Study Protocol

Study Title:

A Phase IIb, Open-Label, Randomized Controlled Dose Ranging Multi-Center Trial to Evaluate the Safety, Tolerability, Pharmacokinetics and Exposure-Response Relationship of different doses of Delpazolid in combination with Bedaquiline Delamanid Moxifloxacin in Adult Subjects with Newly Diagnosed, Uncomplicated, Smear-Positive, Drug-sensitive Pulmonary Tuberculosis

Short title:

PanACEA DElpazolid dose-finding and COmbination DEvelopment (DECODE)

Protocol Number:

PanACEA - DECODE – 01/LCB01-0371-20-2-02

Protocol Version No:

2.1

Protocol Date:

08 September 2021

Confidential document

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Sponsor Signature Page

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Protocol Number: PanACEA--DECODE-01/ LCB01-0371-20-2-02
Protocol Version: 2.1, dated 08 September 2021

I hereby approve the protocol PanACEA-DECODE-01, Version 2.1, dated 08.09.2021, and confirm that it contains all necessary information to conduct the study according to the ethical principles laid down in the declaration of Helsinki, Good Clinical Practice and all applicable local regulations.

_____ _____

(Sponsor Delegated Person) Date

Principal Investigator Signature Page

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I hereby confirm that I have read the above protocol and agree to conduct this clinical trial as outlined in the above protocol. I will provide copies of the protocol and access to all the information required to conduct the clinical trial 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 trial requirements.

Principal Investigator Signature	Principal Investigator Printed Name and Qualification

Date

1 PROTOCOL SYNOPSIS

Short protocol Title	PanACEA DElpazolid dose-finding and COmbination DEvelopment (DECODE)
Full protocol title	A Phase IIb, Open-Label, Randomized Controlled Dose Ranging Multi-Center Trial to Evaluate the Safety, Tolerability, Pharmacokinetics and Exposure-Response Relationship of different doses of Delpazolid in combination with Bedaquiline, Delamanid and Moxifloxacin in Adult Subjects with Newly Diagnosed, Uncomplicated, Smear-Positive, Drug-sensitive Pulmonary Tuberculosis
Name of IMP	Delpazolid (DZD) 400 mg tablets
Non-IMP study drugs	Bedaquiline (BDQ), Delamanid (DLM), Moxifloxacin (MXF)
Indication	Pulmonary Tuberculosis (TB)
Objectives	**Safety and Tolerability Objective:** To describe the safety, tolerability and exposure-toxicity relationship of DZD given over 16 weeks, in combination with standard-dose BDQ, DLM and MXF, compared to standard-dose BDQ, DLM and MXF alone **Efficacy Objectives:** *Primary Efficacy Objective:* • To establish an exposure-response model for DZD, given over 16 weeks in combination with standard-dose BDQ, DLM and MXF, on the change in liquid culture MGIT time to positivity (TTP) *Secondary Efficacy Objective:* • To assess dose and exposure-response relationships for DZD, based on secondary efficacy endpoints, including month-2 culture status in liquid media and on solid media, and time to culture conversion in liquid and on solid media • To assess the relative efficacy of increasing DZD doses compared to the background regimen without DZD, based on primary and secondary efficacy endpoints. • To assess the proportion of patients who suffer relapse within 12 months post randomization, out of those patients completing 16 weeks of therapy and achieving sustained sputum culture conversion, defined as two successive negative liquid media cultures at or before WK08, with no positives to follow until the week 16 visit. **Pharmacokinetics Objectives:** *Primary Pharmacokinetics Objective:* • To describe the pharmacokinetics (PK) of DZD through development of a population PK model

	Secondary Pharmacokinetics Objective: • To describe the PK of BDQ, DLM and MXF including their main metabolites **Mycobacteriology Identification and Characterization Objectives:** • To assess the minimum inhibitory concentrations (MIC) of BDQ, DLM, MXF, DZD of the infecting strain, at baseline and on representative isolate(s) grown at or after WK08. • To investigate the frequency of acquired mutations in the infecting strain over treatment • In patients with recurrent disease: comparison of initial and recurrence isolate by whole genome sequencing to discriminate relapse from reinfection
Study Design	This will be an open label Phase IIb dose-finding, randomized, controlled study with a duration of 16 weeks of experimental therapy of DZD – Bedaquiline/Delamanid/ Moxifloxacin (BDM) in adult patients with newly diagnosed, smear positive, uncomplicated, drug sensitive pulmonary tuberculosis (TB) to evaluate the safety, efficacy, tolerability, pharmacokinetics and exposure/response-relationship of different doses of delpazolid in combination with bedaquiline, delamanid and moxifloxacin. Participants will be randomized to one of five arms containing BDM with different doses of DZD: • Arm 1 (D_0): 15 patients will receive **0 mg of DZD** • Arm 2 (D_{400}): 15 patients will receive **400 mg DZD orally once daily** • Arm 3 ($D_{800\text{-}OD}$): 15 patients will receive **800 mg DZD orally once daily** • Arm 4 (D_{1200}): 15 patients will receive **1200 mg DZD orally once daily** • Arm 5 ($D_{800\text{-}BD}$): 15 patients will receive **800 mg DZD orally twice daily**
Decision rule for managing post week 16 treatment or follow - up	Patients will stop therapy after the week 16 visit, if they achieve early sputum culture conversion (SCC) to negative. This is defined as two successive negative liquid media cultures, the first of which is at or before week 08, with no positive cultures to follow. Results for the negative cultures need to be available by the week 16 (day 112) visit, with a time window of +6 days to accommodate for late availability of culture results, in order for patients to stop therapy. Post treatment follow-up visits at weeks 18, 26, 38 and 52 will serve to determine whether they have achieved lasting cure. Patients with a positive culture or other indication as described under 11.4 during the follow up period will undergo a standardized assessment for potential poor treatment outcome. If this procedure results in a diagnosis of recurrent disease, they will be referred to the national TB program. Patients who do not achieve SCC as defined above will be referred to the national TB program for standard of care TB treatment according to national guidelines, to complete at least 6 months of treatment in total. In addition to the rule above, additional positive sputum LAM testing results late in treatment may be used to enhance detection of patients at risk for unfavourable outcome,

	who would NOT qualify for treatment stop after 16 weeks. The sponsor will produce a centralized decision rule when kits for this assessment become available.
Time Schedule	Per Subject: 52 weeks in total, including a 36-week follow-up Study duration: Anticipated Recruitment Period: Q3 2021 – Q1 2022. Study duration: Planned End Date (LPLV): Q2 - Q3 2022
Population	A total of 75 male or female subjects, aged between 18 and 65 years with newly diagnosed, drug sensitive, uncomplicated, smear-positive, pulmonary TB will be included
Sample size	To be analysed: N = 15 patients per arm with a total of N = 75 patients, and a wide range of DZD doses (from 0mg to 800mg BID) has been determined as an adequate sample size for population PK modelling, and for exposure-response modelling to detect a clinically meaningful dose-dependent relationship.
Dosage and Administration	**Bedaquiline:** will be dosed as per the licensed dose: 400 mg orally once daily for the first 14 days, then 200 mg orally three times a week. **Delamanid:** will be dosed as per the licensed dose: 100 mg orally twice daily (totalling 200mg daily) **Moxifloxacin:** will be dosed as per the licensed dose: 400 mg orally once daily **Delpazolid:** not licensed: Current experience in humans up to Phase 2a. Dose according to randomization to dosing arm: • Arm 1 (D_0): 15 patients will receive 0 mg of DZD • Arm 2 (D_{400}): 15 patients will receive 400 mg DZD orally once daily • Arm 3 ($D_{800\text{-}OD}$): 15 patients will receive 800 mg DZD orally once daily • Arm 4 (D_{1200}): 15 patients will receive 1200 mg DZD orally once daily • Arm 5 ($D_{800\text{-}BD}$): 15 patients will receive 800 mg DZD orally twice daily All above drugs will be given for 16 weeks.
Evaluation Criteria	**Primary Endpoints** The safety of DZD will be assessed by evaluation of AEs during treatment and follow-up phase, including assessments of vital signs, physical examinations, weight, detailed neurological examinations, colour vision and visual acuity tests, 12-lead ECG, and routine clinical laboratory tests (including chemistry, haematology and urinalysis data). *Primary Safety Endpoint* • Proportion of patients experiencing expected oxazolidinone class toxicities, including peripheral or optical neuropathy, incident leukopenia, anemia or thrombocytopenia, or adverse events in line with tyramine pressor response, of Grade 2 or higher, possibly, probably or definitely related to DZD *Primary Efficacy Endpoint*

	The efficacy of DZD will be evaluated by measuring the change in mycobacterial load over time on treatment as quantified by time to positivity in BD MGIT 960® liquid culture described by non-linear mixed-effects methodology **Secondary Endpoints** *Secondary safety endpoints* • All adverse events • Adverse events of Grade 3 severity or higher • Adverse events possibly, probably or definitely related to study drugs • Treatment discontinuations or interruptions related to adverse events/serious adverse events • Frequency, severity and type of ECG alterations • Changes in ECG intervals of PR, RR, QRS, QT, Fridericia-corrected QT [QTcF] • Proportion of participants with QTcF > 500ms in ECGs on treatment • Proportion of participants with a prolongation of QTcF of grade 3 and above as defined under 14.6. *Secondary Efficacy Endpoints:* • Proportion of patients who suffer relapse, defined as recurrent disease caused by a strain identical to the baseline isolate, within 12 months post randomisation, out of patients completing 16 weeks of therapy and achieving stable culture conversion to negative (SCC) as defined below by week 08, with no positives to follow by the week 16 visit. • Time to recurrent TB, and to relapse, within 12 months post randomisation • Time to stable sputum culture conversion (SCC) to negative on liquid media (defined as two negative cultures without an intervening positive culture). Time will be measured as time on treatment until the first negative culture. • Proportion of participants who achieved SCC at each time point during treatment • Proportion of participants with negative sputum culture on solid media at WK 08 and other timepoints • Proportion of participants developing drug resistance among those not converting to negative culture **Pharmacokinetic Endpoints** A population PK model will be developed for DZD. The following secondary parameters will be derived for DZD, for BDQ, DLM and their main metabolites, and for MXF: • Area under the plasma concentration curve from morning dosing to 24 hours (AUC 0-24) on day 14 (WK2) • The observed maximum concentration (C_{max}) on day 14 • Time to reach C_{max} (T_{max}) on day 14 • The minimum observed plasma concentration (C_{min}) at day 14 (24 hours following the last dose for intake once daily (QD) and 12 hours following the last dose for twice daily intake (BID)) • Apparent oral clearance (Cl/F)

	• Apparent volume of distribution (Vd/F) • Terminal half-life ($t_{1/2}$) **Mycobacteriology Identification and Characterization Endpoints** Sputum cultures grown from various timepoints will be assessed as follows: • Minimum inhibitory concentrations (MIC) of BDQ, DLM, MXF, DZD, from a baseline isolate, and a representative isolate obtained at or after WK08, if any. • Frequency of acquired mutations in the infecting strain over treatment assessed by whole genome sequencing • Comparison between bacterial strain causing recurrent disease, and the strain at baseline by whole genome sequencing, to discriminate relapse from re-infection **Exploratory endpoints** Exploratory endpoints will be analysed depending on laboratory capacity, availability of test kits and budget, and may not be tested in all trial sites equally. • Rate of change in molecular bacterial load assay (MBLA) during treatment • Time to stable culture conversion to negative in MBLA (defined as two negative MBLAs without an intervening positive) • Rate of change in bacterial load measured by quantification of sputum lipoarabinomannan (LAM) during treatment
Analysis timepoints	Analysis for the primary endpoints, and secondary endpoints unrelated to disease recurrence or relapse, will be performed after data are available (allowing for time for culture growth) after the last patient completed their WK16 (or otherwise last treatment) visit. A partial database lock will be performed for this purpose, with a final database lock at the end of study after all long-term outcome data are available.
Safety data review trigger rules	An independent data safety monitoring board (DSMB) will be convened for the trial. The DSMB will review safety data at regular intervals, but will also perform expedited review if the following conditions are met: • Four or more patients experience a grade 3 or higher AE (CTCAE version 5.0) in the same organ system that are at least possibly related to one of the study drugs, and qualify as “unexpected” by being more severe than in previous experience with the drug in question. • Two or more patients experience a grade 4 or higher AE (CTCAE 5.0) in the same organ system that are at least possibly related to one of the study drugs, and qualify as “unexpected” by being more severe than in previous experience with the drug in question • One patient experiences a grade 5 AE (death) that is at least possibly related to one of the study drugs

2 SCHEDULE OF EVENTS

Time points	Screening	Treatment																	Follow-up			
		Treatment visit days each ± 2 days (except for WK2 visit, which may occur with a window of -2 days only																	Follow-up visit 1 ± 4 days	Follow-up visit 2 – 4 [A] each ± 7 days		
	Day -8 to 0	Day 01	Day 07	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 91	Day 98	Day 105	Day 112	Day 126	Day 182	Day 266	Day 364
Visit	SCR	WK 00	WK 01	WK 02	WK 03	WK 04	WK 05	WK 06	WK 07	WK 08	WK 09	WK 10	WK 11	WK 12	WK 13	WK 14	WK 15	WK 16	FU 1 WK 18	FU 2 [A] WK 26	FU 3 [A] WK 38	FU 4 WK 52
Informed Consent	x																					
Check in-/exclusion criteria	x																					
Height	x																					
Demographics	x																					
Medical history	x																					
Serum pregnancy test[B]	x										x[B]								x[B]			
HIV test (+ CD4 count + viral load)[C]	x																					
Urine drug screening[D]	x																					
Chest X-ray	x																					
Enrolment & Randomization		x																				
Hospitalization				x[K]																		
Sputum[E]	1 x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	3x	2x	2x	2x	2x
2x per visit: MGIT, MBLA, (LJ culture, ZN smear), rest: storage[E]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination, vital signs[F]	x	x[G]	x	x[G]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x[G])	(x[G])	(x[G])
Neurological examination/vision testing[G]	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)	(x)	(x)
ECG (12-lead; pre-dose)[H]	1x	3x	1x[F]	1x[F]	1x[F]	1x[F]	1x[F]	1x[F]	1x[F]	1x[F]	1x[F]		1x[F]		1x[F]			1x[F]				
Drug Susceptibility Testing[I]	x	x														(x)						
Screening/Safety Lab[J]	x[J]		x	x[J]	x	x	x	x	x	x	x		x		x			x	x			
Study treatment[A]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Intensive PK sampling [K]				x																		
Biomarker/PG blood sample[L]	x			x		x			x				x		x			x				
Concomitant Medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X
Adverse Events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X

Key: WK: week of treatment; MGIT: liquid media (BD mycobacterium growth indicator tube; LJ: Loewenstein - Jensen solid media; MBLA: molecular bacterial load assay; PK: pharmacokinetics, X: refers to all visits mentioned above, ZN: Ziehl-Neelsen stain, PG: pharmacogenomics

A) *Continuation Treatment and follow-up visits: Participants of all 5 arms will complete their experimental treatment on Day 112. Omission of anti-TB treatment after this and conduct of follow-up visits 2-3 will be conditional on culture conversion with first negative at or before WK08 (see decision rule). Participants who do not convert to negative culture will receive standard TB treatment according to their national TB program through governmental clinics, and will not have visits FU 2 and FU3. FU 4 will be conducted for all participants.*

B) *Serum pregnancy test: blood samples of 7.5mL; for women of childbearing potential only.*

C) *HIV Testing: blood samples of 7.5mL will be taken; CD4 count if HIV positive. If the patient is on ARVs at the time of screening, perform viral load count. Genotypic resistance testing mandatory if viral load is insufficiently controlled (>1,000 copies/µl) and patient would need to be switched back to a non-dolutegravir regimen after the study, recommended otherwise.*

D) *Urine drug screening: for opiates, amphetamines, cannabinoids, cocaine, benzodiazepines and barbiturates. Results on other drugs generated by a combination test may be used as well.*

E) *Sputum sampling: 3 sputa will be collected per time point during treatment phase, 1 spot sputum at SCR and 2 at FU 1-4 each. Two spot sputum samples during treatment and follow-up phase will be processed for mycobacterial culture MGIT liquid culture at all time-points. LJ will be performed at WK00, WK08, WK12, WK16, and FU1-FU4 from two samples each. An isolate from baseline, and all isolates from all cultures positive after WK08 will be stored. The third sputum sample may be a spot or an early morning sputum, and will be stored for future biomarkers work. A Ziehl-Neelsen stained sputum smear will be performed at SCR and WK16 from concentrated sputum and at additional visits as required by the national TB programs.*

F) *Physical examination and vital signs: Additional Blood pressure and heart rate recordings WK00 at 1h, 2h and 3h (± 10 mins) after intake of IMP and WK02 during hospitalization at 1h, 2h, 3h, 4h, 6h (± 10 mins) after intake of IMP with a meal. During follow up visits FU 2-4 symptom orientated physical examination only or to follow up on (S)AEs.*

G) *Detailed neurological examination: to include central and peripheral system testing (i.e. cranial nerves testing, vibration sensitivity, position, pin prick, light touch) and vision testing (Snellen, Ishihara). During follow up visits FU 2-4 symptom orientated neurological examination only or to follow up on (S)AEs.*

H) *ECG: to be recorded after 10 minutes supine rest, before intake of study treatment. Triplicate ECG at WK00, single ECGs at SCR, WK01 – 09, WK11, WK13 and WK16. If a QTcF of >480ms, or a QTc-prolongation over baseline of >50ms is seen in a single ECG during experimental treatment, two more ECGs should be registered, to obtain a more precise, average QTcF measurement.*

I) *Microbiology/Susceptibility Testing: rapid test for RIF and INH susceptibility at SCR. Minimum inhibitory concentration (MIC) for study drugs at baseline, and from a representative isolate if any growth at or after WK08.*

J) *Screening/Safety lab: full blood count (blood samples of 2mL), ALT, AST, gamma-glutamyl transferase (yGT), Alkaline Phosphatase (AP), Lipase, direct and total bilirubin, albumin, serum creatinine, electrolytes (Na+, K+, Serum Calcium, Magnesium, Cl-, $PO4^{3-}$), random glucose (blood samples of 7.5mL for blood chemistry). Urinalysis including dipstick for pH, protein, glucose, ketones, urobilinogen, blood and leukocytes. Urine microscopy on abnormal dipstick results as per the investigator's decision.*

At SCR and WK02 (and at additional visits, if clinically indicated): Coagulation aPTT, PT, INR (blood samples of 3mL)

K) *Intensive PK sampling (WK02): In all participants, blood samples of 8ml will be taken for measurements of DZD and other study drugs' concentrations over time. Study drugs will be dosed with food. Time of intake of food, drugs and of blood sampling will be recorded. Participants will be hospitalized and receive standard meals at days of intensive PK sampling. Intensive PK time points (7 samples in total) at WK02: 0 (within 1h before dosing), 1, 2, 4, 8, 12, (± 10 mins) and 24 hours (± 30 mins) after dosing*

L) *Host biomarker sample storage: Conditional on patient consent to sub-studies. One 5ml blood sample will be taken for plasma/ storage, one 2.5ml blood sample will be collected in PAXgene bottles for transcriptomic analysis, one 10ml sample will be collected for immunological analyses (not all sites) with safety blood draw. Plasma and PAXgene whole blood samples should be stored at -70°C. At screening, one sample for pharmacogenomics sub-studies will be stored (5ml).*

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3 ABBREVIATIONS

ACTG	Acquired Immunodeficiency Syndrome Clinical Trials Group
ADL	Activities of Daily Life
ADR	Adverse Drug Reactions
AE	Adverse Event
ALT	Alanine Aminotransferase
AP	Alkaline Phosphatase
aPTT	Activated prolonged Thrombin Time
ARV	Antiretroviral
AST	Aspartate Aminotransferase
AUC	Area Under the Plasma Concentration Curve
AUC(0-t)	Area under the Plasma Concentration Curve from zero to t
BDM	Bedaquiline, Delamanid, Moxifloxacin
BDMD	Bedaquiline, Delamanid, Moxifloxacin, Delpazolid
BDQ	Bedaquiline
BID	Bis in die (twice daily)
BMI	Body Mass Index
BP	Blood Pressure
C_{max}	Maximum Observed Plasma Concentration
CD4	Cluster of Differentiation 4 (T Helper Cell)
CIOMS	Council for International Organizations of Medical Sciences
CoA	Certificates of Analyses
CRF	Case Report Form
CRO	Contract Research Organisation
Ct	Cycle threshold in GeneXpert MTB/RIF® test
CTCAE 4.0	Common Terminology Criteria for Adverse Events 4.0
DLM	Delamanid
DMP	Drug Management Plan
DOTS	Directly Observed Treatment Short course
DSMB	Data Safety Monitoring Board
DS	Drug sensitive
DZD	Delpazolid
eCrCl	estimated creatinine clearance
EDTA	Ethylene Diamine Tetraacetic Acid
EBA	Early Bactericidal Activity
eCRF	electronic Case Report Form
EMB	Ethambutol
FDA	Food and Drug Administration (USA)
GCP	Good Clinical Practice
HED	Human Equivalent Dose
hERG	Human Ether- à -go-go-Related Gene
HIV	Human Immunodeficiency Virus

HRZE	Isoniazid, rifampicin, pyrazinamide, ethambutol
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
INH	Isoniazid
INR	International Normalized Ratio
IRB	Institutional Review Board
IWRS	Interactive Web Randomisation System
kg	kilogram
LAM	Lipoarabinomannan
LCB	LegoChem Biosciences, Inc.
LZD	Linezolid
m	meter
MBLA	Molecular Bacterial Load Assay
MDR	Multidrug-Resistant
MGIT	Mycobacterium Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MOP	Manual of Procedures
MXF	Moxifloxacin
MTB	Mycobacterium tuberculosis
NIMR-MMRC	National Institute for Medical Research Mbeya- Medical Research Centre
NOAEL	No Observed Adverse Events Level
OVX	Ovariectomy
PCR	Polymerase Chain Reaction
PK	Pharmacokinetics
PT	Prothrombin Time
PTM	Pretomanid
PZA	Pyrazinamide
QD	quaque die (once per day)
QRS	Electrocardiographic QRS Interval
QTcB	QT-Interval corrected by Bazett's formula
QTcF	QT-Interval corrected by Fridericia's formula
RIF	Rifampicin
SA	South Africa
SAE	Serious Adverse Event
SA GCP	South African Good Clinical Practice
SAR	Serious Adverse Reaction
SAP	Statistical Analyses Plan
SSCC	Serial Sputum Colony Count
SUSAR	Suspected Unexpected Serious Adverse Reaction
STEP	Selection Trial with extended Post-treatment follow up
STZ	Sutezolid

TEAE	Treatment-Emergent Adverse Event
TB	Tuberculosis
T_{max}	Time to Reach C_{max}
TSC	Trial Steering Committee
TTP	Time to Positivity in Liquid Media
UGT	Uridine Diphosphate (UDP)-Glucuronosyltransferase
ULN	Upper limit of normal
VZ/F	Apparent Volume of Distribution
WHO	World Health Organization
XDR	Extensively drug-resistant

4 BACKGROUND INFORMATION

4.1 The need for new TB drugs

While progress has been made in recent years in controlling tuberculosis (TB) globally, TB has remained a persistent problem in the developing countries of Africa, Asia and Eastern Europe. TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS [1]. The current first-line anti-tubercular agents have been in use for over 20 years and are relatively ineffective in controlling TB as a public health problem. The long treatment duration and treatment-related toxicity result in poor compliance. As a result of poor treatment compliance, drug resistance is becoming more common. Multidrug-resistant TB (MDR-TB) is a public health emergency, especially in sub-Saharan Africa where human immunodeficiency virus (HIV) infection is endemic. Prevailing challenges such as lack of universal MDR-TB diagnosis, lengthy and toxic treatments that cure only 52% of patients, complicated with development of extensively drug-resistant (XDR) TB, are major impediments in MDR-TB control. Models predict that if the current status is kept up, MDR-TB incidence and associated death trend will increase dramatically and will overturn and become the dominant form of TB by 2050 [2]. Novel approaches to counter-attack the threat are highly needed.

A step in the right direction is a new TB regimen composed of bedaquiline (BDQ), pretomanid (PTM) and linezolid (LZD), which was superior to the current first-line regimen of isoniazid, pyrazinamide, rifampicin and ethambutol (HRZE) in mouse studies. In the TB Alliance's NiX-TB trial, the regimen was given to patients with extensively drug-resistant TB (XDR-TB) for six months, and has achieved lasting cure in 19 out of 20 patients that have completed the follow-up period to date [3, 4]. However, safety of this regimen is an important concern. Toxicity attributed to LZD, specifically, led to treatment interruptions in 71% of patients, which can be considered acceptable only in the XDR patient population with an otherwise dire prognosis.

