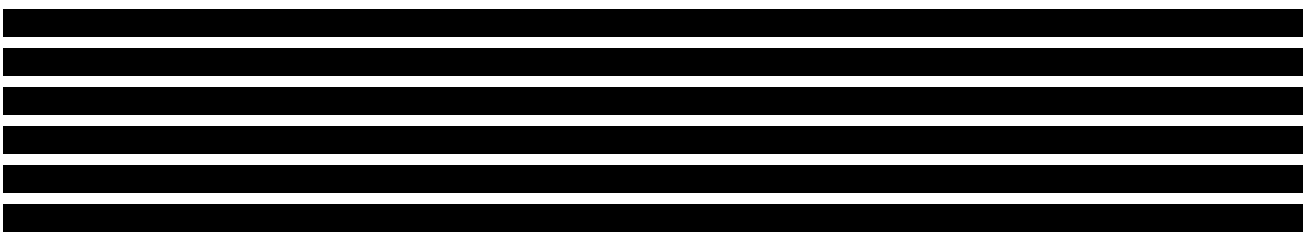

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List of Abbreviations and Definitions of Terms

AE	Adverse Event
AFB	acid-fast bacteria
ART	Antiretroviral Therapy
ALP	alkaline phosphatase
ALT	Alanine Aminotransferase
API	Active Pharmaceutical Ingredient
ASL	Airway Surface Liquid
AST	Aspartate Aminotransferase
AUC	Area Under the Plasma Concentration Time Curve
bd	Twice daily
BMI	Body Mass Index
CF	Cystic Fibrosis
CFU	Colony Forming Units
COPD	Chronic Obstructive Pulmonary Disease
(e)CRF	(electronic)Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
(PA) CXR	(Posteroanterior) Chest X-ray
DBP	Diastolic Blood Pressure
DR	Drug Resistant
DS	Drug Sensitive
EBA	Early Bactericidal Activity
EC	Ethics Committee
EWD	Early Withdrawal
FEV1	Forced Expiratory Volume in the first second
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl transferase
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
HRT	Hormone Replacement Therapy
HRZE	Isoniazid/Rifampicin/Pyrazinamide/Ethambutol
IB	Investigator Brochure
ICF	Informed Consent Form
IF	Incidental Findings
IMP	Investigational Medicinal Product
ISF	Investigational Site File
IV	Intravenous
IUATLD	International Union Against Tuberculosis and Lung Disease
MDR	Multi Drug Resistant
MGIT	Mycobacterial Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
M.tb	*Mycobacterium Tuberculosis*
NTM	Non tuberculosis mycobacteria

NEB	Nebulisation
NO	Nitric oxide
NO_2	Nitrogen dioxide
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OECD	Organisation for Economic Co-operation and Development
OCP	Oral Contraceptive Pill
PI	Principal Investigator
po	per os/orally
PoC	proof of concept
qd	once daily
QTcF	QT interval corrected with the Fridericia formula
PTB	Pulmonary tuberculosis
RR	Rifampicin resistant
SAE	Serious Adverse Event
SABA	short-acting beta agonist
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SIFT-MS	Selected Ion Flow Tube Mass Spectrometry
SOC	System Organ Class
SpMet	Methaemoglobin saturation
t	Time
TASK	TASK Applied Science (Pty) Ltd
TB	Tuberculosis
tds	three times a day
TEAEs	Treatment-Emergent Adverse Events
TMF	Trial Master File
TTP	Time to Sputum Culture Positivity
ULN	Upper Limit of Normal
WHO	World Health Organization
WOCBP	Women of Child-Bearing Potential
WONCBP	Women of Non Child-Bearing Potential
XDR	Extensively Drug Resistant

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. Protocol Synopsis

1.1. Synopsis

Sponsor:	Thirty Respiratory Limited (30 Respiratory) 1 Red Place W1K 6PL London United Kingdom
Funder:	Thirty Respiratory Limited (30 Respiratory)
Study Drug:	RESP301
Protocol Number	RESP30X-EBA
Protocol Title:	A Phase 2 Study to Evaluate the Early Bactericidal Activity, Safety and Tolerability of Nebulised RESP301 in Adults with Newly Diagnosed, Rifampicin Susceptible Pulmonary Tuberculosis
Treatment Indication:	Rifampicin Susceptible Pulmonary Tuberculosis
Test Product, Dose and Mode of Administration:	The Investigational Medicinal Product (IMP) will be supplied as RESP301 6 mL solution, packaged in two differing 3.0 mL vials: • Active vial: Sodium nitrite [redacted] mannitol [redacted] solution • Diluent vial: Citric acid [redacted] These precursor solutions are mixed at point of use for inhaled administration via nebulisation (NEB), resulting in the release of Nitric oxide (NO) throughout the respiratory system, from the upper airways to deep into the lungs. Assuming a 100% conversion rate, theoretically [redacted] of NO is delivered. However due to intra- and inter-patient variations, the delivered dose is estimated to be ~40%-60% of this dose (section 3.2). Dosing is once daily (qd), twice daily (bd) or three times a day (tds) dependent on the study treatment arm, for 14 days.
Control Product Dose and Mode of Administration:	The Control Product will be supplied as isoniazid 75 mg (H), rifampicin 150 mg (R), pyrazinamide 400 mg (Z), ethambutol 275 mg (E) (i.e., HRZE) combination tablets. Dosing is qd for fourteen days based on participants body weight rounded up to the nearest whole number: • 38 to 54 kg: 3 tablets • 55 to 70 kg: 4 tablets • 71 kg and over: 5 tablets The participants' screening weight will be used to determine the HRZE dose and this dose will be administered for the duration of the study.
Primary Study Objectives: Activity	Stage 1: To determine the Early Bactericidal Activity (EBA) of tds dosing of inhaled RESP301 6 mL dosed via NEB over 14 days of treatment. Stage 2: To determine the EBA of inhaled RESP301 6 mL dosed via NEB administered qd, bd alone, or tds in combination with HRZE, over 14 days of treatment to optimise the dosing schedule.
Study Design:	The study is a single-centre, open-label, parallel arm, randomised clinical study 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 approximately 15 participants will be recruited per treatment arm (total of approximately 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 1.

Table 1: Stage 1 Treatment Arms

Treatment Arm	Treatment	Approximate Number of participants
1 (Active):	Inhaled RESP301 6mL via NEB tds	15
2 (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		

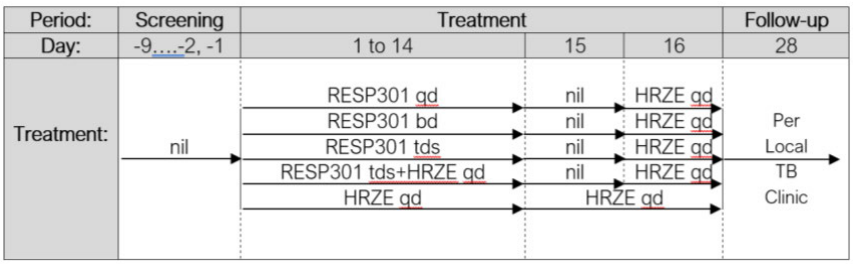
On completion of Stage 1, recruitment will be paused and an interim analysis performed. Dependent on these results a Steering Committee will determine whether the study should proceed to Stage 2.

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

Table 2: Stage 2 Treatment Arms

Treatment Arm	Treatment	Approximate Number of participants
2 (Control):	HRZE po qd	10
3 (Active):	Inhaled RESP301 6 mL via NEB qd	15
4 (Active):	Inhaled RESP301 6 mL via NEB bd	15
5 (Active):	Inhaled RESP301 6 mL via NEB tds plus HRZE* po qd	15
HRZE: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg. NEB: nebulisation, bd: twice daily, po: per os/orally, qd: once daily, tds: three times a day		

The per participant involvement in the study is illustrated in Figure 1.

	Figure 1: Trial schematic  HRZE: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg. NEB: nebulisation, bd: twice daily, po: per os/orally, qd: once daily, tds: three times a day The participant will be admitted to the EBA unit during screening and discharged on day 16 of the study. During the treatment period daily study "visits" will occur where study assessments are performed, unless the participant is withdrawn early from the study. The follow-up visit will occur for all participants (completed treatment or early withdrawals). On discharge from the EBA unit, participants will be referred to the local community TB clinics to continue standard anti-tuberculosis chemotherapy according to national guidelines. The participants will be provided with a referral letter and sufficient TB medication to cover the time from attending their last visit at the EBA unit until their scheduled visit at the TB clinic. This will be documented in the participants source documents.
Patient Population:	A total of approximately 75 male and female participants, aged between 18 and 65, inclusive, with sputum positive newly diagnosed, rifampicin susceptible pulmonary tuberculosis (PTB), will be enrolled onto the study. Approximately 20 participants will be enrolled in Stage 1 and 55 participants enrolled in Stage 2. Participants who withdraw (section 4.4) may be replaced. Participants will be enrolled at the TASK centre in South Africa.
Eligibility Criteria	<u>Inclusion Criteria</u> Participants are required to meet all the following criteria to be randomized. 1. Provide written, informed consent prior to all study-related procedures and agree to undergo all study procedures. 2. Male or female, aged between 18 and 65 years, inclusive. 3. Body weight (in light clothing and with no shoes) between 40 and 90 kg, inclusive. 4. Newly diagnosed pulmonary TB. 5. Either GeneXpert *Mycobacterium tuberculosis (M.tb)* positive with a quantitative readout of medium or high, OR acid-fast bacteria (AFB) sputum smear positive with a grade of at least 1+ on the IUATLD/WHO scale (Appendix 3:), on at least one pre-treatment sputum sample.

	6. Rifampicin susceptible pulmonary TB as determined by molecular testing. 7. Ability to produce an adequate volume of sputum as estimated from a pre-treatment overnight sputum collection sample (estimated 10 mL or more). 8. A chest X-ray taken during the screening period or up to 2 weeks before screening which, in the opinion of the investigator, is consistent with pulmonary TB. 9. Spirometry performed during screening with a Forced Expiration Volume in the first second (FEV1) of ≥ 40%. 10. Be of non-childbearing potential or willing to use effective methods of contraception, as defined in section 4.3.4. <u>Exclusion Criteria</u> Participants will be excluded from participation if they fulfil any of the following criteria. Medical History: 1. Evidence of clinically significant conditions or findings, other than TB, that might compromise safety or the interpretation of study endpoints, per discretion of the investigator. 2. Clinical evidence of extra-thoracic TB, that might compromise safety or the interpretation of study endpoints, per discretion of the investigator. 3. History of allergy to any of the study IMP as confirmed by the clinical judgement of the investigator. 4. Alcohol or drug abuse, that in the opinion of the investigator, is sufficient to compromise the safety or cooperation of the participant. 5. HIV positive AND: (a) CD4 < 350 cells/mm3 (b) <u>OR</u> are receiving antiviral therapy (ART). 6. Methaemoglobin saturation (SpMet) >3%. 7. Female participant who is pregnant or breast-feeding. 8. Participants planning to conceive a child within the anticipated period of study participation and for at least 90 days after the last dose of IMP in the study. Treatment History 9. Participation in other clinical studies with investigational agents within 8 weeks prior to screening. 10. Treatment received for this episode of TB with any drug active against *M.tb* [fluoroquinolones (including moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin), bedaquiline, pretomanid, delamanid, ethionamide, isoniazid, ethambutol, amikacin, cycloserine, rifabutin, rifampicin, streptomycin,

	kanamycin, pyrazinamide, para-aminosalicylic acid, rifapentine, thioacetazone, capreomycin, thioamides]. 11. Treatment with immunosuppressive medications such as TNF-alpha inhibitors within 2 weeks prior to screening, or systemic corticosteroids for more than 7 days within 2 weeks prior to screening. 12. Treatment with nitric oxide and other nitric oxide donor agents (e.g., prilocaine, sodium nitroprusside and nitro-glycerine), phosphodiesterase inhibitors (e.g., sildenafil) and lung surfactant drugs (e.g., beractant, calfactant, poractant), within 30 days prior to screening. Laboratory Safety Testing 13. Participants with the following toxicities at screening as defined by the enhanced Common Terminology Criteria for Adverse Events (CTCAE) toxicity table version 5.0, 27Nov2017 (14. Appendix ***1***) (a) creatinine >1.5 times upper limit of normal (ULN) (b) haemoglobin <8.0 g/dL (c) platelets <50x10^9 cells/L (d) serum potassium <3.0 mmol/L (e) aspartate aminotransferase (AST) >3 x ULN (f) alanine aminotransferase (ALT) >3 x ULN (g) alkaline phosphatase (ALP) ≥ 2.5 x ULN (h) total bilirubin >1.5 x ULN (i) total white cell count <2 cells x 10^9/L. NOTE: Investigations may be repeated once during screening if the initial result is unfavourable.
Study Duration	Participants who complete the study will participate for a maximum of 37 days (screening period of up to 9 days, followed by a treatment period of 16 days and a follow-up visit at Day 28 from first dose of IMP). The overall duration of participants involvement in the study is planned to be approximately 1.5 years (first participant in to last participant out, including the pause between stages 1 and 2 for the interim analyses and continuation decision).
Sample Size	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 studies of this type and accounts for the possibility of up to 3 dropouts per arm which 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.

