Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Nunn AJ, Phillips PPJ, Meredith SK, et al. A trial of a shorter regimen for rifampin-resistant tuberculosis. N Engl J Med 2019;380:1201-13. DOI: 10.1056/NEJMoa1811867

STREAM: A randomized non-inferiority trial to evaluate a short standardized regimen for the treatment of rifampicin-resistant tuberculosis

This supplement contains the following items:

1. Original protocol (4.0), final protocol (6.2), summary of changes.
2. Original statistical analysis plan (1.0), final statistical analysis plan (1.1), a summary of changes is included at the front of version 1.1.

Summary of protocol changes

Version 4.0 to 5.0 (March 2013)

- Modification to the enrolment criteria which previously only allowed enrolment of sputum smear positive pulmonary TB patients. Protocol version 5.0 allowed HIV-co-infected patients who are smear negative but are shown to be positive pulmonary TB patients on GeneXpert

- Addition of Fluorescein Diacetate (FDA) vital staining as facultative (optional) test prior to LPA or GeneXpert screening in sites with low prevalence of MDR. To be used when the patient has been on anti-tuberculosis treatment in the previous 3 months; only those patients whose specimens are positive on vital staining are eligible.

- Removal of the stratification factor of 4 weeks or more previous MDR-TB treatment

- Removal of the limit of 8 weeks as the maximum amount of treatment that can be missed and then made up in the regimen.

Version 5.0 to 5.1 (January 2014)

- Additional 12 lead ECG monitoring added: 12-lead ECG required at weeks 1-4, then every 4 weeks to week 52.

Version 5.1 to 6.2 (February 2015)

- Mandated that all patients should be followed up to week 132 (previously the requirement was that as many patients as possible should be followed up to that time point)

STREAM

The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB

ISRCTN 78372190

Version 4.0
March 2011
(Based on MRC CTU template protocol version 3.15)

Authorised by:

Name:	Professor Andrew Nunn	Role:	Co-Chief Investigator
Signature:		Date:	16 March 2011
Name:	Dr. Sarah Meredith	Role:	Co-Chief Investigator
Signature:		Date:	16 March 2011

SIGNATURE PAGE

Principal Investigator Signature:

The signature below confirms agreement by the individual at the clinical site responsible for signing the clinical trial agreement, that this is the trial protocol, STREAM (Version 4.0) dated March 2011. The trial will be conducted in accordance with this protocol and with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP).

I agree to conduct the trial according to these regulations and guidelines and to appropriately direct and assist the staff under my control, who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.
Any amendments to this protocol that have a direct influence on the participants in the trial will be approved by the relevant ethics committees, regulatory authorities and the sponsor before implementation.

PRINCIPAL INVESTIGATOR'S NAME: _____

SIGNATURE: _____

DATE: _____

GENERAL INFORMATION

This document describes the STREAM trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but centres entering patients for the first time are advised to contact the MRC Clinical Trials Unit (MRC CTU), London to confirm they have the most up-to-date version. Clinical problems relating to this trial should be referred to the relevant Chief Investigator.

Compliance

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, in accordance with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), and the applicable regulatory requirements in the participating countries.

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SAE NOTIFICATION

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Or

Email: stream@ctu.mrc.ac.uk

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ABBREVIATIONS AND GLOSSARY

AE	Adverse Event
AR	Adverse Reaction
CF	Consent Form
CI	Chief Investigator
CRF	Case Report Form
CTA	Clinical Trials Authorisation
DAIDS	Division of Acquired Immunodeficiency Syndrome
DCF	Data Clarification Form
DOT	Directly Observed Treatment
DST	Drug Susceptibility Test
DTM	Domiciliary Treatment Monitor
ECG	Electrocardiogram
EQA	External Quality Assurance
FDA	Fluorescein diacetate staining
GCP	Good Clinical Practice
GLC	Green Light Committee
HE	Health Economics
HIV	Human Immunodeficiency Virus
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITM	Institute of Tropical Medicine
ITT	Intention To Treat
KNCV	Royal Netherlands Tuberculosis Foundation
LCMS	Living Conditions Monitoring Survey
LPA	Line Probe Assay
LQAS	Lot Quality Assurance Sampling
MDR	Multi-Drug Resistant
Genotype MTBDRPlus	Rapid test for *M. tuberculosis* Complex and its resistance to Rifampicin and/or Isoniazid
Genotype MTBDR*s/*	Rapid test for *M. tuberculosis* Complex and its resistance to fluoroquinolones and/or aminoglycosides/cyclic peptides and/or ethambutol
MIC	Minimum Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units–Variable Number of Tandem Repeats
MRC CTU	Medical Research Council Clinical Trials Unit
NE	Notable Event
NTP	National Tuberculosis Programme
PI	Principal Investigator
PIS	Patient Information Sheet
QA	Quality Assurance
QT Interval	A measure of the time between the start of the Q wave and the end of the T wave in the ECG complex.
QTc	QT interval corrected for heart rate
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SRA	Stringent Regulatory Agency
SSA	Site Specific Assessment
STREAM	The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs

	for Patients with MDR-TB
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TBCAP	Tuberculosis Control Assistance Program
TM	Trial Manager
TMG	Trial Management Group
TMT	Trial Management Team
TREAT TB	Technology, Research, Education, and Technical Assistance for Tuberculosis
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
The Union	International Union Against Tuberculosis & Lung Disease
USAID	United States Agency For International Development
WHO	World Health Organisation
XDR	Extensively Drug Resistant
ZN	Ziehl-Neelsen

ABBREVIATION OF DRUG NAMES

K	Kanamycin
C	Clofazimine
M	Moxifloxacin
E	Ethambutol
H	Isoniazid
Z	Pyrazinamide
P	Prothionamide

1 SUMMARY

1.1 Abstract and summary of trial design

1.1.1 Type of design
A non-inferiority multi-centre international parallel group randomised controlled trial.

1.1.2 Disease / patients studied
Patients with Multidrug-Resistant Tuberculosis (MDR-TB).

1.1.3 Trial objectives
The primary objectives of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on the study regimen is not inferior to that on the control (WHO approved MDR-TB) regimen.
2. To compare the proportion of patients who experience grade 3 or greater adverse events, during treatment and follow-up, in the study regimen as compared to the control regimen.

The secondary objectives of the STREAM trial are:

1. To determine the proportion of patients with a favourable efficacy outcome on the study regimen in each country setting.
2. To compare the economic costs incurred during treatment by patients and by the health system, in the study regimen as compared to the control regimen.

1.1.4 Trial intervention
The study regimen is based on the regimen described by Van Deun 2010[1]; it consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for nine months (40 weeks), supplemented by kanamycin, isoniazid and prothionamide in the four months (16 weeks) of the intensive phase. All drugs are given daily (seven days a week) except for kanamycin which is given thrice-weekly after 12 weeks. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks respectively as described in section 7.3.2.

Patients will be randomised to either the study regimen or the locally-used WHO-approved MDR-TB regimen.

1.1.5 Duration
Patients on the study regimen will receive nine months (40 weeks) of treatment (16 weeks intensive phase, 24 weeks continuation phase). In the event of delayed smear conversion the intensive phase of the study regimen can be extended by 4 or 8 weeks, allowing a maximum total duration of 48 weeks treatment.

Patients on the control regimen will receive the locally-used WHO-approved MDR-TB regimen.

All patients in the study will be followed up to 27 months post-randomisation. Those randomised early in the study will be followed up to 33 months. The primary analysis will be based on the data accrued to 27 months; the 33 month follow-up data will be used in a secondary analysis.

1.1.6 Outcome measures

The primary efficacy outcome is status at the end of follow-up i.e. the proportion of patients with a favourable outcome 27 months after randomisation (as defined in section 11). The primary safety outcome is the proportion of patients experiencing a grade 3 or greater adverse event during treatment and follow-up.

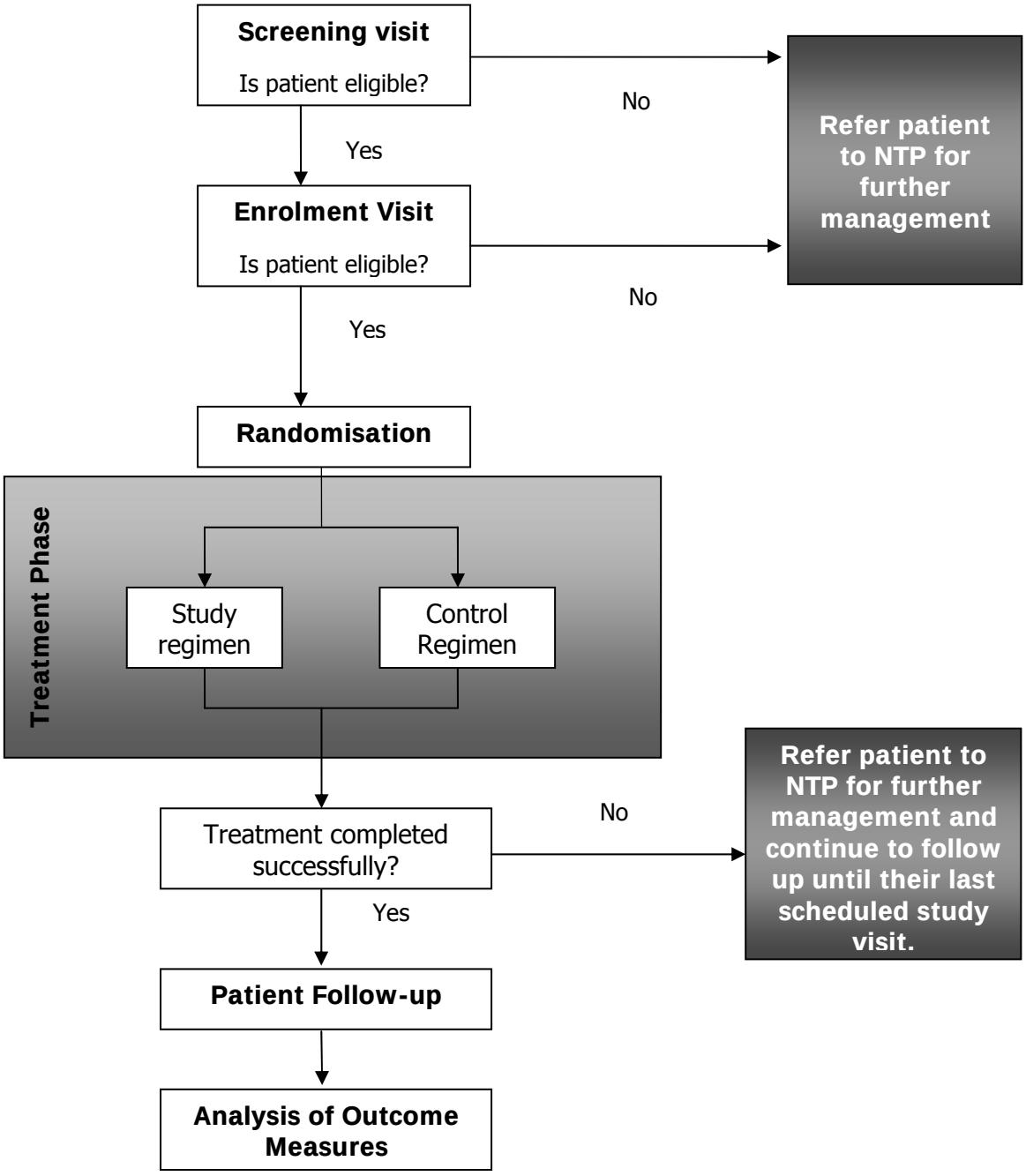
The secondary outcomes include:

- Time to sputum (smear and culture) conversion
- Time to unfavourable outcome
- Time to cessation of clinical symptoms
- Efficacy status at end of follow-up (33 months for those with extended follow-up)
- All cause mortality during treatment and follow-up
- Change of regimen for adverse drug reactions;
- Number of adverse reactions occurring on treatment
- Adherence to treatment
- Acceptability of regimen in to all stakeholders in terms of:
    - Costs to the health system related to delivering the regimen and conducting follow up tests
    - Household costs
    - Patient treatment and support experiences (frequency of health facility visits, side effects)
    - Health worker experiences of delivering treatment and support

1.1.7 Sample Size

A total of at least 400 participants from sites in 4 or 5 countries will be randomised to either the study regimen or the control regimen in the ratio 2:1.

Figure 1: Trial entry, randomisation, treatment and follow-up



2 BACKGROUND

2.1 Introduction

Despite the availability of an efficacious and affordable six-month chemotherapy regimen and the definition of an efficient strategy to deliver treatment under direct observation to the majority of TB patients, TB control worldwide is impeded by two major issues: (i) the emergence of multidrug resistance (MDR) and (ii) co-existent HIV infection. The former hampers dramatically the efficacy of widely implemented standard short-course chemotherapy, thus limiting the success of efforts to fight against tuberculosis worldwide[2, 3], and the latter is associated with a decrease in survival and increase in recurrence. Most recent global estimates of the global MDR-TB burden suggest 511,000 new MDR-TB cases were generated in 2007[4], and since 2002, at least one case of extensively drug-resistant tuberculosis (XDR-TB) has been reported from 45 countries[5]. The overlapping of the HIV and MDR-TB epidemics in some settings raises serious difficulties for the control of the two epidemics[6]. The current recommended treatment approach for MDR-TB is based largely on expert opinion and there is a lack of good evidence on optimal management.

2.1.1 Relevant studies/trials

World Health Organisation (WHO) guidelines for the treatment for MDR-TB recommend an intensive phase of treatment based on at least four drugs known to be effective and given for a minimum of 18 months after culture conversion[7]. In the most recent WHO TB surveillance report[4], the size of most country cohorts in 2004 was too small to give reliable estimates of treatment outcomes in patients with MDR-TB. Of the nine countries with 100 or more patients, treatment success rates ranged from 73% in the Philippines and 71% in Latvia to 38% in Romania and 25% in Morocco. Results reported by some of the most important projects following these guidelines were disappointing, with cure rates rarely exceeding 80% even in the most favourable sub-group of previously untreated cases.

Further reports of treatment outcomes of patients with MDR-TB are only available from a small number of localised cohort studies, most with limited follow-up. It is likely that these studies represent some of the better rates of treatment outcomes from more well-controlled programmes. Of 238 patients enrolled on treatment for MDR-TB in Taipei from 1992 to 1996, 68 (29%) left treatment prior to its completion[8]. Among 76 MDR-TB patients (74% HIV positive) registered in the Lesotho national TB programme (NTP) between July 2007 and April 2008, 21 (29%) had died with 52 (68%) alive but still on treatment by October 2008[9]. Among 76 patients in a community-based treatment programme in Lima, Peru between August 1996 and February 1999, 17 (22%) died during treatment or in follow-up. Treatment was given for a median of 23 months with a median of six drugs[10]. Among 204 patients assessed retrospectively who began treatment for pulmonary MDR-TB in Latvia between January and December 2000, 135 (66%) patients were cured or completed therapy, 14 (7%) died, 26 (13%) defaulted, and treatment failed in 29 (14%) [11]. A recent meta-analysis reported on average 62% successful outcome[12].