PanACEA has taken the opportunity to build on knowledge derived from NiX-TB, and aims to take steps to test a similar regimen to be used in a larger population of TB patients. We have access to drugs of similar class and effectiveness (e.g. delamanid (DLM), a nitroimidazole and delpazolid (DPZ), an oxazolidinone), and safety data available up to now suggests that these drugs may have a more favourable safety profile compared to their counterparts (LZD and PTM). DLM is approved for use in Europe, Japan, and several other countries. DZD is a new, investigational oxazolidinone that will most likely have similar or better efficacy as LZD, however is much less toxic as indicated by in vitro and early human data [5].

PanACEA proposes to investigate the combination of BDM - DZD: BDQ, DLM, moxifloxacin (MXF) and DZD. A more in-depth discussion of the advantages and potential of DZD will be continued in the section below.

4.2 Delpazolid previous experience

DZD (LCB01-0371), discovered in 2010, is an oxazolidinone antibiotic that binds to the V region of the bacterial 23S rRNA, interfering with the early compound formation of 30S and 50S rRNA, thus inhibiting protein biosynthesis being developed by LegoChem Biosciences Inc.

DZD demonstrated good safety in Phase I studies up to 21 days, and good activity in a Phase 2a 14-day EBA study in drug-sensitive TB patients [6] with a total of 239 patients exposed. The early bactericidal activity (EBA) study tested daily doses of 400 mg BID, 800 mg QD, 1200 mg QD and 800 mg BID. Good antibacterial activity was seen, but the study results did not permit to choose the best dose for further clinical trials.

Preclinical studies suggest that DZD has low potential for metabolic interactions with other medications. Mainly, inhibition of Monoaminooxidase (MAO) was seen in secondary pharmacology testing in vitro, of similar (MAO-A) or lower inhibitory magnitude (MAO-B) than in the licensed oxazolidinone, linezolid.

Unlike other oxazolidinone drugs, DZD is eliminated mainly by the renal route. The ensuing relatively short half-life leads to the hope that the main, severe oxazolidinone class toxicities in chronic dosing, suppression of hematopoiesis and occurrence of peripheral neuropathy, will not be seen with DZD as these are mostly associated with high drug concentrations at the end of the dosing interval [7]. This hope seems to be confirmed so far in human studies, which have dosed for up to 21 days.

LCB has animal toxicity data available for 6 months of DZD dosing in rats, and 9 months of dosing in dogs, which according to regulatory guidance will permit long-term dosing.

A further detailed description of all study drugs is provided in section 6.

4.3 The TB Burden in Tanzania and South Africa

Tanzania, an East African country with a population of 56 million inhabitants and a 2016 per capita GDP of $879 US, is a country with a high burden of drug sensitive (DS) TB. In 2016 the incidence for all forms of TB was 287 per 100,000. Thirty-four percent of tested TB patients are HIV positive. The incidence of TB in Tanzania is slowly declining. The rate of MDR-TB among new cases of TB is still low (1.3%, 95% CI 0.47-2.1), but higher for retreatment cases (6.2%, 95% CI 5.1-7.4). Overall, treatment success is high, with 90% success-rate amongst new and relapsed cases in 2015, and 80% treatment success for previously treated cases. The MDR/RR-TB cases that started on second-line treatment in 2014 have had treatment-success in 76% of all cases (143 total) [8].

South Africa has a population of 56 million persons and a per capita GDP of $5,273 (US) in 2016. The country has one of the highest TB burdens globally, with a 2016 incidence of 781 per 100,000. 59% of tested patients are HIV positive, which is, as a major risk factor for TB one of the biggest contributors to the TB epidemic in SA. MDR-TB is more frequent than in Tanzania, at 3.4% (95% CI 2.5-4.3) of all newly diagnosed cases, and 7.1% (95% CI 4.8-9.5) of retreatment cases. Unfortunately, MDR-TB and XDR-TB have been on the rise in SA in recent years. In 2016 there

were 967 cases of XDR-TB, with 628 cases starting on treatment. So far, the treatment-success in XDR-TB has been approximately 27% [9].

5 RATIONALE FOR THE STUDY

New TB treatment regimens are vital to combat the TB epidemic, and most importantly, the drug-resistant TB epidemic.

In MDR-TB, only 52% of MDR cases achieve cure [1]. A model of MDR-TB in Vietnam showed that under current diagnostic and treatment practices, MDR-TB incidence will increase by 17%, and deaths by 22%, within ten years [10]. In a similar model in China, MDR-TB will become the dominant form of TB by 2050 [2].

To effectively combat this epidemic, more potent drug combinations with regards to sterilizing capacity are necessary. These can be created through introduction of novel drugs with a low pre-existing resistance.

As evidenced by the success of the Nix-TB regimen composed of BDQ, a nitroimidazole, and LZD in patients with high levels of drug resistance, this drug combination is effective in curing TB within 6 months of treatment.

DZD in combination with BDM, is key in advancing a TB regimen with low pre-existing resistance and improved tolerability compared to LZD containing regimens.

DZD has a good safety profile, as has been assessed both pre-clinically and clinically for up to 21 days in healthy volunteers.

Previously DZD has been studied in patients in an EBA monotherapy study. DZD showed good activity in this study [11], but the question of selecting the best possible dose is not answered yet, since a reliable dose-response was not observed then.

A 14-day EBA is not long enough to assess the safety of different doses of DZD, since oxazolidinone class toxicity usually becomes apparent at a later stage in treatment.

Based on this, we believe it is more valuable to evaluate DZD over a dosing period which will generate enough accurate data on efficacy and safety to link exposure to safety observations. A time-period of dosing for 4 months is minimal to get a reliable estimate of toxicity, given that oxazolidinone class toxicity occurs not immediately during therapy [7].

A U.S. FDA guidance recently described an incompletely understood dose-toxicity and dose-response relationship as the cause for failure of many drug development programmes. It suggested testing several doses in phase IIb, and then continuing one or two selected doses [12].

PanACEA developed the concept this of this DZD dose selection study to establish an exposure-response model and an exposure–toxicity model if specific toxicity is observed. Based on data from this study, a DZD dose will be selected for further assessment in following studies.

6 INVESTIGATIONAL PRODUCT

6.1 Delpazolid

Delpazolid (LCB01-0371) belongs to the oxazolidinone class of antibiotics and is currently under development for the indication treatment of TB, especially to improve upon the limitations of existing oxazolidinone compounds.

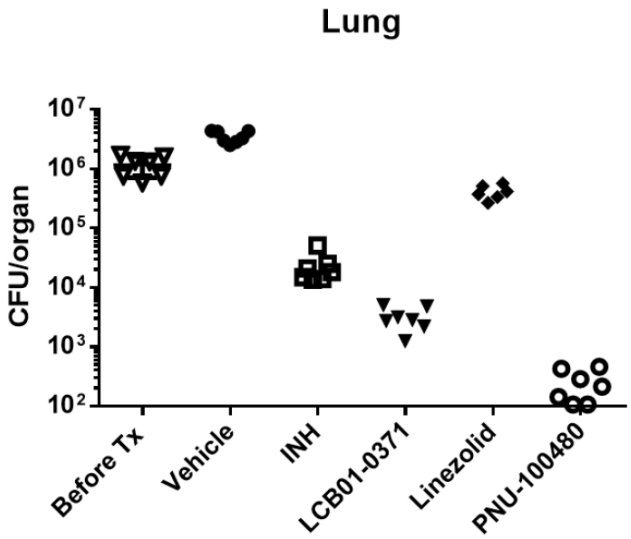
Oxazolidinones are a class of antimicrobial drugs that act by blocking protein translation, preventing the formation of the protein initiation complex. DZD binds to the V region of bacterial 23SrRNA and is interfering with the early compound formation of 30S and 50S rRNA. Therefore, it inhibits the protein biosynthesis at a very early stage leading to inhibition of bacterial proliferation and survival. Nonclinical studies demonstrated antibacterial activity towards various clinical pathogens, including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and *Mycobacterium tuberculosis* (*M. tuberculosis*).

Currently, DZD is available in two formulations: a tablet, containing 400 mg of DZD, to be used in this trial, and a parenteral injection.

6.1.1 Delpazolid: Efficacy in animal models

The efficacy of DZD in comparison to Linezolid, PNU-10480 and isoniazid was evaluated in a *M. tuberculosis* mouse model (figure 1). Mice were treated with DZD (150 mg/kg), Linezolid (150 mg/kg), PNU-10480 (150 mg/kg) or isoniazid (25 mg/kg) for 4 weeks. DZD decreased the CFU of M. tuberculosis by 3.08 log in the lung compared to a 1.11 log reduction (Linezolid), 4.21 log reduction (PNU-10480) and 2.23 log reduction (isoniazid).

Figure 1. In Vivo Bactericidal Activity of LCB01-0371 in C57BL/6 Mice Infected with M. Tuberculosis



6.1.2 Delpazolid: Pharmacokinetics and product metabolism in animals

Several in vitro studies were conducted in mice, rats and dogs to assess the pharmacokinetics of DZD.

DZD is mainly eliminated via urinary excretion, followed by fecal excretion. Plasma protein binding is low.

No inhibition of the hepatic enzymes CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 over the concentration range tested was observed. Treatment with DZD (at 2 to 200 µM for 48 hours) did not increase mRNA expression of drug metabolizing enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, UGT1A1, UGT1A4, UGT1A9, and UGT2B7) and transporters (MDR1, BCRP, MRP2, NTCP, OATP1B1, OCT1) using human hepatocytes. Furthermore, there was no evidence that DZD inhibited the transport activities of MDR1, BCRP, MRP1, MRP2, OCT1, OCT2, NTCP, OAT1, OAT3, OATP1B1, OATP1B3, and OATP2B1 transporters. Significant inhibition was only observed against monoamine oxidase (MAO)-A enzyme.

The potential of DZD and linezolid to bind to MAO-A and MAO-B receptors was tested in vitro using human recombinant enzymes. DZD showed inhibition of MAO-A and MAO-B with IC_{50} values of 12.6 µM and 27.0 µM, respectively. Linezolid showed inhibition of MAO-A and MAO-B with IC50 values of 26.3 µM and 1.51 µM, respectively.

Further details about DZD absorption, bioavailability, distribution and metabolism can be found in the Investigator's Brochure.

6.1.3 Delpazolid: preclinical animal toxicity studies

Overall, preclinical toxicology studies were performed as single and repeat-dose studies up to 4 weeks in rats and dogs and further repeat-dose studies up to a duration of 26 weeks in rats, and up to 39 weeks in dogs. No accumulation after treatment with multiple doses of DZD occurred.

Repeat-dose studies in rats

In the 4-week oral toxicity study in rats, groups of rats received drug levels of 0, 30, 60 and 140 mg/kg/d. All main study animals survived to their scheduled terminal or recovery necropsies with the exception of 2 males. One control male was euthanized on day 26 due to a possible handling injury and a male at 140 mg/kg/d was euthanized on day 30 due to test article related gastrointestinal ulceration. No test article related effects for clinical findings, body weight, food consumption measurement, ophthalmoscopic examinations, haematology, coagulation evaluations, macroscopic examinations and organ weight measurement were noticed at dose levels of 30 and 60 mg/kg/d. At 140 mg/kg/d, test article related decreases in mean body weight and food consumption occurred. Furthermore, test article-related clinical findings and effects in hematology, coagulation and urinalysis parameters were observed as were adverse clinical chemistry findings. Therefore, 60 mg/kg/d was the no-observed adverse effect level (NOAEL) in this group.

In the 26-week repeat oral dose toxicity study in rats dose levels of 10, 25, 50,100 → 75 (from week 11 of dosing) mg/kg/d and a control group (0.5% Methylcellulose and 0.1% Tween 80 in water for injection) were selected. A recovery period of 4 weeks was added, to evaluate the reversibility of potential toxicity. For observation of systemic exposure, a toxicokinetic study was also conducted and the vehicle or test substance was administered to all groups twice a day with a total daily dose of 20 mL/kg/d. As observed in the 4-week study, no accumulation or saturation of DZD was evident. T_{max} of DZD ranged from 0.50 to 1.00 hours. Systemic exposure was similar in both sexes and increased in proportion to dose between 10 and 75 mg/kg/d. No death or toxicologically significant test substance-related clinical findings were observed in the test substance dosing groups. No test substance-related toxic effects in food consumption, ophthalmology, urinalysis, clinical chemistry and bone marrow examinations were observed. Test substance-related adverse observations: decrease in mean body weight in males in the 100 → 75 mg/kg/d group and decrease in red blood cell, haematocrit and mean corpuscular haemoglobin in males in the 50 and 100 → 75 mg/kg/d groups were observed. Furthermore, there was a decrease in absolute testis weight at 50 and 100 → 75 mg/kg/d and decrease in epididymis weight at 25, 50 and 100 → 75 mg/kg/d noted, as well as signs of atrophy in the testis and epididymis at 25, 50 and 100 → 75 mg/kg/d in the histopathological examinations and also cell debris in lumen and oligospermia at these dose levels. Therefore, a NOAEL of 10 mg/kg/d for male rats and 100 → 75 mg/kg/d for female rats was considered.

In the 28-day intravenous rat study a dose of 60, 120 and 240 mg/kg/d were administered. Three deaths at 240 mg/kg/d occurred and were treatment related. Microscopic changes (decreased hematopoietic cellularity in the bone marrow) were noted in males at 120 and 240 mg/kg/d and in females at 240 mg/kg/d. Decreased food consumption at 240 mg/kg/d was observed (120 mg/kg/d in females during the first week only) and atrophy and/or secretory depletion in a wide variety of other tissues were considered secondary to the decreased food consumption. All changes in the male sex organs were fully reversible. Therefore, the NOAEL was considered to be 120 mg/kg/d.

Repeat-dose studies in dogs

In the 4-week oral toxicity study in beagle dogs, the test article was administered twice daily at dose levels of 5, 10 and 20 mg/kg/d, as was the vehicle in the control group. The toxicokinetic parameters calculated for males and female were similar. The combined mean $AUC_{0\text{-}24}$ values on day 27 were 13.8, 29.8 and 65.4 hr ug/mL at 5, 10 and 20 mg/kg/d, respectively. Combined mean C_{max} values on day 27 were 2.21, 5.5 and 11.6 ug/mL/d, respectively. No deaths occurred during the study.

The NOAEL was 20 mg/kg/d for male dogs and 10 mg/kg/d for female dogs due to adverse effects on clinical signs, body weight, food consumption, hematology/clinical chemistry and pathology.

In the 39-week repeated oral dose toxicity study with a recovery period of 4 weeks to assess the reversibility of adverse events, 0, 2, 5 and 10 mg/kg/d were administered to male and female dogs. 20 mg/kg/d was given until week 5, followed by a 2-week withdrawal period and continued at 15 mg/kg/d. The decrease was necessary due to gastrointestinal symptoms in females. The systemic

exposure of the test substance was determined through a toxicokinetic study. The AUC_{0-24} and C_{max} were increased in proportion to the dose level and similar between both sexes. The mean T_{max} was similar across dose levels and varied between 0.50 and 0.88 hours. No differences between sexes was noted and accumulation did not occur. There were no deaths during the experimental period. No test-related adverse effects were observed in any test substance dosing group for ophthalmologic examination, electrocardiogram, urinalysis, organ weights, necropsy or histopathology. Gastrointestinal symptoms (females only), decrease in body weight and food consumption (males and females) were noticed at 20 mg/kg/d and all subjects recovered after a change from 20 to 15 mg/kg/d.

In the 28-day intravenous infusion toxicity and toxicokinetic study in Beagle dogs, doses of 5, 15 and 30 mg/kg/d were administered. One female was killed at 30 mg/kg/d. Bone marrow and lymphoid tissue depletion was observed, as were gastrointestinal tract and other tissue changes due to inappetence. Eosinophilic round inclusions within centrilobular hepatocytes were observed at all doses (at lowest dose only in males). In summary, the NOAEL was 15 mg/kg/d.

Genotoxicity

No evidence of genotoxicity was observed in a bacterial reverse mutation assay, in a L5178Y/TK+/- mouse lymphoma mutagenesis assay and in a rat micronucleus assay.

Reproductive toxicity

Reproductive toxicity studies at dose levels of 0, 15, 30 and 60 mg/kg/d were performed in rats following oral administration and the reproductive performance, in utero development, dams and embryo-fetal development were examined. The NOAEL (reproductive performance and in utero development) was 15 mg/kg/d in males due to decreased sperm motility and abnormal sperm. In females, the NOAEL was 60 mg/kg/d.

The NOAEL for maternal animals was considered to be 30 mg/kg/d based on body weight, and the NOAEL for embryo-fetal development in rats was 15 mg/kg/d based on decreased fetal body weight at higher doses tested.

In rabbits, embryo-fetal development was observed at dose levels of 1, 3 and 10 mg/kg/d, with test substance related effects evident at 10 mg/kg, and the high- and low-dose levels were determined to be 3 and 0.3 mg/kg/d.

6.1.4 Delpazolid: Efficacy and safety in clinical studies

DZD has been clinically tested up to Phase 2A with the oral tablet formulation (7 Phase 1 studies- 1 study withdrawn and 1 Phase 2a study) and up to phase 1 (1 study) with the intravenous formulation. A total of 239 subjects received DZD, for a maximum administration period of 21 days.

In the single ascending dose study LCB01-0371-11-1-01, the multiple ascending 7-day dose study LCB01-0371-12-1-02 and the multiple ascending 21-day dose study LCB01-0371-14-1-01 the safety, tolerability, and pharmacokinetics of DZD were examined.

Altogether, there were no signs of significant myelosuppression or neuropathy. All ECG results were normal or assessed as not clinically significant. No deaths or SAEs occurred.

In the single-dose phase 1 study DZD was dosed up to 3200 mg. 20 adverse events occurred in 15 subjects. Most AEs were mild; moderate AEs only occurred at 3200 mg.

In the 7-day repeat-dose study, DZD was dosed up to 1600 mg BID. Most AEs were mild. Moderate AEs occurred in the 1600 mg BID group and this group was terminated after the first dose. The maximum tolerated dose in this study was 1200 mg BID.

In the 21-day repeat-dose study, DZD was dosed at a maximum of 1200 mg BID. Most AEs were mild, except for AEs of decreased neutrophil count, abnormal haematology test and abnormal liver function. 1 subject in the 1200 mg BID group showed a low haematocrit 17 days after drug administration and the study drug was discontinued at the discretion of the investigator. A non-compartmental PK analysis was also performed in 28 out of 40 subjects. Accumulation was almost non-existent with multiple dosing.

A food-effect study LCB01-0371-13-1-03 demonstrated a delayed absorption, a decrease in the maximum blood concentration and a decrease of the AUC_{last} by 10%. It was concluded that diet does not have a meaningful influence on treatment.

In another dose escalating phase 1 study LCB01-0371-16-1-01, DZD was administered intravenously to 36 healthy male participants to investigate the pharmacokinetics, safety and tolerability. A maximum dose of 1200 mg i.v. was administered. No severe or life-threatening AEs occurred. However, dose escalation was stopped at 1200 mg i.v. due to probably drug related AEs. Furthermore, 2 subjects from the 1200 mg i.v. group experienced moderate nausea and were withdrawn from the study. Therefore, 800 mg i.v. is the maximum defined dose for i.v. administration. Infusion site-related AEs were the most frequent AEs and appeared to be increased with the administered dose.

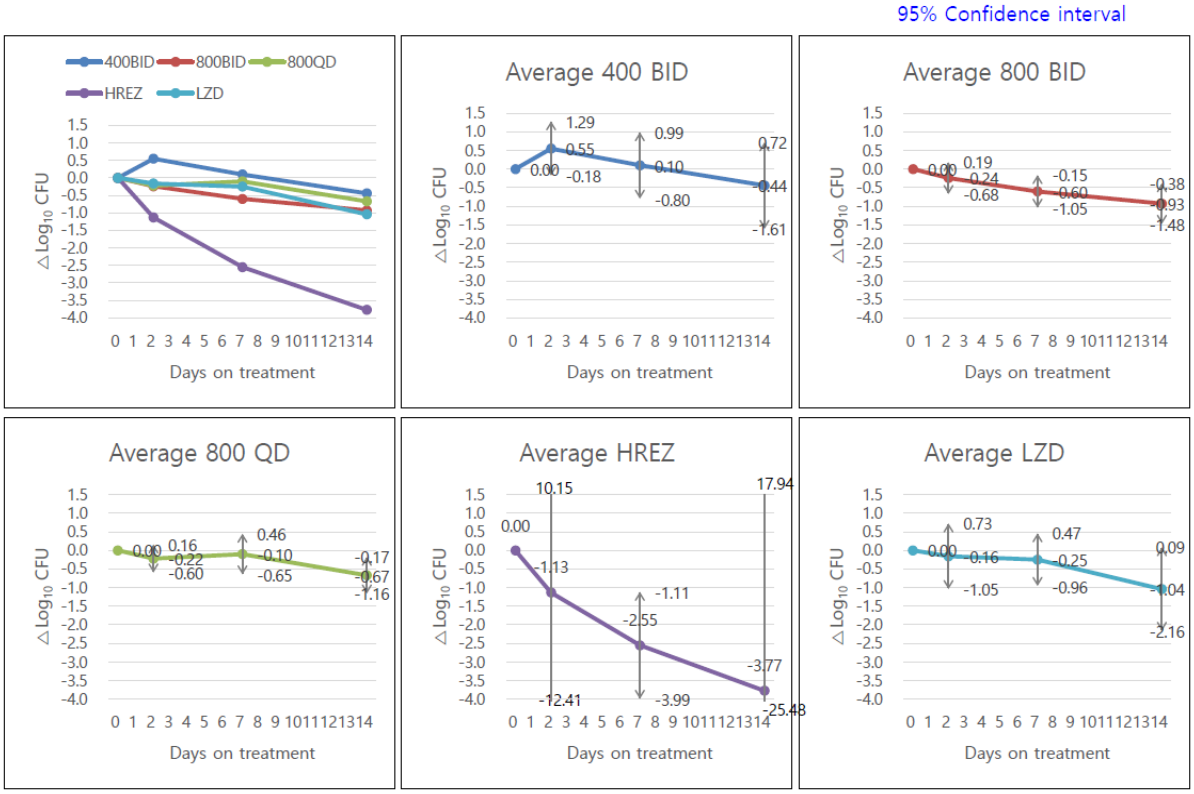
In the phase 2a early bactericidal activity study, all DZD doses administered showed killing of sputum bacilli.

The average daily change in log-CFU in DZD treatment groups were -0.044 ± 0.016 for 800 mg QD group, -0.053±0.017 for 400 mg BID group, -0.043±0.016 for 800 mg BID group and -0.019±0.017 for 1200 mg QD group. The average daily CFU change in the control groups was -0.192±0.028 for the HRZE (isoniazid 75 mg, pyrazinamide 400 mg, RIF 150 mg, ethambutol hydrochloride 275 mg contained per tablet) group and -0.154±0.023 for Linezolid 600 mg BD group.

Using a model based approach, the DZD patients achieved a daily decline in log-colony-forming units (CFU) over the 14 days of 23%, 27%, 22% and 10% of the results obtained from the patients receiving the HRZE combination in the 800 mg BID, 400 mg BID, 800 mg BID, and 1,200 mg BID groups respectively. The results are similar to previously reported EBA results for oxazolidinones

[13]. The ADR occurrence rate in study groups were 18.75% (3/16 subjects, 8 cases) in 400 mg BID group, 18.75% (3/16 subjects, 6 cases) in 800 mg BID group, and 37.50% (6/16 subjects, 13 cases) in 1200 mg QD group, while there was no ADR in 800 mg QD group. That of control groups were 50.00% (4/8 subjects, 9 cases) in HRZE and 50.00% (4/8 subjects, 7 cases) in LZD 600mg group. The most frequently reported ADR was gastrointestinal disorder, especially nausea and diarrhoea.

Figure 2. Phase 2a EBA study: daily decline in log-CFU with different treatments



6.1.5 Delpazolid: interpretation of preclinical animal and clinical human findings

Comparing effects of DZD in rats and dogs and humans at the longest application period, DZD had an effect of the gastrointestinal system, presenting with decreased body weight in rats and in dogs, decreased food consumption in dogs, and gastrointestinal symptoms in dogs and humans. In humans, these symptoms occurred during fasting conditions and most likely will be improved under fed conditions, which we are planning to use in this study.

6.1.6 Delpazolid: Discussion of risk for tyramine pressor syndrome in this study

Oxazolidinones, like LZD, are known to have a weak reversible monoaminooxidase (MAO) inhibitory effect in vitro due to their structural similarity to the reversible MAO inhibitor toloxatone [14]. Therefore, oxazolidinones can block the metabolization of dietary tyramine and

thus may act as pressor enhancers, as documented for LZD in rats [15] and in rare cases in humans [16].

Tyramine is a naturally occurring trace amine, which acts as catecholamine releasing agent. When intestinal MAO-A is inhibited, higher levels of tyramine can be absorbed in the intestines, leading to indirect increase of norepinephrine signalling at nerve endings, which might cause arterial hypertension and hypertensive crisis.