1.2. Evaluations

	Objective	Outcome Variable/s	Statistical Analysis
Primary: Activity	Stage 1 To determine the EBA of inhaled RESP301 6 mL tds dosed via NEB over 14 consecutive days. Stage 2: To determine the EBA of inhaled RESP301 6 mL dosed via NEB administered qd, bd, or tds in combination with HRZE, over 14 days of treatment to optimise the dosing schedule.	Overnight sputum collection from which viable mycobacteria in the sample is quantified: • as TTP using the Mycobacteria Growth Indicator Tube (MGIT) (BactecTM MGIT™ 960) system. The samples from participants receiving HRZE alone serve to confirm that the laboratory assays result in the expected EBA for HRZE, thereby validating the method.	The EBA_{TTP} (0-14) in the MGIT (Bactec™ MGIT™ 960) system as determined by the predicted rate of change in the TTP in sputum over the period Days 0 to 14 will be analysed using linear, bi-linear, or non-linear functions using nonlinear mixed effects modelling of TTP over time. Predicted estimates of rates of change including uncertainties for the potential differences in treatment effects between the study arms will be determined and graphically illustrated.
Secondary: Activity	Stage 1 To determine the EBA of inhaled RESP301 6 mL tds dosed via NEB over 14 consecutive days. Stage 2: To determine the EBA of inhaled RESP301 6 mL dosed via NEB administered qd, bd alone, or tds in combination with HRZE, over 14 days of treatment to optimise the dosing schedule.	Overnight sputum collection from which viable mycobacteria in the sample is quantified: • as CFU on a solid media culture. The samples from participants receiving HRZE alone serve to confirm that the laboratory assays result in the expected EBA for HRZE, thereby validating the method.	The EBA_{CFU} (0-14) as determined by the predicted rate of change in log10CFU per mL sputum over the period day 0 - 14 will be described using linear, bi-linear or non-linear functions using nonlinear mixed effects modelling as dictated by the data of log_{10}CFU over time and in relation to drug exposure. Predicted estimates of rates of change including uncertainties for the potential differences in treatment effects between the study arms will be determined and graphically illustrated.

	Objective	Outcome Variable/s	Statistical Analysis
Secondary: Safety and tolerability	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.	Measurement of: • Adverse Events • Concomitant Medication • Safety Laboratory (Haematology, Chemistry, Urinalysis) • Methaemoglobin • Physical Examinations • Vital Signs • Body Mass Index (BMI).	Incidence of Treatment Emergent Adverse Events (TEAEs) will be presented by severity, drug relatedness, seriousness, leading to early withdrawal and leading to death. Clinical laboratory measurements and vital signs will be summarized using descriptive statistics as actual values and changes from baseline. Physical examinations and concomitant medications will be listed.
Exploratory	To explore the EBA variability due to potential *M.tb* sterilisation in sputum samples as a result of NO contamination in sputum samples.	Overnight sputum collection from which viable mycobacteria in the sample is quantified as TTP using the MGIT (BactecTM MGIT™ 960) system.	Comparison of EBA_{TTP}(0-14) versus EBA_{TTP}(0-15) and the predicted EBA_{TTP}(14-15) versus observed EBA_{TTP}(14-15) analysed using linear and non-linear functions using mixed effects modelling of TTP over time and analysis of variance.

1.3. Study Flow Chart for Stages 1 and 2

Period	Screening			Treatment															FU	EWD
Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20 [A]	
Day	(-9 to -3)	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	28	
Written Informed Consent	X																				
Demography	X																				
Medical & Treatment History	X																				
Physical Examination [B, C]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs [C]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																				
IMP Administration [D], and Compliance Check				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Concomitant Medication [E]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events [F]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Eligibility Assessment [G]	X																				
EBA unit inpatient	X [H]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X [I]		
Randomisation [J]		X																			
Chest X-ray	X																				
Spot or Overnight Sputum [K, L]	X																				
Overnight Sputum [K, M]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Safety labs (Haematology, Chemistry, Urinalysis) [C]	X			X							X						X			X	X
Methaemoglobin (SpMet) [N]	X			X							X						X				
Coagulation Blood Screen	X																				
HIV Test and CD4 Count	X																				
Urine Drug Screen	X																				
β-HCG Serum Pregnancy Test [O]	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: Follow-up visit: a window of ±3 days allowed.
B: Physical examinations: Full will be complete at screening and targeted (symptom-directed) thereafter.
C: Vital signs, safety laboratory: on days where there is IMP dosing, to be performed pre-dose.
D: 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. Patient identification is part of the IMP administration process. Experimental arms will not receive IMP on Day 15, only control arm will receive HRZE.
E: Concomitant Medication: collected from 14 days prior to first dose of IMP.
F: Adverse Events: Collected from first dose of IMP to Follow-Up visit.
G: Eligibility Assessment: Continuous from screening to just prior to first IMP dose.
H: EBA unit inpatient: admission, anytime from initial screening to day -2, whichever is in the participant's best interest.
I: EBA unit inpatient: discharge.
J: Randomisation: May occur any time after eligibility has been confirmed to just prior to first IMP dose.
K: Spot or Overnight Sputum: A screening and the last positive sputum sample (if >7 days after treatment start) on *M.tb* culture will be retained by the mycobacteriology laboratory for possible future testing. These samples contain the mycobacterium genetic material only, no participant genetic material is retained.
L: Spot or Overnight Sputum: For eligibility assessments.
M: 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 1 hour after any RESP301 dose that overlaps with the overnight sample collection window where applicable. If sputum collection is paused, participants to drink a cup of water before restarting (section 5.3.2).
N: Methaemoglobin: Screening single measurement. Days 1, 8 and 14: continuous measurement for an hour ± 10 mins from start of treatment on the first dose of that day only.
O: β-HCG Serum Pregnancy Test: All female participants (section 4.3.4).

2. Introduction

2.1. Background Information

2.1.1. Tuberculosis

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M.tb*) infection, remains a concerning global health problem. TB is present in all countries and age groups. In South Africa, the estimated burden of bacteriologically confirmed pulmonary TB among people aged 15 years or older in 2017–19 was 852 cases per 100 000 population. (Moyo S, 2022 Aug) Worldwide a total of 1.6 million people died from TB in 2021, with TB the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS). Eight countries in Africa and Asia accounted for more than two thirds of the global TB cases reported in 2021, with an estimated 10.6 million people falling ill with TB worldwide. However, the COVID-19 pandemic had a damaging impact on access to TB diagnosis and treatment and the burden of TB disease. The most obvious and immediate impact was a large global drop in the reported number of people newly diagnosed with TB. Reductions in the reported number of people diagnosed with TB in 2020 and 2021 suggest that the number of people with undiagnosed and untreated TB has grown, resulting first in an increased number of TB deaths and more community transmission of infection and then, with some lag-time, increased numbers of people developing TB. The burden of drug-resistant TB (DR-TB) is also estimated to have increased between 2020 and 2021, with 450 000 new cases of rifampicin-resistant TB (RR-TB) in 2021. (WHO, 2022). Whilst official numbers have yet to be published, it is accepted in scientific circles that TB is once again the leading cause of death due to infectious disease.

Whilst drug-sensitive (DS)-TB is a treatable and curable disease, it is treated with a standard 4-month or 6-month course of four antimicrobial drugs. For this treatment to be effective and not lead to an increased likelihood of developing drug-resistance, adherence is essential. (WHO, 2022) However, the duration of treatment plus the treatment side effects often does result in poor adherence. Overall, the current long-standing first-line antituberculosis agents are relatively ineffective in controlling the TB epidemic, especially in high-burden countries.

Drug-resistant (DR)-TB also poses a major threat to the control of the TB epidemic. Strains that are resistant to one or more of the standard TB treatments have been documented in every country surveyed by the WHO, who note that multidrug resistant (MDR)-TB, resistance to rifampicin and isoniazid, remains a public health crisis and a health security threat. Globally, there were an estimated 450 000 incident cases of MDR/Rifampicin resistant (RR)-TB in 2021, up 3.1% from 437 000 in 2020. An estimated 191 000 deaths occurred due to MDR/RR-TB in 2021. Globally in 2021, the estimated proportion of MDR/RR-TB cases with even more extensive resistance, was 20%. (WHO, 2022)

Significant progress has been made in recent years in identifying more efficacious, safer medicines and shorter treatment regimens. However current treatment regimens for MDR/RR-TB patients are far from satisfactory. Compared with treatments for DS-TB, these regimens require a longer course of treatment (minimum 6 months), a higher pill burden, and the use of medicines with a higher toxicity profile. In addition, patients may develop significant adverse events and have poorer treatment outcomes. Globally, although treatment success rates have increased, almost 15% of patients with MDR/RR-TB or more advanced forms of DR-TB die from the disease. (WHO, 2022).

2.1.2. Early Bactericidal Activity Clinical Studies

Achieving clinical proof of concept (PoC) for a new drug represents a major achievement and triggers significant investment into its further clinical development. It also results in parallel optimisation of production of the active pharmaceutical ingredient (API) and drug product. In TB, the assessment of the early bactericidal activity (EBA) of antituberculosis agents in sputum over the first few days up to 2 weeks of treatment is the established method for the early clinical evaluation of new antituberculosis agents and regimens. (EMA, 2017) (FDA, 2022) (Jindani A, 1980) The primary endpoint of EBA studies is the daily rate of change of colony forming units (CFU) of *M.tb* in sputum (measured as $\log_{10}$CFU/mL sputum/day) or the prolongation of time to positivity (TTP) in liquid culture (measured in hours/day) in patients with newly diagnosed smear-positive pulmonary TB. (Diacon AH, 2014) The latter provides an indication whether a novel treatment has antituberculosis properties in humans and gives an early indication of the dosage required. Pharmacokinetic analyses offer the opportunity to study the relationship between dose, drug concentrations and between-participant variabilities due to covariates such as age, bodyweight, renal function etc. Preliminary safety and tolerability data can also be collected for the duration of the treatment. (Diacon A, 2011; Donald PR, 2003)

2.2. RESP301

Refer to the Investigator Brochure (IB) for full information.

2.2.1. Mechanism of Action

Nitric Oxide (NO) is produced by many cells in the human body and is an important physiological first line of defence against a range of pathogens including bacteria, viruses, fungi, and yeasts. (Fang FC, 1997) (Schairer DO, 2012) (Kao YJ, 2001) The physiological role of NO and the enzymatic pathway for its synthesis via NO synthase have been clearly established for many years. (Moncada S, 1999 Apr) An alternative non-enzymatic synthetic pathway for NO synthesis is used in the human entero-salivary system and involves the acidification of dietary nitrates and nitrites to form nitric oxide in the stomach.

RESP301 is a nitric oxide generating solution.

As a powerful antimicrobial agent, NO has both a direct antimicrobial action and an important role in initiating the innate immune response to infection (Bogdan C, 2015) in the lungs. (Bogdan C, 2001) The mode of action is determined by NO concentration, cell type, and local context.

At high concentrations in the micromolar range (≥1 μM) and above, NO:

- directly impairs bacterial enzyme activity by irreversibly binding iron sulphur cluster residues and inhibit cellular respiration by binding heme groups within cytochrome oxidases. (Wink DA, 1998) (Thomas DD, 2008) (Radi R, 2018)
- reacts with oxygen and superoxide spontaneously to generate further species that can cause protein damage, through thiol and tyrosine nitrosylation, DNA damage through base deamination, and damage to membranes and lipid structures through lipid peroxidation. (Hogg N, 1999) (Toledo JC, 2012)

At low concentrations in the nanomolar range (≤1 μM), NO regulates and can promote the growth and activity of immune cells by e.g., guanylate cyclase activation. (Tripathi P, 2007) (Schairer DO, 2012)

NO is thus an integral, highly conserved, part of the host immune response, with very limited evidence of bacterial resistance to its antimicrobial effect. (Andreakis N, 2011) (Joerink M, 2006) (Privett BJ, 2012)

Inhaled Nitric Oxide gas is already marketed as a therapeutic agent in the US (INOmax) where it is indicated to improve oxygenation in neonates with hypoxic respiratory failure. (FDA, 2015)

[redacted]

2.2.2.Non-clinical studies

Nonclinical studies to assess the pharmacological, pharmacokinetic, and toxicological properties for sodium nitrite, which is the drug substance in RESP301 [redacted]

In addition to the information available in the public domain, the sponsor has performed several supportive non-GLP nonclinical studies. Both these supportive sources are summarised below.

[redacted] 4

Please refer to the RESP301 IB for full details.