2.1.2 Population

The study population will be patients diagnosed with MDR-TB who fulfil the inclusion/exclusion criteria outlined in sections 5.1 and 5.2.

2.1.3 Investigational regimen

The **study regimen** (represented by 4KCMEHZP/5MEZC) will be given in two phases:

1. An intensive phase of kanamycin, clofazimine, moxifloxacin, ethambutol, isoniazid, pyrazinamide and prothionamide given daily for four months (16 weeks) which can be

extended by 4 weeks at a time up to a maximum of 48 weeks if smear conversion is not achieved within 16 weeks. Kanamycin is only given thrice-weekly from week 12 onwards.

2. A continuation phase consisting of moxifloxacin, ethambutol, pyrazinamide and clofazimine given daily for five months.

The only change from the regimen described by Van Deun 2010[1] is that moxifloxacin has been substituted for gatifloxacin because gatifloxacin was withdrawn by the original marketing authorisation holder and generic sources investigated did not meet WHO requirements for quality, safety and efficacy.

The **control regimen** will be the locally-used WHO-approved MDR-TB regimen. Country-specific regimens are described in the STREAM Patient Management Guide.

2.2 Rationale

Given the urgent need to increase access to treatment for MDR-TB, careful evaluation of treatment strategies is vital to ensure the most effective and feasible approaches are implemented, particularly in low-income settings where most cases of MDR are found. New drugs with novel mechanisms of action for the treatment of MDR-TB (including TMC-207 and OPC-67683[13]) are being evaluated but it will be several years until any become available for public use. Clinical trials utilising these new compounds in treatment regimens are also clearly warranted. However, maximising the utility of existing drugs is essential for the protection of new compounds for use in alternative regimens.

Van Deun et al (2010)[1] reported very successful results in a cohort of over 200 patients treated with a 9 month regimen on a population of patients in Bangladesh with MDR-TB[1]. A regimen given for less than 12 months with a high success rate would represent a considerable advance over standard practice.

The first objective of the STREAM trial is to assess whether the study regimen, which is designed to be as similar as possible to that regimen used in Bangladesh, is not inferior to the control regimen. Its practical, programme-based study design will also ensure that if the results are favourable they will be generalisable to routine programme settings.

In addition, health system and patient costs associated with implementation will be documented. These will be analysed in association with the clinical outcomes of the trial using the TREAT TB Impact Assessment Framework[14] in order to provide as much information as possible for subsequent policy and practice decision-making.

It was necessary to substitute moxifloxacin for gatifloxacin in the study regimen because the original manufacturer of gatifloxacin withdrew their product from the market due to reports of associated dysglycaemia, and it was not possible to identify a generic source of gatifloxacin that met WHO manufacturing norms and standards for quality, safety and efficacy. If, therefore, the study regimen is found to be inferior to the control regimen, one possible explanation will be that moxifloxacin was less effective than gatifloxacin. However, moxifloxacin and gatifloxacin have similar bactericidal activity[15] and the trial will therefore test the regimen that is closest to the standardised regimen developed by Van Deun[1] that is available in routine program setting.

2.2.1 Risks and benefits

A nine-month regimen is substantially shorter than those recommended by the WHO guidelines[7] and could therefore increase the risk of treatment failure or relapse. However, this

has not been observed in the setting where the regimen has already been used where there was a relapse-free cure rate of 88%, 95% confidence interval (83%, 92%)[1].

Most second-line drugs have unpleasant associated toxicities; isoniazid and moxifloxacin will be included in the study regimen at higher doses than are usually administered. However, the regimen given in Bangladesh was well tolerated[1] and the shorter duration of chemotherapy in the study regimen may result in fewer severe adverse drug reactions than in the control regimen. Study patients will be closely monitored for drug toxicity including an assessment of the impact of the moxifloxacin dose on QT interval.

A summary of the safety information on the higher dose of moxifloxacin and the safety monitoring that is to be undertaken in the trial is provided in Appendix 2.

Restriction of the injectables and prothionamide to the intensive phase may also explain why no acquired resistance to these drugs was observed in the failure or relapse cases in the Van Deun[1] study. Although used with only one second-line drug in the continuation phase, acquired fluoroquinolone resistance did not occur, probably due to the relatively high fluoroquinolone dose used; the initial resistance to flouroquinolones rarely resulted in an adverse bacteriological outcome. Moreover, study criteria limiting inclusion to cases sensitive to both fluoroquinolones and second-line injectable drugs will almost certainly prevent amplification of resistance leading to extensively drug-resistant tuberculosis.

3 OBJECTIVES AND OUTCOMES

3.1 Objectives

3.1.1 Primary Objectives

The primary objectives of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on the study regimen is not inferior to that on the control (WHO approved MDR-TB) regimen.

2. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment and follow-up in the study regimen as compared to the control regimen.

3.1.2 Secondary Objectives

The secondary objectives of the STREAM trial are:

1. To determine the proportion of patients with a favourable efficacy outcome on the study regimen in each country setting.

2. To compare the economic costs incurred by patients and by the health system during treatment in the study regimen as compared to the control regimen.

3.2 Outcome measures

The primary efficacy outcome measure is the proportion of patients with a favourable outcome as defined in section 11, Statistical Considerations.

The primary safety outcome measure is the proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria[16], during treatment and follow-up.

Secondary outcome measures include:

- Time to sputum (smear and culture) conversion
- Time to unfavourable efficacy outcome
- Time to cessation of clinical symptoms
- Efficacy status at end of follow-up (33 months for those with extended follow-up)
- All cause mortality during treatment and follow-up
- Change of regimen for adverse drug reactions;
- Number of adverse reactions occurring on treatment
- Adherence to treatment
- Acceptability of regimen in to all stakeholders in terms of:
    - Costs to the health system related to delivering the regimen and conducting follow up tests
    - Household costs
    - Patient treatment and support experiences (frequency of health facility visits, side effects)
    - Health worker experiences of delivering treatment and support

4 SELECTION OF SITES

Country selection is based on background disease burden of TB, MDR-TB, and TB-HIV co-infection. Sites within countries are selected to ensure sufficient numbers of MDR-TB cases to meet recruitment targets.

4.1 Site inclusion criteria

Participating sites are required to meet the following criteria:

- Experience in treating MDR-TB patients
- Full support from the National Tuberculosis Control Programme and a willingness to consider use of the study regimen after the trial completion (dependent on the trial results)
- A local Principal Investigator (PI) who is a TB specialist and experienced in the treatment of MDR-TB who will oversee the patients throughout the trial, (there may be more than one PI per country).
- Suitable treatment site staff and facilities
- Treatment site staff willing to enrol all eligible patients into trial. This site would ideally function as a single coordinating/enrolling facility and work with satellite sites for treatment and follow-up.
- Acceptable plans for close supervision of patients in treatment and follow-up
- Willing to offer HIV testing to all patients wishing to participate in the trial and routinely available HIV clinical management services (including provision of antiretroviral therapy (ART))
- A network of well-functioning smear microscopy laboratories and a reference laboratory already performing cultures, with a system of quality assurance
- Ability to export strains and sputum for testing to in ITM, Antwerp, if required
- Ability to get authorisation of importation for the medicines which will be provided through The Union procurement unit
- Agreement to use specified standardised bacteriological methods
- Availability of rapid genotypic line-probe drug susceptibility testing (LPA DST) for rifampicin, second-line injectables and fluoroquinolones of the required quality (or ability to quickly build capacity for this testing)

4.2 Local Trial Management

The staff members concerned in the management of the study patients at each site will form a Local Management Committee, under the direction of the local Principal Investigator(s). This committee (including a member of the laboratory staff) will meet at regular intervals to discuss the progress of the trial at the centre. A brief report of the discussions will be sent to the STREAM Trial Manager.

5 SELECTION OF PATIENTS

Patients will be recruited into the trial from tuberculosis clinics in the catchment area of the main site. The target population is all patients with sputum smear positive pulmonary TB and evidence of resistance to at least rifampicin (by conventional drug susceptibility testing (DST) or Hain Genotype LPA[17]).

5.1 Patient inclusion criteria

A patient will be eligible for entry to the study if he/she:

1. Is willing and able to give informed consent to be enrolled in the trial treatment and follow-up (signed or witnessed consent if the patient is illiterate)
2. Is aged 15 years or older (national ethics committees may require a higher minimum age)
3. Has smear-positive pulmonary tuberculosis with initial laboratory result of resistance to rifampicin by line probe assay or other DST
4. Is willing to have an HIV test and, if positive, is willing to be treated with ART in accordance with the national policies.
5. Agrees to use effective barrier contraception or have an intrauterine contraceptive device during treatment phase if a pre-menopausal woman
6. Has an identifiable address and expects to remain in the area for the duration of the study
7. Is willing to adhere to the follow-up schedule and to study procedures

5.2 Patient exclusion criteria

A patient will not be eligible for entry to the study if he/she:

1. Is infected with a strain of *M. Tuberculosis* resistant to a second-line injectable drug by line probe assay
2. Is infected with a strain of *M. Tuberculosis* resistant to a fluoroquinolone by line probe assay
3. Has tuberculous meningitis or bone and joint tuberculosis
4. Is critically ill, and in the judgment of the investigator, unlikely to survive more than 4 months.
5. Is known to be pregnant or breast-feeding
6. Is unable to attend or comply with treatment or follow-up schedule
7. Is unable to take oral medication
8. Has AST or ALT >5 times the upper limit of normal
9. Has any condition (social or medical) which in the opinion of the investigator would make study participation unsafe.
10. Is taking any medications contraindicated with the medicines in either the trial or control regimen
11. Has a known allergy to any fluoroquinolone antibiotic
12. Is currently taking part in another trial of a medicinal product
13. Has a QTc interval of ≥500msec at screening

Confirmation of drug susceptibility test results may not be available until after enrolment in the study. All patients who are not eligible for the STREAM trial will be referred to the National Tuberculosis Control Programme (NTP) for further management according to local guidelines.

5.3 Number and source of patients

It is proposed to enrol a minimum of 100 patients in each of four or five countries.

5.4 Screening procedures

Written informed consent **must** be obtained from the patient before any protocol-specific screening procedures are carried out.

5.4.1 Screening visit

At the first (screening) visit, the study, including potential risks and benefits of participation, will be explained to prospective patients. This will include a general overview of the trial purpose and procedures as well as the samples to be collected at this visit. Each patient will be asked to sign (or provide a thumb print in the presence of a witness if illiterate) for the screening procedures and will be given a copy of the signed consent form and a patient information sheet to take home.

After giving consent for screening, patients will be assigned a screening number by entering their name on to the next line of a screening register and evaluated for their eligibility according to the inclusion and exclusion criteria. The following will be done:

- Sputum sample for smear, culture and LPA for rifampicin resistance and, if shown to be resistant to rifampicin, also for second-line injectable and fluoroquinolone
- Blood sample for HIV antibodies and liver function tests (AST and LFT)
- Collect details of the patient's address and, if available, phone number

Patients already on treatment at the time of screening will continue with that treatment until enrolment.

If patients are successfully screened, further information and testing is required at the Enrolment visit (see Section 6.1).

6 ENROLMENT PROCEDURE

6.1 Evaluations at the Enrolment visit

Patients will need to be re-assessed for eligibility when returning after their screening visit. The time between the screening and enrolment visits should be kept as short as logistically possible, but should be no more than four weeks; those returning after four weeks will have to be re-screened prior to enrolment.

Patients attending the enrolment visit will be given further information about the trial and what would be expected of them in terms of follow-up visits and procedures. If they are still willing to take part, they will be asked to sign an enrolment consent form (or give a thumb print in the presence of a witness if illiterate), and will be given a signed copy to take home together with the Patient Information Sheet. Patients who are ineligible or do not wish to take part will be referred to the National Tuberculosis Programme (NTP) for further management.

Once an eligible patient has given consent to participate in the trial, the following will be done:

- Interview to obtain demographic details, medical history (prior diagnoses and treatment, concomitant disease and medication and current symptoms) and key information on asset ownership to document socio-economic status
- Record contact information, all patients must have an identifiable address as part of the inclusion criteria
- Clinical examination including height, weight and vital signs (temperature, systolic and diastolic blood pressure (BP) and pulse rate)
- Simple hearing test
- Urinalysis
- A urine pregnancy test (if pre-menopausal woman)
- Serum creatinine, serum potassium, blood glucose, haemoglobin and CD4 count for HIV positive patients
- Posteroanterior (PA) chest X-ray
- ECG before and after the first dose of trial treatment.

All patients providing their consent to participate in the study will also be asked to provide their consent for the biostorage of additional specimens for biomarker tests. These samples will be stored for the discovery and validation of TB drug effect biomarkers. Those providing their consent for biostorage of their specimens will be requested to give blood (at enrolment and at 16 weeks). No human genetic testing on these samples will be performed.

6.2 Allocation of treatment (Randomisation)

Patients will be randomised to the study regimen or the control regimen using either a web-based randomisation system or secure alternative. Access to the web based system will be controlled through an authorised user name and password. Before treatment allocation the patient's eligibility will need to be confirmed and their centre, previous MDR-TB treatment and HIV status entered. Following this, the patient will be automatically assigned a trial number and allocated to either the study regimen or control regimen. The patient's trial number and treatment allocation should be entered onto the enrolment CRF.

Randomisation will be in a 2:1 ratio in favour of the study regimen to allow more data on efficacy and safety to be collected on this regimen. Randomisation will be stratified by (1) site, (2) whether the patient has had previous MDR-TB treatment for at least four weeks and, (3) HIV status for sites with high TB-HIV co-infection rates. Separate randomisation lists for

each combination of strata will be prepared in advance, for each site, using varying block sizes by a statistician independent of the study.

7 TREATMENT OF PATIENTS

7.1 Introduction

All patients will be randomised to receive either the study regimen or the control regimen which is the locally used WHO approved MDR-TB regimen.

7.2 Trial Intervention

7.2.1 Study regimen

The study regimen consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for nine months (40 weeks), supplemented by kanamycin, isoniazid and prothionamide in the first four months (16 weeks). All drugs are given daily (seven days a week) except for kanamycin which is given thrice-weekly after 12 weeks. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks respectively as described below in section 7.3.2.

Table 1: Study regimen doses

Product	Weight group		
	Less than 33 kg	33 kg to 50 kg	More than 50 kg
Moxifloxacin	400 mg	600 mg	800 mg
Clofazimine	50 mg	100 mg	100 mg
Ethambutol	800 mg	800 mg	1200 mg
Pyrazinamide	1000 mg	1500 mg	2000 mg
Isoniazid	300 mg	400 mg	600 mg
Prothionamide	250 mg	500 mg	750 mg
Kanamycin	15 mg per kilogramme body weight (maximum 1g)		

All drugs should be given in a single daily dosage under directly observed treatment (DOT) by a treatment supervisor. Treatment supervisors may be clinic staff or family members or other members of the community, depending on local circumstances.

7.2.2 Control regimen

The **control regimen** will be the locally-used WHO-approved MDR-TB regimen. Country-specific regimens are described in the STREAM Pharmacy Plan.