The potential interaction of foods with a high tyramine content and irreversible MAO-inhibitors is well documented. The interaction with drugs, having reversible MAO-inhibitory effects, like Linezolid, and DZD in studies to date, and oral tyramine doses within the range of normal dietary intake (up to 100mg per meal) showed no significant changes in blood pressure [17, 18]. Reversible MAO-A inhibitors, e.g. moclobemide have been extensively studied and their PK, clinical PK and toxicological profiles have been thoroughly defined. Here, just minimal interaction with exogenous amines, like tyramine have been found [19, 20].

A specific tyramine pressor test study was conducted for linezolid in humans, to examine the quantity of oral tyramine intake required to produce a blood pressure increase by 30mmHg. It was found that very high doses of tyramine, of above 100 mg, were required to produce such a blood pressure increase, similar to the specific MAO-A inhibitor, moclobemide which was used as a control [16]. Based on these findings, the actual recommendations for tyramine quantities for patients receiving LZD are <100mg per meal [21, 22] and moclobemide is prescribed without dietary tyramine restrictions in the UK, but with a general precaution in the product label to avoid excessive amounts of tyramine-rich foods [23].

No studies with DZD were so far conducted to address this issue.

In this study, we have decided to approach the issue with some caution and restrict the use of certain foods with the highest tyramine content: certain cheeses, certain meats, beers and wine, and soy sauce (see Appendix 20.2 for restricted foods).

In addition, we have added scheduled post-IMP blood pressure measurements at the visit Day 01 and Week 02 to detect eventual increases in blood pressure.

7 NON-IMP DRUGS USED IN THE STUDY

For clarity, all licenced drugs in this study (all drugs other than DZD), are referred to as ´non-IMP study drugs´. BDQ, DLM and MXF are meant by this term. When reference is made to ´study drugs´ all drugs including DZD are meant.

7.1 Bedaquiline

BDQ, developed by Janssen Therapeutics, is a diarylquinoline compound with a novel mechanism of action against MTB. The inhibition of mycobacterial adenosine triphosphate synthase. The Food and Drug Administration (FDA), on 28 December 2012, granted accelerated approval to SIRTURO® (BDQ) tablets as a part of combination therapy in adults with MDR-TB. It is the first new anti-TB

drug to be approved after 40 years. It is also the first to be introduced specifically for the treatment of MDR-TB in combination with other drugs. Since 2018, SIRTURO® is approved and part of the national standard recommended treatment regimen for Rifampicin-resistant and MDR-TB in South Africa [24], and is approved in Tanzania.

Very recently, WHO has recommended BDQ to be part of therapy of MDR-TB [25].

7.1.1 Bedaquiline: Efficacy in clinical studies

In vitro, BDQ has potent activity against drug-susceptible and drug-resistant *MTB* isolates and is also bactericidal against dormant (non-replicating) MTB in a murine TB model, BDQ is as active as the triple combinations of INH, RIF, and pyrazinamide (PZA), with BDQ accelerating clearance of bacilli and displaying synergy with PZA. Drug regimens that include BDQ are considered the most effective experimental combinations for treatment of DS-TB in the presence of PZA, or in regimens without PZA that include a nitroimidazole where there is a higher likelihood of PZA resistance [26, 27].

In vivo, BDQ has been evaluated in DS-TB patients in a Phase 2a study and in MDR-TB patients in Phase IIb studies, and there is now a large post-registration database containing data mostly from use of the drug in South Africa. A Phase 3 study is currently recruiting (STREAM stage 2), but results are not expected until 2022.

7.1.2 Bedaquiline: Pharmacokinetics

BDQ is well absorbed with a T_{max} of 4-6 hours irrespective of the dose [28]. Dose-proportionality of C_{max} and AUC is seen up to 700 mg with single doses and 400 mg with multiple doses. The average $t_{1/2}$ of BDQ is 132 days and is 112 days for its M2 metabolite. Administration with food increases the bioavailability by 95%. In individuals of black race, concentrations of BDQ are appreciably lower (52%) compared to individuals of non-black race [29]. BDQ is metabolized by oxidative metabolism via the CYP3A4 isoenzyme to its N-desmethyl metabolite, M2. BDQ has an extensive $t_{1/2}$, therefore it accumulates over time. In adults, drug accumulation is mitigated by using the current loading dose of 400 mg once daily for two weeks followed by a reduction in dose and dosing frequency to 200 mg three times weekly for six additional weeks.

7.1.3 Bedaquiline: Safety and tolerability

The primary safety concerns about BDQ included an increase in late mortality in the phase 2b trial, which also identified QT interval prolongation. At 24 weeks, the mean change in QTcF in the BDQ arm was a 15.4 ms increase, with 3.3 ms increase in the placebo arm [30]. Despite of the fact that most of the phase 2b deaths were attributed to TB by the investigators, the phase 2b deaths led to a black box warning on the package insert. More recently, however, results using BDQ in 2,093 patients in France, Georgia, Armenia, and South Africa were presented in a review by the WHO BDQ guideline development group, published in 2017 [31]. MDR-TB patients who received BDQ for a mean of 6.37 months were significantly more likely to survive (adjusted odds ratio for death (OR) 0.39; 95% CI 0.31-0.59; p<0.001; adjusted hazard ratio (HR) 0.48; 95% CI 0.39-0.59) compared to those who did not. This effect was present across resistance profile categories and history of

previous TB disease and may alleviate some concerns stemming from the unclear safety situation after the phase 2b study.

7.2 Delamanid

DLM is a nitroimidazole that inhibits the synthesis of mycolic acids, a crucial component of the cell wall of *MTB*. It represents a promising new weapon in the arsenal for treatment of MDR-TB and was developed by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). DLM has received regulatory approvals in several countries, and is currently recommended by WHO for the treatment of MDR-TB in specific cases [32-34].

7.2.1 Delamanid: Efficacy in clinical studies

Delamanid was licensed by the European Medicines Agency (EMA) based on results from a phase 2b study, with a primary endpoint of 2 months culture conversion [35]. DLM was added to an optimized background regimen for the patients' MDR-TB, compared with placebo.

In liquid media, 45.4% of 141 patients had negative sputum samples at month 2, compared with 29.6% of 125 patients who received placebo.

In the phase 3 study, DLM shortened the time to culture conversion by 6 days (median: OBR + DLM: 51 days; OBR + placebo: 57 days). The standard of care arm performed much better than in past studies, with 53.5% of patients achieving two-month culture conversion; vs 58.4 % when DLM was added. In the DLM arm, 81.4% of patients achieved cure or treatment completion compared to 81.2% in the placebo arm. Additional drug resistance was acquired during treatment by 6.5% of patients on the placebo arm, and by 1.9% of patients on the DLM arm. The conclusion was that DLM showed modest improvement of treatment outcomes, but due to the unexpected high efficacy of the standard treatment did not show similar improved activity as in the earlier phase 2b study.

7.2.2 Delamanid: Safety in clinical studies

Twelve phase I trials of DLM in healthy adults have shown a favourable side effect profile, with the most commonly reported side effects emerging while on treatment being dizziness, nausea and headache. The total number of adult individuals exposed to DLM in these safety trials has been 949, and overall, the side effects were similar in the groups receiving DLM plus optimized background regimen and the groups receiving placebo plus optimized background regimen.

DLM has been studied in a Phase 2a study in DS-TB patients and placebo-controlled phase 2b and phase 3 trials in MDR-TB. Both in the phase 2b and in the phase 3 studies, the safety profile of DLM in MDR-TB patients was favourable [36]. Serious treatment emergent AEs were similar in patients treated with DLM or placebo on a background regimen in the phase 3 trial that was recently reported. Eight (2.3%) patients in that trial discontinued due to AEs on DLM, compared to three (1.8%) on placebo. The proportion of patients experiencing hepatotoxicity was 6.5% in the DLM arm and 7.1% in the placebo arm, suggesting that DLM did not add to hepatotoxicity of

the background regimen. In the DLM arm, 18 (5.3%) patients experienced a prolongation of their QT interval, as opposed to 5 (2.9%) on placebo.

The QTc prolongation seen in patients treated with DLM is characterized by a slow increase over the first 6-10 weeks of treatment followed by stability, thereafter, appearing to correlate with plasma concentrations of the major metabolite, DM-6705. Both hypoalbuminemia (<2.8 g/dl) and hypokalaemia have been found to be risk factors for DLM associated QTc prolongation.

In the phase 3 study, the mean placebo-corrected QTcF change was 5.3 ms (90% CI: 2.8 - 7.9) at week 8, and 2.5 ms (-0.3 - 5.3) at week 26. Low albumin concentration (DLM is metabolized by albumin) was not associated with higher QTcF prolongation in that study.

7.2.3 Delamanid: Pharmacokinetics

In adults, DLM reaches a peak concentration (T_{max}) four hours post-dose, and the apparent terminal elimination half-life ($t_{1/2}$) is 30-38 hours. DLMs bioavailability is increased 2- to 4-fold when it is taken with food. The $t_{1/2}$ of its main metabolites is approximately 150-600 hours. The PK profile of DLM is similar for healthy volunteers and for patients with TB. Among adult patients with MDR-TB, a dose of 100 mg twice daily achieves an AUC_0-24h of 7234 h*ng/mL (CV% 32) at two weeks and an AUC_0-24h of 7925 h*ng/mL (CV% 38) at two months [35]. Once daily dosing of 200 mg of DLM (four 50-mg tablets), previously resulted in a mean ± SD AUCτ of 5,950 ± 1,440 ng·h/mL and a C_{max} of 476 ± 119 ng/mL (Day 15) in healthy subjects [37].

DLM is metabolized to M1, a unique metabolite formed by cleavage of the 6-nitro-2,3-dihydroimidazo[2,1-b] oxazole moiety, in plasma albumin in vitro. The metabolic activities in dogs and humans are higher than those in rodents. In animals and humans eight metabolites (M1-M8) produced by cleavage of the imidazooxazole moiety of DLM were identified in the plasma after repeated oral administration [38]. DLM was initially catalyzed to M1 and subsequently metabolized by three separate pathways, which suggested that M1 is a crucial starting point. The major pathway in humans was hydroxylation of the oxazole moiety of M1 to form M2 and then successive oxidation to the ketone form (M3) mainly by CYP3A4. M1 had the highest exposure among the eight metabolites after repeated oral dosing in humans, which indicated that M1 was the major metabolite. Non-hepatic formation of M1 and multiple separate pathways for metabolism of M1 suggest that clinically significant drug-drug interactions with DLM and M1 are limited [38].

Albumin in serum primarily regulates the metabolism of DLM, with the cytochrome P450 3A4 enzyme also contributing modestly, so concurrent administration with medications known to inhibit or induce this enzyme may modestly alter drug levels [38, 39]. In addition, administration of DLM to patients with albumin levels <2.8 g/dL is not recommended. DLM and its major metabolites do not show meaningful inhibition of CYP isoenzyme activity, including CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. DLM is not an inducer of CYP1A2, CYP2C9 nor CYP3A4/5. In DDI studies of DLM used in healthy volunteers in combination with tenofovir, efavirenz and/or lopinavir-ritonavir, no significant DDIs were seen; when given

with lopinavir/ritonavir, a modest increase in DLM area under the concentration-time curve of 25% was observed [37]. Co-administration of DLM with first-line TB drugs resulted in lower DLM exposures (47 and 42% for the AUC and C_{max} values, respectively) as well as decreased exposure of the three primary metabolites. DLM did not affect RIF, pyrazinamide nor INH exposure while the ethambutol (EMB) AUC0-τ and C_{max} values were about 25% higher with DLM co-administration. Although there was a decrease in the DLM concentrations when co-administered with TB drugs, this was posed to be likely related to decreased DLM absorption (because of ingestion of many tablets in a short time frame) and not to CYP induction. Overall, the drug-drug interactions between DLM and selected antiretroviral agents (including the strong CYP inhibitor ritonavir) and a combination of anti-TB drugs was judged as not clinically significant [37].

7.3 Moxifloxacin

MXF belongs to the group of fluoroquinolones (FQ). FQs are a mainstay of MDR-TB treatment, and MXF is considered one of the most potent drugs in second line MDR-TB therapy, recently reviewed by WHO [40] with only moderate pre-existing resistance in the community. MXF is a potent QTc prolongation agent and is frequently used as a positive control in QTc studies of new drugs.

7.3.1 Moxifloxacin: Efficacy in clinical studies

Efficacy studies looking at the added value of MXF included the Rifaquin study by Jindani et al, which showed that a 6-month regimen including rifapentine and MXF (in which INH was replaced by daily MXF for 2 months followed by one weekly dose of both MXF and 1200 mg of rifapentine for 4 months) was as effective as standard of care [41]. In the REMox study, a 4-month regimen containing MXF was further investigated. Although the 4-month regimen (replacing either INH or EMB with MXF in two different arms) did not show non-inferiority in comparison to standard of care, the potential of MXF itself was shown, with more rapid decline in bacterial load compared to standard of care in both MXF-containing arms [42].

7.3.2 Moxifloxacin: Pharmacokinetics

MXF is easily and rapidly absorbed after oral administration. The bioavailability of MXF following oral dosing exceeds 90% (52). MXF is widely distributed, with some tissue concentrations reported in excess of plasma levels. MXF is metabolized in the liver via glucuronide and sulfate conjugation by cytosolic enzymes glucuronosyltransferase and sulfotransferase [43]. The major human uridine diphosphate (UDP)-glucuronosyltransferases (UGTs) responsible for formation of the glucuronide metabolite are UGT1A1, UGT1A3, and UGT1A9 (UGT1A1 being the main isoform). MXF is a substrate of p-glycoprotein, and the drug transporter protein plays an important role in its absorption, distribution and elimination [44]. The cytochrome P450 (CYP450) enzyme system is not involved in the metabolism of MXF nor is it affected by the drug (58). Neither MXF nor its metabolites inhibit the CYP450 enzymes. Similarly, MXF was shown not to be an inhibitor of any of the major human UDP-glucuronosyltransferases. The sulphate conjugate accounts for 38% of the oral dose and is excreted in faeces; about 14% of an oral dose is converted to the glucuronide conjugate and is excreted in urine [45]. Peak plasma levels of the sulphate and glucuronide

metabolites are <10% and about 40% those of the parent drug, respectively. Overall, about 45% of an oral dose is excreted unchanged as parent drug, and about 51% as known sulphate and glucuronide metabolites, which are biologically inactive. Co-administration with food may slightly prolong time to maximum concentration and may reduce the maximum serum concentration (C max) by 16%; these effects are thought to be insignificant, and MXF may therefore be administered with or without food [46].

7.3.3 Moxifloxacin: Safety and tolerability

Data on the long-term use of MXF show that it has an excellent safety profile [47]. Comprehensive safety data is available from Phase II to Phase IV studies performed by Bayer Healthcare, comparing MXF to a comparator group of patients receiving various other antibiotics. 23.9% of patients suffered adverse drug reactions (ADRs) due to oral application of MXF, while 22.0 % of patients in the comparator group suffered such reactions [48]. Nausea (7.5%) and diarrhoea (5.8%) are the most frequent individual AEs reported for MXF, but also for the comparator group. Nausea is more common in the MXF group. All other AEs differ by less than 1% between the treatment groups. Headache (4.4%) and dizziness (3.6%) were third and fourth most frequently encountered AEs in patients with oral MXF. Among 13.368 patients who received the drug via oral route, 3 fatal ADRs were registered. Among 1.144 patients who received the drug via intravenous route, no fatal ADRs were registered. In PO active-controlled studies, 38 of the 10,613 MXF-treated patients (0.4%) and 51 of the 10,685 comparator recipients (0.5%) had at least 1 AE with fatal outcome. Myocardial infarction and septic shock (reported in 5 patients each) are the most frequent AEs leading to death. Serious adverse drug reactions (SADRs) were reported in 59 (0.6%) and 53 (0.5%) of MXF and comparator-treated patients, respectively. The most common SADRs are abdominal pain and vomiting, reported with an incidence <0.1%.

7.4 Combination of drugs used in the study

Three of the drugs used in this study have the potential to increase cardiac QT intervals, which has in the past discouraged large-scale use of these drugs together in a regimen. We provide recent data below to suggest that these drugs have been combined safely:

Best evidence on this question is available from the prospectively randomized ACTG 5343 (DELIBERATE) trial. This trial compared MDR-TB treatments containing either BDQ or DLM alone, or both drugs in combination, over a treatment period of 24 weeks.

The study found a mean change in QTcF from baseline of 11.9 ms (95% CI: 7.4 - 16.5) in the BDQ arm, 8.6 ms (4.0 - 13.2) in the DLM arm, and 20.7 ms (16.1 - 25.4) in the BDQ+DLM arm [49].

The combined effect on the QTcF interval of co-administration of bedaquiline and delamanid was clinically modest and no more than additive. This study demonstrated the cardiac safety of the combined use of these drugs in patients with MDR-TB taking MBT with normal baseline QTcF values.

These data from a randomized study are corroborated by a meta-analysis by the group of Migliori et al. analysed combined treatment of drug-resistant TB with BDQ and DLM in Armenia, France,

India, Latvia, Russian Federation, South Africa, South Korea, and The Netherlands. This review is the largest up to date, and included 87 cases of 7 different studies, of which 54.5% of patients suffered from XDR-TB and 27.3% were HIV co-infected. The study concluded the following: Most M/XDR patients were concomitantly treated with BDQ and DLM, and in most cases for a duration > 6 months. The sputum culture conversion rate after 6 months of treatment was considerably higher (81.4%) than historical M/XDR-TB patient cohorts. Out of 87 patients, 23 (26.4%) showed >1 episode of QT prolongation >450 ms in men or 470 ms in women, or a QT increase >60 ms from baseline values, however only 2.3% of treatments were interrupted for the occurrence of life-threatening cardiac adverse events [50].

Médecins Sans Frontières published data on 28 patients receiving BDQ and DLM in combination from South Africa, India and Armenia [51]: 25 of 28 patients received at least one additional QT-prolonging agent, clofazimine or MFX, and 2 patients received both. The key finding from this cohort was that no patient exceeded a QTcF interval of 500 ms, which is the cut-off point best supported as a safety issue by clinical data. Only 4 patients had a change of QTcF >60 ms over their baseline QT interval, and all of them had received clofazimine as QT prolonging agent. In no patient, study drug was permanently discontinued. Of 16 SAEs in 7 patients, 6 were considered possibly related to DLM, BDQ or to both drugs. Eleven SAEs were considered possibly related to other TB drugs. The only fatal event in this cohort was associated with immune reconstitution inflammatory syndrome (IRIS), characterized by acute renal failure and hypoglycaemia. The most common SAE in the cohort was gastrointestinal (4/16, 25%), a common side effect of a number of MDR-TB drugs that may have been part of the regimen (thioamides or para-aminosalicylic acid, PAS). Nervous system disorders accounted for 4/16 SAEs (25%) and psychiatric disorders 2/16, (12%). Both SAEs are commonly associated with cycloserine, another drug commonly used in MDR-TB regimens. Of note, 23 (82%) patients in this report received LZD as part of their regimen.

Further published case reports include 7 patients: 2 single XDR patients were summarized in a review of case series [52] and 5 more XDR patients' outcomes were published as a separate case series, with patients living in Russia (2), India (2), or the Netherlands (1) [53]. One of 2 in the first case review and 2 of 5 patients in the second case review experienced QTcF >500 ms; in one case prolongation reverted on repeat examination; the second patient was started on concomitant verapamil and had an unspecified dose adjustment. All 3 of the patients exceeding an absolute QTcF prolongation of 500ms had received CFZ as additional QT-prolonging agent in their regimen.

Recent safety data summarized below shows that MXF can also be safely combined with a BDQ-nitroimidazole combination without exacerbating QT prolongation:

The TB Alliance NC005 study assessed regimens containing BDQ, PTM and PZA, with and without MXF [54]. Published data of QT prolonging effects of PTM 200mg given alone in a 14-day EBA study [55] and DLM in its Phase III study [36] suggests that DLM would not cause more QT prolongation than PTM.

In the regimen containing BDQ+PTM+PZA, the mean QTcF change from baseline was 21.9 ms (95% confidence interval 18.2-25.7), very much in line with that seen for BDQ + DLM in the ACTG 5343 DELIBERATE study mentioned above. In patients receiving MXF in addition to BDQ+PTM+PZA,

QTcF prolongation was unchanged at 21.9 (95% CI 18.7-25.0); so, in this study, there was no difference in QTcF due to the addition of MXF. In the control arm (INH + RIF + PZA + EMB) the mean increase from baseline was 10.2 ms (95% CI 7.0-13.4)[56].

Serious treatment-emergent AEs were equally distributed in the BDQ+PTM+MXF+ PZA group (60 patients) and the BDQ+PTM+PZA group without MXF (59 patients), with 4 events occurring in each arm. The INH+RIF+PZA+EMB arm also had 4 events in 61 patients. In all arms, most patients experienced at least one treatment-emergent AE: 57 of 60 in the MXF-containing arm, 50 of 59 in the arm without MXF, and 44 of 61 in the control arm.

8 STUDY OBJECTIVES

8.1 Safety and Tolerability Objective:

Overall, the primary objective is to describe the safety, tolerability and exposure-toxicity relationship of DZD given over 16 weeks in combination with standard-dose BDQ, DLM and MXF compared to standard-dose BDQ, DLM and MXF alone.

The aim is to identify the optimal dose of DZD to be used in subsequent studies that provides the best **efficacy at acceptable safety** of the drug when given daily over 4 months in patients with newly diagnosed, uncomplicated, smear-positive, drug-sensitive, pulmonary tuberculosis.

8.1.1 Efficacy Objectives

Primary Efficacy Objective:

- To establish an exposure-response model for DZD, given over 16 weeks in combination with standard-dose BDM, on the change in liquid culture MGIT time to positivity (TTP)

Secondary Efficacy Objective:

- To assess dose and exposure-response relationships for DZD, based on secondary efficacy endpoints, including month-2 culture status in liquid media and on solid media, and time to culture conversion in liquid and on solid media
- To assess the relative efficacy of increasing DZD doses compared to the background regimen without DZD, based on primary and secondary efficacy endpoints.
- To assess the proportion of patients who suffer relapse within 12 months post randomisation, out of those patients completing 16 weeks of therapy and achieving sustained sputum culture conversion, defined as two successive negative liquid media cultures at or before WK08, with no positives to follow by the week 16 visit.

8.1.2 Pharmacokinetics Objectives

Primary Pharmacokinetics Objective:

- To describe the pharmacokinetics (PK) of DZD through development of a population PK model

Secondary Pharmacokinetics Objective:

- To describe the PK of BDQ, DLM and MXF including their main metabolites

8.1.3 Mycobacteriology Identification and Characterization Objectives

- To assess the minimum inhibitory concentrations (MIC) of BDQ, DLM, MXF, DZD of the infecting strain.
- To investigate the frequency of acquired mutations in the infecting strain over treatment
- In patients with recurrent disease: comparison of initial and recurrence isolate by whole genome sequencing, to differentiate between relapse and reinfection

9 TRIAL ENDPOINTS

9.1 Primary Endpoints

9.1.1 Primary Safety endpoint

Participants will be regularly assessed for AEs during treatment and follow-up phase, including assessments of vital signs, physical examination, weight, detailed neurological examination, colour vision and visual acuity tests, 12-lead ECGs and routine clinical laboratory tests (including chemistry, haematology and urinalysis data).

The safety of DZD will be investigated by evaluation of:

- Proportion of patients experiencing expected oxazolidinone class toxicities, defined as peripheral or optical neuropathy, or incident leukopenia, anemia or thrombocytopenia, or adverse events in line with tyramine pressor response, all of Grade 2 or higher, possibly, probably or definitely related to DZD

9.1.2 Primary Efficacy Endpoint

The efficacy of DZD will be evaluated by measuring the change in mycobacterial load over time on treatment as quantified by time to positivity in BD MGIT 960® liquid culture described by non-linear mixed-effects methodology

9.2 Secondary Endpoints

9.2.1 Secondary safety endpoints

- All adverse events
- Adverse events of Grade 3 severity or higher
- Adverse events possibly, probably or definitely related to study drugs
- Treatment discontinuations or interruptions related to adverse events/serious adverse events
- Frequency, severity and type of ECG alterations
- Changes in ECG intervals of PR, RR, QRS, QT, Fridericia-corrected QT [QTcF]

- Proportion of participants with QTcF > 500ms in ECGs on treatment

- Proportion of participants with a prolongation of QTcF > 60ms in ECGs of grade 3 and above as defined under 14.6 *Table 2*

9.2.2 Secondary Efficacy Endpoints

Further efficacy of DZD will be evaluated by assessment of:

- Proportion of patients who suffer relapse, defined as recurrent disease caused by a strain identical to the baseline isolate, within 12 months post randomisation, out of patients completing 16 weeks of therapy and achieving stable culture conversion to negative (SCC) as defined below by week 08, with no positives to follow by the week 16 visit.
- Time to recurrent TB, and to relapse, within 12 months post randomisation
- Time to stable sputum culture conversion (SCC) to negative on liquid media (defined as two negative cultures without an intervening positive culture). Time will be measured as time on treatment until the first negative culture.
- Proportion of participants who achieved SCC at each time point during treatment
- Proportion of participants with negative sputum culture on solid media at WK 08 and other timepoints
- Proportion of participants developing drug resistance among those not converting to negative culture

9.2.3 Pharmacokinetic Endpoints

A population PK model will be developed for DZD. The following secondary parameters will be derived for DZD, for BDQ, DLM and their main metabolites, and for MXF:

- Area under the plasma concentration curve from morning dosing to 24 hours (AUC 0-24) on day 14 (WK02)
- The observed maximum concentration (C_{max}) on day 14
- Time to reach C_{max} (T_{max}) on day 14
- The minimum observed plasma concentration (C_{min}) at day 14 (24 hours following the last dose for QD and 12 hours following the last dose for BID)
- Apparent oral clearance (Cl/F)
- Apparent volume of distribution (Vd/F)
- Terminal half-life ($t_{1/2}$)

Analyses may be limited by available budget, which may lead to parameters for certain drugs not being reported.