2.2.2.1. Primary Pharmacodynamics

[redacted]

[redacted]

2.2.2.2. Anti-microbial activity

Mycobacteria are divided into two major groups: *M.tb* species and non-tuberculous mycobacteria (NTM), which comprise all the other mycobacteria species that do not cause tuberculosis. Since many mycobacterium species are known to cause pulmonary disease and multi-drug resistance is common, RESP301 has been tested against a range of *M. tuberculosis* and NTM species (including *M. abscessus* and *M. avium*). Co-infection with *M. abscessus* and *Pseudomonas aeruginosa* is frequently encountered in patients with cystic fibrosis, therefore RESP301 has also been tested against Pseudomonas species.

Anti-bacterial effects of NO have also been widely demonstrated in the literature against a wide range of common pathogens including Mycobacteria, *Pseudomonas*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Klebsiella* species listed in Table 3. (Cutruzzolà F, 2016) Antimycobacterial activity, in particular, is affected by direct NO action and the generation of NO in macrophages plays a key role in intracellular mycobacterial killing. (Jamaati HE, 2017) Consistent with this, administration of intermittent high dose gaseous NO (Bentur L, 2020) (Bogdanovski K, 2020) (Yaacoby-Bianu K, 2018) in cystic fibrosis (CF) patients with pulmonary NTM infection resulted in decreased *M. abscessus* lung infection load, improved airway function and resulted in improved quality of life.

Table 3: Publications Demonstrating Nitric Oxide Anti-Bacterial Activity

Finding/s	Publication
NO has been shown to be effective against a wide range of bacterial pathogens, including clinical isolates of *Staphylococcus aureus*, methicillin-resistant *S. aureus, Escherichia coli*, Group B *Streptococcus, Pseudomonas aeruginosa, M. abscessus.*	(Ghaffari A., 2006) (Schairer DO, 2012) (Ghaffari A, 2018)
NO exerts anti-bacterial activity against a wide range of common pathogens including Mycobacteria.	(Chan ED, 2001)
NO exerts antimicrobial effects in multiple ways depending on its concentration. For bacterial killing NO levels of 1.25-5 micromolar have been reported to be necessary	(Schairer DO, 2012)
A wide range of concentrations of NO from sub-nanomolar to micromolar (over a 1000-fold different concentration range) mediates different physiological/biological responses which depend both on NO concentration and kinetic release profile . At low concentrations, as generated by certain types of host cells for periods of time, NO acts as a signalling molecule on immune cells, promoting their growth and activity.	(Tripathi P, 2007) (Wink DA, 1998) (Thomas DD, 2008)

Finding/s	Publication
At high concentrations, such as during the respiratory burst of a neutrophil, NO covalently binds DNA, proteins, and lipids, thereby inhibiting or killing target pathogens. Thus, the body's NO-generating capacity performs at two levels, with either low concentrations over a period of time, or a short burst of high-volume NO. Both are important for the antimicrobial defence function. At concentrations greater than 1 µM, reactive nitrogen oxide species cause oxidative and nitrosative damage by altering DNA, inhibiting enzyme function, and inducing lipid peroxidation.	(Wink DA, 1998)
Few bacteria can escape the antimicrobial effect of NO.	(Andreakis N, 2011) (Joerink M, 2006) (Privett BJ, 2012)

NO: Nitric Oxide

The nonclinical *in vitro* and *in vivo* studies summarised in Table 4 have been performed to demonstrate the effects of acidified nitrite formulation RESP301 on a variety of respiratory pathogens, with reference to its action against *M. abscessus* in the context of CF.

Table 4: Summary of Main Nonclinical Microbiology Studies conducted with RESP301

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Study	RESP301	Agent	Result
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]			

These studies established that therapeutic doses of RESP301 demonstrate profound *in vitro* and *in vivo* activity against *M. abscessus*:

- [redacted]

The nonclinical *M.tb* studies performed are summarised in Table 5.

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted] dosing

CFU: colony forming units

2.2.2.3. Nonclinical Safety Pharmacology

The safety pharmacology of NO has been well characterised in the literature. This includes effects on the cardiovascular system, respiratory system, central nervous system and blood oxygenation (Table 6).

Table 6: Publications Demonstrating Nitric Oxide Nonclinical Pharmacology

Finding/s	Publication
<u>Cardiovascular:</u> Nitrites cause vasodilation thereby reducing BP by a cyclic guanosine monophosphate-mediated mechanism. A compensatory increase in HR has been seen in the dog with shortened PR and QT intervals. However, this occurred in a dose dependent manner and the corresponding human equivalent doses are substantially more than those proposed clinically. The morphology of the ECG as well as quantitative measurement of the corrected QT intervals and QRS complex duration in dogs were not affected. Tolerance or increased sensitivity to the effects on BP and HR was not observed.	(Bryan NS, 2015) (Rubin LJ, 2006) (Tepper J, 2014)
<u>Respiratory:</u> In dogs and rats there were no adverse respiratory effects.	(Tepper J, 2014)
<u>Central Nervous System:</u> Effects on the CNS were not identified following oral administration of sodium nitrite or inhalation of sodium nitrite or nitric oxide gas.	(Calabrese, 2007)
<u>Blood oxygenation:</u> Dogs given a single dose displayed increased respiration, HR, ECG changes and methaemoglobinaemia within 1 to 2 hours of administration. This however, occurred in a dose dependent manner.	(WHO, 1996)

BP: Blood Pressure, CNS: Central Nervous System, ECG: electrocardiogram, HR: Heart Rate

The observed effects, documented in these publications, on the cardiovascular, central nervous systems (if any) along with the potential for development of methaemoglobinaemia all occurred at doses in excess of those proposed clinically. Hence, the potential for development of methaemoglobinaemia and effects on the cardiovascular, respiratory, and central nervous systems following the proposed use of RESP301 are considered to be low.

2.2.2.4. Pharmacodynamic Interactions

There are no known pharmacodynamic interactions with RESP301.

2.2.2.5. Pharmacokinetics and Product Metabolism in Animals

The pharmacokinetic (PK) profile of sodium nitrite is based upon data from the literature.

Absorption

(Tepper J, 2014) conducted a study in the Sprague Dawley rat whereby nebulised sodium nitrite was administered by inhalation (for up to 120 minutes) at 10, 30 and 75 (female) / 90 (male) mg/kg/day for 26 weeks. Plasma nitrite Time at which C_{max} is observed (T_{max}) was observed immediately after the end of inhalation, and for the nitrate metabolite this was delayed until approximately 1-hour post-dose. Pre-dose plasma concentrations of nitrite were generally below the lower limit of quantitation of the assay in all animals on Day 1 and in vehicle-treated animals throughout the study.

Toxicokinetic assessments were carried out on study days 1, 28, 85, and 176 and no significant differences were observed in maximum observed plasma concentration (C_{max}) and area under the plasma concentration time curve (AUC) values for nitrite spanning the multiple assessments (see reference for details), which suggests that the reported values were at steady state. Plasma concentrations of nitrite decreased rapidly with a terminal half-life between 0.3 and 1.6 hours at steady state, while the half-life of the nitrate metabolite was consistently longer (between 1.4 and 11.8 hours). Similarly, C_{max} and AUC values of the nitrate metabolite were also lower in males. At 1-hour post-dose, increases in C_{max} and AUC were generally proportional to dose for both nitrite and nitrate. No accumulation of nitrite was observed, but slight accumulation of the nitrate metabolite was observed at the end of the 26-week study. Generally, C_{max} and AUC for plasma nitrite were lower in males than in females even though males received 90 mg/kg/day while females received 75 mg/kg/day. In addition, methaemoglobin levels were lower in male rats. The mechanistic basis for these possible sex differences is currently unknown.

Table 7: Mean steady state plasma nitrite toxicokinetic parameter values on Day 26 following a 2-hour inhalation administration of sodium nitrite to rats for 26 consecutive weeks.

Dose, mg/kg/d	Sex[a]	$t_{1/2}$,h	T_{max},h	C_{max}, mg/mL	$AUC_{0\text{-}24\ h}$, h-mg/mL
10	F	0.3	2	1.23	1.95
	M	MS	2	1.14	MS
30	F	1.6	2	4.32	12.4
	M	0.3	2	3.22	4.96
75	F	0.9	2.2	14.3	38.7
90	M	0.6	2	17.4	34

Abbreviations: $t_{1/2}$, half-life; T_{max}, time to C_{max} from the start of exposure; C_{max}, maximum concentration; AUC, area under the curve; F, female; M, male; MS, missing (insufficient data points for determination); TK, toxicokinetic.
[a] Three animals/sex/time point.
Reference: (Tepper J, 2014)

Refer to IB for information on other routes of administration.

Distribution
Intratracheal and intravenous administration of radiolabelled 13N-nitrite to mice and rabbits showed an even distribution throughout the soft tissues and organs within 5 minutes after injection, most of it in the form of nitrate. (Parks N, 1981) Subsequent studies have determined the apparent volume of distribution to be 93 L (64–124 L), which suggests that nitrite distributes well into tissues when compared with blood. (Kortboyer JM, 1997)

Metabolism
In man, nearly all nitrite is converted to nitrate then excreted in urine. (Hunault CC, 2009) Further metabolites of nitrite are NO and reactive oxygen species formed during the conversion of nitrite to minor metabolites.

Following inhalation of NO, the gas combines with haemoglobin, forming nitrosyl-haemoglobin (NOHb) from which, nitrites (NO_2) and nitrates (NO_3) are generated. In the presence of oxygen, rapid oxidation of NOHb into MetHb occurs; this is subsequently reduced by methaemoglobin reductase into ferrous Hb and nitrate. Following inhalation, a small amount is discharged in the oral cavity by way of the salivary glands. Nitrite is converted to N_2 gas in the stomach. Nitrate in the intestine is partly reduced to ammonia (NH3), re-absorbed in the body, and converted to urea. (European Medicines Agency, 2021)

Excretion
There is evidence to suggest that nitrites and nitrates are primarily excreted via the urine. Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for > 70 % of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration. (European Medicines Agency, 2021)

2.2.2.6. Toxicology

[redacted]) In addition, repeated dose studies in the mouse and rat along with genotoxicity, carcinogenicity and reproductive toxicology studies have been performed under the direction of the National Toxicology Program (USA). Review of the respective publications suggests that these studies were performed in accordance with Good Laboratory Practice and conducted within an Organisation for Economic Co-operation and Development (OECD) member state. For the remaining publications, it cannot be verified whether the studies cited complied with GLP regulations; however, it is assumed that the studies conducted by the investigator would have been conducted in compliance with the standards prevailing at the time.

[redacted].

These are summarised below.

Single-Dose Toxicity Studies

In the rat, inhalation of sodium nitrite at up to ~11 mg/kg (0.95 mg/L or 13.8 µM solution) for 4 hours was well tolerated with increased methaemoglobin. (OECD SIDS, 2005) The oral lethal dose at 50 percent (LD50) values for rats and mice are 180 and 215 mg/kg, respectively (Smyth HF, 1969)(OECD SIDS).

Repeat-Dose Toxicity Studies

Rats

[redacted] It was found that methaemoglobin was increased in all dose groups as a function of dose. No cumulative effect of repetitive dosing on methaemoglobin levels was observed at the doses evaluated. In the decedents, mild lung oedema, moderate congestion as well as moderate vacuolation of the vomeronasal organ was observed. Among animals surviving the full treatment period, histopathology included minimal perivascular mixed cell infiltrates of the lung in both the control and animals at the highest dose (72/101 mg/kg, the only groups examined). Based mainly on the transient increases in methaemoglobin (methaemoglobin increased in all dose groups as a function of dose, with no cumulative effect observed), a NOAEL of 18 mg/kg/day was established.

In a GLP compliant study described by Tepper et al. (Tepper J, 2014) sodium nitrite was administered with the aid of a nebuliser to Sprague Dawley rats for 26 weeks. The results showed that effects suggestive of chronic toxicity were not observed. Cyanosis and methaemoglobinaemia were observed primarily at the high dose. Lethality occurred in several of the high-dose rats (75/90 mg/kg) and was considered subsequent to acute hypoxia secondary to methaemoglobin levels greater than 50%. Other than acute methemoglobinemia, few significant findings were observed after 26 exposures. Given this data, the toxicity appears primarily due to methemoglobinemia; and therefore, the inhaled NOAEL, after 26 weeks of dosing, was considered to be 30 mg/kg/d in rats. It should however be noted that no adverse respiratory tract findings occurred up to 75/90 mg/kg/day where the corresponding human equivalent doses are at least 4-fold higher than that proposed clinically.

Dogs

Nebulised sodium nitrite (acidified with citric acid; pH 5.5) was administered to dogs for 7 days with a NOAEL of 12 mg/kg/day (Aries Pharmaceuticals, 2009). No remarkable clinical observations were noted over the 7-day period. Methaemoglobin levels in blood were minimally above basal levels at 12 mg/kg/day and appreciably at 54 mg/kg/day. Gross necropsies, including histopathology of the lungs, of the treated groups were similar to controls. Inhalation of sodium nitrite up to 54 mg/kg/day produced only mild and/or transient changes in methaemoglobin; therefore, for this 7-day study, a NOAEL of 12 mg/kg/day was established.