7.2.3 Medicines supplies

The medicines given in the study regimen will be supplied through The Union Procurement Unit, supplies for the control regimen will be provided by the participating countries. Details of drug supplies, storage and pharmaceutical information are provided in the STREAM Pharmacy Plan.

7.2.4 Treatment cards

Following randomisation, the patient and/or a treatment supervisor will be given the relevant Treatment Card and a prescription to take to the pharmacy. The treatment supervisors will be instructed about observing the patient swallowing their oral medication every day (directly observed treatment) and recording treatment taken on the treatment card. Treatment Cards should be returned at each visit and a new card issued.

7.3 Treatment Procedures

7.3.1 Dispensing and supervision of medicines

Local policy will be followed as to whether the patient will be admitted to hospital during the intensive phase irrespective of the regimen.

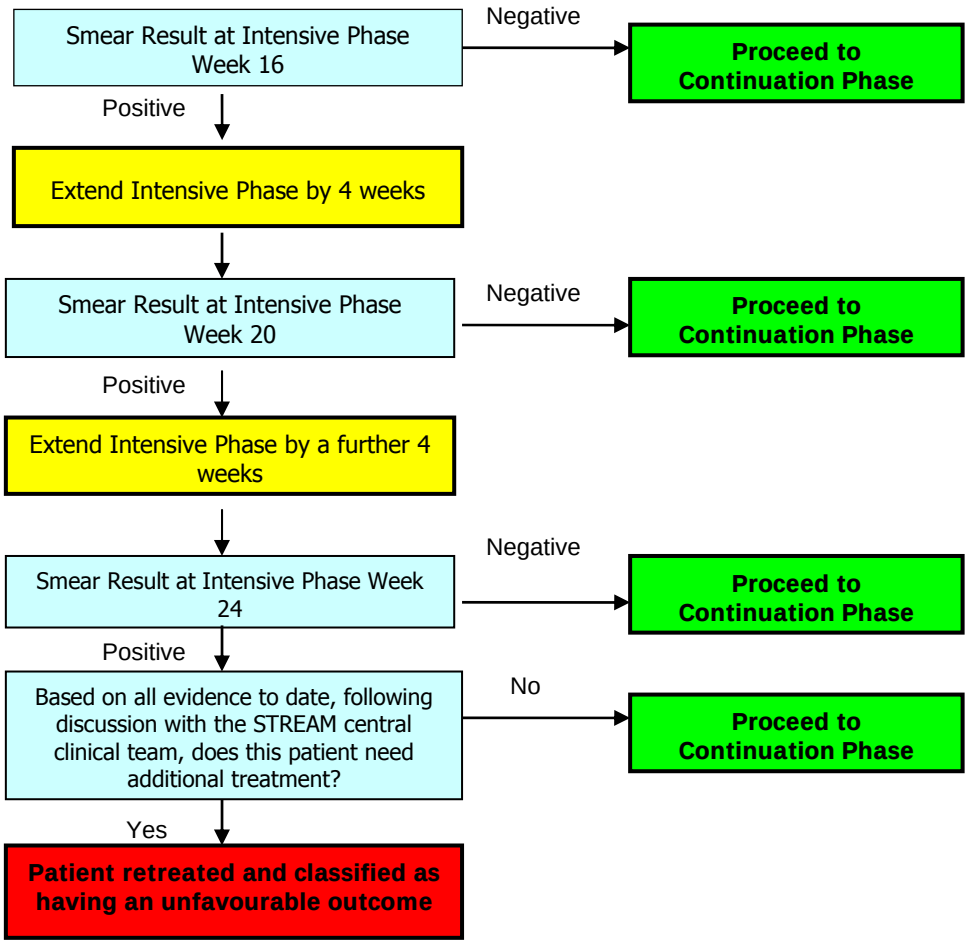
All medicines in the study regimen must be given on seven days per week under strict conditions of direct observation of treatment (seen to be swallowed) by a trained treatment supervisor for the whole treatment period. For the control regimen, sites will be strongly encouraged to follow the same standard. Full details of the medicines, regimen, including dosages, for each patient and of the procedure to be followed are also given on each Treatment Card. Treatment supervisors and/or patients will maintain a record of the treatment taken.

The pharmacy staff will maintain a product dispensing log and provide, on a regular basis, a reconciliation report (between products in stock, products delivered and remaining stock).

7.3.2 Transition from Intensive to Continuation phase in the study regimen

For patients allocated to the study regimen, the following algorithm will be used to determine when a patient can proceed from the intensive to the continuation phase of the regimen.

Figure 2: Transition from Intensive to Continuation phase for patients on the study regimen



As a consequence of extending the intensive phase, patients on the study regimen may receive treatment for up to 48 weeks.

The procedure for transition from the intensive to the continuation phase in the control regimen will be according to local policy.

7.3.3 Procedure following missed treatment

Any days missed (up to 8 weeks in total) in either the intensive or the continuation phase can be made up by extending this phase of the regimen by the number of days missed at the discretion of the treating clinician.

If the clinician considers the patient to be failing they should contact the STREAM central clinical team to discuss whether management of the patient needs to be modified.

Patients in either regimen who miss more than 8 weeks in total will be referred to the NTP for further management. They should however still be followed up to 27 months post-randomisation although this could be with reduced frequency of attendance if agreed with the central clinical team.

7.3.4 Adherence assessment and counselling

At each visit, patients will be counselled about the importance of taking their medication and the dangers of developing further resistance if they fail to do so.

7.3.5 Pregnancy

It is possible that some of the drugs in the study regimen, if given to a pregnant woman, will harm the unborn child. Pregnant women must not therefore take part in this trial; neither should women who plan to become pregnant during the trial. Women who could become pregnant must use barrier contraception while on treatment unless they have an intrauterine contraceptive device. Women who are pre-menopausal will be asked to have a pregnancy test before taking part to ensure that they are not pregnant. Any woman who finds that she has become pregnant while taking part in the trial should immediately tell her research doctor who will contact a member of the STREAM clinical team to discuss management of the patient.

All pregnancies occurring at any point during treatment or follow-up will be followed for outcome even when the pregnancy outcome occurs after the end of 27 months of follow-up.

7.4 Non-trial treatment

Drugs that are known to prolong the QT interval should not be used. The following list includes some examples, but is not comprehensive.

Antiarrhythmics Class IA, e.g. quinidine, hydroquinidine, disopyramide,
Antiarrhythmics Class III, e.g. amiodarone, sotalol, dofetilide, ibutilide
Certain neuroleptics, e.g. phenothiazines, pimozide, sertinodole, haloperidol, sultopride
Tricyclic antidepressive agents
Certain antimicrobials, e.g. sparfloxacin, erythromycin IV, pentamidine
Certain antimalarials, e.g. halofantrine
Certain antihistamines, e.g. terfenadine, astemizole, mizolastine
Others: cisaprid, vincamine IV, bepedril, diphemanil

8 ASSESSMENTS AND FOLLOW-UP

8.1 Assessment Schedule

Patients will be required to attend the clinic every four weeks for assessments throughout the trial, both during and after treatment. See section 8.1.1 Assessment Schedule for details of the various assessments required for each of those visits post-randomisation.

Assessments made during treatment will include sputum smear and culture, a clinical examination and investigation of potential known adverse drug effects.

The intensive phase of treatment may be extended for late smear conversion or missed treatment (section sections 7.3.2 and 7.3.3 above); the continuation phase may also be extended for missed treatment. Two sputum samples will be collected for smear and culture at the last visit of the intensive and continuation phases of treatment and at the 27 month follow-up visit. Because early morning samples are preferred, at the conclusion of each visit, patients should be given a sputum container for sample collection to be presented at their next visit. When a second sputum sample is required or if an early morning specimen is not available, a spot sample will be taken at the time of clinic attendance.

At enrolment and from twelve weeks and every twelve weeks thereafter, patients will be interviewed to document the costs e.g. transport and hospitalisation costs, incurred by them in adhering to the regimen. System costs will also be estimated.

8.1.1 Assessment Schedule

Observation/ Investigation	Screening (Pre-trial)	Enrolment	Treatment Phase		Post-Treatment Phase
			Intensive Phase	Continuation Phase	Follow-up
Written informed consent	X	X			
Demographics		X			
Medical History		X			
Clinical Examination	X	X	X	X	X
Clinical assessment (including AEs and concomitant medication during treatment)		X	X	X	X
Height		X			
Weight		X	X	X	X
Simple hearing test		X	If clinically indicated		
HIV antibody test	X				
CD4 (in HIV positive patients)		X	According to national guidelines		
Haemoglobin		X			
AST and ALT	X		X		
Serum creatinine		X	X		
Serum potassium		X	X	If clinically indicated	
Blood glucose		X			
Urinalysis		X	X		
Urine: HCG Pregnancy test		X	If clinically indicated		
Chest X-ray		X			
ECG		X[§]	Weeks 1-4 & 12	Weeks 24 & 36	
Sputum smear and culture[‡]	1	2	1[*]	1[*]	1[*]
Rifampicin (& 2nd line) LPA	1				
Patient's costs		X	X	X	X
Blood sample for storage (if patient consents)		X		X[π]	

X indicates assessments required at particular visits

[*] One sample will be collected per visit, except at the final visit of each phase of treatment and at the 27 month follow-up visit, when two samples will be collected.

[‡] all positive strains post-randomisation onwards will be shipped to ITM Antwerp for full drug susceptibility testing.

[§] one ECG will be done prior to, and another, after administering the first dose of treatment

[π] one sample will be collected for storage at 16 weeks, for patients consenting to sample storage

8.2 Post-treatment schedule

After completion of treatment, the patient will be reminded of the need for follow-up visits by the Principal Investigator, or recruiting physician, and be informed of the date of their next visit date.

Patients will be assessed at 4 weekly intervals throughout the study, irrespective of whether on treatment or in the post-treatment follow-up phase.

Sputum for smear and culture will be collected at every visit; at most visits this will be a single specimen, unless otherwise indicated in section 8.1.1. Because early morning samples are preferred, at the conclusion of each visit patients should be given a sputum container for sample collection to be presented at their next visit. When a second sample is required or if an early morning specimen is not available, a spot sample will be taken at the time of clinic attendance.

During the follow-up visits, the following procedures will be undertaken:

- Sputum collection for smear and culture examination
- Patients will be asked about any adverse events that may have occurred after their last visit and any concomitant medications they may have received.

There may be times when the PI requests additional tests for a patient depending on their disease progression at a particular visit.

Patients randomised early in the study will be followed up to 33 months to ascertain if there are later relapses occurring; these data will not be included in the primary analysis

8.3 Procedures for assessing safety

Throughout this study, patients will be closely monitored for signs and symptoms of drug toxicity. All toxicities leading to the study therapy being temporarily or permanently discontinued and all Grade 3 or greater toxicity effects will require thorough investigation with relevant clinical and laboratory tests, as clinically indicated. These should be repeated as needed until final resolution or stabilisation of the toxicity. All symptoms and laboratory findings will be graded according to severity using DAIDS criteria. Laboratory events will be reported only if clinically significant. If the patient has a medical diagnosis at enrolment whose signs or symptoms worsen during the study to a Grade 3 or greater, this is a notable event that must be reported. SAEs and other notable events will be reported as they occur to the MRC CTU, as well as other bodies required to be notified in each country. For details of safety reporting see Section 13.

In order to minimise potential risk of QT prolongation with moxifloxacin, all patients will have a 12-lead ECG immediately prior to randomisation and will be ineligible if the QTc interval is ≥500msec. An ECG will be recorded 2 and 4 hours after the first dose of trial treatment, initially but the timing of ECG monitoring will be reviewed as the study progresses. Further ECGs will be performed weekly for the first 4 weeks and at 12, 24 and 36 weeks after the first dose of trial treatment. A 24-hour ECG (Holter monitor) will be undertaken at the end of the first week in any participant whose QTc at week 1 is ≥ 450msec (the upper limit of normal for men, the upper limit of normal for women being ≥ 470msec). For further details, see Appendix 2).

Any QTc prolongation to ≥500msec while on treatment is considered a notable event and should be reported immediately to MRC CTU (See Section 13).

8.4 Other study considerations

8.4.1 Interruptions to treatment

The study or control regimens may be interrupted at the discretion of the local PI for:

- A serious adverse event,
- A QTc measurement of ≥500msec or
- The investigator decides to withhold treatment in the interest of the safety and well being of the participant

If treatment is interrupted for a suspected serious drug reaction, attempts should be made to identify the drug concerned. After resolution of the suspected adverse reaction, treatment may be gradually re-introduced until the allocated regimen has been re-instituted.

In the event that the local PI considers that treatment needs to be modified or changed, he or she should inform the coordinating centre by submission of an SAE form and discuss treatment plans with a member of the central clinical team.

All patients will continue to be followed-up to 27 months post-randomisation whether or not they have stopped taking their allocated treatment.

8.4.2 Missed visits

For each patient, clinic staff will obtain or confirm contact information. In the event that a patient misses a scheduled appointment, a Home Visitor will try within the week following the missed appointment to establish communication with the patient and/or treatment supervisor through all possible means which they have approved and while protecting their confidentiality (e.g., by telephone if this is possible, writing to the patient and contacts, and/or visiting the patient's home or workplace). Permission for this contact must be obtained in the initial consent form. All attempts to locate a patient following each missed appointment will be documented in the source document. The need to attend all scheduled follow-up visits will be emphasised to all study patients at every visit.

Every site will develop its own method for tracing and retaining patients in the trial. See Section 8.4.3 for procedures to follow if the patient returns to the clinic following a missed visit.

A patient will be deemed to be missing if he/she does not attend the treatment centre to take the treatment as prescribed for a period of one week.

If the patient has not resumed treatment **within seven days**, i.e. the patient has missed at least two weeks of treatment; a note should be made in their clinic notes with details of attempts to contact the patient.

8.4.3 Visit after a Missed Appointment

Patients who miss their scheduled appointment will be contacted and arrangements made for a new appointment. If patients are not successfully reached by phone/text messaging, a home visit will be arranged to ensure contact.

Patients returning after missed appointments will have procedures for the visit closest to their total time in follow up performed (e.g. if a patient returns to the clinic at or near to week 16 after missing their visits for weeks 8 and 12, the visit for that day should be recorded as week 16). Subsequent visits will continue as scheduled. However, treatment to be prescribed should be determined by the actual number of days already taken and not by time in the study.

8.4.4 Loss to follow-up

If the patient does not return to the clinic before the study is closed, the final form will be completed at the time of study closeout. The form should indicate that the patient was lost to follow-up. The "loss to follow-up" designation cannot be made for any patient until at least 3 months after the patient's scheduled 27 month visit.

If a patient can be contacted and declines further study participation, information on their reasons will be asked. An attempt will be made to have him/her come to the clinic for a final visit, or at least obtain a sputum sample for the assessment of the primary efficacy outcome.

8.4.5 Follow-up of patients discontinued from treatment

For patients who are discontinued from treatment or whose treatment is changed or who are referred to the NTP, every effort should be made to continue to follow them up (at a reduced frequency if necessary which has been agreed with the STREAM central clinical team), unless the patient has specifically withdrawn consent for further follow-up. In this event, a final status form should be completed.