9.2.4 Mycobacteriology Identification and Characterization Endpoint

Sputum cultures from various timepoints during the study will be assessed as follows:

- Minimum inhibitory concentrations (MIC) of BDQ, DLM, MXF, DZD of the infecting strain, at baseline and on representative isolate(s) grown at or after WK08, if any.
- Frequency of acquired mutations in the infecting strain over treatment by whole genome sequencing
- Comparison between bacterial strain causing recurrent disease, and the strain at baseline by whole genome sequencing, to discriminate relapse from re-infection.

These assessments will be performed dependent on availability of lab capacity and budget, so may not be completed for all possible timepoints.

9.2.5 Exploratory Endpoints

The following exploratory endpoints will be analysed depending on laboratory capacity, availability of test kits and budgets permit and may not be tested in all trial sites:

- Rate of change of bacterial load measured by molecular bacterial assay (MBLA) during treatment
- Time to stable conversion to negative in MBLA (defined as two negative MBLAs without an intervening positive)
- Rate of change in bacterial load measured by quantification of sputum lipoarabinomannan (LAM) during treatment

These assessments will be performed dependent on availability of lab capacity and budget.

10 TRIAL DESIGN

10.1 Summary

This will be an open label Phase IIb, randomized, controlled, dose-finding, multi-centre study in participants with newly diagnosed, smear positive, uncomplicated, DS pulmonary TB.

10.2 Trial population

Participants will be randomized to one of five arms containing a BDM backbone with different doses of DZD, ranging from 0mg DZD (no DZD present in the regimen) up to 1,600mg (800mg BD). 75 participants will be randomized into this study, with 15 participants per arm.

In the case of unforeseen delays or a rate of dropouts or non-evaluable participants that is higher than anticipated, it may be necessary to recruit more participants than planned into the entire study (see sample size considerations, Chap. 15.1).

Participants will be randomised by centralized allocation, using a probabilistic minimisation algorithm, which will stratify participants by bacterial load in sputum as measured by GeneXpert cycle threshold, site and HIV status.

Participants will visit the study clinic on a weekly basis for sputum collection, safety monitoring and receipt of study medication.

After the completion of 16 weeks of experimental treatment, participants in the experimental arms, who did not achieve two successive negative liquid media cultures with the first at or before WK08, with no positives to follow by the week 16 visit, will be referred to their local health care facility to complete their course of anti-TB treatment according to the national TB program.

10.3 Inclusion Criteria

Participants are required to meet **all** the following criteria in order to be randomized:

1. Provide written, informed consent prior to all trial-related procedures including HIV testing.
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, previously untreated, drug susceptible pulmonary TB: presence of MTB complex and rapid molecular tests result confirming susceptibility to RIF and INH such as GeneXpert and/or HAIN MTBDR *plus*.
5. A chest X-ray (no older than 2 weeks) which, in the opinion of the Investigator, is consistent with TB.
6. Sputum positive on microscopy from concentrated sputum for acid-fast bacilli on at least one sputum sample (at least 1+ on the IUATLD/WHO scale).
7. The participant is willing to forgo consumption of foods high in tyramine for the period of taking study medication (See Appendix, section 20.2, page 95).
8. The participant is either unable to conceive/father children AND/OR his/her partner is unable to conceive/father children AND/OR they will consent to be using effective methods of contraception when engaging in heterosexual intercourse, as defined below:
    a. Non-childbearing potential:
        i. Female participant/sexual partner of male participant: Bilateral oophorectomy, and/or hysterectomy or bilateral tubal ligation more than 12 months ago and/or has been postmenopausal with a history of no menses for at least 12 consecutive months
        ii. Male participant/sexual partner of female participant: Vasectomised or has had a bilateral orchidectomy minimally three months prior to screening
        iii. Male participants having a pregnant female partner or a male sexual partner: At least one barrier method has to be used in this case.
    b. Effective contraception methods:

i. Female participants: Two methods, including methods that the patient's sexual partner(s) use. At least one must be a barrier method. Contraception must be practised for at least until 12 weeks after the last dose of DZD.

ii. Male participants: Two methods, including methods that the patient's female sexual partner(s) use. At least one must be a barrier method. Effective contraception must be ensured for at least 16 weeks after the last dose of DZD.

Note: hormone-based contraception alone may not be reliable when taking RIF during continuation phase; therefore, hormone-based contraceptives alone cannot be used by female participants/female partners of male participants to prevent pregnancy.

10.4 Exclusion Criteria

Participants for whom **one of the following** criteria is met will be excluded from trial:

1. Circumstances that raise doubt about free, unconstrained consent to study participation (e.g. prisoner or mentally handicapped person)
2. Poor general condition where delay in treatment cannot be tolerated or death within four months is likely.
3. Poor social condition which would make it unlikely that the patient would be able to complete follow-up
4. The patient is pregnant or breast-feeding.
5. The patient is infected with HIV with a CD4 count <220 cells/mm^3. If >220 cells/mm^3, patients will be included only if **any** of the following is applicable:

    - The patient is antiretroviral (ARV) naïve and able to postpone commencing HIV treatment for 2 months after the trial has started and then restrict regimens to those containing dolutegravir (see section 12.6.2 on ARVs)

      or

      The patient is ARV experienced (has been on ARV´s a minimum of 5 months) and able to switch to a dolutegravir-based regimen.

    - The patient is treated with nucleosidic reverse transcriptase inhibitors (are permitted as concomitant medication).
    - The patient is treated with protease inhibitors as part of antiretroviral treatment regimens, which will be stopped at least 3 days before the start of study treatment (WK01, day1) for a patient to be eligible.

- The patient is treated with Efavirenz as part of antiretroviral treatment regimens which would have to be stopped 14 days before the start of study treatment (WK00, Day 01) for a patient to be eligible.

6. The patient has a known intolerance to any of the study drugs or concomitant disorders or conditions for which study drugs or standard TB treatment are contraindicated.
7. The patient has a history of, or current evidence of clinically relevant cardiovascular metabolic, gastrointestinal, neurological, psychiatric or endocrine diseases, malignancy, or any other condition that will influence treatment response, study adherence or survival in the judgement of the investigator, especially:
    a. Neuropathy, or significant psychiatric disorder like depression or schizophrenia; especially if treatment for those has ever been required or is anticipated to be required
    b. Clinically significant evidence of extra-pulmonary TB (e.g. miliary TB, TB meningitis, but not limited lymph node involvement)
    c. Serious lung conditions other than TB, or significant respiratory impairment in the discretion of the investigator
    d. Any diabetes mellitus
    e. Cardiovascular disease such as myocardial infarction, heart failure, coronary heart disease, arrhythmia, tachyarrhythmia, or pulmonary hypertension
    f. Arterial hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg on two occasions during screening).
    g. Long QT syndrome or family history of long QT syndrome or family history of sudden death of unknown or cardiac-related cause
    h. Alcohol or other drug abuse that is sufficient to significantly compromise the safety or cooperation of the patient, that includes substances prohibited by the protocol or has led to significant organ damage at the discretion of the investigator.
8. Any of the following laboratory findings at screening:
    a. Serum amino aspartate transferase (AST) and/or alanine aminotransferase (ALT) >3x the upper limit of normal (ULN),
    b. Serum alkaline phosphatase or y-glutamyl transferase > 2.5x the ULN,
    c. Serum total bilirubin level >1.5x the ULN
    d. Estimated creatinine clearance (eCrCl; using the Cockroft and Gault formula [57] lower than 30 ml/min
    e. Serum albumin < 2.8 g/dl
    f. Haemoglobin level <7.0 g/dl
    g. Platelet count <50,000/mm^3,

h. Serum potassium below the lower level of normal for the laboratory

i. Blood glucose at screening of less than 70mg/dL (3.9mmol/L)

9. ECG findings in the screening ECG: (one or more):

   a. QTcF of >0.450 s

   b. Atrioventricular (AV) block with PR interval > 0.20 s,

   c. QRS complex > 120 milliseconds

   d. Any other changes in the ECG that are clinically relevant as per discretion of the investigator

10. Restricted medication:

   a. Treatment with any other investigational drug within 1 month prior to enrolment or enrolment into other clinical (intervention) trials during participation.

   b. Previous anti-TB treatment with drugs active against MTB within the last 3 months prior to screening.

   c. Unable or unwilling to abide by the requirements regarding restricted medication or have taken restricted medication as described under section 12.6, page 59. Restricted medication includes the following drug classes:

      - Anti-TB drugs other than study drugs
      - Medication that lowers the threshold for epileptic seizures
      - Medication that prolongs the QTc interval
      - Drugs that affect monoaminooxidase or serotonin metabolism
      - CYP 450 inhibitors or inducers, including grapefruit containing foods / beverages and St. John's Wort

10.5 Schedule of events

The trial consists of the following periods:

- Screening period: Visit SCR (Day -8 to 0)
- Experimental Treatment Period: Weekly visits from Visit WK00 (Day 01) to WK16 (Day 112 ± 2 days).
- Follow-up period: Visit WK18 (Day 126 ± 4 days), WK26 (Day 182 ± 7 days), WK38 (Day 266 ± 7 days), WK52 (Day 364 ± 7 days)

A tabular overview of the individual timing and details of the respective procedures and laboratory samples to be done at each visit can be found in the Schedule of Events, p.13.

10.5.1 Screening Period

Visit SCR (day -8 to 0)		
Administrative & regulatory		• Written informed consent • Assign participant screening number
Clinical		• Demographic data: Date of birth, ethnic group, sex • Medical history, incl. treatment history and current concomitant medication • Physical examination, Vital signs (temperature, blood pressure, pulse, pulse oxymetry and respiratory rate) after 10 mins supine rest • Weight (kg)/height (m) • Neurological examination, vision testing
Laboratory	Blood	• Serum pregnancy test (blood sample of 7.5mL), if female of childbearing potential • HIV test and CD4 count (blood sample of 7.5mL) • Host biomarker and pharmacogenomics blood sample, if consent for this sub study is given • Screening laboratory (blood sample of 12.5mL)
	Urine	• Urine drug screening • Urinalysis
Microbiology		• Spontaneously produced sputum • Ziehl-Neelsen smear from concentrated sample • Susceptibility testing for RIF and INH by rapid test
Other		• Inclusion and exclusion criteria • Chest X-ray (if no chest X-ray is available no older than 2 weeks) • ECG (12 lead, single) after 10 mins supine rest
Study drugs		• N/A

10.5.2 Treatment Period

Visits WK 00 – WK 16 (Day 01 – Day 112, each ±2 days with exception of day 14 (-2 days window))	
Administrative & regulatory	• Randomization (before/at WK00 only)

		• Enrolment (WK00 only) • Hospitalization* (WK 02 only)
Clinical (before intake of IMP)		• Physical examination, vital signs, weight Additional blood pressure and heart rate measurements at WK00: 1h, 2h and 3h (± 10 mins) after intake of IMP, at WK02 during hospitalization: 1h, 2h, 3h, 4h, 6h (± 10 ins) after intake of IMP with a meal • Neurological examination/vision testing (not WK 00) • Concomitant medication • Adverse events • Hospitalization: WK02 visit only, for intensive PK sampling after dosing together with standardized meal.
Laboratory (before intake of IMP)	**Blood**	• Safety Lab (WK01-09, WK11, WK13, WK16): haematology and biochemistry (blood sample of 9.5mL), Coagulation only at WK 02 and if clinically indicated (additional blood sample of 3mL); *to be sampled before study treatment administration* • Serum pregnancy test (blood sample of 7.5mL), if female of childbearing potential (WK09 only) • Host biomarker blood sampling only at WK02, WK04, WK07, WK11, WK13 and WK16, if consent for this sub study is given • Intensive PK sampling DZD (WK02 only): 0 (within 1h before dosing), 1, 2, 4, 8, 12, (± 10 mins) and 24 hours (± 30 mins) after dosing
	Urine	• Urinalysis (WK01-09, WK11, WK13, WK16): per dipstick, additional microscopy on abnormal dipstick results
Microbiology		• Sputum (3 samples per visit, one of which may be an early morning sample – to be specified in the microbiology laboratory manual) • AFB smear from screening and WK16 samples • Culture in BD MGIT liquid media from 2 spot sputum samples • Culture on LJ solid media from 2 samples (WK00, WK08, WK12 and WK16 only) • Susceptibility testing: minimum inhibitory concentration (MIC) for drugs the participant is receiving will be tested on a representative MTB isolate from baseline and from WK08 or later, if any growth detected • Storage of the 3rd sputum sample

	• Storage of an isolate obtained at baseline, isolates from treatment visits, and from all cultures positive after WK08 will be stored as specified in the TB Lab Manual • Analysis by MBLA and/or sputum LAM, if capacity/budget permits
Other	• ECG (12-lead, triplicate at WK00, single at WK01-WK09, WK11, WK13, WK16): pre-dose, after 10 minutes lying down, before any other assessment. If a QTcF of >480ms, or a QTc-prolongation over baseline of >50ms is seen in a single ECG during experimental treatment, two more ECGs should be registered, to obtain a more precise, average QTcF measurement.
Study drugs	• drug dispensing of weekly supply as specified in the Drug Management Plan (DMP) • intake of the morning dose of study drug • drug accountability (except for Wk00) • Compliance assessment

10.5.3 Follow-up Period

Visits FU 1 at WK18 (Day 126 ±4 days): all patients **FU 2,3 at WK26, WK38 (Day 182, Day 266, each ±7 days): only patients with sputum culture conversion who end treatment after week 16.** **FU 4 at WK52: all patients (Day 364 ± 7 days): all patients** All reasonable efforts should be made to achieve on-site visits being conducted. Should this not be possible, a home visit by a study team shall be performed, or, if this is again not possible, telephonic visits will be conducted to obtain health status, information on TB treatment outcome and eventual re-treatment, and pregnancy status if the patient is a woman of childbearing potential.		
Administrative & regulatory		• N/A
Clinical		• Physical examination, vital signs at FU 1 (WK18); at FU 2-4 (WK26, WK38, WK52) symptom orientated physical examination only or to follow up on (S)AEs • Neurological examination/vision testing at FU 1 (WK18); at FU 2-4 (WK26, WK38, WK52) symptom orientated neurological examination only or to follow up on (S)AEs • Concomitant medication • Adverse events
Laboratory	**Blood**	• Safety laboratory (blood sample of 9.5mL) at FU 1 (WK18) • Serum pregnancy test (blood sample of 7.5mL), if female of childbearing potential at FU 1 (WK18) only
	Urine	• Urinalysis at FU 1 (WK18) only
Microbiology		• Spot sputum (2 sample) • Ziehl-Neelsen smear from concentrated sample • Culture in BD MGIT liquid media • Culture on LJ solid media • Analysis by MBLA and/or sputum LAM, if capacity/budget permits
Study drugs		• Standard of care TB treatment from government health system outside of the study, if patient did not meet the definition for sustained culture conversion by week 08.

11 TRIAL ASSESSMENTS

The following trial assessments will be performed at the timing indicated in the Schedule of Events table on page 13. Below is a detailed description of what each assessment entails.

11.1 Demographic and background assessments

- Written informed consent
- Demographic data: Date of birth, ethnic group, sex
- Medical history, incl. treatment history and current medication
- Chest X-ray (X-ray images performed at non-study facilities may be accepted, if there is reasonable certainty that the patients ID is correct, and if they are not older than 2 weeks)
- Method of birth control, both for male and female subjects.

11.2 Efficacy assessments

The assessments of efficacy will be done at multiple fixed time-points during treatment and follow-up phase as follows:

Spot sputum

- Method: Participants will be shown images of what good quality sputum samples should look like, which reportedly has led to better sample quality and higher detection rates of MTB.
- Before producing a sample, participants should first rinse their mouth with clean water. Then, they should deeply inhale three times and cough deeply to produce a good quality sample, trying to avoid saliva and nasal secretions. 1 sample is collected at screening visit (SCR), 3 samples are collected per experimental treatment phase visit and two samples during follow-up phase at each visit.
- From two spot sputum samples at visits WK00 to WK16, cultures will be done in MGIT liquid media. At WK00, WK08 and WK12, LJ solid media cultures will be performed from two samples as well. Additionally, the MBLA, a novel assay to measure mycobacterial load by quantifying 16S rRNA from sputum samples, will be performed on stored aliquots of sputum samples collected at all study visits; and evaluation of LAM concentration will be performed as experimental determination of bacterial load, both depending on test availability.
- During follow-up phase at visits FU 1-4 the two spot sputum samples will be processed for mycobacterial culture MGIT liquid culture, Ziehl-Neelsen stained sputum smear and for LJ on solid media.
- If the volume of sputum sampled is too small for all tests to be done, culture in MGIT liquid media will be prioritised as these data are required for evaluation of the primary study end-point.

- The infecting isolate will be stored from a positive culture of a sample obtained prior to treatment start, from visits during experimental treatment, and from every positive culture obtained at or after visit WK08, for assessments of MICs and typing by molecular bacteriology, to be specified in the Laboratory Manual.
- The methodology will be described in detail in the Laboratory Manual, which will be supplied to the trial sites before study start.

11.3 Safety assessments

Electrocardiography

ECGs are to be performed after a period of at least 10 minutes of recumbent (lying down) rest; and before any other procedures are done.

At visit WK00, a 12-lead ECGs will be performed in triplicate. At all other visits, a single ECG will suffice, unless the following is observed:

If a QTcF of >480ms, or a prolongation over baseline of >50ms is seen in a single on-treatment or unscheduled ECG, two more ECGs should be registered, to obtain an average QTcF measurement that will be more precise than the estimate from a single ECG.

12-lead ECG analysis will be performed. The following ECG variables will be assessed for safety endpoint: Heart rate, PQ interval, QRS, QT, and QT corrected for heart rate using Fridericia correction (QTcF), morphological abnormalities.

- Method: Single ECGs will be recorded for at least 10 seconds pre-dose at 50mm/second or greater. Timing and registration technique for ECGs will be standardized for all participants. Patients should be lying down (recumbent) for at least 10 minutes prior to each 12-lead ECG evaluation. For each participant, the ECG should be collected at around the same time of day, preferably within 2 hours before dosing.
- ECGs will be analysed immediately by site investigators to ascertain patient safety.

Screening/Safety laboratory tests

Samples for laboratory tests are to be taken before the day's dose of study treatment is administered.

- Urine:
  - Urine drug screening by a rapid test for opiates, amphetamines, cannabinoids, cocaine, benzodiazepines and barbiturates.
  - Urinalysis*: Dipstick for pH, protein, glucose, ketones, urobilinogen, blood and leukocytes.
  - Urine microscopy on abnormal dipstick results as per the investigator's decision.

- Blood:
    - Serum pregnancy test, if female of childbearing potential (SCR, WK09 and WK18)
    - HIV test (CD4 count if HIV positive, additionally viral load, if patient is on ARVs, additionally genotypic resistance testing, if viral load is >1,000 copies/µL) and patient would have to be switched to a non-dolutegravir ART regimen after end of study (SCR)
    - Haematology*: Complete blood count with differential and platelets
    - Biochemistry*: ALT, AST, gamma-glutamyl transferase (yGT), alkaline Phosphatase (aP), lipase, direct and total bilirubin, albumin, serum creatinine, electrolytes (Na+, K+, Serum Calcium, Magnesium, Cl-, PO43-), random glucose
    - Coagulation: aPTT, PT, INR

*performed during safety laboratory assessment, complete list of tests done during screening only

Physical examination

Details about the conduct of physical examination will be laid out in the Manual of Procedures (MOP)

- Weight (kg)/height (m)
- Physical examination
- Additionally, the Hunter criteria for early detection of serotonin syndrome will be assessed:
    - Spontaneous clonus
    - Inducible clonus
    - Ocular clonus
    - Tremor
    - Hyperreflexia
    - Agitation
    - Diaphoresis
    - Hyperthermia above 38ºC

Serotonin syndrome is diagnosed according to the following decision rule, which will prompt stopping a subject from receiving study drug if fulfilled. The Hunter Serotonin Toxicity Criteria are fulfilled if any of the following is fulfilled:

- Spontaneous clonus
- Inducible clonus PLUS agitation or diaphoresis
- Ocular clonus PLUS agitation or diaphoresis

- Tremor PLUS hyperreflexia
- Hypertonia PLUS temperature above 38ºC PLUS ocular clonus or inducible clonus

In the case of presence of newly appearing symptoms “inducible clonus”, “ocular clonus”, or “tremor”, which would not fulfil Hunter criteria on their own, close observation of the participant (ideally daily) is warranted while continuing on medication, and the sponsor medical expert should be notified.

- Vital signs (temperature (degrees Celsius), blood pressure (systolic and diastolic, in mmHg), pulse (bpm), pulse oxymetry, and respiratory rate (breaths/minute)). Measurements for blood pressure and pulse rate will be performed in the supine position after at least 10 minutes rest using an oscillometric method. As far as possible, measurement will always be performed on the same arm.
- On visits WK00 and WK02, additional blood pressure measurements will be performed:
  WK00: after 1h, 2h and 3h (± 10 mins) after IMP is taken;
  WK02: 1h, 2h, 3h, 4h, 6h (± 10 mins) after intake of IMP

Detailed neurological examination

The detailed neurological examination will be laid out in the Manual of Procedures (MOP)

- Neurological examination including the following assessments:
  - Cranial nerves
    - Test both pupillary responses to light
    - Eye movements in all directions
    - Palatal movement
    - Tongue movement
  - Reflexes: Biceps, Achilles’ and patellar tendon reflexes bilaterally
  - Sensory: Vibration sensitivity, pin prick, light touch

Vision testing

The vision testing will be laid out in the Manual of Procedures (MOP)

- Visual acuity testing according to Snellen
- Vision testing for colour deficiencies according to Ishihara

Compliance assessment for food and beverage restrictions (at SCR and during experimental treatment WK00 – WK16)

The investigator will assess the risk to the participant by questioning about their usual diet and drinking habits, and about their understanding of which beverages they usually take are not permitted in the study.

The investigator will have to come to the conclusion at screening that the participant will likely comply, for a participant to be eligible to study participation.

11.4 Assessments when Recurrence is Suspected

During follow-up, patients who are experiencing complaints, or have laboratory events suggesting a recurrence of disease, will be evaluated in a standardized manner that has been described for the TBTC study 31 [58].

Triggers for the assessment will be the following events:

- A positive culture confirmed as M. tuberculosis from a sputum specimen collected at or after the WK16 visit
- Worsening signs and/or symptoms consistent with tuberculosis at or after WK16
- The site investigator is considering an extension of tuberculosis therapy beyond 16 weeks

The following procedures shall be conducted:

- Assessment of symptoms
- Review interval medical history
- Weight, vital signs and physical examination
- Obtain 3 sputum specimens for smear and MGIT plus LJ culture, 2 specimens at least 4h apart. If M. tuberculosis is isolated, the isolate is to be stored frozen.
- Chest radiograph
- Review participant' s contact information, and update if indicated
- Contact sponsor medical expert to review procedures, results and decide on management

The outcome of this assessment will be twofold:

- to define whether the patient will have met the endpoint of "relapse", conditional on a matching of baseline and recurrence isolate, if available;
- to transfer the patient to the government TB treatment programme to receive treatment for recurrent disease

11.5 Pharmacokinetic assessments

The exact time of drug administration will be recorded on the day of PK sampling and will be defined as 0h for the following PK sampling procedures. The timing of drug administration on the two preceding days will also be recorded.

Delpazolid

All patients will undergo intensive PK sampling at Day 14 (WK02) visit. A sample will be drawn within 1h before administration of the DZD morning dose (0 h).

The DZD morning dose will be administered with a standardized meal. Blood draws thereafter will be performed at 1, 2, 4, 8, 12 (± 10 mins) and 24 hours (± 30 mins) after DZD morning dose. The exact time of meal and DZD administration at the day of PK sampling will be recorded. Standardized food and fluid intake will be monitored and noted throughout the PK days.