Tepper and colleagues (Tepper J, 2014) makes brief mention of a 28-day inhalation toxicity study using dogs restrained in slings for up to 4 hours. While mortality was observed at very high methaemoglobin levels (data not presented), no evidence of pulmonary toxicity was observed. The inhalation procedure was deemed too stressful to the dogs to be utilized for a study of 26 weeks duration.

Further information on the above repeat dose toxicity studies, plus on studies of other routes of administration, are available in the IB.

Genotoxicity

In vitro studies

During a screening assay for a series of food additives, the potential for sodium nitrite was evaluated in the Ames test (at up to 5000 µg/per plate) using the *S.Typhimurium* tester strains TA92, TA94, TA100, TA1535 and TA1537 with and without rat liver microsome fraction (S9) (Ishidate M, 1984). No significant (<2-fold) increase in the number of revertant colonies was observed in treated plates when compared to controls; hence, sodium nitrite was not considered to be mutagenic.

In a separate study, sodium nitrite at 100 to 10,000 µg/plate was evaluated and found to be mutagenic in the *S. typhimurium* strain TA100, with and without induced hamster and rat liver S9 enzymes but not mutagenic in strain TA98 (National Toxicology Program, 2001).

In vivo studies
In a rat micronucleus assay, sodium nitrite was administered by intraperitoneal injection at 6.25 to 200 mg/kg to males three times at 24-hour intervals; on the basis of the observed trend towards to a small increase in the frequency of micronucleated polychromatic erythrocytes that was observed during this initial study, sodium nitrite was considered to be mutagenic. (National Institutes of Health, 2001) However, results of a subsequent rat bone marrow micronucleus test, demonstrated that sodium nitrite was not mutagenic in vivo at up to 50 mg/kg. A similar study in male mice also showed that doses of 7.81 to 250 mg/kg were non-mutagenic. Moreover, in a separate study, no significant increase in the frequency of micronucleated normochromatic erythrocytes was observed following repeated administration of sodium nitrite via the drinking water (at 375 to 5,000 ppm) for 14 weeks.

Carcinogenicity
No evidence of carcinogenicity was reported from inhaled nitric oxide gas administered to rats at 20 ppm for 20 h/day for 2 years (0.02 mg/L) (European Medicines Agency, 2021). Based on the evidence provided, use of RESP301 during the clinical studies as proposed, does not pose a carcinogenic risk.

Reproductive and Developmental Toxicity
Taking findings from both mouse (National Toxicology Program, 2001)and rat (National Toxicity Programne, 1990) into consideration, no effects on male and female reproductive parameters were noted at human equivalent doses that are at least 4-and 6-fold higher respectively than the doses proposed clinically.

Overall, no significant effects on embryofoetal development were noted in the mouse (Shimada T, 1989), hamster, or the rat (US Food and Drug Administration, 1972); the human equivalent doses evaluated were either below or similar to those proposed clinically.

Local Tolerance

[redacted]

[redacted]

2.2.2.7. Conclusion

The nonclinical studies have demonstrated the anti-*M.tb* activity, safety and tolerability of RESP301, making it a promising candidate for TB treatment and acceptable to move into human clinical studies.

2.2.3. Clinical studies

[redacted]

2.2.3.1. RESP301 development program

[redacted]

Table 8: RESP301 Clinical Studies

Study	Status	Title	No of participants receiving at least 1 dose of RESP301	Comment
RESP301-005 (CORVIS)	Completed	Phase 2 study, entitled, "Community patients at Risk of Viral Infections including SARS-CoV-2".	39	Single ascending dose study
RESP301-003 (NOMab)	Ongoing	Phase II, open label single centre study assessing the efficacy, safety and tolerability of RESP301, a nebulized nitric oxide generating solution, in patients with *M. abscessus*-pulmonary disease	5	
RESP301-007 (PROTECT-Surg)	Ongoing	A Phase 2, Platform, Randomized Clinical Study entitled, "Preventing pulmonary complications in surgical subjects at risk of COVID-19".	79	
RESP301-002 - NOCOv2	Terminated early	An open-label, adaptive randomized, controlled multicentre study to evaluate the efficacy and safety of RESP301+SOC vs SOC in hospitalized participants with COVID-19 WHO grade 3&4.	14	Early terminated due to changes in the standard of care during the study and limited patient availability
RESP301-006 (PREVENT)	Terminated early	Phase 2/3 study, entitled, "Prevent Viral Exposure and Transmission study: a SARS-CoV-2 PEP Study"	0	Whilst approved by the Ethics Committee and Regulatory Authority, was never initiated due to lower than anticipated numbers of patients meeting the eligibility criteria

As of 30 March 2023, a total of 175 AEs have been reported in 51 patients across the RESP301 development programme, of which 60 were deemed highly probably related to RESP301. Of note 52 (87%) of these AEs were reported in RESP301-005 (CORVIS study) in patients with COPD/Bronchiectasis with cough being the most frequently reported AE in this group.

RESP301 has been generally well tolerated, but there appears to be a small sub-population of patients, notably Chronic Obstructive Pulmonary Disease (COPD) patients with airway hyperresponsiveness complicated by very poor underlying respiratory function (FEV1 less than 30% of predicted) who may be at increased risk of AEs (specifically bronchoconstriction). This appears to be rapidly reversed with short acting bronchodilators (SABA) and may be prevented by pre-dosing with a SABA, as demonstrated in the Single Ascending Dose portion of the CORVIS study. Given this, patients with a FEV1 of less than 40% prior to dosing with the IMP will be excluded from this study.

In addition:

- 19 AEs were considered probably related to RESP301
- 18 possibly related
- 21 unlikely related
- 57 were deemed not related to RESP301

AEs have been seen most frequently in the respiratory, thoracic, and mediastinal disorders System Organ Class (SOC).

A total of 9 SAEs have been reported by 6 patients in the development programme, 8 of the 9 were deemed unrelated to RESP301. The one SAE of severe bronchospasm, which was reported as a suspected unexpected serious adverse reaction (SUSAR), was deemed highly probably related/related to RESP301. The subject was a 70-year-old male with a medical history of severe chronic obstructive pulmonary disease (diagnosed from 2016, with an FEV1 30% of predicted and treated with salbutamol 100 mcg and umeclidinium 55 mcg) and type 2 diabetes (diagnosed from 2002). Post administration of RESP301 he reported chest tightness shortness of breath and was promptly treated with oxygen, salbutamol and oral prednisolone and the event resolved completely within four hours and follow up at 24 hours revealed no sequelae. The event was deemed serious as it was considered to be a medically important event. The subject also reported cough during and after nebulisation.

Table 9: Most Frequent Related Adverse and Serious Adverse Events Across RESP301 Development Program

Adverse Event / Serious Adverse Event	Treatment Related (includes Definitely, Highly Probably Related and Probably Related)
Cough (includes increased cough/dry cough, intermittent dry cough, worsening cough, slight increase in cough)	40 (23.1%, 28 mild, 11 moderate, 1 severe)
Dyspnoea and Shortness of Breath (includes increased shortness of breath, SOB on exertion, worsening SOB on exertion)	11 (6.4%, 3 mild, 8 moderate)
Chest tightness	9 (5.2%, 2 mild, 7 moderate)
Bronchospasm (and wheeze)	2 (1.7%, 1 severe, 1 moderate)
Sore throat (includes throat irritation, worsening of)	2 (1.2%, 2 mild)
SOB: Shortness of Breath	

There is no PK data available for RESP301 as human clinical studies are still ongoing at the time of writing.

[redacted]

Previous trials have shown once participants have experience with the nebuliser their tolerance improves.

For further information refer to the IB.

2.2.3.2. Inhaled Sodium Nitrite Published Literature

Inhaled sodium nitrite, formulated similarly to RESP301, has been assessed during inhalation studies in man and was well tolerated based on clinical studies obtained from published literature. (Rix PJ, 2015) (Borlaug BA, 2016) (Parakaw T, 2017). Key points are noted in Table 10 with more detail in the IB and references.

To support its use, safety reviews for both sodium nitrite and nitric oxide by the inhaled and/or oral route have been performed by regulatory agencies from the chemical, occupational and food safety industries e.g., ECHA, EFSA and NIOSH.

Table 10: Key Points* of Inhaled Nebulised Sodium Nitrite Clinical Data from Literature

Summary	Relevant Results	Reference
The pharmacokinetics, pharmacodynamics, and tolerability of nebulized sodium nitrite (AIR001) following single and repeated inhalation were assessed in three individual studies, in which a total of 82 healthy male and female subjects were treated.	Pulmonary absorption of nitrite was rapid and complete, and plasma exposure dose was proportional through the MTD dosage level of 90 mg, without accumulation following repeated inhalation. At higher dosage levels, DLTs were orthostasis (observed at 120 mg) and hypotension with tachycardia (at 176 mg), but venous methaemoglobin did not exceed 3.0 % at any time in any subject. Neither the tolerability nor pharmacokinetics of nitrite was impacted by conditions of mild hypoxia, or co-administration with sildenafil.	(Rix PJ, 2015)
Double-blind, randomized, placebo-controlled, parallel-group study designed to study the effects of nebulised inhaled sodium nitrite on cardiovascular haemodynamics at rest and during exercise in subjects with heart failure with preserved ejection fraction.	The inhaled nitrite was well tolerated. No subject developed hypotension, cough, and bronchospasm or reported headache or other adverse effects after inhalation of nitrite. No subject developed clinically meaningful methaemoglobinaemia. Nitrite (NO_2^-) levels increased to 11.1±4.9 µmol/L in subjects receiving active drug ($p<0.0001$ vs baseline). The calculated half-life of nitrite in this study was 0.72±0.74 hours.	(Borlaug BA, 2016)
Measurement of platelet aggregation, P-selectin expression, platelet-leukocyte aggregates and phosphorylated vasodilator-stimulated phosphoprotein following sodium nitrite inhalation in healthy subjects.	Nitrite administered by inhalation decreased platelet activation in healthy subjects through the sGC/cGMP pathway.	(Parakaw T, 2017)

* Key points only, see publications and IB for more information.
DLT: Dose Limiting Toxicities, MTD: Maximum Tolerated Dose

2.2.3.3. Inhaled Nitric Oxide Published Literature

The active product of RESP301, NO gas, is a licensed product used:

- in adults and neonates with pulmonary hypertension (Europen Medicines Agency, 2021).
- for the treatment of acute cyanide poisoning in IV formulation (30 mg/mL) (Hope Pharmaceuticals, 2018)
- as a food additive.

To support its use, safety reviews for both sodium nitrite and nitric oxide by the inhaled and/or oral route have been performed by regulatory agencies from the chemical, occupational and food safety industries e.g., ECHA, EFSA and NIOSH.

Inhaled NO has been assessed during inhalation studies in man and was well tolerated based on clinical studies obtained from published literature. Key points are noted in Table 11 with more detail in the IB and references.

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted] • [redacted] • [redacted] • [redacted] • [redacted]	[redacted])

2.2.3.4. Conclusion

The clinical studies have demonstrated safety and tolerability of RESP301 and, together with the nonclinical studies which demonstrate RESP301 expected anti-*M.tb* activity in humans, make RESP301 a promising candidate for TB treatment and acceptable to move into human clinical studies.

2.3. HRZE

Participants randomized to treatment with the control product will receive standard, intensive phase pulmonary TB treatment as recommended in the South African National TB Treatment Guidelines, which is HRZE (H=isoniazid: R=rifampicin Z=pyrazinamide: E=ethambutol) 75/150/400/275 mg oral daily. The HRZE group is included as a control for the EBA quantitative mycobacteriology and to evaluate whether HRZE in this population gives similar EBA results to that demonstrated in prior studies with this combination.

Please see a HRZE package insert e.g., Rifafour e-275® or other fixed dose combination, for HRZEs known and potential risks and benefits. (Sanofi-Aventis South Africa, 2018)

2.4. Known and Potential Risks and Benefits of the RESP301

The following are recognised as clinically significant potential risks of RESP301 treatment:

- [redacted]

[redacted]

[redacted]

- [redacted]

Table 12 summarises the advantage of RESP301 over inhaled NO.

Table 12: Advantages of RESP301 over inhaled NO

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]		

2.5. Overall Benefit/Risk Assessment

The totality of the nonclinical and clinical safety data, including toxicology studies, support the safety of this proposed study. The potential risks associated with RESP301 will be monitored carefully throughout the conduct of the study. The safety monitoring practices employed by this protocol (physical examinations, vital signs, SpMet, haematology, serum chemistry, urinalysis, and adverse event (AE) monitoring), shall allow early detection of any treatment-attributable AEs and minimize risks to the participants enrolled in the study.

Participants will remain under constant medical attention and will be housed and monitored in the EBA unit from admission through the duration of the treatment period; this will allow a continuous monitoring of the health conditions of each participant, any of whom can be withdrawn at any stage of the study and removed from study treatment should his/her condition suggest to the investigator that this would be in his/her best interest. Upon discharge, participants will be given the initial doses of standard TB treatment

and immediately referred to the national TB treatment program at their local TB clinic. The investigators' primary responsibility is to ensure participant safety.