8.4.6 HIV

Patients who are known to be HIV infected or who are found to be HIV infected at trial screening will be enrolled into the study and follow the routine study procedures, if they fulfil all other study eligibility criteria.

Newly-diagnosed HIV positive patients will be given appropriate counselling about the medical consequences of their diagnosis and about the need to take responsible precautions to reduce the risk of infecting others. They will be referred to appropriate medical and social HIV treatment services, and will be given the option of not proceeding to the randomisation stage of the STREAM trial if they wish to re-consider their options.

HIV co-infected patients in the STREAM trial will be managed or co-managed by clinicians with appropriate expertise in HIV medicine. It will be important therefore for the Principal investigator at each participating site to establish links with the national AIDS programme and/or organisations that provide treatment in their country, and to establish the national criteria for ART eligibility for HIV-infected TB patients. Wherever possible, patients in the STREAM trial who are co-infected with HIV will be managed in a joint treatment clinic to ensure close co-ordination of management of the two conditions, and to ensure that appropriate decisions can be made concerning the management of drug interactions and side-effects.

Guidelines for selection of drugs in ART regimens, use of appropriate opportunistic infection prophylaxis, management of interactions between TB and HIV drugs, management of toxicity, and the timing of initiation of HIV and MDR-TB treatment will be provided in STREAM Patient Management Guide.

8.5 Trial Closure

The trial will be considered closed when the last patient has completed their final visit and all follow-up and laboratory reports have been received.

The trial may be terminated early by the Trial Steering Committee (TSC), on the advice of the Independent Data Monitoring Committee (IDMC) (See sections 19.2 and 19.3). In addition, MRC CTU and the sponsor have the right to close this trial and/or a centre, at anytime,

although this should occur only after consultation between involved parties and with the agreement of the TSC.

At trial closure, the local and central Research Ethics Committees/Institutional Review Boards and the regulatory authorities that approved the trial should be informed. It is the responsibility of the sponsor to inform the Main REC within 90 days of the 'end of the trial' that the trial has closed.

Should a site be closed prematurely, all trial materials (except documentation that has to remain stored at site) must be returned to MRC CTU. The Principal Investigator will retain all other documents, for at least 15 (fifteen) years, until notification is given by MRC CTU for destruction. Patients currently on treatment will be referred to the National Tuberculosis Programme for completion of treatment and further management.

8.6 Bacteriology

The following bacteriological tests will be performed at the local reference laboratory: smear, culture and diagnostic line probe assays. At each visit, one early morning sputum sample will be tested for AFB smear and culture (at certain visits a second spot specimen will be collected at the clinic see Assessment Schedule in 8.1.1). Patients will be given a sputum pot to bring an early morning sputum sample to their next scheduled visit. In case a patient has forgotten to bring a morning sputum sample, a spot sample will be collected (two spot specimens for those visits requiring two samples).

The selected methods and techniques for use by the sites may not be the most sensitive ones, but they are simple and applicable at any site with high reproducibility, thus allowing a high degree of standardisation. Long-term follow-up will compensate for imperfect sensitivity. These methods are:

- hot Ziehl-Neelsen (ZN) or auramine fluorescence technique for all study smears
- decontamination without neutralisation or centrifugation and inoculation on acidified Ogawa (Kudoh medium) for all study cultures
- Hain Genotype MTBDRPlus line probe assay DST (LPA) from smear-positive sputum for screening of suspects. If this test or other DST shows at least resistance to rifampicin, the Hain Genotype MTBDRsl LPA will be performed to exclude significant fluoroquinolone and second-line injectable resistance

All isolated strains will be sent to the study reference laboratory at ITM Antwerp, to confirm species identification and sensitivity status. This includes diagnostic strains and strains during and after treatment (in case of failure or relapse), and also isolated positive cultures of unclear significance. Strains from recurrences will be tested for DST as well as fingerprinting, to confirm their identity and to compare their resistance pattern with the originally isolated strain. ITM will store all study strains at -80°C.

The techniques to be used are:

- slow phenotypic DST using the proportion method on Löwenstein-Jensen medium for first line drugs and agar-based Middlebrook 7H11 medium for second line drugs; for difficult strains, the 99% minimum inhibitory concentration (MIC) and DNA sequencing can be used to arrive at the most correct result
- fingerprinting: spoligotyping, with confirmation by MIRU-VNTR analysis (mycobacterial interspersed repetitive units–variable number of tandem repeats) if the same spoligotype is found

A detailed description of the various laboratory tests is found in the STREAM Laboratory Manual.

8.7 Other assessments:

Data relevant to the health economic assessments will be collected as explained below:

8.7.1 Health system costs

Health system costs will be obtained through:-

- An analysis of health worker time involved in prescribing, monitoring, and supervising the study regimen in each country, and the control regimen in those countries where randomisation takes place
- Health worker salary and benefits data from the Ministry of Health based on grade of staff rather than named individuals
- An analysis of additional, short-term technical assistance time allocated to implement the study regimen. This is distinct from existing or additional staff time required to deliver the study regimen
- Salary and benefits data for technical assistance
- Records of drug, consumable and equipment procurements
- Standard costs of supplies from government purchasing units or other appropriate sources
- Study implementation financial records

Costs will be assessed as one-off costs required for establishing the study regimen and as costs for recurrent costs for sustaining it.

8.7.2 Patient and household costs

Data on patient and household costs will be collected through interviews with patients at intervals of 12 weeks after initiation of treatment. The interviews will include questions on fees paid to the health system, drugs and laboratory test costs, transport, food and accommodation costs incurred as a result of the treatment process as well as time lost from economic activities due to illness or care-seeking. The TBCAP/TB & Poverty Subgroup/KNCV costing tool will be adapted for use across all sites.

8.7.3 Socio-economic status

The socioeconomic status of patients will be assessed through asking patients about asset ownership. The assets will be determined based on existing poverty analyses or similar sources (living conditions monitoring surveys (LCMS) or census data) for the country or region within the country. These questions about asset ownership will be included in the demographic assessment at enrolment and again every 12 weeks after the initiation of treatment.

9 DISCONTINUATION FROM TREATMENT

In consenting to the study, patients are consenting to study treatment, follow-up and data collection. If a patient wishes to discontinue their allocated study treatment they should not be withdrawn from follow-up unless they expressly request it. Patients should be told about the importance of remaining on follow-up, or failing this, of allowing routine follow-up data to be used for study purposes.

The treating clinician will be discouraged from restarting treatment without evidence of treatment failure or recurrence of MDR-TB. As soon as the treating clinician has any indication of a treatment failure or recurrence they should contact the STREAM central clinical team to discuss whether the patient should be retreated. Guidelines for retreatment, in the STREAM patient management guide, will be used to inform the decision which will be made on a case by case basis using all the available bacteriological and clinical data. If the decision is made not to retreat, then the case should be reassessed as further data accumulates with further discussions with the STREAM central clinical team as necessary.

9.1 Discontinuation of allocated regimen

The Investigator must make every reasonable effort to keep each patient on their allocated regimen and in follow-up for the whole duration of the study. However, if it is necessary to discontinue a patient's allocated regimen, every reasonable effort will be made to ensure the patient continues to be followed-up.

The following are justifiable reasons for the Investigator to discontinue a patient's allocated treatment:

1. Unacceptable toxicity.
2. Patient refuses to take study drugs.
3. Serious violation of the study protocol (including persistent patient attendance failure, non-adherence to treatment and persistent non-compliance).
4. The Investigator decides to discontinue a patient's treatment for clinical reasons not related to the study regimen.
5. Evidence of treatment failure based on consistently positive bacteriology usually accompanied by signs and symptoms of disease.
6. Pregnancy: women who become pregnant will stop trial treatment, and be treated according to the National TB Programme.

9.2 Salvage regimens

Salvage regimens may be needed for MDR-TB patients who participate in the STREAM trial but fail treatment. Three treatment strategies for MDR-TB are recommended by WHO:
1) standardised treatment,
2) standardised treatment followed by individualised treatment, and
3) empirical treatment followed by individualised treatment.

As patients in the trial who need salvage regimens have already been treated for MDR-TB using second line drugs, salvage regimens need to take treatment history and DST results into account. Salvage regimens might be different for patients on the trial regimen and those on the control regimen because drugs used in the trial intervention are different from those of the control regimen.

A standardised salvage treatment strategy for the trial may not be feasible because patients have been treated with different drugs for various durations, have different drug susceptibility patterns at base line, and have different risks of increased resistance while enrolled in the

STREAM trial. The risk of development of resistance to fluoroquinolones and/or injectable agents among those who fail is likely to be higher than relapse. For patients with similar past treatment history, a standardised salvage regimen may be used, followed by individualised modification of regimen tailoring to update DST results.

The key principle in designing a MDR-TB regimen is to include at least 4 drugs of either certain or almost certain effectiveness. Given that the number of drugs could be used for salvage regimens is limited and that these reserve drugs are less potent, drugs of uncertain effectiveness may be included. Further details of anti-tuberculosis drugs that may be used for salvage regimens are provided in the STREAM Pharmacy Plan.

Salvage regimens for the control group should take into account the regimens used of the control group and DST results, as well as history of treatment prior to enrolment.

In countries where there is capacity for surgical intervention, the feasibility of surgery should be evaluated.

9.3 Patient transfers

For patients moving out of the area, every effort should be made to continue to follow them if at all possible; this could include follow-up at another participating trial centre.

9.4 Early stopping of follow-up

If patients explicitly state that they do not wish to contribute further data to the study, MRC CTU should be informed in writing of the patient's decision and a withdrawal form should be completed. Such patients who discontinue the study may not re-enrol and will not be replaced.

10 DATA MANAGEMENT

Data will be recorded on paper case report forms (CRFs) and entered into a database either at each local site or at a central location. At each visit, details of clinical findings, procedures, tests and results will be recorded in the patient's case notes and on the appropriate CRF. The CRF top copy will be sent for data entry, and the duplicate retained in the patient's Trial Folder. Entries made in the CRF must be either verifiable against source documents, or have been directly entered into the CRF, in which case the entry in the CRF will be considered as the source data. The Investigator Site File and all source data should be retained until notification is given by the sponsor for destruction.

Instructions on data capture, cleaning and subsequent storage can be found in the STREAM CRF Completion Guide and Data Management Plan.

11 STATISTICAL CONSIDERATIONS

The primary objective of the study is to demonstrate that the study regimen is not inferior in efficacy to the WHO-approved standardised MDR-TB regimens currently used in the participating countries. If the response of patients on the study regimen is found to be better than that of those on the control regimen, a test will be performed to assess whether the response on the study regimen is superior to that of the control regimen.

11.1 Outcome Measures

11.1.1 Primary Efficacy Outcome

The primary outcome is efficacy status at 27 months after randomisation defined as follows:

Favourable

A patient's outcome will be classified as **favourable** if they have a negative culture result 27 months after randomisation not having been previously classified as unfavourable. If the patient is unable to produce sputum at 27 months, the outcome will be classified as **favourable** if they have a negative culture result at the last visit at which they were able to produce sputum.

Unfavourable

A patient's outcome will be classified as **unfavourable** if:

1. they are discontinued from their allocated study treatment and subsequently restarted on a different MDR-TB regimen,
2. treatment is extended beyond the scheduled end of treatment for any reason other than making up of missed treatment,
3. they are restarted on any MDR-TB treatment after the scheduled end of treatment,
4. they change their allocated study treatment for any reason other than the replacement of a single drug,
5. they die at any point up to 27 months post-randomisation,
6. they have a positive culture result at the end of follow-up, 27 months post-randomisation.

An extension of the intensive phase of the study or control regimen does not constitute an unfavourable outcome, as long as the extension is in accordance with either the algorithm described in section 7.3.2 for patients on the study regimen or the locally-used WHO-approved MDR-TB regimen for patients on the control regimen. Similarly, the discontinuation of one or more drugs that are not replaced does not constitute an unfavourable outcome.

A patient whose failure or recurrence specimen is a different strain to their enrolment specimen (re-infection) will not be classified as unfavourable, but as **unassessable**.

A patient who is either discontinued from their allocated regimen and not retreated for MDR-TB, or lost to follow-up from the trial, having not been otherwise classified as unfavourable (based on the definitions above) will be regarded as **unassessable** and will be excluded from the primary analysis provided their last two cultures, from specimens taken on separate occasions, are negative. Any patient who is discontinued from their allocated regimen or lost to follow-up from the trial who does not fulfil these criteria will be classified as **unfavourable**.

These definitions apply to both the study and control regimens and are not dependent on the duration of treatment.

Patients will be excluded from the per-protocol analysis if they fail to take treatment on 20% or more of scheduled treatment days.

11.2 Sample Size

A meta-analysis of treatment outcome in patients with MDR-TB found an overall favourable outcome of 64% (95% CI 59-68) in patients given individualised treatment and 54% (95% CI 43-68) in patients given standardised treatment[12]. A reasonable estimate of the efficacy of the control regimen would therefore be 65%.

Based on the experience with the study regimen[1, 18] in other settings, a reasonable estimate of the efficacy of the study regimen would be between 75% and 85%.

11.2.1 Power to demonstrate non-inferiority in the primary efficacy outcome

Based on a 2:1 allocation ratio in favour of the study regimen, Table 2 gives the total number of patients required to demonstrate non-inferiority under the specified scenarios using a margin of non-inferiority of 10%. These totals allow for 20% of patients being classified as unassessable in a per-protocol analysis and are based on a one-sided level of significance of 2.5%.

Table 2: Power to demonstrate non-inferiority in the primary efficacy outcome

Power	Percentage favourable outcomes in control regimen	Difference in percentage favourable outcomes in study regimen compared to the control regimen		
		0%	5%	10%
80%	60%	1060	464	255
	65%	1005	435	238
	70%	928	398	214
90%	60%	1419	620	340
	65%	1345	583	318
	70%	1242	533	287

Therefore, 398 patients would be required (266 on the study regimen and 132 on the control regimen) to demonstrate non-inferiority with 80% power assuming 70% favourable outcomes in the control regimen and 75% in the study regimen and 20% unassessable. A larger difference in response rates of 10% would require fewer patients and could also be demonstrated with greater than 90% power with a total enrolment of approximately 400 patients.

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden and duration and the expected increase in adherence in reducing the regimen from 24 to 9 months.

If the difference in response rates in favour of the study regimen is more than 10% it may be possible to demonstrate superiority of that regimen over the control.

At least 400 patients will need to be enrolled across all countries to give sufficient power to demonstrate non-inferiority. Patients will be randomised to the study and control regimens in the ratio 2:1.

11.2.3 Power to demonstrate non-inferiority in the primary safety outcome

Assuming a sample size of 400 on a 2:1 allocation ratio in favour of the study regimen, Table 3 gives the power available to demonstrate non-inferiority in the primary safety outcome under different proportions of grade 3 or 4 events on the control and study regimens. These calculations assume a margin of non-inferiority of 10% and a one-sided level of significance of 2.5%. All randomised patients will be included in the safety analysis.