Procedures for sample connection, storage and transportation will be described in the Laboratory Manual. Assaying of DZD, BDQ and DLM plus main metabolites, and MXF will be conducted centrally at the Department of Pharmacy, Radboudumc, the Netherlands, with validated LC-MS/MS methods.

Pharmacokinetic parameters will be assessed using non-compartmental techniques and non-linear mixed effects methodology. The latter will be utilized to develop a population PK model describing DZD, which will be linked to safety and efficacy parameters. Secondary PK parameters to be reported are listed in section 9.2.3.

11.6 Additional biomarker assessments

The main evaluation of treatment response biomarkers in this study focus on sputum microbiology (via serial MGIT and LJ cultures); and the experimental assessments for LAM, and the MBLA). Collection and appropriate storage of plasma and whole blood samples will permit exploratory evaluation of host immunological and transcriptomic biomarkers as sub-studies. In addition, a sample for pharmacogenomics analyses will be stored at screening (see section 20.3.3). Sampling for these sub-studies will be conditional on participant approval to storage, and genetic testing.

12 STUDY TREATMENT

12.1 Study drug regimens

The study will recruit into the following arms:

Arm 1 (D_0): BDM (bedaquiline, delamanid, moxifloxacin)

Arm 2 (D_{400}): BDM + DZD (bedaquiline, delamanid, moxifloxacin, delpazolid 400 mg OD)

Arm 3 (D_{800}): BDM + DZD (bedaquiline, delamanid, moxifloxacin, delpazolid 800 mg OD)

Arm 4 (D_{1200}): BDM + DZD (bedaquiline, delamanid, moxifloxacin, delpazolid 1200 mg OD)

Arm 5 ($D_{800\text{-}BD}$): BDM + DZD (bedaquiline, delamanid, moxifloxacin, delpazolid 800 mg BID)

BDQ, DLM, MXF are approved drugs. Product Information Leaflets for these drugs will be provided to investigators.

DZD is not an approved drug. It will be manufactured and tested according to Good Manufacturing Practice (GMP) requirements. The Sponsor will provide Ethical Review Boards and site investigators with a set of documents, as required by the local regulatory authority, that provides information on the manufacture and quality control of the raw substance and the medicinal product; Certificates of Analysis (CoA) of the batches to be used; and an Investigational Brochure (IB) that summarizes preclinical and clinical data with respect to DZD.

12.2 Study drug dosage and administration

The experimental and control treatment as described above will be administered daily for 16 weeks (see Table 1. Daily Dosing of Study Medication), together with food.

- Bedaquiline: will be dosed as per the licensed dose: 400 mg orally once daily for the first 14 days, then 200 mg three times a week.
- Delamanid: will be dosed as per the licensed dose: 200 mg orally in two daily doses of 100 mg.
- Moxifloxacin: will be dosed as per the licensed dose: 400 mg orally once daily
- Delpazolid - not licensed:
    - Dose: will be according to randomization to dosing arm:
        - Arm 1: 15 participants will receive 0 mg of DZD
        - Arm 2: 15 participants will receive 400 mg DZD orally once daily
        - Arm 3: 15 participants will receive 800 mg DZD orally once daily
        - Arm 4: 15 participants will receive 1200 mg DZD orally once daily
        - Arm 5: 15 participants will receive 800 mg DZD orally twice daily

The daily dose of study drug for participants will be dependent on the subjects' experimental arm as shown in the following table:

Table 1. Daily Dosing of Study Medication

	Bedaquiline (Sirturo®) 100 mg tablet	**Delamanid 50 mg tablet**	**Moxifloxacin 400 mg tablet**	**Delpazolid 400 mg tablet**
Arm 1 (D_0): BDM	*Day 01-14:* 4 tablets to be taken orally once per day in the mornings *Day 15-112:* 2 tablets to be taken orally 3 times per week in the mornings	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings	
Arm 2 (D_{400}): BDM-DZD	*Day 01-14:* 4 tablets to be taken orally once per day in the mornings *Day 15-112:* 2 tablets to be taken orally 3 times per week in the mornings	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings
Arm 3 ($D_{800\text{-}OD}$): BDM-DZD	*Day 01-14:* 4 tablets to be taken orally once per day in the mornings *Day 15-112:* 2 tablets to be taken orally 3 times per week in the mornings	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings	*Day 01-112:* 2 tablets to be taken orally once per day in the mornings
Arm 4 (D_{1200}): BDM-DZD	*Day 01-14:* 4 tablets to be taken orally once per day in the mornings *Day 15-112:* 2 tablets to be taken orally 3 times	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings	*Day 01-112:* 3 tablets to be taken orally once per day in the mornings

	per week in the mornings			
Arm 5 ($D_{800\text{-}BD}$): BDM-DZD	*Day 01-14:* 4 tablets to be taken orally once per day in the mornings *Day 15-112:* 2 tablet to be taken orally 3 times per week in the mornings	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)	*Day 01-112:* 1 tablet to be taken orally once per day in the mornings	*Day 01-112:* 2 tablets to be taken orally 2 times per day (mornings and evenings)

DZD can be taken with or without food. The bioavailability of BDQ and DLM strongly increases when taken with food. MXF can be taken with or without food. As a result, all anti-TB drugs have to be taken with food and a glass of water in the mornings and evenings.

During pharmacokinetic sampling days, the anti-TB drugs are taken with standardized meals.

As specified under sections 10.4 (page 42) and 12.6.2 (page 60), HIV patients in need of ART will be treated with a dolutegravir-based ART regimen during the study. Dolutegravir in a fixed-dose combination, containing Dolutegravir (50 mg), Lamivudine (300mg) and Tenofovir Disoproxil Fumarate (300mg), will be provided following local clinical practice/procedures and specific recommendations on initiating these drugs. Therefore, this is considered as concomitant medication and not study medication (see also section 12.6).

12.3 Study drug management

A Study Drug Management Plan (DMP) will describe all aspects of drug management in this study, including details on packaging, labelling, storage conditions, distribution, drug-dispensing, drug accountability and other drug-related procedures. A team of pharmacists, including a pharmacist at each study site, will be responsible for study drug management.

The Sponsor will supply the study drugs to allow completion of this study including spare drugs.

The overall intent of the labelling of the study drugs is to ensure the protection of the trial participants, to ensure traceability and identification of the drugs and trial at all times, to facilitate the proper use of the drugs, and to comply with national and international regulations on investigational drugs. The study drugs will be labelled for the study according to *The Rules Governing Medicinal Products in the European Union,* Annex 13 of GMP, and to national labelling requirements of the study sites.

All study medication must be kept in secure cabinets or rooms with access restricted to designated study personnel. Medication will be stored at appropriate conditions at designated temperatures,

protected from light and moisture. A temperature monitoring system will be in place to monitor appropriate storage conditions and record possible deviations.

The dispensing of study drugs for individual patients will be performed and recorded as described in the DMP. Dispensing at each site will be supervised by a pharmacist. Dispensing will only be performed by licensed and trained study personnel.

12.3.1 Quality check and product release

DZD tablets will be released for the study by a designated qualified person. At the study sites, trial pharmacists will verify once more the identity and quantities of all drugs supplied. This will be an administrative check.

12.3.2 Study Drug Accountability

The study pharmacists will be responsible for maintaining accurate records of receipt and destruction of study medication in the "Study Drug Inventory Log". The records should reflect the overall quantity of study drugs at site. Furthermore, the pharmacist will maintain individual records of study drug dispensing and return for each patient (Study Drug Dispensing and Accountability Forms).

Upon completion of the study, all unused DZD will be returned to the sponsor or a CRO designated by the sponsor or is destroyed, once drug accountability is completed and checked by the monitor.

All other unused study medication may be used by the sites in routine patient care, if the brand of the drug is approved by host country's regulatory authority and after approval of the sponsor. If this is not the case, the same provisions for return and destruction as described for DZD will take place.

12.3.3 Retention of Testing Samples

Sufficient quantities of each study medication will need to be kept to reconfirm specifications should the need arise. These samples should be retained until the analysis of trial data is complete or as required by applicable regulations, whichever is longer. After this period, retention samples will be destroyed following national regulations.

12.4 Treatment after End of Study Therapy (Post-study Access to Treatment)

The strategy recommended for participants to continue their TB treatment within the government health system is informed by two previous phase 2b TB trials with a 2-month experimental treatment, and the solution found for patients in Tanzania in collaboration with the national TB program in those past trials [27, 56, 59].

Patients who achieve two successive negative liquid media cultures, with no positives to follow by WK16 visit, the first of which is at or before WK 08, will NOT receive standard of care TB-treatment according to national guidelines as continuation phase to complete 6 months of treatment. Their planned post-treatment follow-up visits at WK 18, WK26, WK38 and WK52 will serve to determine

whether they have achieved lasting cure. Patients who will NOT fulfil at least one of these criteria will receive standard of care TB-treatment according to national guidelines until WK26 at a government health facility (continuation Phase).

They will be invited to return to the WK52 follow-up visit to determine their wellbeing and treatment outcome.

The review of culture results, and decision to discontinue or continue treatment will be made on the day before the WK16 visit. Should sufficient culture results to take this decision not be available by this timepoint, but available culture results indicate good response to treatment in the view of the investigator, this decision, and the start of continuation phase treatment, may be delayed until latest to WK16 (day112) + 6 days.

Should positive results become available after a decision is taken to discontinue treatment after WK16, the investigator should discuss these with the sponsor medical expert to come to a conclusion of whether the management of the patient should be changed, which should be done only in exceptions.

12.5 Measurement of Adherence to Study Treatment

Study treatment intake will be observed by study staff during the study visits in the morning and will be administered at home on the other days. Facility-based directly observed treatment (DOT) or community-based DOT (i.e. a friend or relative of the participant will act as a treatment supervisor) will be in place in order to maximize adherence. The method and person chosen will be noted in the participant's source documents. Furthermore, treatment adherence will be assessed by pill counting at every visit.

12.6 Concomitant and Prohibited Medication

12.6.1 Drug-Drug-Interactions

BDQ and, to a lesser extent, DLM are metabolized by CYP3A4. MXF is metabolized by uridine diphosphate (UDP)-glucuronosyltransferases (UGT).

A change in activity of these hepatic enzymes can change the drug concentrations in blood and tissue and thereby influence safety and efficacy readouts. All drugs that would lead to a substantial change in activity of these enzymes are prohibited; and participants may not be included into the study if receiving such drugs at screening or if it is likely that these drugs will be needed during study treatment. Patients who are enrolled and on study medication, and a need arises to treat with any of those drugs during the treatment phase of the trial need to be discussed with the sponsor medical expert. Their experimental treatment may be stopped, and they may be continued on standard TB treatment according to national guidelines. An overview of drugs allowed as concomitant medication and prohibited medication can be found in the following description:

12.6.2 Antiretroviral treatment (ART)

Drug-drug interactions:

Nucleosidic reverse transcriptase inhibitors are permitted as concomitant medications. Most patients will have 2 drugs of this category in their regimen. The third drug of choice during study treatment is dolutegravir due to its limited potential for drug-drug-interactions with the TB drug regimen under study; and participants who are on ART or starting on ART will receive this drug as part of their ART regimens.

Protease inhibitors as part of antiretroviral treatment regimens: because of the unfavourable interaction potential [60, 61], protease inhibitors need to be stopped at least 3 days before the start of study treatment (Day 01) for a patient to be eligible. Patients will be switched to dolutegravir-based ART regimens.

Efavirenz as part of antiretroviral treatment regimens: will have to be stopped at least 14 days before the start of study treatment (Day 01) for a patient to be eligible; so that the CYP3A4 enzyme induction caused by efavirenz can wear off before starting study drugs [62, 63].

If a patient has taken efavirenz within 14 days of the planned Day 01, they will not be eligible for participation in the study, unless three or fewer daily doses have been taken and there is approval from the sponsor medical expert that the patient may be enrolled.

Patients will be switched to dolutegravir-based ART regimens during the study. Dolutegravir will be provided as part of the local available fixed-dose combination, containing also Lamivudine (300mg) and Tenofovir Disoproxil Fumarate (300mg). Dolutegravir is an HIV-integrase inhibitor recommended as first-line treatment together with a nucleoside reverse transcriptase inhibitor (NRTI) backbone in many international guidelines [64, 65]. The main route by which dolutegravir is metabolized is by phase II metabolism in the liver involving glucuronidation by uridine diphosphate glucuronosyltransferase (UGT) 1A1. Minor contributions in glucuronidation come from UGT1A3 and UGT1A9. A small portion (<10%) is metabolised by the cytochrome P450 (CYP) 3A4 through oxidation (phase I metabolism). In theory, drugs that induce or inhibit UGT1A1 or CYP3A4 can lead to drug interactions, but clinically significant interactions via CYP3A4 have not been reported. Dolutegravir itself has no clinically relevant inhibition or induction effects on main CYP-enzymes, and can therefore be applied together with our study medication.

ART experienced patients:

The occurrence of TB in a patient on ARVs may be a symptom of an ARV treatment failure; which could be due to resistance of the HIV strain, or to suboptimal compliance.

The study related switch of ARV regimen to dolutegravir would be beneficial to such patients; as dolutegravir is very likely a new drug for them with no viral resistance. However, in case of treatment failure, it needs to be ensured that they will not be switched back to their previous, failing ARV regimen after end of study participation.

Thus, ART experienced patients will have HIV viral load testing during screening. Should the viral load exceed 1,000/µl, this will be rated insufficient virologic control. If there is a foreseeable need for switching patients back to an ARV regimen that does not contain dolutegravir after the end of study participation, a genotypic HIV resistance screening against the ART regimen taken at screening is to be performed, in all other cases of insufficient virologic control, it is highly recommended.

The patient's ART regimen after end of study is to be designed based on the results of resistance testing and on local availability of drugs.

ART naïve patients:

Patients who, at the time of screening for this study, are not on ARVs may enter the study if they have a CD4+ count of ≥220 \µl, and the enrolling physician feels that ARVs can be safely withheld for two months. After two months of study TB treatment, patients may start on ARVs.

Based on a review of available studies, we conclude that withholding ARVs in patients with >50 CD4 cells/µl is safe – based mainly on the NIMR/WHO TDR study from Tanzania, that was the largest and most rigorous (double blinded, placebo controlled) study. This study found no difference in survival or IRIS in patients with >220 CD4 cells/µl, whether ARVs were started after 2 weeks or after 6 months of anti-TB treatment [66, 67].

The SAPiT study in South Africa randomized 642 South African HIV+TB patients with CD4 counts <500/µl to 1 of 3 times to initiate ART at either, 1) <4 weeks, 2) 8-12 weeks, or 3) 6 months. The median CD4 count was 145; 231 subjects had >200 CD4 cells (relevant to this proposed study). SAPiT was terminated early due to reduced mortality in combined groups 1&2 vs group 3 (HR= 0.44) [68]. However, 12.4% of patients in groups 1&2 had IRIS, vs 3.8% in group 3 (P<.001). A second report from SAPiT found reduced mortality in group 1 vs 2 only in patients with CD4 counts <50 (HR= 0.32), whereas there was no effect on mortality in patients with ≥50 CD4 cells [60]. However, patients with ≥50 CD4 cells in group 1 had a 2-fold increased risk of IRIS vs group 2 (P= .02). In many of these cases TB-IRIS was severe and required hospitalization. Thus, for the patients we propose to recruit in this trial (CD4 >220/µl) SAPiT found 8-12 weeks to be the optimal time point to start ART.

12.6.3 Prohibited medication: anti-tuberculosis agents

Use of drugs with an action on MTB complex, in addition to study treatment, is prohibited while participants are receiving study treatment in both groups, intervention and control arm. This includes, but is not limited to *amikacin and other aminoglycosides, cycloserine, EMB, INH, LZD, para-aminosalicylic acid, RIF, rifabutin, rifapentine pyrazinamide, streptomycin, kanamycin, thioacetazone, capreomycin, fluoroquinolones, and thioamides*. Significant concomitant use of one or more of these agents will be evaluated by the sponsor medical expert and may lead to exclusion of the patient from treatment. Excluded patients will be taken off-IP and will be follow-up according to protocol. These patients' data will be analysed in an ITT-analysis.

12.6.4 Prohibited medication: drugs that can induce epileptic seizures

Around 4-10% of all persons experience an epileptic seizure during their lifetime [62, 64, 69]; therefore unprovoked seizures are not a rare event.

Since epileptic seizures in a participant might thus either be independent of study treatment, but could also constitute a safety signal of DZD.

Drugs that lower the threshold for epileptic seizures and provoke such an event could generate a false safety signal and are thus prohibited in study participants.

These include, but are not limited to *atomoxetine, aminophylline, theophylline, tramadol, penicillins, tricyclic antidepressants, selective serotonin reuptake inhibitors, and monoamino oxidase inhibitors*.

12.6.5 Prohibited medication: QT prolonging medication

Concurrent treatment with QTc-prolonging agents (other than the study drugs) is prohibited for participants in all treatment arms in this study until visit WK 16, as this could influence the safety assessments of novel treatment combinations and put participants at risk. QTc- prolonging agents include but are not limited to *amiodarone, bepridil chloroquine, chlorpromazine, cisapride, clarithromycin, disopyramide dofetilide, domperidone, droperidol, erythromycin, halofantrine, haloperidol, ibutilide, levomethadyl, lumefantrine, mefloquine, mesoridazine, methadone, pentamidine, pimozide, procainamide, quinidine, quinine, sotalol, sparfloxacin, terfenadine, thioridazine, and voriconazole* during study participation.

Participants having received such drugs within 30 days prior to dosing, are not eligible for this study. Exceptions may be made for individuals who have received 3 daily doses or less, and at least 5 elimination half-lives of the drug have passed before first dose of study treatment. Such exceptions should be discussed with the sponsor medical expert.

Due to the extended half-life of BDQ and DLM metabolites, QTc-prolonging drugs which are administered after end of study treatment should be given with caution and with ECG controls. We advise the sponsor medical expert be contacted about this prior to such administration.

Significant concomitant use of one or more of these agents will be evaluated by the sponsor medical expert and may lead to exclusion of the patient from treatment. Excluded patients will be taken off-IP and will be follow-up according to protocol.

12.6.6 Prohibited medication: drugs affecting monoamine oxidase (MAO)

Drugs affecting monoamine oxidase (MAO), or are metabolized by MAO: If participants require treatment, or have had treatment within 30 days before start of study treatment, with drugs which are mainly metabolized by, or affecting activity of MAO - A or B; such as α-methyldopa, or MAO inhibitors, then these participants are excluded from treatment. These include, but are not limited to *rasagiline, safinamide, selegiline, moclobemide, tranylcypromine, phenelzine and isocarboxazid*.

Significant concomitant use of one or more of these agents will be evaluated by the sponsor medical expert and may lead to exclusion of the patient from treatment.

Exceptions may be made for individuals who have received 3 daily doses or less, and at least 5 elimination half-lives of the drug have passed before first dose of study treatment. Such exceptions should be discussed with the sponsor medical expert.

12.6.7 Prohibited medication: Serotonin agonists

Participants may not be included into the study or will be excluded from treatment if they are taking drugs that have agonistic effects to serotonin action. These include, but are not limited to, *serotonin reuptake inhibitors such as fluoxetine, citalopram, or serotonin agonists such as sumatriptane, dextromethorphan, or opiate analgesics (mainly fentanyl)*.

Significant concomitant use of one or more of these agents will be evaluated by the sponsor medical expert and may lead to exclusion of the patient from treatment. Excluded patients will be taken off-IP and will be follow-up according to protocol. These patients' data will be analysed in an ITT-analysis.

12.6.8 Prohibited medication: CYP450 inhibitors/inducers

Patients requiring treatment with any drug(s) or substance(s) known to be strong inhibitors or inducers of cytochrome P450 enzymes, or specific inhibitors/inducers of CYP3A4 may not be included in the study. This also applies to patients who having received such medications in the 30 days prior to starting study treatment. This may be subject to exceptions after consultation with the sponsor medical expert for participants who have received 3 days or less of one of these drugs or substances, if there has been a wash-out period equivalent to at least 5 half-lives of that drug or substance.

Also, such drugs may not be given to participants while they are on study treatment.

HIV protease inhibitors or *efavirenz* are drugs that influence CYP 450 activity; and provisions on how to deal with those drugs is made above under 12.6.2.

12.6.9 Antimalarials

If a patient is diagnosed with uncomplicated malaria (microscopic confirmation) Atovaquone/Proguanil is an option for the treatment of uncomplicated malaria that is compatible with study treatment.

Severe malaria is a life-threatening emergency, treatment of which has priority over continuation of participants in the trial. The currently recommended first choice of intravenous artesunate is compatible with study treatment.

- The following antimalarials are incompatible with BDQ, DLM and MXF due to their inherent QT prolonging effect: *Primaquine* (recommendation: to delay primaquine admission until after end of study treatment, in case of a plasmodium vivax or ovale infection), *Artemether/lumefantrin (ALU), Quinine, and Chloroquine*.

12.6.10 Dosing precautions

Iron supplements: orally administered iron supplements decrease the absorption of many drugs, therefore should be administered 4h before or 2h after study treatment.

12.6.11 Antinausea and antipeptic drugs

The anti-nausea drug of choice in patients on study treatment is metoclopramide due to a low potential for interaction (for background, see Appendix, chapter 21.1, page 98). However, since metoclopramide shows potential to affect the QT interval, if given i.v. in higher doses, and may cause akathisia and seizures, it should therefore be used very restrictively only by low dose oral administration.

The anti-peptic drugs of choice for participants in this study are ranitidine (1st choice) or omeprazole (2nd choice; see Appendix, chapter 21.2)

Orally administered *aluminium or magnesium containing antacids* interfere with bioavailability of many drugs and are therefore prohibited in this study.

12.6.12 Substance abuse

Patients who are regular abusers of opiates are not eligible for this study. Other substance abuse is covered by exclusion criterion 7, and special caution is to be taken to exclude persons whose abuse can lead to seizures during withdrawal, including but not limited to alcohol, met-/amphetamines or tramadol.

12.6.13 Food restrictions

Dietary tyramine can, in the presence of substantial inhibition of enteric monoaminooxidase A, cause an increase in blood pressure. Study participants need to agree to forgo the consumption of certain foods while taking study medication. A list of those foods is provided in Appendix 20.2, page 95.

13 PARTICIPANT ENROLMENT

Patients will be invited to be screened for inclusion in the trial if they are suspected to have pulmonary TB or have an established diagnosis by smear microscopy, GeneXpert or chest X-ray, done within the government or private health sector.

Participants will be enrolled and assigned to randomized study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

13.1 Recruitment procedures

Recruitment can be enhanced by individual and community awareness of the study and/or TB in general, through the following materials, but not limited to these:

- Public announcements through advertisements, posters and radio announcements

- Information leaflets distributed to healthcare providers, to be handed to newly diagnosed TB patients

Recruitment materials will be used only upon approval of the ethics committees relevant for this trial.

13.2 Informed consent

Patients will be given a patient information sheet about the trial and the anticipated benefits, where, among other information, the potential risks associated with the protocol procedures will be explained. The investigator or a person designated by the investigator will inform the patient or the patient's legally acceptable representative. The language used will be as non-technical as possible, and the patient will not unduly be influenced to participate in the trial.

Written informed consent must be obtained from every patient prior to any procedures being done specifically for the study. For illiterate patients, study information is given in the presence of an impartial, literate witness, who will read the information sheet to the patient or will witness the complete reading of the information sheet to the patient. The patient will give consent by thumb printing the ICF. The witness states that free, informed consent has been given by his/her signature on the ICF. An original or a photocopy of the signed informed consent form will be provided to the patient or to the patient's legally acceptable representative, depending on locally applicable regulations. A signed original will be kept with the patient's medical records at the site.

Participants will be informed that screening includes collecting sputum samples for smear and culture, chest X-ray and ECG registration, blood and urine samples, pregnancy testing, if applicable, and testing for HIV. Patient will be encouraged to receive their HIV results but may choose not to be informed. HIV seropositive individuals will be referred to the local HIV services. Participants will be informed that agreeing to be screened does not mean that they must join the trial, however that participation in the trial will require good adherence. Patients will be informed that they will be free to withdraw from the study at any time, and that withdrawal will have no negative effects on them receiving standard care afterwards.

The site will document the name and position of those study personnel at the site who have received appropriate Good Clinical Practice (GCP) training in advance are responsible for obtaining informed consent. No other staff will perform the informed consent process.

The informed consent template will be provided to the investigator by the Sponsor. If any modifications to the informed consent form are proposed by the site, the consent form must be submitted to the Sponsor for approval prior to submission to the ethics committee. The informed consent form will be revised whenever new important information becomes available. Each consent form revised during the course of the study will need to be reviewed by an ethics committee. The ICF can only be used after approval of the ethics committee. After approval of a revised informed consent, all active participants must sign the revised informed consent form to be able to continue study participation.