3. Study Rationale and Objectives

3.1. Study Rationale

As described in section 2.1.1, TB, caused by *M.tb* infection, remains a concerning global health problem. As a powerful antimicrobial agent as demonstrated in the nonclinical studies and literature to date (section 2.2), the NO generated by RESP301, has shown anti-*M.tb* activity, safety and tolerability, making RESP301 a promising candidate for TB treatment. [redacted].

3.2. Dose Rationale

RESP301 is intended for inhaled administration via a nebuliser, [redacted]

[redacted]82 healthy volunteers following six days of administration was 90 mg every 8 hours. (Rix PJ, 2015)

RESP301 treatment will occur for 14 days as per standard EBA study format (section 2.1.2). However, overnight sputum sample collection will occur on Day 15 when no RESP301 dose is administered, to allow testing for any residual NO effect on *M.tb* in sputum samples from prior RESP301 dosing.

3.3. Study Objectives

3.3.1. Primary Objectives

Stage 1

To determine the EBA of tds dosing of inhaled RESP301 6 mL dosed via NEB over 14 days of treatment.

Stage 2

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

3.3.2. Secondary Objective

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.

3.3.3. Exploratory Objective

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

4. Study Design

4.1. Summary of Study Design

The study is a single-centre, open-label, parallel arm, randomised clinical study in two sequential stages, with no stratification. The study endpoints are primarily laboratory based and the microbiology staff will be blinded to treatment allocation. A total of approximately 15 participants will be recruited per treatment arm (total of approximately 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 1.

Table 13: Stage 1 Treatment Arms

Treatment Arm	Treatment	Approximate Number of participants
1 (Active):	Inhaled RESP301 6mL via NEB tds	15
2 (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 Steering Committee will determine whether the study should proceed to Stage 2, based on minimum activity criteria, and safety, noted in section 8.3.

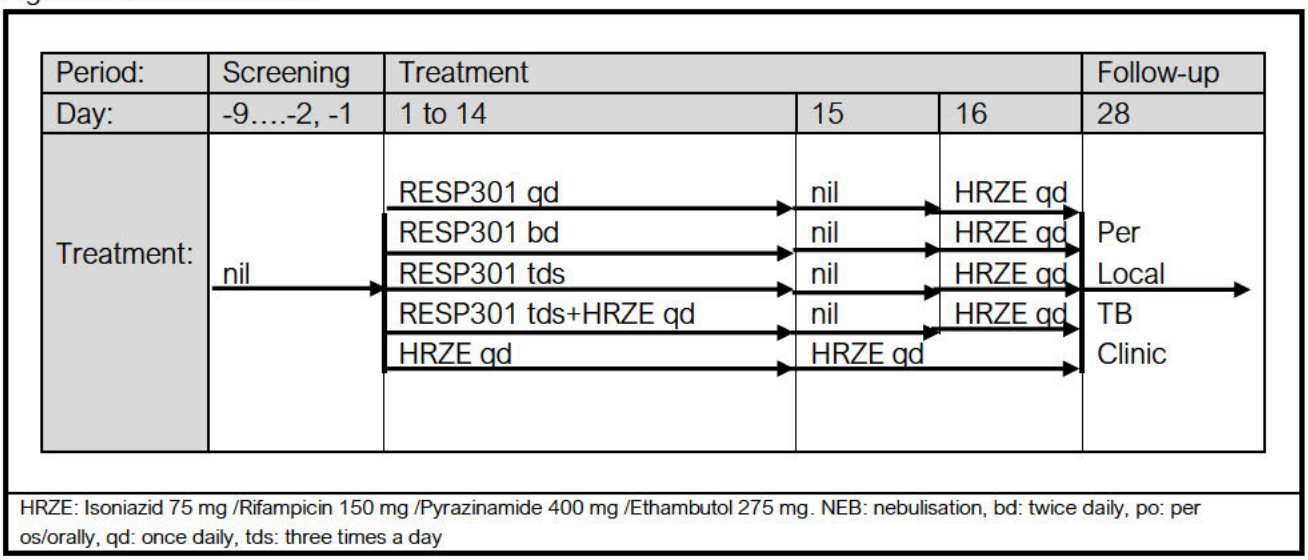
Stage 2: Treatment arms and participant numbers are described in Table 2.

Table 14: Stage 2 Treatment Arms

Treatment Arm	Treatment	Approximate Number of participants
2 (Control):	HRZE po qd	10
3 (Active):	Inhaled RESP301 6 mL via NEB qd	15
4 (Active):	Inhaled RESP301 6 mL via NEB bd	15
5 (Active)	Inhaled RESP301 6 mL via NEB tds plus HRZE* po qd	15
HRZE: Isoniazid 75 mg /Rifampicin 150 mg /Pyrazinamide 400 mg /Ethambutol 275 mg. NEB: nebulisation, bd: twice daily, po: per os/orally, qd: once daily, tds: three times a day		

The per participant involvement in the study is illustrated in Figure 1.

Figure 2: Trial schematic



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

The participant will be admitted to the EBA unit during screening and discharged on day 16 of the study. During the treatment period daily study "visits" will occur where study assessments are performed, unless the participant is withdrawn early from the study. The follow-up visit will occur for all participants (completed treatment or early withdrawals).

Before discharge from the EBA unit, participants will be referred to the local community TB clinics for standard anti-tuberculosis chemotherapy according to national guidelines. The participants will be provided with a referral letter and sufficient TB medication to cover the time from attending their last visit at the study clinic until their scheduled visit at the TB clinic. This will be documented in the participants source data.

4.2. Study Population

A total of approximately 75 male and female participants, aged between 18 and 65, inclusive, with sputum positive newly diagnosed, rifampicin susceptible PTB, will be enrolled onto the study. Approximately 20 participants will be enrolled in Stage 1 with approximately an additional 55 participants enrolled in Stage 2. Participants who withdraw (section 4.4) can be replaced.

Participants will be enrolled at a single site in South Africa: TASK, South Africa

4.2.1. Inclusion Criteria

Participants are required to meet all the following criteria to be randomized.

1. Provide written, informed consent prior to all study-related procedures and agree to undergo all study procedures.
2. Male or female, aged between 18 and 65 years, inclusive.
3. Body weight (in light clothing and with no shoes) between 40 and 90 kg, inclusive.
4. Newly diagnosed pulmonary TB.
5. [redacted]
6. Rifampicin susceptible pulmonary TB as determined by molecular testing.

7. Ability to produce an adequate volume of sputum [redacted]
8. A chest X-ray taken during the screening period or up to 2 weeks before screening which, in the opinion of the investigator, is consistent with pulmonary TB.
9. Spirometry performed during screening with a FEV1 [redacted]
10. Be of non-childbearing potential or willing to use effective methods of contraception, as defined in section 4.3.4.

4.2.2. Exclusion Criteria

Participants will be excluded from participation if they fulfil any of the following criteria.

Medical History:

1. Evidence of clinically significant conditions or findings, other than TB, that might compromise safety or the interpretation of study endpoints, per discretion of the investigator.
2. Clinical evidence of extra-thoracic TB, that might compromise safety or the interpretation of study endpoints, per discretion of the investigator.
3. History of allergy to any of the study IMP as confirmed by the clinical judgement of the investigator.
4. Alcohol or drug abuse, that in the opinion of the investigator, is sufficient to compromise the safety or cooperation of the participant.
5. HIV positive AND:
   [redacted]
6. Methaemoglobin saturation (SpMet) [redacted]
7. Female participant who is pregnant or breast-feeding.
8. Participants planning to conceive a child within the anticipated period of study participation and for at least 90 days after the last dose of IMP in the study.

Treatment History

9. Participation in other clinical studies with investigational agents within [redacted] screening.
10. Treatment received for this episode of TB with any drug active against *M.tb* [redacted]
11. Treatment with immunosuppressive medications [redacted]
12. Treatment with nitric oxide and other nitric oxide donor agents [redacted]

Laboratory Safety Testing

13. [redacted]

[redacted]

NOTE: Investigations may be repeated once during screening if the initial result is unfavourable.

4.3. Restrictions

The following restrictions apply for the study. This does not include eligibility restrictions which are self-explanatory in the inclusion/exclusion criteria (section 4.2 Study Population).

4.3.1. Foods and Beverages

HRZE tablets will be administered with a full glass of water fasted (1 hour before, or 2 hours after a meal). However, if gastrointestinal irritation occurs, the tablets may be taken with food. If aluminium containing antacids are taken, administer them one hour after the HRZE tablet/s dose.

4.3.2. Type of Pulmonary Tuberculosis

To be eligible for the study the study the participant must be:

- Newly diagnosed and have untreated pulmonary TB AND
- [redacted]
- Their *M.tb* strain must be rifampicin susceptible (molecular test) AND
- They must have the ability to produce an adequate volume of sputum [redacted]

The tests required to determine this are detailed in Table 15. [redacted] results must be available and assessed by the investigator showing eligibility prior to first dose of IMP.

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

If the first spot sputum does not show a favourable result, the tests may be repeated on freshly collected spot sputum or overnight sputum and that result used for the eligibility assessment. If results are discordant, the tests may be repeated during screening at the investigator's discretion. Overnight sputum may be collected for a number of screening days if the screening results are delayed. It will not be collected for more than the allowed pre-treatment time period.

4.3.3. Concomitant Medications and Procedures

Concomitant Medication and Procedures are defined as any medication taken/procedure performed within 14 days prior to first dose of IMP and during the study until the follow-up visit (all participants including early withdrawal/discontinuations). This includes for new and/or worsening medical conditions.

Concomitant medications should be kept to a minimum during the study. However, if necessary for the participant's welfare and are unlikely to interfere with the IMP, they may be given at the discretion of the investigator once the prescribing information has been consulted and reviewed carefully. The determinations listed in the respective contraindicated, warning, and precaution sections must be respected to prevent any potentially serious and/or life-threatening drug interactions. Drugs that may cause methaemoglobinaemia may be used with caution.

The following concomitant medications are prohibited throughout the study. Please confirm any medication you are uncertain of with the Medical Monitor.

- [redacted]

Concomitant medications and procedures are to be reported on the concomitant medication and procedures page of the electronic case report form (eCRF). For concomitant medication the reported information is to include the drug name (trade name if known, otherwise generic), treatment period, dosing regimen, route of administration, and its indication (diagnosis if known, otherwise separate symptoms). Any changes in the dosage of concomitant medication must also be reported on the concomitant medication page of the eCRF. The corresponding diagnosis should be completed as medical history (before first dose of IMP) or as an AE (at or after first dose of IMP).

4.3.4. Pregnancy and Contraception

To be included in the study the following restrictions apply:

- all participants to be of non-childbearing potential or using effective methods of birth control, as defined below and
- women of childbearing potential (WOCBP) are to receive an injectable or other contraceptive method (Table 16) prior to or during screening, and at least 2 days prior to first dose of IMP. Should a WOCBP be receiving an Oral Contraceptive Pill (OCP) it is required that this is stopped for the duration of the study, and they transition to an injectable or other allowed contraceptive method (Table 16). The site staff will educate the participant on how the

transition should occur. It is recommended that female partners of male participants who are WOCBP also follow these guidelines.
- all female participants are to have a negative β-HCG serum pregnancy test at screening (result available before first dose of IMP).

During and after the study:
Participants (female and male) should not conceive a child during IMP treatment or within 90 days after their last dose of IMP.

Women in the following categories are considered WOCBP (fertile):
- following menarche
- from the time of menarche until becoming post-menopausal unless permanently sterile (see below).

Women in the following categories are considered Woman of Non-childbearing Potential (WONCBP):
- premenopausal female with permanent infertility or permanent sterilization due to one of the following (for the purpose of this study):
- *Documented hysterectomy
- *Documented bilateral salpingectomy
- *Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), investigator discretion should be applied to determining study entry.
- postmenopausal females are defined as no menses for 12 months without an alternative medical cause.
- A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT).
- Females on HRT and whose menopausal status is in doubt must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

*Documentation can come from the site personnel's review of the participants medical records, medical examination, or medical history interview. If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of IMP, additional evaluation should be considered.

The following Highly Effective Contraceptive Methods are required for the study.

Male participants: condoms must be used in addition to recommended female partner hormonal contraception, if applicable. Male condom and female condom should not be used together (due to risk of failure from friction).
Female participants: Refer to Table 16.

Oral contraceptive pill, periodic abstinence (calendar, sympto-thermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception for this study.

4.3.5. HIV

To be included in the study the participant must be:

- HIV negative OR
- HIV positive [redacted]

HIV infection (HIV positive status) is defined as a documented result of any licensed rapid HIV test, ELISA or molecular test at any time prior to entry and confirmed by a second test using a different method. Confirmatory tests may include licensed Western blot, antibody test, or by HIV-1 antigen or plasma HIV-1 RNA viral load of >1,000 copies/mL.

Absence of HIV infection (HIV negative status) is defined as a documented result of any licensed rapid HIV test, ELISA or molecular test within 60 days prior to entry.