Table 3: Power to demonstrate non-inferiority in the primary safety outcome

Proportion grade 3 or 4 on control regimen	Assuming same proportion in study regimen	Assuming an absolute 5% lower proportion on the study regimen
10%	88%	99%
15%	75%	99%
20%	65%	96%
25%	58%	93%
30%	53%	89%
35%	50%	86%
40%	48%	83%

11.3 Interim Monitoring and Analyses

There will be no formal interim analyses of the data, but the Independent Data Monitoring Committee (IDMC) will review efficacy and safety data every 6 months after commencement of recruitment or as required, including an early assessment of QT data after 3 months. The IDMC will give particular attention to the QTc data at these times and at other times as necessary, with technical assistance provided by a cardiologist to enable them to interpret the results and their implication on the study. Further details of the role and function of the IDMC is given Section 19 and in the STREAM IDMC charter.

11.4 Preliminary Analysis Plan

After data cleaning, analysis will proceed according to a pre-specified analysis plan. In general, the primary analysis will be based on both per protocol and intention-to-treat (ITT) populations. Patients randomised who have no molecular or bacteriological evidence of tuberculosis disease at enrolment, who are not resistant to rifampicin, or become pregnant and have their treatment stopped will be excluded from both analyses.

For the primary analysis, the difference in rate of favourable outcomes between the study and control regimens with 95% confidence interval will be estimated. The analysis will be stratified by the randomisation stratification factors. For the non-inferiority comparison, the analysis will be repeated on a per protocol sub-population.

The rate of unfavourable outcomes with 95% confidence intervals will be estimated for each country. The primary safety outcome is the occurrence of a Grade 3 or greater adverse event. This analysis will be repeated in subgroups according to HIV infection status and drug resistance patterns.

A detailed analysis plan that covers both the final analysis and the planned interim analyses will be developed and approved prior to the completion of the study. Results concerning time to sputum conversion will be shared with the TREAT TB transmission modelling team in order that the longer term impacts of reducing treatment times may be assessed.

12 TRIAL MONITORING

Before the trial can be initiated, the prerequisites for conducting the trial must be clarified and the organisational preparations made with the trial centres. MRC CTU must be informed immediately of any change in the personnel involved in the conduct of the study.

The purposes of trial monitoring are to verify that:

- The rights and well-being of human subjects are protected
- The reported trial data are accurate, complete, and verifiable from source documents
- The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with the principles of GCP, and with the applicable regulatory requirement

12.1 Risk assessment

A risk assessment was carried out during feasibility for this trial. The outcome of this assessment and its components are detailed in a separate document, the STREAM Monitoring Plan.

12.2 Monitoring Plan

A detailed monitoring plan was developed prior to study start that reflects the specific needs of the trial as determined by the risk assessment. This plan specifies the responsibilities and qualifications of monitors, central monitoring procedures, and the site monitoring visit procedures. Site visits by MRC CTU will be made in accordance with MRC CTU SOPs to assure the quality and accuracy of data collected and entered in the database, to determine that the applicable regulatory requirements are met and that rights and well being of trial subjects are protected.

On-site monitoring visits will be made at a frequency determined by the risk assessment and pre-defined triggers, including 'for-cause' monitoring as detailed in the monitoring plan. These visits will be made by the Trial Manager, Data Manager and/or other members of MRC CTU Trial Team.

12.3 Clinical site monitoring

12.3.1 Direct Access to Data

Participating investigators must agree to allow trial-related monitoring and audits, ethics committee review and regulatory inspections by providing direct access to source data/documents as required. Patients' consent for this is obtained as part of the trial consent process.

During the trial the MRC CTU TM is responsible for monitoring data quality in accordance with MRC CTU SOPs. Before the study start, the Local Trial Coordinator will be advised of the anticipated frequency of the monitoring visits and will receive reasonable notification before each monitoring visit. Responsibilities of the monitors are outlined in the Monitoring plan.

During the course of this trial, the TM will maintain contact with the study sites on a regular basis. This will include a training/initiation visit prior to participant enrolment; a monitoring visit soon after screening/enrolment begins and further visits as detailed in the monitoring plan. Closeout visits will be conducted after trial participation is completed. The sites will be contacted in advance to schedule each visit. All participant records, CRFs, and other source

documents for the patients enrolled in this study will where possible be made available for review by the monitor. A site-visit log will be maintained at the study site to record all site visits made by authorised individuals.

12.3.2 Quality Assurance Procedures

QA procedures at MRC CTU include a systematic review of the trial protocol by the Protocol Review Committee (PRC), the preparation of a risk assessment and Quality Management Plan. A review of these documents is undertaken by the Quality Management Committee (QMC) which is the QA function of MRC CTU. Internal audits of the Trial Master File will be conducted as directed by the QMC. Audits of sites may be conducted by or on behalf of the sponsor.

Good Clinical Practice (GCP) training, and where appropriate Good Laboratory Practice (GLP)[18] training will be provided for all staff involved in the trial; this will form part of the capacity strengthening component of the trial.

12.3.3 Laboratory Quality Assurance

Details of the arrangements for laboratory quality assurance (QA) are found in the STREAM Laboratory Manual.

ITM Antwerp will assess and prepare the sites' reference labs before start of the trial, and assure quality of the sites' microscopy, cultures and DST throughout the trial and LPA during the screening. In addition, detailed registration of tests' performance will be standardised.

The following QA procedures will be used:

- microscopy: internal control of newly prepared lots of staining solutions and external quality assurance (EQA) by rechecking at the site following international recommendations based on lot quality assurance sampling (LQAS) sampling. All smears will be stored for review by Antwerp lab as required. FDA smears will use internal positive controls.
- cultures: monitoring of false negative and contamination rates, besides parallel testing of an aliquot of 10% of follow-up sputa sent in CPC preservative to Antwerp.
- LPA DST: a water blank in each run, to check for cross-contamination; strip-inbuilt controls for QA of amplification and colour reaction.
- ITM Antwerp DST and fingerprinting QA: participation in WHO/Union international proficiency testing rounds; annual audits and certification under the Belgian QA programme.

12.4 Central Monitoring

Central monitoring of data at MRC CTU will be conducted by CRF review where appropriate and range and consistency checks programmed into the database. MRC CTU will raise any concerns they may have about the data captured by use of query forms sent to the site, as detailed in the Data Management Plan.

The Trial Master File will be stored at MRC CTU and will be maintained by the TM throughout the trial. All trial specific documents will be centrally tracked and copies obtained from the sites for all communication with regulatory bodies. Details about maintaining trial files and any other monitoring that will be carried out centrally are in the Monitoring Plan and other study documentation and plans as appropriate.

13 SAFETY REPORTING

GCP requires that both investigators and sponsors follow specific procedures when notifying and reporting adverse events/reactions in clinical trials. These procedures are described in this section of the protocol. Section 13.1 lists definitions, section 13.2 gives details of the institution/investigator responsibilities and section 13.3 provides information on MRC CTU responsibilities.

13.1 Definitions

The definitions of the EU Directive 2001/20/EC Article 2 based on GCP apply in this protocol. These definitions are given in Table 4.

Table 4: Definitions

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 • other medically important condition • combination of above, specify

13.1.1 Clarifications and Exceptions

*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.

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

13.1.2 Trial Specific Exceptions to Expedited SAE Notification and Reporting

Data on disease relapse or progression are collected as part of the primary outcome of the trial and are not considered to be SAEs.

13.1.3 Additional Notable Events

Pregnancy while on protocol treatment, QTc measurement ≥500 msec while on treatment and any toxicity that leads to a planned change of allocated treatment is defined as a notable event and should be reported as an SAE.

13.2 Institution/Investigator Responsibilities

The severity (i.e. intensity) of all AEs/ARs (serious and non-serious) in this trial should be graded using the DAIDs criteria. A version summarised for this trial is available in Appendix 2.

13.2.1 Investigator Assessment

(a) Seriousness

When an AE/AR occurs the investigator responsible for the care of the patient must first assess whether the event is serious using the definition given in Table 4. If the event is serious and not exempt from expedited reporting, then an SAE form must be completed and the MRC CTU notified.

(b) Causality

The Investigator must assess the causality of all serious events/reactions in relation to the trial therapy using the definitions in Table 5. There are 5 categories: unrelated, unlikely, possibly, probably and definitely related to trial treatment. If the causality assessment is "unrelated" or "unlikely to be related" to trial treatment the event is classified as an unrelated SAE. If the causality is assessed as possible, probable or definitely related then the event is classified as a Serious Adverse Reaction (SAR).

(c) Expectedness

If the event is a SAR the Investigator must assess the expectedness of the event. The definition of an unexpected adverse reaction (UAR) is given in Table 3. A list of expected toxicities associated with the drugs being used in this trial is provided in the STREAM Pharmacy Plan. If a SAR is assessed as being unexpected it is a Suspected Unexpected Serious Adverse Reaction, or SUSAR.

(d) Notification

The MRC CTU should be notified within one working day of the investigator becoming aware of an event that requires expedited reporting. Investigators should notify the MRC CTU of all SAEs and other notable events defined above occurring from the time of randomisation until 30 days after the last protocol treatment administration. SARs and SUSARs must be notified to the MRC CTU to the end of follow-up (i.e. no matter when they occur after randomisation).

(e) SAEs exempt from expedited reporting

Relapse of tuberculosis is a study outcome and does not require to be reported as an SAE.

Table 5: Definitions of causality

Relationship	Description	Event Type
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

Notification Procedure:

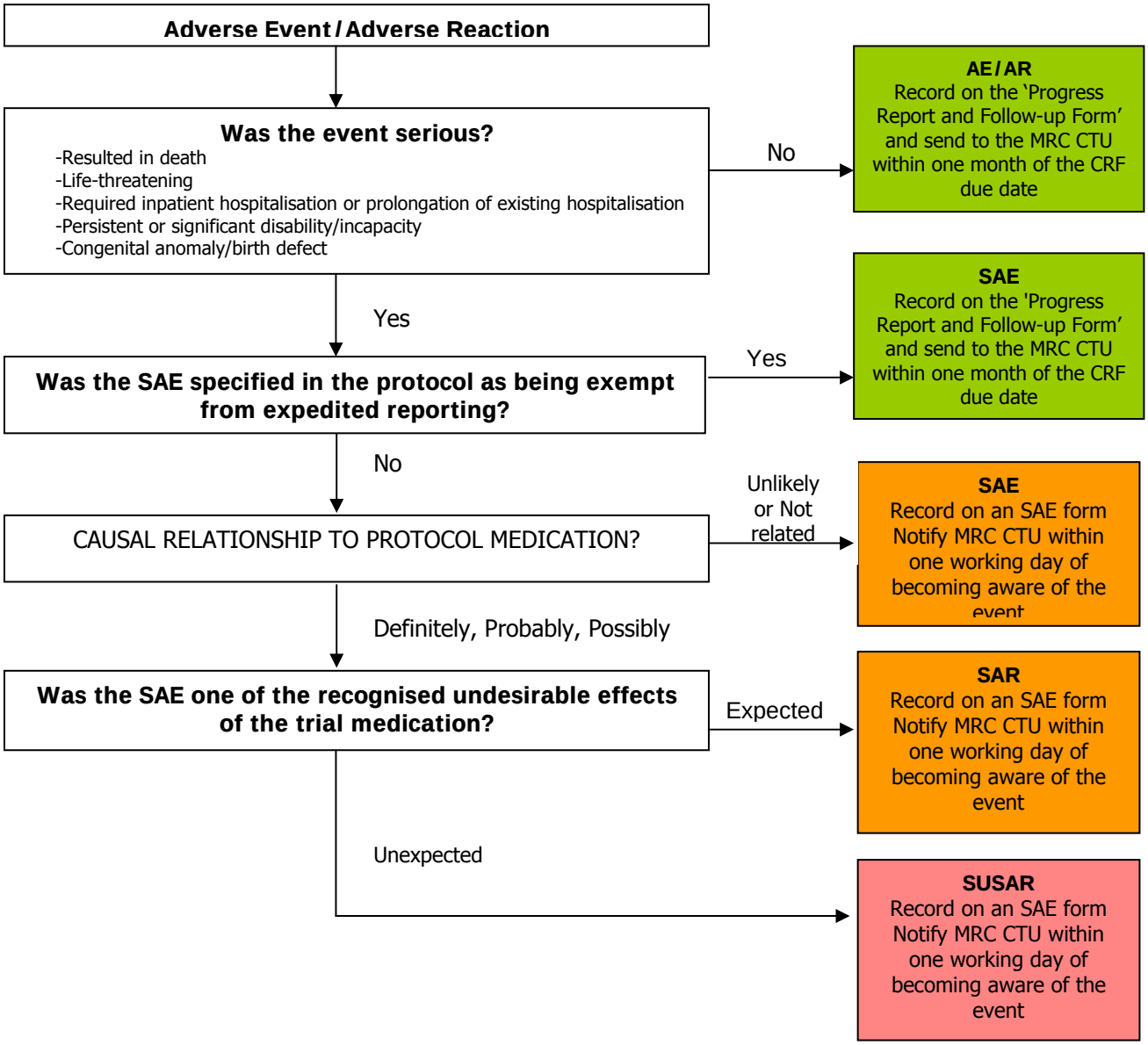
1. The SAE form must be completed by the Investigator (consultant named on the signature list and delegation of responsibilities log who is responsible for the patient's care), with due care being paid to the grading, causality and expectedness of the event as outlined above. In the absence of the responsible investigator the form should be completed and signed by a member of the site trial team delegated to do so. The responsible investigator should subsequently check the SAE form, make changes as appropriate, sign and then re-fax to the MRC CTU as soon as possible. The initial report shall be followed by detailed, written reports as appropriate.

2. Send the SAE form by fax or email to the MRC CTU within one working day.
   Fax Number: + 44 (0) 20 7670 4829 Email: stream@ctu.mrc.ac.uk

3. Follow-up: Patients must be followed-up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of protocol treatment if necessary. Follow-up information should be noted on a further SAE form by ticking the box marked 'follow-up' and faxing to the MRC CTU as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The patient must be identified by trial number, date of birth and initials only. The patient's name should not be used on any correspondence.

4. Staff at the investigator site must notify the research ethics committee of the event (as per the institutions standard local procedure).

Figure 3: Safety Reporting Flowchart



CRF: Case report form
SAE: Serious adverse event
SmPC: Summary of product characteristics
IB: Investigator's brochure
SAR: Serious adverse reaction
SUSAR: Suspected unexpected serious adverse reaction

13.3 MRC CTU Responsibilities

Medically qualified staff at the MRC CTU or the Co-CI's medically qualified delegate will review all SAE reports received. The causality assessment given by the local clinical investigator cannot be overruled and in the case of disagreement, both opinions will be provided in any subsequent reports. The investigator's assessment of expectedness may be modified by the medical reviewer.

The MRC CTU is undertaking the duties of trial management and is responsible for providing the Sponsor's research ethics committee and the regulatory authorities that have approved the trial with the safety reports that they require.

The MRC CTU will provide the Independent Data Monitoring Committee (see section 19) with aggregated reports of SAEs for their review and will keep all investigators informed of any safety issues that arise during the course of the trial. After receipt and review of these reports, MRC CTU will also notify the Union.

The Union will also be notified of all reportable (serious and unexpected and drug related/unknown relationship) events.