13.3 Exclusion of particular groups

Minors below 18 years are excluded from the study. TB in children is different in diagnosis and disease course from adult disease. Therefore, this age group will not participate in this trial.

13.4 Vulnerable participants

Patients who are unable to give free, informed consent (e.g. mentally impaired persons, prisoners) will be excluded from the trial, as their uncoerced agreement to study participation and procedures cannot be ascertained, and compliance may be suboptimal.

13.5 Procedures to assign participants to treatment groups

Patients who have given informed consent and who have been found eligible for participation (meeting all inclusion criteria and no exclusion criterion) based on results from screening visit, will be randomly assigned to one out of five treatment arms with a 1:1:1:1:1 allocation. Randomization must be performed when all screening results are available but not before eligibility for study participation is proven.

Randomization will be performed through centralized assignment of patients by means of an Interactive Web Response System (IWRS), a web-based randomization system. Patient characteristics will be used to balance the composition of the treatment arms with regards to prognostic factors.

These characteristics will be:

- site
- HIV status
- bacterial load in sputum as measured by GeneXpert cycle threshold

13.6 Methods of avoiding bias

This study will be open-label, patients and physicians will be aware of treatment allocation. To ensure unbiased assessment of efficacy endpoints, the personnel assessing patients' outcomes, like the microbiology laboratory staff or the sponsor medical expert discussing the possibility of recurrent disease, will remain blinded to treatment assignment throughout the whole study. Every effort will be made to maintain this blinding.

Further, there will not be any cumulative summary of study data by randomized treatment arms generated, except for data to be provided to the independent data safety monitoring board (DSMB) by the unblinded statistician. Such analysis may not be distributed outside of the DSMB.

Determination of MICs for the experimental treatment allocated to the patients will only be made after all other assessments of patient samples have been completed in the respective laboratory or will be done at a different specialised laboratory.

13.7 Participant withdrawal

A patient may decide to withdraw consent and therefore withdraw from the trial at any time and for any reason. The investigator may also withdraw a patient from study treatment or the entire trial for any of the following reasons:

- A major protocol violation caused by the patient
- If, for any reason, the investigator concludes that continued participation in the trial would not be in the participant's best interest.
- Requires medication that is prohibited by the protocol

The investigator will also withdraw a patient upon request of the Sponsor or if the study is terminated. If a serious adverse event occurs, the Principal Investigator will discuss it with the sponsor. A patient who is discontinued due to an AE, will be followed up as described under "Adverse Events".

In general, patients who withdraw or are withdrawn from treatment (but still maintain consent to continue in the study) shall continue to follow study assessments as laid down in the schedule of events, in order to make them evaluable in the ITT population.

When a patient is withdrawn from the study, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page of the case report form (CRF). When a patient withdraws from the study on his/her own decision, it is up to the patient to provide a reason thereof. The investigator shall at least ask for the reason and point out that the collection of all withdrawal reasons is of great importance for the sponsor from a medical and scientific point of view.

On the date of withdrawal and in the interest of participants' safety, if consent to this is still maintained, participants should have all assessments scheduled for Day 112 (WK16) completed at the day they leave the study. These include vital signs, complete physical examination, including neurological examination and vision testing, safety lab, 12-lead ECG, concomitant medication and assessments of AEs.

Recent safety laboratory results should be available to make sure that no adverse findings are missed that could put the participant at risk. In a female participant of childbearing potential, a serum pregnancy test should be requested, if possible.

If a continuation following the schedule of assessments is not possible, and if the participant agrees, a follow-up visit should be scheduled 14 days (+/-4 days) after withdrawal. During this visit all procedures as described in section 10.5.3, page 48, "Follow-up Period", of the protocol should be carried out, i.e. vital signs, symptom related physical examination, follow-up of former AEs and assessments of new AEs, incl. blood tests to follow up on past abnormal results and new AEs, as per investigators discretion.

For patients who withdraw from the study, every attempt should be made to perform all or as many as possible of the follow-up assessments.

Patients who do not return for final assessments will be contacted by site personnel in an attempt to complete the follow-up assessments.

All attempts gearing towards the completion of the study visits should be documented in the patients's study file.

13.8 Adjustment of sample size during the study

The number of patients, who withdraw or are withdrawn from the study for any reason after intake of at least one dose of study medication will be monitored by the Sponsor. If dropouts due to withdrawal are higher than expected, the sample size will be reviewed and might be revised by the responsible biometrician. Due to these revisions, adjustments to recruitment numbers might be necessary, which could lead to recruitment of more participants than currently planned.

13.9 Safety: expedited safety data review

An independent data safety monitoring board (DSMB) will be convened for the trial. The DSMB will review safety data at regular intervals as defined in the DSMB Charter, but will also perform expedited review if the following conditions are met:

- Three or more patients experience a grade 3 or higher AE (CTCAE 5.0) in the same organ system that are at least possibly related to one of the study drugs, and qualify as "unexpected" by being more severe than in previous experience with the drug in question.
- Two or more patients experience a grade 4 or higher AE (CTCAE 5.0) in the same organ system that are at least possibly related to one of the study drugs, and qualify as "unexpected" by being more severe than in previous experience with the drug in question.
- One patient experiences a grade 5 AE (death) that is at least possibly related to one of the study drugs

13.10 Additional safety considerations: individual patient stopping criteria for safety

- Hepatotoxicity stopping criteria: these follow the FDA guidance on evaluation of drug-induced liver injury [66]. More detail on patient management in case of liver toxicity including intensifying the assessment schedule, is provided under 20.1, page 93.

  A patient should discontinue study drug if:

  - ALT or AST >8xULN
  - ALT or AST >5xULN for more than 2 weeks
  - ALT or AST >3xULN and (total bilirubin (TBL) >2xULN or INR >1.5

- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

As soon as symptoms resolve and elevated ALT/AST are below 2x ULN, reintroduction of study treatment is to be discussed with the sponsor medical expert. Study treatment will be reintroduced (all drugs simultaneously). Close, at least biweekly monitoring of symptoms and laboratory parameters is needed to identify repeated DILI. Should this occur, the study regimen will be permanently stopped and the participant be continued on a regimen that is considered non-toxic.

All participants who discontinue study treatment should be followed up as described for follow-up of adverse events classified as "severe" (grade 3 and above).

- ECG stopping criterion: Participants will stop treatment if their on-treatment ECG shows a prolongation of the Fridericia corrected QT interval (QTcF) on average in triplicate ECGs to grade 3 as defined under 14.6.

  In this case, the patient will be hospitalized and monitored closely with at least daily ECGs until the abnormality abates. Study drugs and other potentially offending drugs will be withheld. Blood electrolytes (mainly Mg^{+}, Ca^{++}, K^{+}) will be measured and abnormalities corrected, after discussion with the sponsor medical expert.

  As soon as the AE improves to grade 2 or less, study drugs might be restarted in close discussion with the sponsor medical expert, unless QTcF prolongation resulted in dangerous arrhythmias (grade 4 QTcF prolongation as defined under 14.6 *Table 2* – in this case, the patient will be discontinued from study treatment..

- Neuropathy stopping criterion: Participants will stop treatment with DZD in case they develop clinically significant signs of motor or sensory neuropathy, i.e. loss of muscle strength, loss of sensation, loss of vibration sensitivity, or loss of visual acuity or colour vision.

  In this case, treatment with BDQ, DLM and MXF may continue as scheduled. Since neuropathy may be associated to MXF in rare events, a discontinuation of MXF may be advised in severe cases of neuropathy or if neuropathy does not improve after DZD is discontinued.

- Hypertension (tyramine pressor effect) stopping criterion:

  BP systolic >160 mmHg, or diastolic >100 mmHg:

  - **Re-assessment:** Participants who develop significant hypertension with systolic blood pressure (BP) averages of three measurements of ≥ 160 mm Hg, and/or diastolic BP of ≥ 100 mm Hg, but less than 180/110 mmHg, will be re-assessed on 2 separate occasions.
    They should be re-counselled as to the foods and drink to be avoided with study treatment, as non-compliance to this could be an important aspect to the hypertension.

- If this evaluation supports the conclusion of a significant increase in blood pressure, the investigator will assess potential causes. If the increase is determined to be associated with study treatment, a de-challenge/re-challenge will be performed: participants will discontinue DZD. If, after ≥ 10 h (> 5 x $t_{1/2}$ of DZD) after the last dose, BP has dropped significantly, a re-challenge with daily BP measurements will be considered.
- Treatment with BDQ, DLM and MXF should continue throughout.
- **Continued hypertension after re-assessment:** participants who develop persistent hypertension ≥ 160/100 mmHg after evaluation and adequate antihypertensive treatment, including those who have undergone a re-challenge with DZD, will discontinue all study treatment and complete TB treatment according to national TB program guidelines. These participants will receive follow-up to determine whether the condition normalizes after discontinuation of study treatment.

BP systolic >180 mmHg, or diastolic >110 mmHg:

- **Immediate stop:** Participants who develop hypertension with systolic blood pressure (BP) averages of three measurements of ≥ 180 mm Hg, and/or diastolic BP of ≥ 110, will stop study treatments immediately, and receive antihypertensive treatment. During follow-up, investigators should attempt to determine whether the condition normalizes after discontinuation of study treatment, in order to better judge relatedness to IMP.

- Pregnancy stopping criterion: a female patient will stop treatment immediately, if a pregnancy test is positive at any time. Further management is described under Chap. 14.11..
- Serotonin syndrome stopping criterion: A patient will stop treatment with DZD if at least one of the following criteria are fulfilled, which are indicative of serotonin syndrome (Hunter Serotonin Toxicity Criteria):
  - Spontaneous clonus
  - Inducible clonus PLUS agitation or diaphoresis
  - Ocular clonus PLUS agitation or diaphoresis
  - Tremor PLUS hyperreflexia
  - Hypertonia PLUS temperature above 38ºC PLUS ocular clonus or inducible clonus
- Convulsions/seizures stopping criterion: A patient will stop study treatment if clinically significant convulsions are observed in the discretion of the investigator.

  In such a case, the following examinations should be done as soon as possible, after stabilisation of the participant' s condition, to assess the aetiology and contributing factors to the event in order to assess relatedness to study drugs.

- Clinical assessment: ventilation pattern, neurological abnormalities, body temperature, blood pressure, heart rate
- Quick bed-side test: glucose
- ECG
- Question on concomitant medications, including herbal or over-the-counter preparations
- Question on alcohol or other drug intake, regular abuse and changes in pattern of use that might have led to a withdrawal
- Re- question on own and family history of seizures (to be done at screening, but repeated in this case)
- Draw safety blood sample, including all safety blood tests that are done as per schedule of events

  PLUS:

  Malaria microscopy and rapid test, if in a malaria endemic area; to exclude cerebral malaria
- Cerebral imaging like computed tomography (CT) or magnetic resonance imaging (MRI) are indicated; to be conducted urgently if cerebral haemorrhage is suspected as the underlying cause. It is to be noted that CT is suboptimally sensitive to describe a stroke during the first 24h but is highly sensitive to describe haemorrhage.
- Electroencephalography (EEG) should be conducted when patient is stable.

In addition, a seizure can be a symptom of CNS infection such as meningitis or encephalitis. If this is suspected, a lumbar puncture may be seen to be in the patient' s best interest, after elevated intracranial pressure has been duly excluded by ophthalmoscopy or cerebral imaging.

The information obtained by these diagnostics will be transmitted to the sponsor medical expert on the immediately reportable event form within 24h, and any updates transmitted and discussed in a timely manner.

14 REPORTING ADVERSE EVENTS

14.1 Definitions

For safety monitoring and reporting purposes, a drug related AE is defined as an adverse event that is judged to be definitely, probably or possibly related to (one of) the study drug(s). Adverse events will be graded for severity according to the CTCAE 5.0 grading system.

- Proportion of adverse events of Grade 3 or Grade 4 severity

- Proportion of adverse events possibly, probably or definitely related to study drugs.
- Proportion of treatment discontinuations or interruptions related to adverse events
- Specific ECG endpoints:
    - Frequency, severity and type of ECG alterations
    - Changes to PR, RR, QRS, QT, Fridericia-corrected QT [QTcF]
    - Proportion of participants with QTcF > 500ms on treatment
    - Proportion of participants who experience a prolongation of QTcF > grade 3

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the summary of product characteristics (or Investigator brochure) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening* • requires hospitalisation or prolongation of existing hospitalisation** • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect • is medically significant***

*The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient 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.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition (including elective procedures that have not worsened) do not constitute an SAE. Due to the seriousness of the disease in this study, some patients may be admitted to hospital for the initial Phase of their trial treatment. This would not qualify as an SAE, although if that hospitalisation had to be prolonged beyond the normal length of admission, then it would be an SAE.

[***]A medically serious event is one which does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. An example might include allergic bronchospasm, haemoptysis, or seizure requiring treatment in an emergency room, that do not result in hospitalization.

14.2 Clarifications and exceptions

Occurrences will be registered as AE from administration of the first dose of study drugs. All events that occur or are elicited before administering any study drugs, e.g. screening laboratory abnormalities qualifying as Adverse Events, will be recorded as medical history. If such events meet criteria for seriousness, they will nevertheless be reported as SAEs.

Abnormalities which are not clinically significant as per the qualified judgement of the investigator do not constitute adverse events and are not recorded and reported as such. However, these abnormalities may be analysed and reported in the study report.

14.3 Additional immediately reportable events

The following events are notable events and need to be reported to the Sponsor within 24 hours of the site becoming aware of the event:

- Pregnancy while on study treatment,
- QTc prolongation meeting a stopping criterion
- any toxicity or condition that leads to a planned deviation from study medication
- any grade 4 event that is defined as probably or definitely related to study treatment by the investigator, and does not meet criteria for seriousness or other immediately reportable events

14.4 Eliciting adverse event information

Patients will be instructed during informed consent to report occurrence of adverse events to the investigator, also between study visits and if not directly asked for.

Patients will be instructed to contact the investigator outside the schedule in case of a new complaint, or worsening of an existing complaint, that they feel is severe.

At every study visit, patients will be asked to describe their complaints, and report new occurrences.

Physical examination findings, weight, vital signs, ECG and laboratory information will be gathered as described by the study schedule of events. Additional investigations will be ordered by the investigator, if they are in the subject`s best interest.

14.5 Assessment of Causality

The Investigator will review each AE and assess its relationship to study treatment based on all available information at the time of the completion of the CRF.

Each sign or symptom reported will be graded on a 5-point severity scale (see below). Additionally, the date and time of onset, relationship to the study medication, duration, action taken, and outcome (resolved, improved, unchanged, worse, fatal, or unknown (lost to follow-up) of each event will be noted.

The following definitions for rating attribution/causality will be applied:

Relationship	**Description**	**Event Type (if Serious)**
Unrelated	There is no evidence of any causal relationship	Unrelated SAE
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).	Unrelated SAE
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).	SAR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	SAR
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

Factors to be considered in judging relatedness include:

- The temporal sequence from drug administration.
- Recovery on discontinuation (de-challenge), recurrence on re-administration (re-challenge).
- Underlying, concomitant, intercurrent disease.
- Concomitant medication or treatment.
- Known response pattern for the classes of drug the participant is receiving
- Exposure to physical and/or mental stresses.
- The pharmacology and PK of the medication used.

14.6 Assessment of Severity

Severity of AEs will be classified following the U.S. National Institutes of Health Common Terminology Criteria for Adverse Events 5.0 (CTCAE), available online at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf , published: November 27, 2017, with the exception of QTc prolongation, which will be graded as specified .in table 3. If the specific event is not contained in this compendium, the following generic definition, which is also contained in CTCAE 5.0 for such events, will apply:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life (ADL)*.
- Grade 3: Severe; significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, work or farming, managing money, etc.*

***Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

Table 2: definition of severity grading of QTcF prolongation

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
(1) Absolute QTcF >480 and ≤500 ms and QTcF change from baseline >0 ms and ≤30 ms; or	(1) Absolute QTcF >480 ms and ≤500 ms and QTcF change from baseline >30 ms and ≤ 60 ms; or	1) Absolute QTcF >500 ms; or	Life-threatening consequence, e.g., torsades de pointes or other associated serious ventricular dysrhythmia.	-
(2) absolute QTcF ≤480 ms and QTcF change from baseline >30 and ≤60 ms	(2) absolute QTcF ≤480 and QTcF change from baseline >60 ms.	(2) absolute QTcF >480 and QTcF change from baseline >60 ms.		-

This deviation from the CTCAE severity grading is necessary due to the specific situation of TB patients; a population in whom a change in QTc over baseline is difficult to assess. Before

treatment starts, they are sick, have elevated temperature and are distressed by their first contact with the trial team, which influences the QT interval, through elevated heart rate and possibly other mechanisms.

In a specific analysis of the Oflotub phase III study, it was confirmed that the elevated heart rates at baseline were associated with lower QTcF; and that specifically at baseline, QTcF correction undercorrects at these high heart rates of TB patients [76].

Further, QTc at baseline and late during treatment were compared. A mean increase in QTcF of 11.2 ms was found in the Gatifloxacin arm, and 9.8 ms in the control arm; despite the fact that for none of the drugs, a correlation between Cmax and QTc prolongation could be described.

Due to this limitation, there is a risk that an incorrect signal of QTcF prolongation over baseline in a patient will occur that in itself will not show a safety hazard to the patient, but will result in lifesaving drugs being withheld. Therefore, assessment of severity of QTcF prolongation and the stopping of treatment in this study as laid out in *Table 2: definition of severity grading of QTcF prolongation*, follows the precedent of the ACTG A5343 trial mentioned above; where a combination of bedaquiline and delamanid was trialled and assessed for its potential to prolong the QT interval [77].

Here, in order to prevent a false signal that might be due to a change in heart rate between assessments, a higher grade QTcF prolongation is defined as a combination of QTcF prolongation from baseline with an elevated absolute value, not a prolongation alone.

14.7 Other AE definitions

The following definitions will be used for AE Reporting:

Action Taken with Study Drugs

- IMP unchanged
- IMP interrupted
- IMP stopped
- Non – IMP study drug unchanged
- Non – IMP study drugs interrupted
- Non – IMP study drugs stopped
- Not applicable

Other Action Taken

- None
- Medication given
- Hospitalisation or prolongation of hospitalisation
- Therapeutic or diagnostic procedure

Outcome

- Resolved
- Improved

- Unchanged
- Worse
- Fatal
- Unknown

Occurrence

- Once
- Intermittent
- Continuous

14.8 Adverse event reporting

Adverse events will be recorded by the investigator from the time a participant signs the informed consent form.

Any AE (serious or non-serious) observed by the investigator or reported by the subject will be recorded in the source data and on the Adverse Event electronic case report form (eCRF). The Investigator will review each AE and assess its relationship to the study treatment based on all available information at the time of the visit. The following information will be recorded for each AE 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; where possible the wording in the CTCAE should be used.
- Date of onset
- Stop date (duration) if applicable
- Severity (grade)
- Action taken with IMP/non-IMP study drugs
- Other action taken
- Outcome
- Relationship to IMP/non-IMP study drugs
- Occurrence
- Seriousness

14.9 Serious adverse event reporting

Any AE that occurs which is serious, or which is potentially serious, but a decision cannot be taken due to a lack of information, must be reported by the investigator to the study monitor and to the sponsor medical expert within 24 hours of the site first being aware of the SAE, whether or not the serious event is deemed drug-related. Medical Review will be done by the Sponsor Medical Expert. Furthermore, any SAE will be reported to Otsuka Novel Products GmbH, with its principal recipient in the United States, and secondary at Erika-Mann-Strasse 21, 80636 Munich, Germany.

In addition, the investigator will provide a detailed, signed, written, and complete SAE Form, including the investigator's estimates of the relationship with the study drug and seriousness of the AE in question to the study monitor and sponsor medical expert within 24 hours of becoming aware of the SAE. The investigator will ensure that the participant in the report is identified by an anonymous code rather than by personal details.

The study monitor will confirm receipt of the SAE Form with the Investigator and review the initial information on the SAE Form for diagnosis, consistency and completeness of data. To update or add information to a reported SAE, the Investigator will provide the study monitor and sponsor medical expert with a newly completed Serious Adverse Event Form, designated as a follow-up report. This will be submitted to the study monitor and Sponsor Medical Monitor within 24 hours of the Investigator receiving the information.

If additional information is needed to complete the profile of the reported SAE, the additional information will be requested from the Investigator, if necessary, to complete the profile of the SAE reported. After evaluation of the SAE Form for expectedness and possible relation to study drug, the sponsor/designee will complete a standardized form by the *Council for International Organizations of Medical Sciences* (CIOMS) for unexpected treatment-emergent SAEs (SUSARs), which are forwarded to the investigator. All SAEs and SUSARs will be submitted to all IECs/IRBs and regulatory bodies in accordance with local requirements and ICH-GCP guidelines.

14.10 Clinical Laboratory adverse events

Changes in the results of the clinical laboratory assessment, 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 if this is a clinically significant change from baseline for that individual patient. 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 negative, clinically significant change from baseline for that patient, it is an adverse event.

14.11 Pregnancy as adverse event

All women of childbearing potential will be instructed to contact the investigator immediately if they suspect they might be pregnant during the trial (for example, missed or late menses). If pregnancy is suspected while the patient is receiving experimental study treatment, this will be withheld immediately until pregnancy can be ruled out with certainty.

If pregnancy is confirmed, experimental treatment will be permanently discontinued in an appropriate manner. The patient should complete her TB treatment course following the host country's national guidelines.

The investigator must immediately notify the sponsor of any pregnancy in a study subject. The pregnancy must be recorded on the pregnancy form and forwarded to the sponsor. Expedited reporting of pregnancy to regulatory authorities is necessary in case of a pregnancy complication fulfilling the criteria for Serious Adverse Events.

Protocol-required procedures for trial discontinuation must be performed unless contraindicated by the pregnancy. Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report to the sponsor for follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants must be followed for a minimum of 6 months. Congenital malformations are to be reported as Serious Adverse Events.

14.12 Disease under study

Symptoms of the disease under study (i.e. TB) experienced by the subject whilst on the study will be assessed by the Investigator. If the symptom has worsened whilst the subject is on the study, and the Investigator assesses it as clinically significant, it will be recorded as an adverse event.

If there is no change and the Investigator assesses the symptom as due to the subject's TB and not clinically significant, it will not be recorded as an AE, and this will be noted in the patient's source documentation. If the Investigator is unsure as to whether the symptom is clinically significant or not, it is to be classified as significant and reported as an AE.

If during follow-up phase, the participants is found to have at least one spot sputum sample that is found to be associated with relapse or re-infection with a different strain of MTB, this is to be recorded as adverse event.

14.13 Follow-up of adverse events

14.13.1 General Follow-up of Adverse Events

During and following a patient's participation in a clinical trial, the Investigator must ensure that adequate medical care is provided to the patient for all AEs, including significant abnormal laboratory values. The investigator should inform the patient when medical care is needed for any AEs, he/she becomes aware of.

All non-serious AEs classified as severe and probably/possibly related to IMP must be followed until the AE has resolved, or until there is no further change likely to the participants' condition. Cases of pre-existing chronic conditions or if the patient dies from another event can be closed with an outcome of "unchanged".

All other non-serious AEs must be followed until the outcome of the event is “improved” (for chronic conditions), “resolved” or until the last scheduled study visit, and all queries on these AEs have been resolved. If the patient dies from another event, the case can be closed with an outcome of “unchanged”.

All SAEs must be followed until the outcome of the event is deemed “resolved”, “unchanged” or “fatal” and until all queries have been resolved. Cases of chronic conditions, cancer or if the patient dies from another event can be closed with an outcome of “unchanged”.

14.13.2 Follow-up of Post-Trial Adverse Events

Any new SAEs reported by the patient to the Investigator that occur after the last scheduled contact, that are determined by the Investigator to be causally related or possibly related to the use of the IMP, will be reported to the study monitor and sponsor medical Expert, IEC/IRB and regulatory authorities on an expedited basis as required.

15 STATISTICAL CONSIDERATIONS

15.1 Sample size determination

15 participants per arm with a total of 75 participants, and a wide range of DZD doses (from 0mg to 800mg BID) has been determined as an adequate sample size for population PK modelling, and for exposure response modelling to detect a clinically meaningful dose-dependent relationship.

Furthermore, the planned sample size of 15 subjects per treatment group is in keeping with other trials of this type and accounts for the possibility of up to 3 drop-outs per group, which based on previous studies of this type conducted at these sites, represents a conservative estimate of the expected drop-out rate.

Previous Phase IIA (EBA) studies indicate that the between patient standard deviation of logCFU can be approximately 0.2. Therefore, assuming similar variability in this trial the expected standard errors of group mean EBA and corresponding width of 95% confidence intervals are 0.052 and 0.101 respectively for a group size of 15 and 0.063 and 0.124 respectively for a group size of 10. This level of precision with a group size of 15 is considered adequate.