Participants with unknown status or known HIV status but without adequate documentation in medical records, should undergo counselling (repeat if applicable) prior to HIV testing and on receipt of the results, followed by HIV testing. Approval for this to be performed will be obtained from participants during the written informed consent process. Participants will be counselled on HIV by the PI, sub-investigator or trained counsellors.

4.4. Treatment Discontinuation and Participant Withdrawal

Participants have the right to voluntarily withdraw from the study at any time for any reason. In addition, the Investigator has the right to withdraw a participant from the study at any time.

A participant must immediately discontinue treatment, if applicable, and be prematurely withdrawn from the study for the following reasons:

- withdrawal of informed consent
- investigator considers it in the best interest of the participant that he/she be withdrawn
- methaemoglobinaemia as defined as follows:
  - [redacted]
- pregnancy
- serious adverse event (SAE) if probably or certainly (Table 21) related to the IMP
- missed doses:
  - [redacted]
    - [redacted]
    - [redacted]
  - [redacted]
    - [redacted]s
- requires a medication that is prohibited by the protocol
- at the specific request of the sponsor or termination of the study by the sponsor
- lost to follow-up
- failure to comply with protocol requirements as determined by the investigator or sponsor

Participants who withdraw from the study before they have received all days of IMP, or whose study endpoints are not evaluable, may be replaced per the discretion of the sponsor. Sample size allows for up to 3 participant withdrawals per arm before replacement is necessary. Data collected on participants who withdraw prior to the final study visit will be entered on the eCRF for all visits conducted.

Although a participant is not obliged to give his/her reason(s) for withdrawing prematurely from a study, the investigator should make a reasonable effort to ascertain the reason(s) and document it on the eCRF, while fully respecting the participants rights. If a participant requests to be withdrawn from the study, this request must be documented in the source documents and signed by the study staff.

All early withdrawal/discontinued participants will be referred to the local community TB clinics for standard anti-tuberculosis chemotherapy according to national guidelines. The participants will be provided with a referral letter and sufficient TB medication to cover the time from attending their last visit at the study clinic until their scheduled visit at the TB clinic. This will be documented in the participants source data.

Early withdrawal/discontinued participants during treatment will have an early withdrawal visit and will be requested to attend the Follow-up visit within 14 days (±3 days) after withdrawal. The exceptions to this are those who withdraw their consent to do so, and those lost to follow-up.

Lost to follow-up is defined as randomized participants who do not complete all study visits and cannot be contacted up to 7 days after Day 28 of the study. Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, minimum of 3 telephone calls, plus email contact if applicable, plus attempt to contact the participant via

their local clinic and community health workers). These contact attempts should be documented in the participant's medical record.

4.5. Study 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:

- [redacted]

[redacted]

[redacted]

4.6. End of Study

End of study for this trial is the date of the final data capture which is the date of the availability and entry into the database of the last Mycobacteriology Sputum Sample Result. This is expected to occur approximately 6 weeks after the Last Patient Last Overnight Sputum Sample collected (Day 14).

5. Investigational Medicinal Product (IMP)

5.1. Study Treatments

[redacted]

Dosing is once daily (qd), twice daily (bd) or three times a day (tds) dependent on the study treatment arm, for fourteen days.

The Control Product will be supplied as isoniazid 75 mg (H), rifampicin 150 mg (R), pyrazinamide 400 mg (Z), ethambutol 275 mg (E) (i.e., HRZE) combination tablets. Dosing is qd for fourteen days based on participants body weight at screening rounded up to the nearest whole number:

- 38 to 54 kg: 3 tablets
- 55 to 70 kg: 4 tablets
- 71 kg and over: 5 tablets

The participants' screening weight will be used to determine the HRZE dose and this dose will be administered for the duration of the study.

After completion of the 14 days of study treatment, participants on a RESP301 containing treatment arm will receive no treatment for one day (Day 15) and will begin standard HRZE od po dosing on the next day (Day 16). HRZE alone treatment arm participants will continue their HRZE treatment. Thereafter all participants will be referred to the local community TB clinics for standard anti-tuberculosis chemotherapy according to national guidelines (section 4.4).

5.2. Randomization and Blinding

5.2.1. Screening

Participants who have given written, informed consent will be screened for the study during Visits 1, 2 and 3 (days -9 to -1), and will be identified by a study generated participant identification code for anonymity (participant number).

5.2.2. Study enrolment and randomization

Randomisation by the delegated study personnel may occur once all the screening results are available and the investigator has determined that the participant is eligible for the study, until just prior to first IMP dose. On randomisation participants will be assigned a treatment number. The randomisation scheme will be generated by the study statistician/data manager. Randomisation will be conducted using permuted block randomisation.

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.

5.2.3. 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 EBA database closure and release.

5.3. IMP Administration

5.3.1. Route and Dosage

Refer to section 5.1.

RESP301

RESP301 is inhaled via nebulisation. [redacted] Participants will be trained in use of the nebuliser by the site personnel prior to using. The methodology will be detailed in the Pharmacy Manual.

HRZE

Will be administered orally.

5.3.2. Dosing Instructions

The methodology will be detailed in the Pharmacy Manual.

On the days of IMP dosing:

Inhaled RESP301 nebulisation:

- all treatment regimens:
  - each dose is to be given using freshly prepared admix solution i.e., inhalation of the RESP301 should start with [redacted]
  - on Day 1 only: a short acting beta agonist is to be administered before starting the nebulisation for each dose, within 30 minutes of RESP301 is suggested
  - inhalation of RESP301 should be completed within [redacted] of starting NEB, pauses may be taken during NEB if not tolerated. The NEB should be stopped during a pause to preserve the remaining IMP until restarted.
  - the first dose should be given in the morning
  - All dosing should occur at approximately the same time each day (window of ± 1 hour of the IMP dose/s on Day 1).
- once or twice daily:
  - When dosing overlaps with overnight sputum sample collection, sputum collection should be paused during and for [redacted] the end of the neb. A cup ([redacted]) of water should be taken after the neb is complete, and prior to continuation of overnight sputum sample to wash away any remaining RESP301 from the airway.
  - the first dose should be given at half dose (half of each vial)
- three times a day (alone or with HRZE):
  - the first 2 doses should be given at half dose (half of each vial)
  - this tds dosing will be [redacted] to prevent overnight disruption to participants and limit overlap of dosing and sputum sampling
- twice or three times a day (alone or with HRZE):
  - to ensure a dose does not affect any *M.tb* present in the overnight sputum sample, should a dose overlap with the overnight sample collection window, sputum collection is

to be paused for [redacted] completion of inhalation of the RESP301 dose. Should the participant need to expectorate sputum during this pause, this will occur into a separate container which will be destroyed. The participant is to drink a cup ([redacted] of water after the neb is complete, and prior to continuation of overnight sputum sample collection

HRZE oral dosing (alone or with RESP301):

- to occur in the morning
- preferably with a full glass of water fasted [redacted] However, if gastrointestinal irritation occurs, the tablets may be taken with food.
- if aluminium containing antacids are taken, administer one hour after the HRZE tablet/s dose.

HRZE plus RESP301 three times a day

- RESP301 tds instructions and the HRZE instructions above to apply.
- oral dose of the HRZE should be given in the morning, followed within [redacted] the start of the first (morning) dose of inhaled RESP301.

The exact dosing times (start time and end time) of each individual IMP will be recorded.

5.3.3. Participant Compliance

The IMP will be administered and compliance checked by the investigator/designated site personnel. This process will be detailed in the Pharmacy Manual. Where applicable the following checks will occur:

RESP301 nebulisation:

- the participant identity matches the dispensed vials
- the number of RESP301 vials (A and B) before and after mixing and nebulisation
- the date and time of preparation of the nebuliser solution (start and stop)
- the date and time of inhalation of the nebulisation solution (start and stop)
- confirmation the nebuliser is empty/minimal solution remains when nebulisation is complete.
- The investigators or appropriately trained delegated study personnel will remain with the participant whilst they are being nebulised to observe correct administration and monitor SpMet.

HRZE tablets:

- Participants will be checked for IMP compliance by the investigators or study personnel via the hand-and-mouth procedure. Both the hand and mouth of the participant will be checked to ensure that the participant has swallowed the IMP.

This information will be recorded in the participant's source documents and eCRF where required. All packaging will be checked to ensure complete administration.

Table 17 contains guidelines on how to manage HRZE dosing for a participant experiencing vomiting.

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]

Please consult the study medical monitor for guidance, caution should be given when considered repeat dosing. This is to be documented in source and in the eCRF.

5.3.4. Manufacturing, Packaging and Labelling

[redacted]

5.3.5. Storage

RESP301 vials will be stored within a temperature range of [redacted]r.

HRZE will be stored at or below a temperature of 25 °C, in well-closed containers, and protected from light.

All investigational products will be kept securely stored by the site pharmacist/registered dispenser in a secured temperature-controlled area with limited access to designated site personnel only. Only qualified personnel will undertake the preparation, handling and safe disposal of investigational products. Logs will be kept for drug accountability (chain of custody) and temperature.

5.4. Dispensing and Accountability

The site pharmacist/delegated dispenser will be responsible for dispensing the IMP. The IMP will be prepared separately from drugs used for other participants and studies to prevent cross-contamination. Accurate accountability and participant identification records will be kept by the site to assure that the IMP will not be dispensed to any person who is not a participant under the terms and conditions set forth in this protocol i.e., delivery to site, inventory at site, use by participant, destruction etc. The investigator/designee will immediately inform the Sponsor of any quality issues arising with respect to the IMP. The Sponsor will take whatever action is required should such a situation arise. The investigator undertakes to use the study medication only as indicated in this protocol.

5.5. Returns and Destruction

Upon completion or termination of the study, and after IMP accountability and monitoring thereof has been completed, all unused and/or partially used:

- RESP301 will be destroyed by the site according to their standard operational procedure or returned to the sponsor for use or destruction per their standard operational procedure.

[redacted]

- HRZE will be stored for future use or donated where possible.

If no supplies remain, this fact will be indicated in the drug accountability section of the final report.

6. Study Assessments

6.1. Demographic and Baseline Assessments

The demographic and baseline assessments (Table 18) will be performed at the time points described in the study flow chart. Where applicable, sample collection, handling and testing will be described in the Laboratory Manual.

[redacted]

Procedure	Details
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted].

6.2. Activity Assessments

The activity assessments (Table 19) will be performed at the time points described in the study flow chart.

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]

6.3. Safety and Tolerability Assessments

The safety and tolerability assessments (Table 20) will be performed at the time points described in the study flow chart.

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]
	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

7. Adverse Events

The investigators are responsible for eliciting adverse events by observing the participant and recording all adverse events observed or reported by the participant during the study.

7.1. Definitions

Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Examples of AE include (but are not limited to): clinically significant abnormal test findings, clinically significant symptoms and signs, clinically relevant changes in clinical examination findings, hypersensitivity, progression / worsening of pre-existing condition or underlying disease, recurrence of pre-existing condition, complication and termination of pregnancy.

The criteria for determining whether an abnormal objective test finding should be reported as an AE are follows:

- Test result is associated with accompanying symptoms
- Test result requires additional diagnostic testing or medical / surgical intervention
- Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy
- Test result is considered to be an AE by the Investigator or Sponsor

Repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test results that are determined to be errors do not require reporting as AEs.

An AE does not include the following:

- Medical or surgical procedures performed; the condition that led to the procedure may be an AE if applicable.
- Pre-existing medical condition, condition or laboratory abnormalities present or detected before the IMP first dose that do not worsen. If clinically significant these should be documented in the medical history page of the eCRF.

Serious Adverse Event (SAE)

Any untoward medical occurrence that at any dose:

- results in death
- is life threatening (any event in which the participant was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization (excludes hospitalisation in EBA unit for study purposes unless this is extended due to an AE)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is a medically important event.

Note: Medical and scientific judgment should be exercised in deciding whether an AE is a medically important event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse. A "suspected transmission of infectious agent by a medicinal product" is also considered a serious adverse event under the SAE criterion "Other medically important condition".

Adverse Drug Reaction (ADR)

An ADR is defined as a response to a medicinal product which is noxious and unintended at any dose and that is considered causally related (possible, probable, or certain) to an investigational medicinal product. A serious ADR (SADR) is an ADR which meets the serious criteria.

Unlisted (Unexpected) Adverse Event

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Expectedness is assessed by the sponsor/sponsor's delegate.

Attribution/Causality

The definitions for rating attribution/causality will be as described in Table 21.

Table 21: Adverse Events Attribution/Causality Ratings

Relatedness Rating	Definition
Not Related	An adverse event, which is not related to the use of the drug.

Relatedness Rating	Definition
Unlikely	An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s) or concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.
Possible	An adverse event, which might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s) or concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
Probable	An adverse event, which might be due to the use of the drug. The relationship in time is suggestive, e.g., suspicion strengthened by onset of symptoms associated with initiation of drug and/or improvement of symptoms when stopping drug. An alternative explanation is less likely, e.g., concomitant drug(s) or concomitant disease(s).
Certain	An adverse event, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s) or concomitant disease(s).