SAE NOTIFICATION

Within one working day of becoming aware of an SAE, please fax a completed SAE form to the MRC CTU on:

Fax: 020 7670 4829

Email: stream@ctu.mrc.ac.uk

14 ETHICAL CONSIDERATIONS AND APPROVAL

14.1 Ethical considerations

The study will abide by the principles of the Declaration of Helsinki.

14.1.1 Research Ethics Committee (REC) review and approval

Before initiating the study at any given site, the study must be approved in writing by the local REC and/or Institutional Review Board (IRB), where appropriate as well as the Ethics Advisory Group of The Union. The study will be conducted in accordance with all conditions of approval by the REC. The local Principal Investigator will forward the approval letter to MRC CTU.

Before starting the trial, the protocol, patient information sheet, consent form, study specific patient cards and any local advertising materials must be reviewed by the MRC CTU Protocol Review Committee; and be approved by the Trial Steering Committee (TSC) and the appropriate Ethics Committee in all participating countries.

It is the local Principal Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information becomes available that might affect the patient's willingness to continue in the trial. The Principal Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented, where appropriate.

The sponsor and Investigators must ensure that the study is carried out in accordance with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), the Declaration of Helsinki and applicable regulations in each country.

14.1.2 Informed consent

No patient may be screened for or enrolled into this study until the investigator has obtained his/her informed consent. Informed consent encompasses all oral or written information given to the participant about the study and the study materials. All such information will be in a language which is understandable to him/her. The information will not include any language in which the participant is made to waive any of her rights or which releases or appears to release the investigator, the investigator's institution MRC CTU, from liability for negligence.

Consent for screening will be based on a template Patient Information Sheet (PIS) which will be provided to all participating sites. The information contained in the PIS will be translated into the relevant local languages and back-translated to ensure consistency of content. Literate patients will be asked to read the form and illiterate patients will have the contents explained to them by a counsellor. Patients will have the opportunity to discuss the PIS with the medical officer/treatment supervisor. They will be assured that their decision to participate in the trial or not will not affect the quality of care they will receive. Once this person is satisfied that the patient has understood the PIS and the consent form, the patient will be asked to give consent.

The patient will sign (or thumbprint) and the investigator or designee will also sign the consent form. A witness will be present during the whole process if the patient is illiterate, and will also sign the form. One copy of the signed consent form will be offered to the participant, a second copy will be filed in the patient's medical notes (where available) and the original signed consent form will be kept in his/her study file. The investigator is also responsible for developing tools that may help in explaining the study to patients, these

materials will also be submitted to MRC CTU at least one week before submission to the local REC.

14.1.3 Randomisation

Prior to the start of any trial procedures, the randomisation process will be explained as part of the patient information sheet at the patient's enrolment visit. Patients will be given a chance to ask any questions they may have before they consent to taking part in the trial.

14.1.4 Patient confidentiality

The confidentiality of all patients enrolled into this study will be protected to the fullest extent possible. All patient information will be kept secure and will be available only to the treatment staff.

Study patients should not be identified by name on any case report form, email or on any other documentation sent to MRC CTU and will not be reported by name in any report, presentation or publication resulting from data collected in this study. Patient's data/specimens will be identified by trial number and/or initials or hospital number only.

The trial will comply with the principles of the Data Protection Act or the equivalent regulation/legislation of the country of the participating centre.

14.1.5 Additional Trial Requirements

Patients may be required to provide additional sputum samples or may be required to come to the clinic for more visits if clinically indicated.

14.1.6 Record Retention

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical trial and the quality of the data produced to be evaluated and verified.

All essential documents (according to GCP and MRC CTU SOPs) required to be held by the Investigator must be stored in such a way that ensures that they are readily available, upon request, to the local Regulatory Agency or sponsor, for the minimum period required by national legislation or for longer if needed by MRC CTU. Records must not be destroyed without prior written approval from MRC CTU.

The medical files of trial subjects shall be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

At the end of the trial, photocopies of pertinent study documentation (such as REC correspondence, etc.) will be kept by MRC CTU. Original copies of the CRFs will be sent to the sponsor or MRC CTU. The duplicate copies will remain in the patient's study file at the participating sites. The signed original informed consent documents for each participant and originals of other study documentation (e.g. drug inventory forms, participant clinic records, original laboratory reports, etc) will be retained by the local PI for a minimum of 15 years (as specified in MRC CTU working instructions on archiving). If those years have passed with no request for the data, the sites may request permission in writing from MRC CTU to destroy the records. No records may be destroyed without written permission from MRC CTU.

14.1.7 Audit

The investigator may be subject to a field audit by The Union or MRC CTU to validate the participation of study patients, to verify the data reported on the Case Report Forms and to confirm the compliance of the conduct of the trial with applicable regulations and requirements and the protocol. This audit could occur while the study is in progress, several years after the study is completed, or when the data are under review. All of the patients' records and other study documentation must be filed and accessible on short notice (3-5 days) during the study and subsequent retention period.

14.2 Protocol Deviations

14.2.1 Protocol Deviations

No waivers will be given by MRC CTU on behalf of the sponsor for patients who do not fulfil the eligibility criteria for this trial. No deviations from, or changes to the protocol should be initiated without prior written REC/IRB, regulatory authority approval/favourable opinion and approval from MRC CTU on behalf of the sponsor.

The reporting procedures and how to handle deviations are detailed in the MRC CTU SOPs and Trial specific Working Practice Documents for protocol deviations.

14.3 Ethical approval

The Union Ethics Advisory Group has given a favourable opinion for the trial and has indicated in broad terms that the trial concept is consistent with ethical requirements. The final protocol will be submitted to the Ethics Advisory Group for assessment. Each participating site will need to submit the protocol to their relevant Ethical Review Committees and/or Institutional Review Boards. All substantial amendments to this protocol will have to be submitted for approval.

A copy of local REC/IRB approval of the protocol and of the Participant Information Sheet (PIS) and Consent Form (CF) on local headed paper and any other written information given to the participant should be forwarded to MRC CTU before patients are enrolled to the trial. Each patient's consent to participate in the trial should be obtained after a full explanation has been given of the treatment options, including the conventional and generally accepted methods of treatment.

The right of the patient to refuse to participate in the trial without giving reasons must be respected. After the patient has entered the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the patient will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the patient must remain free to withdraw at any time from the protocol treatment and trial follow-up without giving reasons and without prejudicing his/her further treatment.

15 REGULATORY ISSUES

All Investigators will be expected to obtain, in writing, approval to participate from their local Regulatory Authority. Copies of the approval (or non-approval) must be submitted to MRC CTU no later than 5 working days from receipt of the same.

A special authorisation of importation for the medicines to be used in the study should be obtained by the responsible of the sites from the National Drug Regulatory Authorities (NDRA) and provided to The Union Procurement Unit.

16 INDEMNITY

The sponsor of the trial is The Union Against Tuberculosis and Lung Disease (IUATLD, Inc.). Insurance for the trial was obtained by IUATLD, Inc.

The local Principal Investigator/Investigator's Institution is liable for negligent harm, for each of the participating sites. Patients will be indemnified for non-negligent harm through a separate policy taken out by the trial sponsor.

All personnel involved in the trial will be expected to be indemnified by their employing authority.

17 ANCILLARY STUDIES

Any ancillary studies will have to be agreed by the Trial Steering Committee (TSC).

18 FINANCE

The trial is sponsored by The International Union Against Tuberculosis and Lung Disease (IUATLD, Inc.) with funding from the United States Agency for International Development (USAID). This trial will be managed and coordinated by the Medical Research Council Clinical Trials Unit (MRC CTU).

Each participating centre will be supported according to the submissions of their budgetary requirements.

Reimbursements will be made according to a Sub Agreement signed between the MRC/CTU and the participating centres.

19 TRIAL COMMITTEES

19.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will consist of representatives from different disciplines involved in the day to day running of the trial. It will include the Co-Chief Investigators, a member with clinical expertise in MDR-TB, members of the MRC CTU involved in the running of STREAM, namely the Trial Manager, the Data Manager, Project Manager and statistician. The group will also include a representative from each of the following: IUATLD, Inc. -TREAT TB Project Coordination Team, ITM in Antwerp, the Impact Assessment team from the Liverpool School of Tropical Medicine and the MRC CTU Data Services team. The TMG will be responsible for the day-to-day running and management of the trial and will convene monthly; it will report to the TSC on progress and issues.

19.2 Trial Steering Committee (TSC)

A TSC with an independent chair and a majority of independent voting members will be responsible for the oversight of the trial and provide advice to the investigators. No important decision should be made in the absence of a Chief Investigator. Additional observers, including other investigators, may be in attendance at the TSC meetings; they may provide additional input as requested. A STREAM TSC charter describes the membership and responsibilities of the TSC which include:

- providing expert oversight of the trial
- making decisions as to the future continuation (or otherwise) of the trial
- monitoring recruitment rates and encourage the TMG to develop strategies to deal with any recruitment problems
- reviewing regular reports of the trial from the trials unit (sent on behalf of the Trial Management Group (TMG))
- assessing the impact and relevance of any accumulating external evidence
- approving any amendments to the protocol, where appropriate
- approving any proposals by the TMG concerning any change to the design of the trial, including additional sub-studies
- overseeing the timely reporting of trial results
- approving the statistical analysis plan
- approving the publication policy
- approving the main trial manuscript
- approving any abstracts and presentations of any results *during* the running of the trial
- approving external or early internal requests for release of data or subsets of data or samples including clinical data and stored biological samples

19.3 Independent Data Monitoring Committee (IDMC)

The IDMC with an independent chair and voting members will review safety and efficacy data regularly. The list of members and their responsibilities is included in the STREAM IDMC charter. The IDMC could in exceptional circumstances recommend termination of the study or termination of one of the treatment regimens because of unacceptably high failure/relapse rates or unacceptable levels of drug toxicity. The IDMC will be asked to give advice on whether the accumulated data from the trial, together with results from other relevant trials, justifies continuing recruitment of further patients.

The IDMC will advise the TSC that the trial should be stopped if in their view there is proof beyond reasonable doubt that one of the trial treatments is clearly indicated or clearly contra-indicated in terms of a net difference in efficacy or adverse events or, there is proof beyond

reasonable doubt from other studies to influence clinic staff in their management of patients that is incompatible with continuing. Such proof would require a difference in failure/relapse rates between two of the study arms significant at the 0.1% level. In arriving at their recommendations, the IDMC will also take account of outcomes reported from all countries. They may recommend modification or closure of the study in a country or sub-group of patients, such as those who are HIV-infected.

The IDMC will convene approximately 6 monthly but may meet more frequently if it becomes necessary to do so. A charter describes in full the responsibilities of the IDMC and the format of their meetings and members will be required to sign this before the first meeting. The data will also be reviewed by the IDMC before the end of TREAT TB grant (September 2013) to comment on justification for continuation of the study beyond the USAID initial agreement period.

20 PUBLICATION

The definition of publication for this purpose is any public representation of the data emerging from this trial. Results of this study will be submitted for publication in a peer reviewed journal. This will be the analysis of the primary objectives of the study and this manuscript together with subsequent manuscripts, including any single centre data, will require the review and agreement of the TSC prior to submission.

Details for producing manuscripts, abstracts, press releases and any other publications including guidelines for authorship are outlined in the STREAM Publication Policy.

21 PROTOCOL EDITS AND AMENDMENTS

Version 1.0 11Jan2011

1. Cover page, authorised by, protocol signed again to revise date of signature for Prof. Andrew Nunn from '*6 January 2010'* to '*11 January 2011'*
2. Cover page, USAID logos added.

Version 2.0 Jan2011

1. Page 49, section 19.1, line 5: representation revised from '*the TREAT TB Project Coordination Team, The Union'* to '*The Union-TREAT TB Project Coordination Team'*
2. Page 55, Appendix 1, Ethiopia, revised from *'Dr. Daniel Kokebu'* to *'Dr. Daniel Meressa Kokebu'*
3. Page 55, Appendix 1, cities added for all sites: Ethiopia *'Addis Ababa';* South Africa *'Cape Town';* Vietnam *'Hanoi'* and *'Ho Chi Minh City'*
4. Page 55, Appendix 1, titles changed from '*Associate Prof.*' to *'Dr'*. for Keertan Dheda (South Africa) and Dinh Ngoc Sy (Vietnam)
5. Page 55, Appendix 1, South Africa, added two investigators *'Dr. Alex Pym, Unit for Clinical and Biomedical TB Research, MRC – Durban, email: Alexander.Pym@mrc.ac.za'* and *'Dr. Francesca Conradie, Right to Care, Sizwe Tropical Diseases Hospital, Edenvale, email: fconradie@witshealth.co.za '*

Version 3.0 Feb 2011

1. Cover page, USAID logo was added to Version 1.0 11Jan2011 but not recorded under changes made above.
2. Page 2, version number changed to version 3.0 Feb2011
3. Page 3, General Information: Sponsor title and address corrected from, '*The International Union Against Tuberculosis and Lung Disease (The Union)… 68 Boulevard Saint Michel, 75006 Paris, FRANCE, Tel: (+33) 1 44 32 03 60, Fax: (+33) 1 43 29 90 87…'* to '*The International Union Against Tuberculosis and Lung Disease (IUATLD, Inc.)… 61 Broadway, Suite 1720, New York, NY, 10006 USA, Tel (main): +1 212 500 5720, Fax: +1 212 480 6040…'*
4. Page 5, table of contents: section 6, '*ENROLMENT PROCEDURE*' changed to '*Enrolment Procedure*' for consistency in formatting.
5. Page 6, table of contents: section 14, '*ETHICAL*' changed to '*Ethical* for consistency in formatting.
6. Page 7, Abbreviations and glossary: '*NAE –notifiable adverse event*' revised to '*NE – notable event*' for clarity.
7. Page 9, section 1.1.3 Trial objectives: primary objective 2 revised from '*during treatment*' to '*during treatment and follow-up*' for clarity
8. Page 9, section 1.1.4 Trial Intervention: first paragraph revised from, '*the trial intervention will be a modified version of the 9-month regimen based on the one described by Van Deun 2010[1] hereafter referred to as the study regimen: ethambutol (E), pyrazinamide (Z), moxifloxacin (M) and clofazimine (C) throughout, supplemented by kanamycin (K), prothionamide (P) and isoniazid (H) in the first four months (4KCMEHZP/5MEZC)*' to '*the study regimen is based on the regimen described by Van Deun 2010[1], it consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for nine months (40 weeks), supplemented by kanamycin, isoniazid and prothionamide in the four months (16 weeks) of the intensive phase. All drugs are given daily (seven days a week) except for kanamycin which is given thrice-weekly after 12 weeks. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks respectively as described in section 7.3.2'* for clarity.