15.2 Populations to be analysed

The final analysis populations will be described in the Statistical Analysis Plan (SAP), which will be signed off before database lock.

15.2.1 Intent-to treat (ITT) population

The ITT population will consist of all randomized patients in the groups to which they were randomly assigned, and who have taken at least one dose of study treatment.

15.2.2 Adequate adherence (AA) population:

The AA population will be the same as the ITT population with the following patients excluded:

- Randomised patients not meeting the eligibility criteria
- Patients having missed 10 or more doses of their allocated treatment in the first 16 weeks of their treatment.

15.2.3 Safety Population

The safety population will be defined as all patients who received any dose of study medication.

15.3 Analysis of safety endpoints

The safety analysis population will be used for all safety analyses.

15.3.1 ECG analysis

QT and QTc data will be analysed categorically based on the number and percentage of patients classified in each category by treatment group. QTc data will be presented for Friderica's corrections.

Post-baseline QT- and QTc-intervals will be classified into the following categories:

- QT/QTc<450 ms
- 450 ms <QT/QTc <480 ms
- 480 ms < QT/QTc < 500 ms
- QT/QTc > 500 ms

QTc-changes from baseline will be classified into the following categories:

- decrease (an increase < 0 ms)
- increase < 30 ms
- >30 ms and < 60 ms
- increase >60 ms

15.3.2 Adverse Events

The incidence of treatment – emergent adverse events, defined as all AEs that occur after the administration of IMP, and their relatedness to experimental treatment will be summarised by treatment group, by system organ class and preferred term, maximum severity, and potential relationship to IMP, as well as TEAEs with an outcome of death, serious TEAEs, and discontinuations due to TEAEs.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary.

15.3.3 Other safety variables

Binary and categorical variables will be tabulated by treatment arm. Continuous variables will be summarised with location (such as mean or median) and precision (such as standard error or inter-quartile range) summary estimates.

Patients with worsening condition from baseline on any variable will be described and tabulated. The following variables will be analysed:

- Laboratory Parameters: a list of safety laboratory parameters collected in the trial is described under section 11.3.
- Ophthalmologic Variables: Visual Acuity (Snellen) and Colour Vision (Ishihara) tests
- Physical Examination incl. neurological examination; esp. presence of Hunter score criteria
- Vital signs

15.4 Analysis of primary efficacy endpoint

The primary efficacy endpoint of this study will be change in mycobacterial load over time on treatment as quantified by time to positivity in BD MGIT 960® liquid culture and described by nonlinear mixed-effects methodology. All patients in the ITT analysis population will be included in this analysis

This endpoint will be analysed by non-linear mixed effects modelling.

15.5 Procedure for accounting for missing, unused and spurious data

Study data will, after being entered, be checked for consistency and completeness through programmed checks by the database, which will raise automatic queries. Further completeness and consistency checks will be performed by data management and any resulting queries will be sent through the database query system so as to leave an audit trail.

15.6 Procedure for reporting any deviations from the original statistical plan

Any deviations from the Statistical Analysis Plan will be reported and explained in the Clinical Study Report.

15.7 Timing of analyses

Analysis for the primary endpoints, and secondary endpoints unrelated to disease recurrence or relapse, will be performed after data are available (allowing for time for culture growth) after the last patient completed their WK16 (or otherwise last treatment) visit. A partial database lock will be performed for this purpose, with a final database lock at the end of study after all long-term outcome data are available.

16 DATA HANDLING AND QUALITY ASSURANCE

16.1 Data collection

The investigator agrees to maintain accurate source documentation and CRFs. For each patient screened, an electronic CRF (eCRF) will be completed, even if the patient drops out at any time point during the study. All eCRF information for the completed visits must be completed.

All applicable eCRF pages must be completed for each participant, who has received any dose of study drug and has completed the study.

For participants who are prematurely withdrawn, the visits up to withdrawal plus the withdrawal visit need to be completed.

In the case of necessary corrections, all changes will be effectuated such that the first version is still legible, and the change is initialled and dated by the correcting person. Each completed eCRF must be reviewed, signed and dated by the responsible Principal Investigator or the sub-investigator in a timely manner.

16.2 Source documents

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

Study data will be collected and entered into the eCRF. Some data may still be captured entirely or partially on paper source documents and will manually be entered into an eCRF, including but not limited to:

- Informed Consents
- Chest X-ray images and reviews
- Sample collection data (will be on Lab Forms except for HIV and malaria test results)
- Safety and microbiology laboratory results and their review by the investigator.

All paper source documents and the electronic source documents pertaining to this trial will be maintained by the investigators. The investigator will be obliged to permit trial-related monitoring, audits, Independent Ethics Committee/Institutional Review Board (IEC/IRB) review and regulatory inspections, providing authorized persons direct access to source data/documents.

16.3 Study monitoring

One or more study monitors will be assigned to the study. The monitor, as a representative of the Sponsor, has the obligation to follow the study closely. The monitor will visit the site at regular intervals and will be in contact by phone and written communication, as required.

Site investigators and designated study personnel will allow the study monitors to inspect study documents, pertinent hospital or clinic records as well as site facilities, as required. All aspects of

the study will be carefully monitored in order to ensure compliance with GCP and all applicable regulatory guidelines. The monitor will be responsible for verification of:

- adequacy of study personnel's qualifications as well as facilities
- informed consent procedures and patient eligibility
- the accuracy and completeness of the (e)CRF entries, source data and other trial-related records against each other
- appropriate study drug storage, usage and accountability
- appropriate adverse event reporting
- maintenance of the essential documents
- all other aspects of the trial relating to protection of the rights and well-being of patients, accuracy of trial data and adherence to the protocol, GCP and applicable regulatory requirements

Source data verification will be carried out according to the *monitoring plan*.

The sponsor will provide a framework for maintenance of quality in performance and reporting of laboratory procedures through the *laboratory monitoring plan*.

The study database will only be locked after the data have been monitored by the sponsor and all queries issued through data cleaning activities have been completed and resolutions documented.

16.4 Inspection of records

The investigator will allow the Sponsor, the Sponsor's Representative, Regulatory Agencies and Ethics Committees access to all study records, if requested. The investigator will promptly notify the sponsor of any audits scheduled by regulatory authorities or ethics committees and promptly forward copies of any audit reports received to the sponsor.

16.5 Records retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in the International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, or for not less than 10 years after trial completion, whichever is longer.

These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

16.6 Confidentiality of personal data

All patient records, lab specimen etc. will be identified in appropriate manner to maintain patients' confidentiality and will be kept in a secure storage area with limited access. Clinical data will not be released without the written agreement of the patient (or their legal guardian), except as necessary for monitoring and auditing by the sponsor or its representative, regulatory authorities or ethics committees.

For those participants who gave consent to store genetic samples for future testing an analysis, their samples will be labelled using anonymous codes. Results of any genetic tests will not be disclosed to anybody not involved with the study, in particular not to immediate relatives without prior consent of the patient.

This confidentiality will be in accordance to the recently instated EU Data protection Law (Regulation (EU) 2016/679).

17 ETHICAL CONSIDERATIONS

17.1 Basic principles

This study will be performed in accordance with the protocol, the principles laid down in the ICH harmonized tripartite guideline regarding GCP (Consolidated Guideline E6, July 2002), the Declaration of Helsinki (Sixth Revision, October 2008), as well as any other applicable national and international regulatory guidelines.

17.2 Involvement of ethical committees

The protocol and the informed consent document to be used in this study must be submitted to the Investigator's Ethics Review Committee/IRB, regulatory authority, and the sponsor IRB for approval. Written documentation of approval of both, the protocol and the informed consent, must be provided to the sponsor before starting the study.

The Investigator will ensure that the purpose of the study is explained to the patient and that written consent is obtained prior to participation in the study. The patient and the Investigator or his/her delegate will sign the consent prior to entry into the study.

The Investigator will promptly report to the Ethics Committee/IRB of all changes in the research activity and all unanticipated problems involving risks to human participants or others and will not make changes in the research without Ethics Committee/IRB approval, except where necessary to eliminate apparent immediate hazards to human participants.

17.3 Regulatory authorities

The Regulatory Authorities will receive the protocol, amendments, reports on SAEs and Serious Unexpected Adverse Drug Reactions (SUSARs), and the Integrated Clinical Trial Report according

to national regulations. Written approval will be obtained from the Regulatory Authorities prior to commencement of the trial.

17.4 Investigators view of the ethical issues and considerations

The investigator(s) participating in this trial, as listed above, have had the opportunity to review the protocol outline. Their concerns and suggestions have been included into the final protocol.

17.5 Falsification of data

Any proven evidence of falsification of data will be dealt with in accordance with the policy of the sponsor and appropriate action will be taken.

18 ADMINISTRATIVE CONSIDERATIONS

18.1 Trial committees

18.1.1 Trial Oversight: Trial Steering Committee (TSC)

The role of the Trial Steering Committee (TSC) is to provide overall supervision of the trial and ensure that the trial is conducted in accordance with GCP and GCLP principles. The Trial Steering Committee will formally report to the Sponsor. TSC specifics will be detailed and justified in the TSC charter. The TSC will at least consist of a sponsor representative person, the sponsor medical expert, the trial statistician, and a principal investigator (PI).

18.1.2 Data Safety Management Board (DSMB)

To include an element of expert advice that is independent of the PIs and the sponsor, an independent Data Safety Monitoring Board (DSMB) will be installed. The DSMB will safeguard the interests of trial participants. The DSMB will review data and will make recommendations to the TSC regarding stopping of certain arms, or the whole trial if trial participation is an undue risk to participants and will use the safety review guidelines in their consideration. The DSMB are not executive, any decision regarding early termination of arms or the whole trial will be made by the TSC and sponsor. A DSMB charter will be established that describes the roles and responsibilities of this independent committee for the trial.

18.2 Trial registration

Before study start, the trial will be registered in clinicaltrials.gov, a WHO recognized clinical trials registry.

In addition, the trial will be registered in the South African National Clinical Trials Registry.

18.3 Financing

The study sponsor is LegoChem Biosciences, Inc. The trial is conducted under the umbrella of PanACEA, the PanAfrican Consortium for the Evaluation of anti-TB Antibiotics. PanACEA is a not-for-profit consortium with the goal of shortening the treatment regimen of drug-sensitive TB.

LegoChem Biosciences ensures that sufficient funding of this study is available.

LegoChem Biosciences, Inc. will contribute sufficient DZD 400mg tablets Biosciences to ensure conduct of this study.

18.4 Patient insurance

The sponsor certifies that it has obtained or will obtain clinical trial insurance in line with the requirements in each country prior to study start. The insurance does not relieve the investigators of the obligation to maintain their own liability insurance as required by applicable law. The sponsor does not assume any obligation for the medical treatment of other injuries and illnesses.

18.5 Patient compensation for trial-related expenditures

Trial participants will receive a flat sum per trial visit (and per day spent in hospital) for out of pocket expenses, which covers loss of salary, travel to/from site and other miscellaneous cost, depending on the vote of the trial sites' ethics committee. This sum will be laid down in the sites' informed consent documents to follow local practice and be approved by sites' ethics committees.

Should a trial participant have higher travelling expenses, these can be reimbursed at the discretion of the investigator. In such cases, receipts and considerations should be documented in the participant's records.

Since these payments are reimbursements of estimated cost, they do not constitute an undue incentive for trial participation.

18.6 Publication Policy

After completion of the study, the data may be considered for reporting at a scientific conference and/or publication in scientific journals. LMU will be responsible for these activities and will collaborate with the investigators to determine how the manuscript is written and edited, the number and order of authors, the journal to which it will be submitted and other related issues.

The results of the study will be published independent of the outcome - positive or negative - of the study.

Under certain circumstances, i.e. when the publication of particular findings (of an epidemiological, sociological or genetics study) may present a risk to the interest of a community or population or a racially or ethnically defined group of people, it may be considered inappropriate to publish findings.

18.7 Protocol amendment policy

Any change to the protocol will be made by means of a protocol amendment. Any changes which affect participant safety or welfare will be submitted to the IEC/IRB and regulatory authorities prior to implementation. The Investigator, IEC/IRB, and sponsor must agree on all amendments. No amendment will be implemented until approved by the relevant authorities 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 for notification purposes as appropriate.

19 REFERENCES

1. WHO, *Global Tuberculosis Report 2017*. 2017, World Health Organization: Geneva.
2. Mehra, M., et al., *Assessment of tuberculosis burden in China using a dynamic disease simulation model.* Int J Tuberc Lung Dis, 2013. **17**(9): p. 1186-94.
3. Murray, S., C. Mendel, and M. Spigelman, *TB Alliance regimen development for multidrug-resistant tuberculosis.* The International Journal of Tuberculosis and Lung Disease, 2016. **20**(12): p. S38-S41.
4. Conradie, F. *The Nix-TB trial of Pretomanid, Bedaquiline and Linezolid to Treat XDR-TB*. in *Conference on Retroviruses and Opportunistic Infections (CROI)*. 2017. Seattle.
5. Choi, Y., et al., *Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects.* J Antimicrob Chemother, 2017.
6. Wallis RS, D.R., Friedrich SO, Venter A, Paige D, Zhu T, Silvia A, Gobey J, Ellery C, Zhang Y, Eisenach K, Miller P, Diacon AH, *Mycobactericidal Activity of Sutezolid (PNU-100480) in Sputum (EBA) and Blood (WBA) of Patients with Pulmonary Tuberculosis.* PLoS One, 2014. **14**(9).
7. Lee, M., et al., *Linezolid for treatment of chronic extensively drug-resistant tuberculosis.* N Engl J Med, 2012. **367**(16): p. 1508-18.
8. WHO, *Country Profile Tanzania WHO 2016* 2016: https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=TZ&LAN=EN&outtype=pdf
9. WHO, *WHO Country profile: South Africa.* 2016.
10. Kendall, E.A., et al., *MDR-TB treatment as prevention: The projected population-level impact of expanded treatment for multidrug-resistant tuberculosis.* PLoS One, 2017. **12**(3): p. e0172748.
11. Wallis, R.S., et al., *Mycobactericidal activity of sutezolid (PNU-100480) in sputum (EBA) and blood (WBA) of patients with pulmonary tuberculosis.* PLoS One, 2014. **9**(4): p. e94462.
12. FDA, *Guidance For Industry: Adaptive Design Clinical Trials for Drugs and Biologics*, C.f.D.E.a.R.C.C.f.B.E.a.R. (CBER), Editor. 2010: Rockville, MD.
13. Cho, Y. and J. Jang, *Development of Delpazolid for the Treatment of Tuberculosis.* Applied Sciences, 2020. **10**: p. 2211.
14. Reck, F., et al., *Novel substituted (pyridin-3-yl)phenyloxazolidinones: antibacterial agents with reduced activity against monoamine oxidase A and increased solubility.* J Med Chem, 2007. **50**(20): p. 4868-81.
15. Humphrey, S.J., et al., *Cardiovascular sympathomimetic amine interactions in rats treated with monoamine oxidase inhibitors and the novel oxazolidinone antibiotic linezolid.* J Cardiovasc Pharmacol, 2001. **37**(5): p. 548-63.
16. Antal, E.J., et al., *Linezolid, a novel oxazolidinone antibiotic: assessment of monoamine oxidase inhibition using pressor response to oral tyramine.* J Clin Pharmacol, 2001. **41**(5): p. 552-62.
17. Rumore, M.M., M. Roth, and A. Orfanos, *Dietary tyramine restriction for hospitalized patients on linezolid: an update.* Nutr Clin Pract, 2010. **25**(3): p. 265-9.
18. Flanagan, S., et al., *In vitro, in vivo, and clinical studies of tedizolid to assess the potential for peripheral or central monoamine oxidase interactions.* Antimicrob Agents Chemother, 2013. **57**(7): p. 3060-6.
19. Warrington, S.J., et al., *Clinical pharmacology of moclobemide, a new reversible monoamine oxidase inhibitor.* J Psychopharmacol, 1991. **5**(1): p. 82-91.
20. Wijnands, S., et al., *Potentiation of the pressor effect of oral and intravenous tyramine during administration of the selective MAO-A inhibitor moclobemide in healthy volunteers.* J Psychopharmacol, 1989. **3**(1): p. 36-44.

21. Pharmacia and Upjohn Company LLC, *Zyvox (Linezolid Package Insert)*. 2018.
22. Marcason, W., *What is the bottom line for dietary guidelines when taking monoamine oxidase inhibitors?* J Am Diet Assoc, 2005. **105**(1): p. 163.
23. Da Prada, M., et al., *On tyramine, food, beverages and the reversible MAO inhibitor moclobemide.* J Neural Transm Suppl, 1988. **26**: p. 31-56.
24. Avorn, J., *Approval of a tuberculosis drug based on a paradoxical surrogate measure.* Jama, 2013. **309**(13): p. 1349-50.
25. WHO, *Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB)*. 2018: Geneva.
26. Diacon, A.H., et al., *Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients.* Antimicrob Agents Chemother, 2010. **54**(8): p. 3402-7.
27. Dawson, R., et al., *Efficacy of Bedaquiline, Pretomanid, Moxifloxacin & PZA (BPaMZ) Against DS - & MDR-TB*, in *Conference on Retroviruses and Opportunistic Infections (CROI)*. 2017: Seattle, WA.
28. van Heeswijk, R.P., B. Dannemann, and R.M. Hoetelmans, *Bedaquiline: a review of human pharmacokinetics and drug-drug interactions.* J Antimicrob Chemother, 2014. **69**(9): p. 2310-8.
29. McLeay, S.C., et al., *Population pharmacokinetics of bedaquiline (TMC207), a novel antituberculosis drug.* Antimicrob Agents Chemother, 2014. **58**(9): p. 5315-24.
30. Diacon, A.H., et al., *Multidrug-resistant tuberculosis and culture conversion with bedaquiline.* N Engl J Med, 2014. **371**(8): p. 723-32.
31. World Health, O., *Report of the guideline development group meeting on the use of bedaquiline in the treatment of multidrug-resistant tuberculosis: a review of available evidence (2016), 28 - 29 June 2016, Geneva, Switzerland*. 2017, World Health Organization: Geneva.
32. WHO, *WHO consolidated guidelines on drug-resistant tuberculosis treatment*, ed. WHO. 2019, Geneva: WHO.
33. Diacon, A.H., N. Lounis, and B. Dannemann, *Multidrug-resistant tuberculosis and bedaquiline.* N Engl J Med, 2014. **371**(25): p. 2436.
34. Nunn, A.J., P.P. Phillips, and S.H. Gillespie, *Design issues in pivotal drug trials for drug sensitive tuberculosis (TB).* Tuberculosis (Edinb), 2008. **88 Suppl 1**: p. S85-92.
35. Gler, M.T., et al., *Delamanid for multidrug-resistant pulmonary tuberculosis.* N Engl J Med, 2012. **366**(23): p. 2151-60.
36. Geiter, L. *Delamanid Global Clinical Database and Phase 3 Trial Results*. in *The 48th Union World Conference on Lung Health*. 2017. Guadalajara.
37. Mallikaarjun, S., et al., *Delamanid Coadministered with Antiretroviral Drugs or Antituberculosis Drugs Shows No Clinically Relevant Drug-Drug Interactions in Healthy Subjects.* Antimicrob Agents Chemother, 2016. **60**(10): p. 5976-85.
38. Sasahara, K., et al., *Pharmacokinetics and Metabolism of Delamanid, a Novel Anti-Tuberculosis Drug, in Animals and Humans: Importance of Albumin Metabolism In Vivo.* Drug Metab Dispos, 2015. **43**(8): p. 1267-76.
39. Shimokawa, Y., et al., *Metabolic Mechanism of Delamanid, a New Anti-Tuberculosis Drug, in Human Plasma.* Drug Metab Dispos, 2015. **43**(8): p. 1277-83.
40. Falzon, D., et al., *World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update.* The European Respiratory Journal, 2017. **49**(3): p. 1602308.
41. Jindani, A., et al., *High-Dose Rifapentine with Moxifloxacin for Pulmonary Tuberculosis.* New England Journal of Medicine, 2014. **371**(17): p. 1599-1608.
42. Gillespie, S.H., et al., *Four-Month Moxifloxacin-Based Regimens for Drug-Sensitive Tuberculosis.* New England Journal of Medicine, 2014. **371**(17): p. 1577-1587.

43. Moise, P.A., Birmingham, M.C., Schentag, J.J., *Pharmacokinetics and metabolism of moxifloxacin.* Drugs of Today, 2000. **36**(4): p. 229.
44. Ito, T., et al., *Transport of Quinolone Antibacterial Drugs by Human P-Glycoprotein Expressed in a Kidney Epithelial Cell Line, LLC-PK₁.* Journal of Pharmacology and Experimental Therapeutics, 1997. **282**(2): p. 955-960.
45. Stass. H, K.D., *Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man.* J Antimicrob Chemother, 1999. **43 Suppl B**: p. 83-90.
46. Lettieri. J, V.R., Agarwal. V, Liu. P, *Effect of food on the pharmacokinetics of a single oral dose of moxifloxacin 400mg in healthy male volunteers.* Clin Pharmacokinet, 2001. **40 suppl 1** p. 19-25.
47. Iannini, P.B. and L.A. Mandell, *An updated safety profile of moxifloxacin.* J Chemother, 2002. **14 Suppl 2**: p. 29-34.
48. Bayer, *Moxifloxacin Investigator' s Brochure*. 2011.
49. Dooley, K.E., et al. *QT effects of Bedaquiline, Delamanid or Both in MDR-TB patients: the DELIBERATE trial*. in *CROI conference*. 2019. Seattle, WA.
50. Emanuele Pontali, G.S., Simon Tiberi, Marina Tadolini, Dina Visca, Lia D'Ambrosio, Rosella Centis, Antonio Spanevello, Giovanni Battista Migliori, *Combined treatment of drug-resistant tuberculosis with bedaquiline and delamanid: a systematic review.* Eur Respir J, 2018.
51. Ferlazzo, G., et al., *Early safety and efficacy of the combination of bedaquiline and delamanid for the treatment of patients with drug-resistant tuberculosis in Armenia, India, and South Africa: a retrospective cohort study.* Lancet Infect Dis, 2018. **18**(5): p. 536-544.
52. Migliori GB, P.E., Sotgiu G, Centis R, D'Ambrosio L, Tiberi S, Tadolini M, Esposito S *Combined Use of Delamanid and Bedaquiline to Treat Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis: A Systematic Review.* Int J Mol Sci. , 2017. **18**(2).
53. Maryandyshev, A., et al., *Bedaquiline and Delamanid Combination Treatment of 5 Patients with Pulmonary Extensively Drug-Resistant Tuberculosis.* Emerg Infect Dis, 2017. **23**(10).
54. Diacon AH, D.R., du Bois J, Narunsky K, Venter A, Donald PR, van Niekerk C, Erondu N, Ginsberg AM, Becker P, Spigelman MK *Phase II dose-ranging trial of the early bactericidal activity of PA-824.* Antimicrob Agents Chemother, 2012. **56**(6): p. 3027-31.
55. Diacon, A.H., et al., *Phase II dose-ranging trial of the early bactericidal activity of PA-824.* Antimicrob Agents Chemother, 2012. **56**(6): p. 3027-31.
56. Dawson R, H.K., Conradie A, Burger D, Murray S, Mendel C. *Efficacy of Bedaquiline, Pretomanid, Moxifloxacin & PZA (BPaMZ) Against DS - & MDR-TB.* in *Conference on Retroviruses and Opportunistic Infections (CROI)*. 2017 Seattle
57. Levey, A.S., et al., *A new equation to estimate glomerular filtration rate.* Ann Intern Med, 2009. **150**(9): p. 604-12.
58. Dorman, S.E., et al., *High-dose rifapentine with or without moxifloxacin for shortening treatment of pulmonary tuberculosis: Study protocol for TBTC study 31/ACTG A5349 phase 3 clinical trial.* Contemp Clin Trials, 2020. **90**: p. 105938.
59. Dawson, R., et al., *Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis.* Lancet, 2015. **385**(9979): p. 1738-47.
60. Abdool Karim, S.S., et al., *Integration of antiretroviral therapy with tuberculosis treatment.* N Engl J Med, 2011. **365**(16): p. 1492-501.
61. Malaty, L.I. and J.J. Kuper, *Drug interactions of HIV protease inhibitors.* Drug Saf, 1999. **20**(2): p. 147-69.
62. Hauser, W.A., J.F. Annegers, and L.T. Kurland, *Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984.* Epilepsia, 1993. **34**(3): p. 453-68.