Severity

Severity rating is to be made per the CTCAE Toxicity Table Version 5 dated 27 November 2017 (Appendix 1). For abnormalities NOT found in the toxicity tables, the scale described in Table 22 is to be used to estimate grade of severity.

Table 22: Adverse Event Severity Ratings

Grade	Severity Rating	Definition
Grade 1	Mild	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required.
Grade 2	Moderate	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/ therapy required.
Grade 3	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.
Grade 4	Potentially Life-threatening	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

Action taken and outcome

The investigator will record the actions taken with the IMP for each AE using the descriptions in Table 23.

Table 23: Adverse Events Action taken with Investigational Medicinal Product

Action Taken	Definition
IMP withdrawn	Participant is permanently withdrawn from the IMP
IMP interrupted	IMP is temporarily withdrawn
IMP unchanged	No action is taken regarding the IMP
Unknown	It is not known what action was taken with the IMP

Action Taken	Definition
Not Applicable	The IMP treatment has been completed prior to reaction /event, or the participant has died
IMP: Investigational Medicinal Product	

The investigator will record the other actions taken, the outcome and the occurrence for each AE using the following descriptions:

Other Action Taken
- none
- medication given
- hospitalisation or prolongation of hospitalisation
- therapeutic or diagnostic procedure.

Outcome
- recovered / resolved
- recovering / resolving
- not recovered / not resolved
- recovered / resolved with sequelae
- fatal
- unknown.

Occurrence
- once
- intermittent
- continuous.

7.2. Reporting

Adverse Event (AE)

Adverse events will be collected by the investigator from the time a participant receives his/her first dose of IMP through to the final Follow up Visit. Any medical event that occurs before the first dose of IMP that is clinically significant is to be recorded as medical history, with not clinically significant events noted in the participant source documents.

Any AE (serious or non-serious) observed by the investigator or reported by the participant will be recorded on the corresponding pages of the eCRF and in the participant's medical record. The investigator will review each AE and assess its relationship to drug treatment based on all available information at the time of the completion of the case report form.

The following information will be recorded for each adverse event reported:
- diagnosis of the AE, if possible. In the case where an overall diagnosis cannot be made, each specific sign and/or symptom will be recorded as individual AEs
- date of onset
- stop date (duration) if applicable
- severity
- action taken with IMP
- other action taken

- outcome
- relationship to IMP
- occurrence
- seriousness.

Serious Adverse Events (SAE)
SAE reporting begins from the time a participant enters screening through to the final visit.

Initial Reporting
- Must be communicated by the investigator to the sponsor, study monitor and medical monitor within 24 hours of the site first being aware of the SAE, whether or not the serious event is deemed associated with the use of the drug.
- In addition, the investigator will provide a detailed SAE report form including estimates of the attribution/causality and the seriousness of the AE to the sponsor, study monitor and medical monitor and regulatory team within 24 hours of becoming aware of the SAE. The investigator will be requested to supply as much detailed information regarding the event that is available at the time of the initial contact (such as examinations carried out and laboratory results).

ANY SERIOUS ADVERSE EVENT MUST BE REPORTED IMMEDIATELY (WITHIN 24 HOURS) TO TASK AT THE FOLLOWING EMAIL ADDRESS: RESP30XSAE@task.org.za

Follow-up Reporting
The Investigator is required to submit follow-up reports to sponsor, study monitor and medical monitor within 24 hours of becoming aware of additional information.
- Additional information may include updated diagnosis, outcome, causality assessment, results of specific investigations and any new significant information that has not been previously reported.
- Anonymized copies of reports should be sent when requested as applicable, such as additional laboratory tests, consultation reports, post-mortem reports, hospital progress reports and autopsy reports.
- For submission of updated SAE report, the investigator will provide a newly completed serious adverse event form, designated as a follow-up report. This will be submitted to the study monitor, medical monitor, and regulatory team within 24 hours of the investigator receiving the information.

The sponsor/investigator/designee will inform regulatory authorities and/or EC of all SAEs in accordance with local requirements and ICH guidelines for GCP.

Clarification on reporting of Serious Adverse Events
- Death is an outcome of an AE, not an AE in itself.
- An AE/SAE may occur even if the participant was not being treated with the IMP at the occurrence of the event.
- Life-threatening means that patient is at immediate risk of death.
- Complications that occur during hospitalisations are typically AEs but can be an SAE. If a complication prolongs the hospitalisation, it is a SAE.

- Participant hospitalisation means that the participant stays overnight in the hospital. Pre-planned hospital stays or hospital stays for non-medical (e.g., social) reasons will not be considered as a SAE.
- A procedure for protocol / disease-related investigations should not be reported as SAE. Hospitalisation or prolonged hospitalisation for a complication such procedures should be reported as SAE.
- Regarding a participant who experiences persistent or significant disability/incapacity, only a persistent or significant or incapacitating disability is implied. The item refers to a substantial disruption of a person's ability to conduct normal life functions. Thus, disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma.
- Congenital anomaly / birth defect includes foetal malformations associated with spontaneous abortions or elective abortions.

Sponsor Evaluation of SAEs
The expected / unexpected status of the serious and related AE will be judged by the sponsor or its designated representative with regards to the reference document (IB).

In all reporting to outside the study team it is important to ensure participant identifiers are removed from all notes, correspondence, results and reports when communicating with the study team noted below.

7.3. Follow up of Adverse Events

All AEs will be followed until:

- Satisfactory clinical resolution or stabilization; or
- Until the end of the follow-up period; and
- Until all queries on these AEs have been resolved.

Certain long-term AEs cannot be followed until resolution within the setting of this protocol. In these cases, follow-up will be the responsibility of the treating physician/referral clinic. This will be confirmed with the Sponsor.

7.4. Post-Study Serious Adverse Events

Any new SAEs reported by the participant to the investigator that occur up to 30 days after the last scheduled contact should be reported to the sponsor on an expedited basis. Any additional SAEs beyond this period that are determined by the investigator to be possibly, probably, or likely related to the use of the IMP will be reported to the sponsor, IEC/IRB and regulatory authorities on an expedited basis as required in accordance with local requirements and ICH guidelines for GCP.

7.5. Clinical Laboratory Adverse Events

Laboratory abnormalities are not necessarily reported as AEs or SAEs. Changes in the results of the clinical laboratory assessment results which the investigator feels are clinically significant will be reported as adverse events.

It is the investigators' responsibility to review the results of all laboratory tests as they become available. This review must be documented by the investigators' dated signature on the laboratory report. For each

abnormal laboratory test result, the investigator needs to ascertain and document if this is a clinically significant change from baseline for that individual participant.

This determination, however, does not necessarily need to be made the first time an abnormal value is observed. The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests. If this laboratory value is determined by the investigator to be a clinically significant change from baseline for that participant, it is considered to be an adverse event.

7.6. Disease under Study

Symptoms of the disease under study (pulmonary TB) experienced by the participant while on the study will be assessed by the investigator. If the symptom has:

- worsened while the participant is in the study; and
- the investigator assesses it as clinically significant

it will be recorded as an adverse event. If it does not meet these criteria, it will not be recorded as an AE and will be noted in the participant's source documentation. All TB related symptoms that meet SAE criteria will be recorded and reported as a SAE.

7.7. Overdose

Overdose of IMP is defined as a dose exceeding the amount allocated within a one-day dosing interval. Overdose of IMP experienced by the participant while on the study, will be assessed by the investigator to determine whether the overdose led to an adverse event, including if the taking of the suspect medicine led to suicidal intention and subsequent overdose of the suspect medicine, or other medication. In this case it will be recorded as an adverse event. If it does not lead to an adverse event, it will not be recorded as an AE and this will be noted in the participant's source documentation.

7.8. Drug Interaction

If the investigator becomes aware that the participant has experienced a drug interaction which has resulted in an adverse event, it will be recorded as an adverse event.

7.9. Pregnancy

If pregnancy is suspected while the participant is receiving IMP, the IMP will be withheld immediately and a pregnancy test performed. If pregnancy is confirmed, the IMP will be permanently discontinued in an appropriate manner and the participant withdrawn from the study. Protocol-required procedures for study discontinuation and follow-up will be performed unless contraindicated by the pregnancy.

Pregnancy reporting will follow the same timelines and reporting structures as for a SAE (see above). Pregnancy will be followed up until the pregnancy outcome is known (birth, termination, etc). SAE reporting will also occur if the pregnancy outcome is a congenital anomaly. This will follow the reporting procedures described above for SAE reporting.

7.10. Monitoring and Safety for Specific Toxicities

Monitoring for specific toxicities is based upon target organs defined in preclinical toxicity studies and clinical studies (see IB).

A number of potential AEs have been described for the RESP301 under study. Specific guidance on more common or alternatively life-threatening AEs are described below. Please contact the medical monitor for consultation when needed.

7.10.1. Allergy

If a grade 3 or higher (CTCAE grading) allergic reaction to IMP occurs, IMP should be discontinued immediately. The patient should be monitored, and standard supportive medical treatment should be applied as required. Mild and moderate (grade 1 and 2) hypersensitivity reactions should be managed supportively, and withdrawal of IP is at the discretion of the investigator. The medical monitor may be consulted as needed. Drug allergies may be manifested by skin rashes of all types, fever, vasculitis, bronchospasm, serum sickness, Stevens-Johnson syndrome, angioedema, and anaphylaxis. Angioedema and anaphylaxis are the most serious hypersensitivity reactions, can occur at any age, and although are more common following injection, can occur after oral ingestion of the drug.

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Allergic reaction	Mild symptoms including bronchospasm – intervention not required	Symptomatic requiring oral or inhaled medication	Requiring urgent intervention, hospitalisation indicated for clinical sequelae,	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death

7.10.2. Methaemoglobinaemia

If a Grade 2 or higher (CTCAE grading) level of Methaemoglobinaemia is observed following administration of IMP, the IMP should be discontinued immediately and continuous transcutaneous monitoring instituted until Methaemoglobin levels have fallen to 1.5%. No active treatment is required and withdrawal of the patient from the study is at the discretion of the investigator. Subsequent administration of the IMP should be accompanied by transcutaneous MetHb monitoring which should be continued for 60 minutes after the end of administration.
If a grade 3 or higher (CTCAE grading) Methaemoglobinaemia occurs with IMP dosing, the IMP should be discontinued immediately. The patient should be monitored, and standard supportive medical treatment should be applied as required.

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Methaemoglobinaemia	-	>ULN 5% sustained for more than 1-minute transcutaneous measurement	Requiring urgent intervention >10%	Life-threatening consequences >40%	Death (>70%)

7.10.3. Overdose

Treatment of overdose should be as follows:

RESP301

- treatment should be supportive and symptomatic
- [redacted].

HRZE

- gastric lavage should be performed as soon as possible. Following evacuation of the gastric contents, the installation of activated charcoal slurry into the stomach may help absorb any remaining medicine from the gastrointestinal tract
- anti-emetic medication may be required to control severe nausea and vomiting
- intensive support measures should be instituted, including airway patency and individual symptoms treated as they arise.

7.10.4. Any other toxicities

As defined by the CTCAE toxicity tables version 5.0 (Appendix 1):

- Grade 1 or 2: Participants who develop grade 1 or 2 AE or laboratory toxicity may continue intake of IMP at the discretion of the investigator.
- Grade 3 or 4: Participants who develop grade 3 or 4 AE or laboratory toxicity will be carefully evaluated by the investigator. Participants may either continue intake of IMP, or be withdrawn from the study if, in the opinion of the investigator, the AE or laboratory toxicity poses a significant risk for the participant to continue participation in the study. Participants should be followed as appropriate until resolution of the AE or toxicity.

For AEs not described in the CTCAE table, the severity definitions defined in Table 22 are to be applied.

7.11. Safety Review Committee

Á Safety Review Committee will be formed which will meet if pre-determined events occur. This will be detailed in the Safety Review Committee Charter and Safety Management Plan which will be released before First Patient Screened.

8. Statistical Analysis

This section is an overview of the key elements of the statistical analysis for this study. Further details on statistical reporting and analyses will be contained in a separate statistical analysis plan (SAP).

This SAP may be revised during the study only to accommodate protocol amendments and to make changes to adapt to unexpected issues in study execution and data collection that could affect planned analyses. In all circumstances, a final SAP should be issued prior to database lock.

Descriptive summary statistics for continuous variables will include the number of participants, mean, standard deviation (SD), median and range. Descriptive summary statistics for categorical variables will include frequency count and percentages. Unless stated otherwise, the denominator for percentage calculations will be the number of participants with non-missing data. Descriptive statistics will be reported for the endpoints.

8.1. Analysis Population/s

The modified Intent to Treat analysis population will contain all randomized participants who receive at least one dose of IMP and have baseline and at least one post-baseline data.

The safety analysis population will include all randomised participants who receive at least one dose of IMP.

8.2. Sample Size

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.

8.3. Interim Review

The objective of stage 1 is to determine the EBA of dosing 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 Steering Committee (SC). This will be detailed in the Steering Committee Charter.