9. Page 9, section 1.1.5 Duration: paragraph 1 revised for consistency from '*patients on the study regimen will receive nine months of treatment (four months intensive phase, 5 months continuation phase). In the event of delayed smear conversion the four month intensive phase of the study regimen can be extended by one or two months, allowing a maximum total duration of eleven months treatment'* to *'patients on the study regimen will receive nine months (40 weeks) of treatment (16 weeks intensive phase, 24 weeks continuation phase). In the event of delayed smear conversion the intensive phase of the study regimen can be extended by 4 or 8 weeks, allowing a maximum total duration of 48 weeks treatment'*.
10. Pages 9-52, section numbers included. These were lost during conversion of Version 2.0 Jan2011, from WORD to PDF
11. Page 10, section 1.1.6 Outcome measures: last part of the paragraph revised from '*grade 3 or greater adverse event during the trial*' to '*grade 3 or greater adverse event during treatment and follow-up*'
12. Page 10, section 1.1.6 Outcome measures: '*number of adverse reactions occurring on treatment*' added to the secondary outcomes.
13. Page 12, section 2.1.3 Investigational regimen: number 1 (lines 2-4) revised for consistency from, *'… given daily for four months which can be extended by one month at a time up to a maximum of six months if smear conversion is not achieved within four months. Kanamycin is only given thrice-weekly from the fourth month onwards'* to '*…given daily for four months (16 weeks) which can be extended by 4 weeks at a time up to a maximum of 48 weeks if smear conversion is not achieved within 16 weeks. Kanamycin is only given thrice-weekly from week 12 onwards'*.
14. Page 14, section 2.2.1 Risks and benefits: first paragraph, line 2, revised from '*cure rate approaching 90%*' to '*cure rate of 88%, 95% confidence interval (83%, 92%)*'.
15. Page 14, section 2.2.1 Risks and benefits: sentence added before the last paragraph stating, *'a summary of the safety information on the higher dose of moxifloxacin and the safety monitoring that is to be undertaken in the trial is provided as Appendix 2'*.
16. Page 15, section 3.1.1 Primary objectives: objective 2 revised from '*during treatment*' to '*during treatment and follow-up*' for clarity
17. Page 15, section 3.2 Outcome measures: last part of the primary safety outcome revised from, '*during the study*' to '*during treatment and follow-up*'
18. Page 15, section 3.2 Outcome measures: '*number of adverse reactions occurring on treatment*' added to the secondary outcomes.
19. Page 17, section 5.1 Patient inclusion criteria: number 5 revised from *'effective barrier contraception during treatment phase…'* to *'effective barrier contraception or have an intrauterine contraceptive device during treatment phase…'*
20. Page 21, section 7.2.1 Study regimen: revised for clarity, from '*the study regimen has two phases: intensive and continuation. The intensive phase consists of kanamycin, clofazimine, moxifloxacin, ethambutol, isoniazid plus pyrazinamide and prothionamide given daily (seven days a week) for 16 weeks but with kanamycin given thrice-weekly after 12 weeks. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks respectively as described below in section 7.3.2'* to '*the study regimen consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for nine months (40 weeks), supplemented by kanamycin, isoniazid and prothionamide in the first four months (16 weeks). All drugs are given daily (seven days a week) except for kanamycin which is given thrice-weekly after 12 weeks. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks respectively as described below in section 7.3.2'*.
21. Page 21, section 7.2.1 Study regimen: the arrangement of drugs in table 1 Study regimen doses was changed for consistency.

22. Page 21, section 7.2.1 Study regimen: paragraph below table 1, the following sentence was added for clarity, 'treatment supervisors may be clinic staff or family members or other members of the community, depending on local circumstances.
23. Page 21, section 7.2.2 Control regimen: first sentence revised from 'locally used WHO approved MDR-TB regimen' to 'locally-used WHO-approved MDR-TB regimen' for consistency.
24. Page 22, section 7.2.4 Treatment cards: line 1, '*their treatment supervisor'* changed to '*a treatment supervisor*' for clarity. In line 2, '*treatment supervisor*' was also changed to '*treatment supervisors*' for clarity. '*For those days the clinic is expected to be closed (Saturdays, Sundays and National holidays) the patients may be given doses to take under the supervision of a designated domiciliary treatment supervisor*' this sentence was deleted since it was already covered in earlier sections.
25. Page 22, section 7.3.1 Dispensing and supervision of medicines: paragraph 2 line 1 revised from, '*all medicines must be given for seven days under…'* to '*all medicines in the study regimen must be given on seven days per week under…'* for clarity. '*For the control regimen, sites will be strongly encouraged to follow the same standard*, also added for clarity.
26. Page 23, section 7.3.2 Transition from intensive to continuation phase in the study regimen: first paragraph line 2, below figure 2, revised for consistency from, '*… receive treatment for up to eleven months'* to '*receive treatment for up to 48 weeks'*.
27. Page 24, section 7.3.5 Pregnancy: line 4, *'unless they have an intrauterine contraceptive device'* was added for clarity.
28. Page 25, section 8.1 Assessment schedule: deleted 'patients will be seen daily by their treatment supervisors who will observe them taking their trial medication'. The next sentence was also revised from, '*patients will be required to attend the clinic for assessments…'* to '*patients will be required to attend the clinic every four weeks for assessments…'*
29. Page 25, section 8.1 Assessment Schedule: paragraph 4, line 1 was revised (for consistency) from *'at enrolment and from the third month and every third month thereafter…'* to *'at enrolment and from twelve weeks and every twelve weeks thereafter…'*
30. Page 25, section 8.1.1 Assessment Schedule: simple hearing test was changed from, '*every visit during treatment*' to '*if clinically indicated'*, 'HIV' was changed to '*HIV antibody test*' and '*samples for storage (if patient consents)*' changed to '*blood samples for storage (if patient consents)*' for clarity
31. Page 25, section 8.1.1 Assessment schedule: table revised to delete penultimate row (DST (if positive)) since this was already covered by the 'Sputum smear and culture[‡]' text in the table and accompanying footnote.
32. Page 25, section 8.1.1 Assessments Schedule, '*sample storage (if patient consents)*' added to the table, with a footnote, '[π] *one sample will be collected for storage at 16 weeks*'
33. Page 25, section 8.1.1 Assessment schedule: sputum smear and culture collected during the post treatment phase changed from *'2'* to '*1**'. The footnote was revised from '* *one sample will be collected at per visit, except at the final visit of each phase when two samples will be collected* to *'*one sample will be collected per visit, except at the final visit of each phase of treatment and at the 27 month follow-up visit, when two samples will be collected*.
34. Page 26, section 8.3 Procedures for assessing safety: paragraph 1 lines 8 and 9 revised for clarity from *'…Grade 3 or greater, this is a notifiable adverse event that must be reported. SAEs and other notifiable adverse events will be…'* to *'…Grade 3 or greater, this is a notable event that must be reported. SAEs and other notable events will be…'*.
35. Page 26, section 8.3 Procedures for assessing safety: paragraph 2 first line revised for clarity from, '…potential risk of QT prolongation, all patients will have an ECG…' to

'…potential risk of QT prolongation with moxifloxacin, all patients will have a 12-lead ECG…'

36. Page 26, section 8.3 Procedures for assessing safety: paragraph 2 last sentence was revised from, *'…any participant whose QTc at week 1 is ≥440msec (upper limit of normal for men the upper limit of normal for women is ≥460msec'* to *'…any participant whose QTc at week 1 is ≥450msec (the upper limit of normal for men, the upper limit of normal for women being ≥ 470msec)'*,'for clarity. The following sentences were also added for clarity: '*for further details, see Appendix 2). Any QTc prolongation to ≥500msec while on treatment is considered a notifiable event and should be reported immediately to MRC CTU (see section 13)*'
37. Page 26, section 8.3 Procedures for assessing safety: paragraph 3 line 1 was revised from, '*any QTc prolongation to ≥500msec while on treatment is considered a notifiable event and…'* to '*any QTc prolongation to ≥500msec while on treatment is considered a notable event and…'*
38. Page 27, section 8.4.1 Interruptions to treatment: line 1, '*any of the following (list is not comprehensive)*' and '*pregnancy*' (third bullet point), were deleted for clarity and '*a QTc measurement ≥500msec or*' was added. Paragraph three, line 2 was revised, for clarity, from *'… coordinating site and discuss it with a member of the central clinical team to agree the treatment'*, to '*… coordinating centre by submission of an SAE form and discuss treatment plans with a member of the central clinical team'*. Paragraph four was moved to section 9.1 (Discontinuation of allocated regimen) for clarity. Last paragraph was revised from, '*all patients will continue to be followed up whether or not…'* to '*all patients will continue to be followed-up to 27 months post-randomisation whether or not…'* for clarity.
39. Page 27, section 8.4.2 Missed visits: paragraph 1 line 3, revised from '*communication with the patient through…'* to '*communication with the patient and/or treatment supervisor through…'* for clarity.
40. Page 27, section 8.4.3 Visit after a missed appointment: paragraph 2 line 2, revised from, *'…e.g. if a patient returns to the clinic at or near to week 16 after missing week 8 and 12 visits, the visit for that day should be visit 16'* to '*if a patient returns to the clinic at or near to week 16 after missing their visits for weeks 8 and 12, the visit for that day should be recorded as week 16*'.
41. Page 28, section 8.4.6 HIV: line 1, '*patients who are known to be HIV positive or who are found to be HIV positive*' changed to '*patients who are known to be HIV infected or who are found to be HIV infected*' for clarity.
42. Page 29, section 8.6 Bacteriology: paragraph 2 line 5 was revised for clarity from *'hot Ziehl-Neelsen (ZN) for all study smears'* to *'hot Ziehl-Neelsen (ZN) or auramine fluorescence technique for all study smears'*
43. Page 30, section 8.7.2 Patient and household costs: line 2 revised from '*…at three monthly intervals after initiation of treatment*' to '*…at intervals of 12 weeks after initiation of treatment'*.
44. Page 30, section 8.7.3 Socio-economic status: line 5 was revised for clarity from '*every three months after the initiation…'* to '*every 12 weeks after the initiation…*'
45. Page 31; section 9.1 Discontinuation of allocated regimen: pregnancy added as another justifiable reason for discontinuing a patient's allocated treatment. Revision made as follows, '*pregnancy: women who become pregnant will stop trial treatment, and be treated according to the National TB Programme'*.
46. Page 32, section 9.2 Salvage regimens: last line in this section, '*capacity of surgical intervention*' corrected to '*capacity for surgical intervention*'.
47. Page 33, section 10 Data Management: line 7 was revised for clarity from '*…the study file and all source data…'* to '*…the Investigator Site File and all source data*'
48. Page 34 section 11.1.1 Primary efficacy outcome: unfavourable outcome 4 was revised for clarity from '… *any reason other than the replacement or discontinuation of a single drug*' to '… *any reason other than the replacement of a single drug'*.

49. Page 34 section 11.1.1 Primary efficacy outcome: sentence added to paragraph 4 for clarity, '*similarly, the discontinuation of one or more drugs that are not replaced does not constitute an unfavourable outcome'*.
50. Page 34, section 11.1.1 Primary efficacy outcome: second-to-last paragraph revised for clarity to '*a patient who is either discontinued…retreated for MDR-TB, or lost to follow-up from the trial…'* for clarity.
51. Page 35, section 11.2.1 Power to demonstrate non-inferiority in the primary efficacy outcome: first paragraph below table 2, last sentence, '*the intention to treat analysis would require fewer patients*' was deleted.
52. Pages 35, section 11.2.1: *'Approximately 400'* changed to *'At least 400'.* The following two sentences were removed: *'100 patients will be required to be enrolled on the study regimen in each country to estimate the rate of favourable outcomes with reasonable precision. It is therefore proposed to enrol a minimum of 100 patients in each of four or five countries.'*
53. Page 37, section 12.3.1 Direct access to data: paragraph 2, line 2, '*Trial Coordinator*' changed to '*Local Trial Coordinator*' for consistency.
54. Page 38, section 12.3.2 Quality Assurance Procedures: paragraph 2 revised for clarity from, '*…training will be provided for all staff involved in the trial as part of the capacity strengthening component of this study. Staff will also be trained in the trial procedures*' to '*…training will also be provided for all staff involved in the trial; this will form part of the capacity strengthening component of the trial*'.
55. Page 38, section 12.3.3 Laboratory Quality Assurance: paragraph 3 line 2 was revised from '*…internal control of newly prepared lots of ZN staining solutions…*' to '*…internal control of newly prepared lots of staining solutions…*'
56. Page 38, section 12.4 Central monitoring: paragraph 1, the following sentence was deleted: '*CRFs will be reviewed remotely as appropriate to determine quality*'.
57. Page 40, section 13.1.3 Additional Notifiable Events: this section was added for clarity, *'pregnancy while on protocol treatment, QTc measurement ≥500msec while on treatment and any toxicity that leads to a planned change of allocated treatment is defined as a notifiable event and should be reported as an SAE*'.
58. Page 40, section 13.1.3: this section was revised from '*additional notifiable events'* to '*additional notable events*' for consistency and line 2 was revised from '*…is defined as notifiable…'* to '*is defined as notable…'* also for consistency.
59. Page 40, section 13.2.1 (d) Notification: line 3 was revised from *'SAEs occurring'* to *'SAEs and other notifiable events defined above occurring'*, for clarity.
60. Page 40, section 13.2.1 (d) Notification: line 3, '…*other notifiable…'* was changed to *'…other notable…'* for consistency.
61. Page 42, section 13.3 MRC CTU responsibilities: second paragraph line 1, corrected from '*MRC CTU is undertaking the duties of trial coordinator...'* to '*MRC CTU is undertaking the duties of trial management…'*
62. Page 44, section 14.1.1 Research Ethics Committee (REC) review and approval: fourth paragraph line 2 corrected from, '*the principles Good Clinical Practice (GCP)*' to '*the principles of Good Clinical Practice (GCP)*'.
63. Page 46, section 14.1.7 Audit: line 5 corrected from, '*…or when the data is under review*' to '*…or when the data are under review*'.
64. Page 47, section 16 Indemnity: sponsor details corrected in paragraph 1 from '*…The Union Against Tuberculosis and Lung Disease (The Union). Insurance for the trial was obtained by The Union'* to '*…The Union Against Tuberculosis and Lung Disease (IUATLD, Inc.). Insurance for the trial was obtained by IUATLD, Inc.*'
65. Page 47, section 18 Finance: sponsor details corrected in paragraph 1 from "*…The Union Against Tuberculosis and Lung Disease (The Union)…*' to '*…The Union Against Tuberculosis and Lung Disease (IUATLD, Inc.)…*'

66. Page 48, section 19.1 Trial Management Group (TMG): line 5 sponsor details corrected from, '*…each of the following: The Union -TREAT TB Project Coordination Team,…*' to '*…each of the following: IUATLD, Inc. -TREAT TB Project Coordination Team,…*'
67. Page 51, section 21 Protocol edits and amendments: protocol edits listed under '*version 3.0 Feb2011 (additional edits)*' or '*version 3.0 Feb2011 (18 Feb 2011 edits)'* were incorporated into one section as changes made to version 3.0 Feb 2011.
68. Page 51, the title for section 21 was revised from '*protocol amendments'* to '*protocol edits and amendments*'
69. Page 55, Appendix 1, Ethiopia: revised from '*St. Peter's Tuberculosis Specialised Hospital*' to '*St. Peter's Tuberculosis Specialised Hospital/Global Health Committee'.*
70. Page 55, Appendix 1, South Africa: revised to list '*Dr. Alexander Pym*' first, followed by *'Dr. Keertan Dheda*'
71. Page 55, Appendix 1, South Africa, changed investigator's name from *'Dr. Alex Pym'* to '*Dr. Alexander Pym'*
72. Page 55, Appendix 1, Vietnam: Dr. Nguyen Thi Ngoc Lan's site was changed from '*Ho Chi Minh City*' to '*Pham Ngoc Thach*' hospital and Dr. Nguyen Huy Dung - the Director of Pham Ngoc Thach hospital was added as a Principal Investigator.
73. Page 60, Appendix 2 High-dose moxifloxacin summary was added to the protocol.