63. Nguyen, A., et al., *A randomized cross-over study to compare raltegravir and efavirenz (SWITCH-ER study).* Aids, 2011. **25**(12): p. 1481-7.
64. Hauser, W.A. and E. Beghi, *First seizure definitions and worldwide incidence and mortality.* Epilepsia, 2008. **49 Suppl 1**: p. 8-12.
65. World Health Organization, *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infections: Recommendations for Public Health Approach*. 2nd ed. 2016, Geneva.
66. FDA, *Guidance for Industry. Drug-Induced Liver Injury: Premarketing Clinical Evaluation*, F.a.D.A. U.S. Department of Health and Human Services, Center for Drug Evaluation and Research, Editor. 2009.
67. Mfinanga, S.G., et al., *Early versus delayed initiation of highly active antiretroviral therapy for HIV-positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): a prospective, international, randomised, placebo-controlled trial.* Lancet Infect Dis, 2014. **14**(7): p. 563-71.
68. Abdool Karim, S.S., et al., *Timing of initiation of antiretroviral drugs during tuberculosis therapy.* N Engl J Med, 2010. **362**(8): p. 697-706.
69. Diletti, E., D. Hauschke, and V.W. Steinijans, *Sample size determination for bioequivalence assessment by means of confidence intervals.* Int J Clin Pharmacol Ther Toxicol, 1992. **30 Suppl 1**: p. S51-8.
70. Wallis, R.S., et al., *Biomarkers for tuberculosis disease activity, cure, and relapse.* Lancet Infect Dis, 2009. **9**(3): p. 162-72.
71. Manfredi, R. and L. Calza, *Severe brain co-infection with Cryptococcus neoformans and Mycobacterium tuberculosis in a young, otherwise healthy student recently immigrated from China.* Int J Infect Dis, 2008. **12**(4): p. 438-41.
72. Khan, Z., et al., *Mycobacterium Avium Complex (MAC) Lung Disease in Two Inner City Community Hospitals: Recognition, Prevalence, Co-Infection with Mycobacterium Tuberculosis (MTB) and Pulmonary Function (PF) Improvements After Treatment.* Open Respir Med J, 2010. **4**: p. 76-81.
73. Jones, M.D., et al., *Discovery of novel intermediate forms redefines the fungal tree of life.* Nature, 2011. **474**(7350): p. 200-3.
74. Peloquin, C.A., *Therapeutic drug monitoring in the treatment of tuberculosis.* Drugs, 2002. **62**(15): p. 2169-83.
75. Garton, N.J., et al., *Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum.* PLoS Med, 2008. **5**(4): p. e75.
76. Desta, Z., et al., *The gastroprokinetic and antiemetic drug metoclopramide is a substrate and inhibitor of cytochrome P450 2D6.* Drug Metab Dispos, 2002. **30**(3): p. 336-43.
77. Harada, T., et al., *Metoclopramide-induced Serotonin Syndrome.* Intern Med, 2017. **56**(6): p. 737-739.
78. Vella-Brincat, J.W.A., et al., *The Pharmacokinetics and Pharmacogenetics of the Antiemetic Cyclizine in Palliative Care Patients.* Journal of Pain and Symptom Management, 2012. **43**(3): p. 540-548.
79. He N, Y.W.e.a., *Inhibitory effects of H1-antihistamines on CYP2D6- and CYP2C9-mediated drug metabolic reactions in human liver microsomes.* Eur J Clin Pharmacol, 2002. **57**: p. 847-51.
80. K;, N. and Z. T;, *Pharmacogenetics*. Vol. 6. 1996.
81. Suzuki, A., et al., *Histamine H1-receptor antagonists, promethazine and homochlorcyclizine, increase the steady-state plasma concentrations of haloperidol and reduced haloperidol.* Ther Drug Monit, 2003. **25**(2): p. 192-6.
82. Akutsu, T., et al., *Identification of Human Cytochrome P450 Isozymes Involved in Diphenhydramine N-Demethylation.* Drug Metabolism and Disposition, 2007. **35**(1): p. 72-78.

83. Hamelin, B.A., et al., *Significant interaction between the nonprescription antihistamine diphenhydramine and the CYP2D6 substrate metoprolol in healthy men with high or low CYP2D6 activity.* Clinical Pharmacology & Therapeutics, 2000. **67**(5): p. 466-477.

20 APPENDIX

20.1 Management and classification of liver toxicity

Since treatments in this study do not include standard of care for DS-TB, we decided to recommend testing for and management of drug-induced liver injury (DILI) following the FDA' s guidance that provides a synthesis of patient protection considerations, and adequate characterisation of the treatment regimen' s risk for DILI [66], rather than follow TB-specific guidelines.

20.1.1 Prevention: Participant awareness

Remind the participant to avoid substances with the potential to cause liver damage: including alcohol and other medications such as paracetamol.

The participant must be informed about signs that might indicate worsening abnormalities or evidence of drug reaction (e.g. loss of appetite, fever, nausea, vomiting, rash, jaundice) and know to present emergently should any of these be noticed.

20.1.2 Closer observation: AST/ALT > 3xULN

It is critical to initiate close observation immediately upon detection and confirmation of early signals of possible DILI, and not to wait until the next scheduled visit or monitoring interval.

A threshold of aminotransferase levels greater than 3xULN is recommended for closer observations, as lesser elevations are common and nonspecific, **if participant is asymptomatic regarding clinical signs of DILI AND bilirubin AND INR are not elevated.**

Other laboratory findings that do not meet these criteria but raise the investigator's concern should be discussed with the sponsor medical expert.

If additional testing, beyond that specified in the trial protocol, is carried out, it is important that the subject's information be added to the CRFs and database.

Close observation includes:

- Repeating liver enzyme, serum bilirubin tests and coagulation tests two or three times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.

- Obtaining a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), but **especially alcohol use, recreational drug use, and special diets**.
- Ruling out acute viral hepatitis types A, B, C, D, and E; hypoxic/ischemic hepatopathy as much as possible; and biliary tract disease. Ultrasound should be performed for the latter.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).

20.1.3 Pausing treatment

Pausing of study treatment should be considered if:

- ALT or AST >8xULN
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

20.1.4 Re-challenge with study treatment:

- As soon as symptoms resolve and elevated ALT/AST are below 2x ULN, reintroduction of study treatment is to be discussed with the sponsor medical expert. Study treatment will be reintroduced (all drugs simultaneously). Close, biweekly monitoring of symptoms and laboratory parameters is then needed to identify repeated DILI. Should this occur, the study regimen will be permanently stopped and the participant be continued on a regimen that is considered non-toxic.
- **Inform the participant**: The participant must be informed about signs that might indicate worsening abnormalities or evidence of drug reaction (e.g. loss of appetite, fever, nausea, vomiting, rash, jaundice) and know to present emergently should any of these be noticed.
- The participant must be informed on avoiding alcohol and other liver toxic co-medications or drugs.

20.1.5 Follow-up of liver toxicities

- All participants who discontinue study treatment should be followed up as described for follow-up of adverse events classified as “severe” (grade 3 and above).

20.2 MAO inhibitor food restriction list

Food Classification	Foods to avoid
Cheeses	**Strong, aged cheeses** (i.e. aged cheddar, Swiss and parmesan) **Blue cheeses** (i.e. Stilton and Gorgonzola), Brie, Camembert, feta, mascarpone.
Meat, Fish or Substitute	**Beef or chicken livers** (aged), **Cured meats** (i.e. meats treated with salt and nitrate or nitrite, such as dry-type summer sausages, pepperoni and salami). **Raw meats and fish if stored outside the refrigerator**
Beverages	Especially **beer from the tap**, **red wine** All alcoholic beverages should be avoided for the reason of hepatotoxicity.
Miscellaneous	Yeast Extracts such as **Marmite, soy sauce**
Leftovers	**Do not eat after 48 hours**.

20.3 Sub-studies

This study provides the opportunity to advance the scientific understanding of TB disease and treatment, within a well-controlled clinical trial generating valuable data on treatment response in a large cohort of participants, with GCP-related quality control and assurance producing high data quality.

It is therefore ethically mandated to use this opportunity to advance the science around TB and TB treatment by making use of this resource, in order to accelerate future developments to the benefit of TB patients and persons at risk of TB diseases.

During this trial, retention samples will be collected as described below. These samples may be used in the following proposed sub-studies, including possible genetic analysis. Collection of these samples will only be done if the site's ethics committee has accepted the conduct of these sub-studies, and if a study participant provides separate consent, on forms separate from consent for the main trial. Study participants will be able to refuse retention sample collection and/or genetic testing while still participating in the main trial.

Sample(s) will be kept until they are all used up or destroyed at the Sponsor' s discretion, which may take up to fifteen years, with extension if needed. If the specimens are going to be stored for longer, we will ask permission from the local ethics committee.

The use of these samples will be decided on by the PanACEA Executive group, upon applications of individual researchers or teams for use of trial samples. Results of these analyses will not be incorporated into the trial reports, and will not be disclosed to investigators before the completion of the trial.

Retention samples will be whole blood for genetic analysis, serum, sputum and urine samples to analyse for potential correlates of treatment success and bacterial load. Storage and analysis of these samples may be performed outside of the countries hosting the trial site(s). The possibility of sample transport and analysis abroad will be included into the information provided to the patient before consenting.

All use of stored samples, which is not identical with intentions and/or methods described in this protocol, shall be submitted to the relevant IRBs before any sample is used.

20.3.1 Rationale: New Markers for Treatment Response

Background: Monitoring of TB treatment response in an individual, as well as for later-stage clinical trials of new regimens, is currently hampered by limitations in the predictive methods used. 2-month culture status is widely accepted as a surrogate marker for the efficacy of a regimen in a Phase II trial. However, on an individual basis, the sensitivity of a positive 2-month culture to predict relapse was less than 50%, thus cannot be used for identifying patients at risk who would benefit from intensified therapy [70]. In Phase II clinical trials, lengthy time to availability of culture results are problematic in terms of adaptive trial designs, which require these data for efficient decisions.

In later stage clinical trials, the gold standard for efficacy is currently the rate of treatment failure and relapse, which requires an 18-month follow up with all consequences including cost, losses to follow-up, slow progress of clinical development etc. [34].

New bacterial and host markers are therefore urgently required and need to be validated for their use in individualized therapy and clinical trials. If feasible, research on these markers will include research on coinfection of TB with other pulmonary pathogens by next generation sequencing, as coinfections may modify TB disease course, and culture is an insufficient method to identify all potential co-infecting pathogens, which was demonstrated recently by the description of *cryptomycota,* a completely new phylum of single-cell fungi [71-73].

Further, the association of pharmacokinetics of standard anti-TB drugs with treatment success is still incompletely understood [74].

In this study, a number of potential biomarkers derived from the pathogen and the host will be examined:

20.3.2 Methods to assess the infecting pathogen:

- Differential Staining methods will be performed on sputum smears to determine the frequency of e.g. mycobacterial persister phenotype as described by Garton et al [75], and to correlate phenotypes with treatment response as measured by the primary and secondary study endpoints.
- Sputum bacterial transcriptomics, proteomics and metabolism may be assessed in order to define and assess innovative endpoints linked to the diagnosis of TB or to TB treatment success.
- Bacterial antigens: novel bacterial antigens may be analysed from the preserved materials in order to validate new tests with a potential to be used as surrogates for treatment success.
- Molecular bacteriology: assessments using molecular bacteriologic measures may be done during screening and during treatment course. This will be done to evaluate novel PCR-based diagnostics, and parameters to determine treatment success such as mycobacterial RNA, and mycobacterial antigens. Sequencing of mycobacterial DNA and RNA may be included.
- Molecular epidemiology: strain typing of the pathogen e.g. through Spoligo-typing, MIRU-VNTR or sequencing of parts or the entire bacterial genome or transcriptome may be done in order to establish molecular strain epidemiology, an association of strains with treatment response, severity of infection, or of the genotype with drug resistance.

20.3.3 Methods to assess Host Markers and Co-infections:

- Serological and Immunological markers: sera, whole blood will be preserved to later allow analysis of host markers for possible surrogates for a diagnosis of active TB, and for primary and secondary endpoints of this study. Markers assessed will include

mycobacterial and other antigens, immunological parameters including those assessed by flow cytometry, and evaluation of concurrent viral and parasitic infections, among others.

- Co-infections: the role of coinfections in TB patients regarding disease symptoms and treatment response shall be investigated. These will be analyzed by standard microbiological methods including culture, staining and antigen detection, but also by molecular detection including next-generation sequencing from stored patient materials.
- Genetic Markers associated with TB treatment outcome: Patient DNA and RNA will be stored identified with a code, to allow for future analysis of host genetic/transcription markers associated with study endpoints and/or PK parameters (pharmacogenomics), among others. Novel technologies for analysis of genome and transcriptome, like next generation sequencing and nucleic acid chip analysis may be used for this purpose.

21 POSSIBLE DRUG INTERACTIONS

21.1 Anti-TB drugs in PanACEA-DECODE and anti-nausea / anti-emetic drugs

21.1.1 Metoclopramide

Background on metoclopramide (MCP): MCP is a phenothiazine-derived anti-emetic. Few data suggest that MCP is a substrate of CYP2D6. More specifically, studies in human liver microsomes suggest that MCP is both a substrate and an inhibitor of CYP2D6 [76]. In vitro, CYPs 1A2, 2C9, 2C19, and 3A4 also metabolized metoclopramide. A pharmacokinetic interaction study between the strong CYP2D6 inhibitor fluoxetine and MCP in humans shows that MCP is a CYP2D6 substrate indeed. MCP interacts with many other CYP2D6 substrates (e.g. antidepressants), resulting in an increased risk of extrapyramidal symptoms and neuroleptic syndrome, and this may (partially) be a reflection of a pharmacokinetic interaction on MCP.

Metoclopramide hydrochloride promotes motility in the upper gastrointestinal tract by sensitizing tissues to the action of acetylcholine, which is independent from intact vagal innervation and does not stimulate biliary, gastric, or pancreatic secretions. It hastens gastric emptying and intestinal transit by increasing tone and amplitude of gastric contractions, relaxing the pyloric sphincter and duodenal bulb, and enhancing peristalsis of the duodenum and jejunum. It also has antiemetic property which is attributed to the central and peripheral dopamine receptor inhibition. Some interactions between MCP and other drugs are mediated by increased gastric motility.

Supraventricular tachycardia, bradycardia, and possible atrioventricular block have been reported very rarely, mostly with intravenous administration of MCP. However, there are no formal cardiological contra-indications for MCP. In Micromedex, concurrent use of metoclopramide and serotonin norepinephrine reuptake inhibitors is contra-indicated, as it may result in an increased risk of extrapyramidal reactions and neuroleptic malignant syndrome. Case reports are available describing serotonin syndrome when MCP was combined with a SSRI, possibly due to a small 5-HT3 receptor blocking effect of MCP [77].

Interaction between MCP and study drugs: a pharmacokinetic interaction between MCP and the drugs in DECODE is unlikely, since none of the study drugs are metabolized via CYP2D6 or interact with CYP2D6. Furthermore, plasma protein binding of MCP is low (~30-40%), making plasma protein displacement less likely and relevant. Apart from the pharmacokinetic interaction, monitoring of the ECG may be a precaution measure, when higher than standard doses of MCP are combined with MXF/DLM/BDQ or when these drugs are combined for a longer period. MCP is a phenothiazine-derivative, and some phenothiazines have been noted to enhance the QT interval prolongation effect of MXF, but QT prolongation is not seen with MCP. Concurrent use of metoclopramide and serotonin norepinephrine reuptake inhibitors is contra-indicated, and MCP may increase the risk of serotonin syndrome, but the risks for this adverse effect seems of minor clinical relevance.

Conclusion: Upon repeated use and with higher doses of metoclopamide, at least ECG should be monitored. No data are available. It is advised that MCP can be combined with the study drugs, but with caution (see arguments listed above). This means that MCP is started at a low dose (10 mg BID orally or rectally), that possible dose increases occur with small steps and with careful monitoring of adverse effects, that the standard dose of 3-4x10mg/day is not exceeded, and that MCP is used as short as possible. A next dose step would be to 10 mg twice daily.

21.1.2 Domperidone

Background on domperidone: domperidone is another phenothiazine-derived anti-emetic. It is metabolized by CYP3A4, CYP1A2 and CYP2E1. QT interval prolongation is a known adverse effect to domperidone. For this reason, domperidone may not be used in this study.

Conclusion: Domperidone is not allowed to be given in this study per protocol due to QT prolongation.

21.1.3 Cyclizine

Background on cyclizine: cyclizine is an antihistaminic anti-emetic with weakly sedating, weakly anticholinergic and strong anti-emetic properties. It is not often used as an anti-emetic. Cyclizine is metabolized in the liver mainly to the largely inactive metabolite nor-cyclizine, formed by a demethylation reaction. Its pharmacokinetics have been poorly studied, and its metabolic pathway is unknown, but it has been suggested that the CYP2D6 enzyme has a role in the metabolism of cyclizine[78]. The antihistamine hydroxyzine, which is the same class of antihistamine (piperazine) as cyclizine, is metabolized by CYP2D6. Cyclizine inhibits CYP2D6 based on a human liver microsome study [79]. The elimination half-life of cyclizine is long (24 h). It can cause tachycardia. Very few interactions have been described.

Interaction between cyclizine and study drugs: a pharmacokinetic interaction between cyclizine and the drugs in DECODE is unlikely, since none of the study drugs are metabolized via CYP2D6 or interact with CYP2D6.

Conclusion: no interaction of the study drugs and cyclizine has been described and it is not anticipated. Cyclizine would have disadvantages as a first-choice anti-emetic in PanACEA-DECODE: there is much less experience with the use of cyclizine as an anti-emetic compared to MCP, cyclizine

has a long elimination half-life which is a disadvantage in case of an interaction, and this old drug may not be available everywhere. For this reason, the use of cyclizine is not recommended in study patients.

21.1.4 Promethazine

Background on promethazine: promethazine is an antihistaminic / phenothiazine-derived anti-emetic. It has strongly sedating, weakly anticholinergic and strong anti-emetic properties. The sedating effect is a disadvantage for its use as an anti-emetic. Promethazine is metabolized in the liver to the main metabolites promethazine-sulphoxide and desmethylpromethazine. It is metabolized predominantly by CYP2D6 [80]. It also inhibits CYP2D6 based on human liver microsome studies [79] and studies in humans [81]. The elimination half-life is 10-14 h. It can cause bradycardia, tachycardia and (when administered i.v.) prolonged QT interval.
Interaction between promethazine and study drugs: a pharmacokinetic interaction with the drugs in DECODE is unlikely, since none of the study drugs are metabolized via CYP2D6 or interact with CYP2D6. Apart from the pharmacokinetic interaction, monitoring for cardiotoxicity may be a precaution when promethazine would be used in this study (possible increased risk of QT interval prolongation). Micromedex on the combination with MXF: use with caution, e.g. start with lowest dose and titrate the dose.

Conclusion: the use of promethazine is not recommended in study patients due to the strong sedative effect, which may put participants at risk of accidents, and the QT-prolonging potential, which has however only been reported for iv use.

21.1.5 Dimenhydrinate

Background on dimenhydrinate: dimenhydrinate is another antihistaminic anti-emetic. It is a salt of diphenhydramine (diphenhydramine theophyllinate). Dimenhydrinate dissociates to diphenhydramine and 8-chlorotheophylline upon administration. Diphenhydramine is an active metabolite and is well absorbed from the gastrointestinal tract with a bioavailability of 42 to 62%. Diphenhydramine experiences extensive first pass hepatic n-demethylation via CYP2D6; minor demethylation via CYP1A2, 2C9 and 2C19; and smaller degrees of metabolism in pulmonary and renal systems. Next to being a substrate for CYP2D6, it inhibits CYP2D6 based on human liver microsome studies and [82] and studies in humans [83]. The drug is widely distributed throughout the body, including the CNS, with protein binding ranging from 78 to 98.5%. Half-life is 4-8 h. It is not commonly used for nausea and vomiting. Eg for chemotherapy-induced nausea and vomiting, diphenhydramine may be a useful adjunct to other anti-emetics but is not recommended as a single agent.
Interaction between dimenhydrinate and study drugs: a pharmacokinetic interaction with the drugs in DECODE is unlikely, since none of the study drugs are metabolized via CYP2D6 or interact with CYP2D6.

Conclusion: dimenhydrinate is not recommended as anti-emetic in this study, since data on actual interactions of dimenhydrinate and licensed drugs are scarce. There is much less experience with the

use of dimenhydrinate as an anti-emetic compared to MCP, it may be a weakly acting drug mainly suitable as an adjunct, and this old drug may not be available everywhere.

21.1.6 Ondansetron

Background on ondansetron: ondansetron is a 5-HT3 (serotonine) receptor antagonist. Hepatic elimination is responsible for 95% of ondansetron clearance and < 5% is recovered unchanged in urine. Ondansetron is moderately hydrophobic organic cation, and human organic cation transporter 1 (OCT1) is responsible for its hepatic cellular uptake. The primary metabolic pathway involves CYP3A (CYP3A4, CYP3A5), whereas CYP2D6, CYP1A2 and CYP2E1 constitute the secondary pathway. Arrhythmia and bradycardia may occur seldomly (<1%) and QT interval prolongation may occur after i.v. administration. Use should be avoided in patients with congenital long QT syndrome, monitoring is recommended in patients with electrolyte abnormalities, bradyarrhythmias, congestive heart failure, and those taking concomitant medications that prolong the QT interval. In Micromedex, simultaneous use with other CYP3A4 substrates and inhibitors is contra-indicated. Moreover, concurrent use with other serotonergic agents may result in increased risk of serotonin syndrome, therefore, **these drugs are prohibited from use in all trial participants**.

Interaction between ondansetron and study drugs: a pharmacokinetic interaction between ondansetron and the CYP3A4 substrates in this study (BDQ, DLM and DZD) has not been described but may be possible, as it is the main metabolic enzyme of ondansetron. In addition, there may be an increased risk of QT interval prolongation and serotonin syndrome.
Conclusion: Ondansetron (a serotonin receptor antagonist) is not allowed to be given in this study per protocol due to increased risk of QT interval prolongation, serotonin syndrome and pharmacokinetic drug-drug interactions (via CYP3A4).

21.2 Anti-TB drugs in PanACEA-DECODE and antacids / H2-antagonists / proton pump inhibitors

21.2.1 Aluminium and magnesium containing antacids

Background on aluminium and magnesium containing antacids: These drugs do not interfere with metabolic enzymes. However, an elevation in the pH of the stomach and or interaction with cations may decrease the absorption to other drugs. **These drugs are prohibited from use in all trial participants.**

Interaction between aluminium and magnesium containing antacids and study drugs: No pharmacokinetic interaction through metabolic enzymes is expected. It is unknown whether lower pH in the stomach may affect the absorption of BDQ, DLM or DZD. When combined with MXF, a 40% decrease in exposure occurs. MXF should be administered at least four hours before or eight hours after an aluminum or magnesium containing product.

Conclusion: no relevant interactions are expected with BDQ, DLM or DZD, but this has not been confirmed in patients. MXF should be separated in time from antacids. **Still these drugs are prohibited from use in all trial participants.**

21.2.2 Ranitidine

Background on ranitidine: ranitidine a histamine H2-receptor antagonist and is metabolized in the liver to at least 3 metabolites. No involvement or clinically relevant inhibition/induction of CYP enzymes is anticipated. In vitro, ranitidine did not inhibit CYP3A activity (Martinez et al., 1999), although in Micromedex CYP3A4 substrates and inhibitors are contra-indicated.

Interaction between ranitidine and study drugs: No pharmacokinetic interaction through metabolic enzymes is expected. It is unknown whether gastric acid secretion affects the absorption of BDQ, DLM and DZD. The bioavailability of MXF is not affected by concurrent administration of ranitidine (Stass et al., 2001).

Conclusion: no pharmacokinetic interactions through metabolic enzymes are expected with ranitidine in this study. It is unknown whether gastric acid secretion affects the absorption of some of the study drugs, nevertheless, ranitidine is the drug of first choice in these patients.

21.2.3 Omeprazole

Background on omeprazole: omeprazole is metabolized by CYP2C19 and CYP3A4. Omeprazole is metabolized directly in part by CYP3A4 to omeprazole sulfone, then transformed into 5-hydroxyomeprazole sulfone by CYP2C19. The metabolism of esomeprazole and omeprazole is essentially similar, except that the rate of 5'-hydroxylation is slower in the case of esomeprazole than in the racemic mixture. It is inhibiting CYP2C19, CYP3A4 and P-gp, and induces CYP1A2 and.

Interaction between omeprazole and study drugs: a pharmacokinetic interaction between omeprazole and BDQ, DLM and DZD may occur, as they are CYP3A4 substrates, and their exposure may be increased as a result. MXF is a P-gp substrate, but this interaction is probably of minor clinical relevance.
Conclusion: limited pharmacokinetic interactions through metabolic enzymes are expected with omeprazole in this study. Therefore, it is proposed to administer omeprazole as second choice in this study. Of note, it is unknown whether gastric acid secretion affects the absorption of some of the study drugs.

21.2.4 Other proton pump inhibitors – esomepromazole, lansoprazole, rabeprazole, pantoprazole

The metabolic profile of these protone pump inhibitors is similar to that of omeprazole. They are all metabolized by CYP3A4 and/or CYP2C19: interactions with study drugs via CYP3A4 therefore cannot be excluded.