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

- [redacted]

8.4. Endpoint Analysis

8.4.1. Primary Endpoint Analysis: Activity

The EBA_{TTP} (0-14) in the MGIT (Bactec™ MGIT™ 960) system as determined by the predicted rate of change in the TTP in sputum over the periods Days 0 to 14 will be analysed [redacted].

The EBA_{CFU} (0-14) as determined by the predicted rate of change in log10CFU per mL sputum over the periods day 0 - 14 will be described [redacted]

[redacted]

8.4.2. Secondary Endpoints Analysis:

Activity

The EBA_{CFU} (0-14) as determined by the predicted rate of change in log10CFU per mL sputum over the periods day 0 - 14 will be described using [redacted].

[redacted].

Safety and Tolerability

Incidence of Treatment Emergent Adverse Events (TEAEs) will be presented by [redacted]

[redacted]

[redacted]

[redacted].

8.4.3. Exploratory Endpoints Analysis: Activity

Comparison of EBA_{TTP}(0-14) versus EBA_{TTP}(0-15) and the predicted EBA_{TTP}(14-15) versus observed EBA_{TTP}(14-15) analysed using [redacted]

9. Records Management

9.1. Data Collection

Electronic Data Capture will be used in this study. All eCRF pages will be completed for each participant who receives any amount of IMP, including EWD participants. For screen failure participants, certain predefined pages of the eCRF will be completed.

The completed eCRFs are the sole property of the sponsor and should not be made available in any form to thirds parties, except for authorized sponsor's representatives or appropriate regulatory authorities, without written permission from the sponsor.

The eCRF being used for this study has been fully certified as being compliant with the ICH GCP guidance requirements.

The investigator or designee will review and approve each completed eCRF. Should a correction be made, the corrected information will be recorded in the eCRF by the authorized person and explained (if necessary). All corrected data will be tracked through an electronic audit trail.

It is the investigator's responsibility to ensure documentation of all relevant data in the participants source file. All data recorded in the eCRF must be documented and referenceable in source data (source documents).

9.2. Source Documents

Source documents are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source documents will include, but are not limited to, progress notes, electronic data, screening logs, and recorded data from automated instruments.

All source documents pertaining to this study will be maintained by the investigators and designees. The investigator will permit study related monitoring, audits, IEC/IRB review and regulatory inspections providing authorized persons direct access to source documents.

9.3. File Management at the Study Site

It is the responsibility of the investigator to ensure that the study site's files are maintained in accordance with ICH and South African GCP Guidelines and the ethical principles that have their origin in the Declaration of Helsinki.

9.4. Records Retention at the Study Site

The investigator is obliged to retain records and data from the study for safety reasons and for audit and inspection subsequent to study completion. The sponsor, or other owners of the data, must retain all the sponsor-specific essential documents pertaining to the study for not less than 25 years or until at least two years have elapsed after the last marketing approval or since the formal discontinuation of clinical development of the IMP.

The sponsor will give written instruction and make financial provisions for the investigator to deposit the documents at an external site for safekeeping for as long as required by regulations and the sponsor.

10. Quality Control and Assurance

10.1. Site Procedures

The investigator undertakes to perform the clinical study in accordance with this protocol, ICH and South African GCP, and the ethical principles that have their origin in the Declaration of Helsinki.

The investigator and designees undertake to complete the source and eCRFs according to the sponsor's requirements, in a timely, accurate and legible manner. eCRF entries will be verifiable to source documentation other than the eCRF.

Site standard operating procedures will be adhered to for all clinical and bio-analytical activities relevant to the quality of the study. Participant compliance will be monitored throughout the study. The investigator will sign and date any analysis results timeously (e.g., laboratory, ECG, etc.) to verify that the results have been reviewed.

The principal investigator (PI) may appoint and delegate other qualified, approved and trained sub-investigators and study staff (the team) to assist with the study. However, the PI maintains responsibility for the overall conduct of the study and will oversee the team and any vendors. Written IEC/IRB and Regulatory Authority approval will be obtained prior to involvement in the study.

The investigator will ensure that any party retained to perform study tasks and all site personnel are adequately trained in ICH and SA-GCP, the protocol, IMP, IB (as applicable) and all study procedures and requirements before commencing any study related duties. This training will be documented.

10.2. Monitoring

The PI is responsible for the validity of all data collected at the study site and must accept the various monitoring procedures employed by the sponsor. The purpose of monitoring is to verify that the rights, safety and well-being of human participants are protected; that study data are accurate, complete and verifiable with source data; and that the study is conducted in compliance with the protocol, International and SA GCP, the ethical principles that have their origin in the Declaration of Helsinki and the applicable regulatory requirements.

Monitors assigned by the sponsor will conduct regular site visits for the purpose of monitoring various aspects of the study. The investigator and site staff will allow the study monitor, independent ethics committees, regulatory authorities, and authorized representatives of the sponsor to:
(1) inspect all essential documents pertaining to the clinical trial (e.g., eCRFs, written informed consent documents and corresponding source documents i.e., original medical records, participant records and laboratory raw data) and
(2) access clinical supplies, dispensing and storage areas.

The investigator and site staff will also:

(1) agree to assist with monitoring activities if requested and
(2) provide adequate time, resources and space for monitoring visits.

The monitor will query any missing, confusing, spurious, or otherwise ambiguous data with the PI or delegated staff. All queries should be resolved in a timely manner (preferably before the next monitoring visit). A monitoring log will be maintained recording each visit, the reason for the visit, the monitor's signature and investigator or designee's confirmation signature.

10.3. Auditing

To comply with International and SA GCP and regulatory agency guidelines, it may be necessary for sponsor-authorized quality assurance personnel and/or authorized personnel from an external regulatory agency to conduct an audit or inspection of the investigational site. The purpose of an audit is to assess participants safety, the quality of data accuracy, adequacy, and consistency, and to assure that studies are in accordance with the applicable guidelines and the protocol. Having the highest quality data from studies is an essential aspect of drug development.

Unless it is an unannounced inspection, the investigator and site staff will be given sufficient notice to prepare for such visits which may be conducted at any stage during the study. The audit may involve the review of all study-related documentation required by GCP to be maintained by each site; drug storage, dispensing and return; all study-related supplies; and source documents against the eCRFs to assure the adequacy and accuracy of the information which has been recorded, including the verification of any AEs and SAEs which have occurred.

In the event of the site being notified of a regulatory inspection, the Sponsor will assist the investigational site with preparation. It is essential that the Sponsor be notified of the inspection as soon as possible.

11. Ethics and Regulatory

11.1. Basic Principles

This research will be carried out in accordance with ICH and South African GCP, the ethical principles that have their origin in the Declaration of Helsinki and the applicable regulatory requirements.

11.2. Independent Ethics Committee/Institutional Review Board Review

The protocol and required study related documents will be reviewed by the site's IEC/IRB. The study will not start until the IEC/IRB has approved the protocol, written informed consent, any written information to be provided to the participant or any modification thereof, plus any other study related documents required for review. The IEC/IRB shall be constituted and shall operate in accordance with ICH and South African GCP, the ethical principles that have their origin in the Declaration of Helsinki. The investigator will maintain an accurate and complete record of all submissions made to the IEC/IRB. The records should be filed in the ISF, and copies will be sent to the sponsor for their TMF.

11.3. Regulatory Authorities

The regulatory authorities will receive the protocol, amendments, reports on SAEs, and the integrated clinical study report according to national regulations. As required by local legislation, written approval will be obtained from the regulatory authorities prior to commencement of the study and implementation of e.g., amendments as applicable.

11.4. Informed Consent

Written informed consent will be obtained from all participants before any study-related procedures (including any screening or pre-treatment procedures) are performed. Investigators may discuss the availability of the study and the opportunity for entry with a potential participant without first obtaining consent. However, informed consent must be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for research, including withdrawal from current medication(s). When this is done in anticipation of, or in preparation for, the research, it is part of the research. Participants who are illiterate will make use of a witness but will still give verbal consent to participate.

The investigators have both ethical and legal responsibility to ensure that each participant being considered for inclusion in this study is given a full explanation of the protocol by means of the informed consent process. This shall be documented on a written informed consent form that shall be approved by the same IEC/IRB responsible for approval of this protocol. Each informed consent form shall include the elements required by international and South African GCP and must adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Once the appropriate essential information has been provided to the participant and fully explained by the investigators (or qualified designees) and it is felt that the participant understands the implications of participating, the IEC/IRB approved written informed consent form will be signed and dated by both the

participant, and/or witness, and the person obtaining consent (investigators or designees), and by any other parties required by the IEC/IRB.

The original signed informed consent form will be kept with the study records and a copy of signed informed consent form will be offered to the participant. Another copy of the signed informed consent form and a source document identifying the study and recording the dates of informed consent and participation will be placed in the participant's medical record.

The monitor will inspect the original completed consent form(s) as per the monitoring plan.

11.5. Confidentiality

All site staff, the sponsor, and any sponsor representatives will preserve the confidentiality of all participants taking part in the study, in accordance with ICH and South African GCP and applicable local legislation/regulations. All participants will agree to the requirement for source data verification with confidentiality of all participant identities maintained. Only participant study number will be used on the eCRF and in all study correspondence, as permitted. No material bearing a participant's name will be kept on file by the sponsor. The written informed consent will contain a clause granting permission for review of the participants' source data by the site staff, the monitor, auditors, inspectors from Regulatory authorities.

12. Incidental Findings

Incidental findings (IFs) in clinical research are findings with potential health or reproductive importance that are discovered while conducting the research but are beyond the aims of the study. IFs in this study may arise from the collecting and analysing of research images or in the tests or information collected to determine eligibility criteria or study endpoint assessments.

The PI is responsible for handling an IF responsibly and promptly and members of the research team will promptly report a suspected IF. If an IF is discovered the PI will inform the participant and, as consented to by the participant, exercise his/her judgment on a case-by-case basis to determine the way forward that is in the best interest of the participant. The PI will make sure that all the necessary documentation for the referral of the participant is put together free of cost to the participant. The sponsor is not liable for the ongoing clinical management of any IFs.

13. Publication Policy

The definition of publication for this purpose is any public presentation of the data emerging from this study.

Results of this research will be submitted for publication as soon as feasible upon completion of the study in the form of a joint publication(s) between compound owners, sponsor, and investigator(s), including site clinical and laboratory investigators, and statisticians as appropriate.

The clinical study will be registered on the open access website https://clinicaltrials.gov and any other public websites required by regulation once approval by local regulators for the conduct of the study has been received.

14. Protocol Amendment Policy

Any change to the protocol will be effected by means of a protocol amendment and will be submitted to the IEC/IRB and Regulatory Authorities prior to implementation as required by them. No amendment will be implemented until approved by the relevant authorities and/or IEC/IRB and signed by all required parties. Exceptions to this are when the investigator considers that the participant's safety is compromised.

Protocol amendments detailing minor administrative changes should be submitted by the investigator to the IEC/IRB and regulatory authorities, either for notification purposes or approval as appropriate.

15. Financial Aspects, Insurance and Indemnity

The study sponsor, who are funding the study, is Thirty Respiratory Limited.

The participants will not receive any incentives for their involvement in the study. The sponsor has made provision to reimburse the participants for time, inconvenience, and out-of-pocket expenses such as travelling to and from the study site and other miscellaneous costs because of their study participation.

Research participants should not bear any financial cost to rectify harms that occur because of study participation. The sponsor will indemnify (provide legal and financial cover for) the investigator against claims arising from the study, excluding claims that arise from malpractice or negligence. The sponsor certifies that it has liability insurance coverage for itself and will provide an associated certificate upon request. The participants taking part in the study will be covered by the insurance taken out by the sponsor for this study.

The insurance does not relieve the investigators of the obligation to maintain their own professional liability insurance (e.g., MPS) as required by applicable law. The sponsor does not assume any obligation for the medical treatment of other injuries and illnesses not related to the study.

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Appendix 1: Common Terminology Criteria for Adverse Events (CTCAE)

Version 5.0 27Nov2017

CTCAE_v5.0_2017-11-27.xlsx (live.com)

Appendix 2: Vital Signs Reference range

Measurement	Normal Reference Range
Blood Pressure diastolic	90-139 mmHg
Blood Pressure systolic	60-89 mmHg
Heart rate	60 – 99 bpm
Respiratory rate	12-20 bpm
Body temperature	35,0– 37, 9°C

Reference: CTCAE Version 5.0 27Nov2017, adjusted per expected TB participants symptoms

Appendix 3: IUATLD/WHO Scale

Finding	Recording
No acid-fast bacilli found in at least 100 fields	negative
1 to 9 acid-fast bacilli per 100 fields	exact figure / 100
10 to 99 acid-fast bacilli per 100 fields	+
1 to 10 acid-fast bacilli per field in at least 50 fields	++
More than 10 acid-fast bacilli per field in at least 20 fields	+++

Reference:
priorities_for_bacteriology_services_in_low-income_countries_(2007).pdf (tbonline.info)
Accessed 25March2023.