22 REFERENCES

1. Van Deun A MA, Salim AMH, Das PK, Sarker MRS, Daru P and Rieder HL. . Short, highly effective and inexpensive standardised treatment of Multidrug-resistant Tuberculosis. Am J Respir Crit Care Med,. May 2010.

2. Laserson KF WC. Reaching the targets for tuberculosis control: the impact of HIV. Bull World Health Organ. 2007; **85**(5): 377-81.

3. Zignol M HM, Wright A, Weezenbeek CL, Nunn P, Watt CJ, Williams BG, Dye C. Global incidence of multidrug-resistant tuberculosis. J Infect Dis. 2006

4. World Health Organization. Global tuberculosis control - epidemiology, strategy, financing.; 2009. .

5. World Health Organization. Anti-tuberculosis Drug Resistance in the World - fourth global report.; 2008.

6. Wells CD CJ, Nelson LJ, Laserson KF, Holtz TH, Finlay A, Castro KG, Weyer K. HIV infection and multidrug-resistant tuberculosis: the perfect storm. J Infect Dis. 2007.

7. World Health Organization. WHO Guidelines for the programmatic management of drug-resistant tuberculosis, ; 2008

8. Chiang C-Y ED, Yu M-C, Bai K-J, Huang R-M, Hsu C-J, Suo J, Lin T-P. Outcome of pulmonary multidrug-resistant tuberculosis: a 6-year follow-up study. Eur Respir J 2006.

9. Seung KJ OD, Keshavjee S, Furin JJ, Farmer PE, et al. . Early Outcomes of MDR-TB Treatment in a High HIV-Prevalence Setting in Southern Africa. PLoS ONE. 2009; **4**(9).

10. Mitnick C BJ, Palacios E, Shin S, Furin J, Alcántara F, Sánchez E, Sarria M, Becerra M, Fawzi MC, Kapiga S, Neuberg D, Maguire JH, Kim JY, Farmer P. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. N Engl J Med. 2003 **348**(2): 119-28.

11. Leimane V RV, Holtz TH, Zarovska E, Skripconoka V, Thorpe LE, Laserson KF, Wells CD. . Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. The Lancet 2005; **365**(9456): 318-26

12. Orenstein E. W. ea. Treatment Outcomes among patients with Multidrug Resistant tuberculosis: systematic review and meta-analysis. The Lancet infectious diseases. 2009; **Vol. 9**(No. 3): 153-61.

13. Chou L HM, Huff B, Jefferys R, Syed J, Wingfield C. . TAG 2009 Pipeline Report,; 2009

14. Mann Gea. Beyond accuracy: creating a comprehensive evidence base for tuberculosis diagnostic tools The International Journal of Tuberculosis and Lung Disease. accepted for publication July 2010.

15. Johnson JL HD, Boom WH, Daley C L, Peloquin CA, Eisenach KD, Jankus DD, Debanne SM, Charlebois ED, Maciel E, Palaci M, Dietze R. . Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006; **10**(6): 605-12.

16. DAIDS RSC. Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events In: RSC DoA, editor.; 2009.

17. Hillemann D R-GS, and Richter E. . Feasibility of the GenoType MTBDRs/assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. Journal of Clinical Mircobiology. 2009; **47**: 1767-72.

18. World Health Organisation. Handbook for Good Laboratory Practice (GLP). 2009.

APPENDIX 1: PRINCIPAL INVESTIGATORS AND PARTICIPATING SITES

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APPENDIX 2: HIGH-DOSE MOXIFLOXACIN SAFETY SUMMARY

1. Rationale

Gatifloxacin (400mg daily for patients <33kg, 600mg for those 33-50kg, and 800mg if > 50kg) was considered to be a critical component of the success of the regimen developed by van Deun et al; the ofloxacin-containing regimens tested were associated with inferior outcomes[1]. Because an internationally acceptable, quality-assured supply of gatifloxacin is not available, it was necessary to substitute a different fluoroquinolone, and moxifloxacin was judged to be the best alternative. Gatifloxacin and moxifloxacin have similar bactericidal activity at the same dose[1] and based on pharmacokinetic modelling there is reason to believe that the higher than standard doses are needed to prevent secondary fluoroquinolone resistance[2].

The standard dose of moxifloxacin is 400mg daily without any weight adjustment. In the STREAM study, as in the regimen developed by van Deun et al, 400mg will be used for patients <33kg, 600mg for those 33-50kg, and 800mg if > 50kg. The main concern about the substitution of moxifloxacin for gatifloxacin is the potential for cardiac toxicity.

2. Cardiac safety of moxifloxacin at the standard dose

Moxifloxacin is an 8-methoxy quinolone, a member of the widely used fluoroquinolone family of anti-bacterial agents, which are some of the most frequently prescribed antibiotics in the world. Fluoroquinolones, in particular moxifloxacin, are known to prolong the QT interval, which occurs when drugs prevent the outward flow of potassium through cardiac voltage-gated potassium channels[3]. This causes a delay in cardiac repolarisation and may increase the risk of torsades de pointes (TdP), a life threatening ventricular tachycardia. However, despite this propensity and it's extensive use, there are very few reported cases of TdP induced by moxifloxacin[4].

QT prolongation is defined as a QT interval above the upper limit of normal: 450ms for men and 470ms for women[5]. However, the best indicator that a drug has the potential to induce arrhythmias is if it causes QTc (QT interval corrected for heart rate) prolongation to greater than 500ms[6].

The QTc increase following moxifloxacin has been well documented. Florian et al. reported an average increase of 10-14ms following a single 400mg dose across several investigations[7]. Tsikouris et al. acquired similar results after conducting an open label cross-over study in 13 healthy participants, including moxifloxacin at 400mg, revealing an average QTc increase of 11ms at 2-hours post dose[8].

Based on all the clinical trial data for moxifloxacin at the standard dose, ventricular tacharrhymias are estimated to occur in <1/1,000 and torsades de pointes and cardiac arrest in <1/10,000[9]. The case reports of TdP potentially related to moxifloxacin have occurred in elderly patients with pre-existing heart conditions[10-12].

Rubinstein's 2002 review reported that there were no cases of cardiovascular morbidity attributable to QTc prolongation recorded in 6000 patients involved in moxifloxacin phase II-IV clinical trials, though there were four cases of arrhythmias (three non-specified) and one case of TdP in one elderly female patient with pre-existing risk factors including hypokalaemia, coronary artery disease, digoxin treatment and a pacemaker. They concluded that the fluoroquinolones in question (including moxifloxacin) were safe but should be closely monitored in patients with pre-existing conditions or those taking concomitant medication[12].

The trial data includes a randomised trial comparing the cardiac safety of moxifloxacin 400mg and levofloxacin 500mg in 387 elderly patients with community acquired pneumonia over 70% of whom had pre-existing cardiac disease; no difference in cardiac safety was detected[13].

3. Higher Doses of Moxifloxacin

Investigations using higher doses of moxifloxacin have been conducted although there is considerably less experience than with the standard dose. Démolis et al. conducted a placebo-controlled crossover study in 18 healthy volunteers in which both 400mg and 800mg prolonged the QT interval compared to placebo, but there was little difference between the two doses: the 400g and 800g doses increased QTc by 4.0% ± 5.1% and 4.5% ± 3.8%, respectively[14]. At two hours post dose, mean QTc intervals were recorded as 394 ±33ms (400mg) and 396 ±28ms (800mg) compared with the placebo mean of 379 ± 24ms. 800mg doses of moxifloxacin were also used in a 4-sequence cross-over study in 48 healthy patients across a spectrum of ages was conducted by Noel et al.[15]. Mean corrected QTc (Bazett) was recorded at 425-430msec post-dose, with the peak between 2-4 hours; 6/47 patients (12.8%) had QTc intervals above the normal limits. All adverse events (6 following moxifloxacin treatment) were described as mild, brief and spontaneously resolving.

In a trial of moxifloxacin-based treatments for H. pylori a total of 94 patients with a mean age of 50 received 800 mg moxifloxacin daily in conjunction with amoxicillin and esomeprazole for 10 days, 102 for 7 days and 98 for 5 days (294 in total) without any cardiac adverse events[16]; no ECG monitoring was undertaken.

Stass et al. conducted a study of moxifloxacin at doses ranging from 50mg – 600mg in 7 healthy subjects[17]. The study drug was well tolerated at all doses, with no clinically relevant changes in electrocardiogram data and only mild adverse events with no deaths or drop-outs.

In addition, there is one case report of a patient with miliary TB whose treatment included 800mg moxifloxacin[18]. Results confirmed that the peak plasma concentration was between 2-4 hours with a mean QTc of 442.

4. Safety Monitoring in STREAM

The available literature suggests that the difference in the effects of moxifloxacin on the QT interval at doses between 400 and 800mg are unlikely to be substantial, while the benefits in relation to prevention of acquired resistance are likely to be integral to the regimen.
The safety measures to be undertaken in STREAM are robust and designed to monitor the possible effects of moxifloxacin at peak concentration and to detect any possible cumulative effects. Any patient with a QTc above 500ms prior to treatment will be excluded from the trial. All patients will be monitored with a 12-lead ECG at 2 and 4 hours post the initial dose to capture the peak QTc increase, with further ECGs at weeks 1-4, 12, 24 and 36. In addition, any patient with a QTc interval ≥450ms (upper limit of normal for males, who have a lower normal range than women) will be more intensively monitored at the end of the first week of treatment using a 24-hour Holter to check that any diurnal variation in QT does not take them over the 500ms threshold. Patients found to have a QTc ≥ 500ms at any point during treatment will discontinue receiving moxifloxacin (unless another cause is identified).

Concomitant medications will be closely monitored throughout the trial, in particular anti-retroviral therapy; however, the recent findings from the SMART trial would suggest that their effects are likely to be small[19]. Although ECG monitoring of this intensity would not be feasible

in routine practice, it is being implemented here both to protect the patients in the trial and to determine the safety of the regimen.

The current data suggests that TdP with moxifloxacin is a rare event. The STREAM protocol is designed to closely monitor patients and those at greatest risk of cardiac toxicity will be excluded. The potential risks of the study regimen should be balanced against both the risks of MDR TB for which outcomes are poor and mortality is high (11% of patients in a systematic review of 33 studies of MDR TB treatment died during treatment)[20], as well as the widely documented and serious adverse effects related to alternative MDR-TB treatment regimens.

5. References

1. Armand Van Deun et al. Short, Highly Effective and Inexpensive Standardized Treatment of Multidrug-resistant Tuberculosis. American Journal of Respiratory and Critical Care Medicine 2010; 182 684-92.

2. Gumbo T et al. Selection of a Moxifloxacin Dose That Suppresses Drug Resistance in Mycobacterium tuberculosis, by Use of an In Vitro Pharmacodynamic Infection Model and Mathematical Modeling. The Journal of Infectious Diseases 2004; 190(1642-1651).

3. Falagas M; Rafailidis P; and Rosmarakis E. Arrhythmias associated with fluoroquinolone therapy. International Journal of Antimicrobial Agents. 2007; 29(374-379).

4. Altin T et al. Torsades de pointes associated with moxifloxacin: A rare but potentially fatal adverse event (case report). The Canadian Journal of Cardiology. 2007; 23(11): 907-8.

5. Haddad P et al. Antipsychotic-Related QTc Prolongation, Torsade de Pointes and Sudden Death. Drugs. 2002; 62(11): 1649-71.

6. Committee for Proprietary Medicinal Products (CPMP). The Assessment of the Potential for QT Interval Prolongation by Non-Cardiovascular Medicinal Products. London, 17 December 1997.

7. Florian J et al. Population Pharmacokinetic and Concentration-QTc Models for Moxifloxacin: Pooled Analysis of 20 Thorough Qt Studies. The Journal of Clinical Pharmacology. 2011.

8. Tsikouris J et al. Effects of Three Fluoroquinolones on QT Analysis After Standard Treatment Courses. ANE. 2006; 11: 52-6.

9. Bayer plc. Avelox (moxifloxacin) Summary of Product Characteristics. Last updated 03/12/2010.

10. Sherzi Sea. Moxifloxacin-induced torsade de pointes (Case Report). 2008; 15: 71-3.

11. Dale K M et al. Moxifloxacin and Torsade de Pointes (Case Report). The Annals of Pharmacotherapy. 2007; 41: 336-40.

12. Rubenstein E and Camm J. Cardiotoxicity of fluoroquinolones. Journal of Antimicrobial Chemotherapy. 2002; 49: 593-6.

13. Morganroth J et al. A Randomized Trial Comparing the Cardiac Rhythm Safety of Moxifloxacin vs Levofloxacin in Elderly Patients Hospitalized With Community-Acquired Pneumonia. CHEST. 2005; 128: 3398-406.

14. Demolis J-L et al. Effect of a single oral dose of moxifloxacin (400mg and 800mg) on ventricular repolarization in healthy subjects. Clincal Pharmacology & Therapeutics. 2000; 8: 658-66.

15. Noel G J et al. Effects of three fluoroquinolones on QT interval in healthy adults after single doses. Clinical Pharmacology & Therapeutics. 2003; 73: 292-303.

16. Sacco F et al. Efficacy of four different moxifloxacin-based triple therapies for first-line H. pylori treatment. Digestive and Liver Disese. 2010; 42: 110-4.

17. Stass H et al. Pharmacokinetics, Safety and Tolerability of Ascending Single Doses of Moxifloxacin a New 8-Methoxy Quinolone, Administered to Healthy Subjects. Antimicrobal Agents and Chemotherapy. 1998; 42(8): 2060–5.

18. Alffenaar J et al. Plasma and Cerebrospinal Fluid Pharmacokinetcis of Moxifloxacin in a Patient with Tuberculosis Meningitis (Letter). Antimicrobal Agents and Chemotherapy. 2008; 52: 2293-5.

19. Soliman E Z et al. Boosted protease inhibitors and the electrocardiographic measures of QT and PR durations. AIDS. 2011; 25(3): 367-77.

20. Orenstein E W et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infectious Diseases. 2009; 9: 153-61.

STREAM

The evaluation of a standard treatment regimen of anti-tuberculosis drugs for patients with MDR-TB

ISRCTN 78372190

PROTOCOL

VERSION 6.2

FEBRUARY 2015

(Based on MRC CTU template protocol version 3.15)

Authorised by:

Name:	Prof. Andrew Nunn
Role:	Co-Chief Investigator
Signature: A J Nunn	Date: 16 - Feb - 2015
Name:	Dr. Sarah Meredith
Role:	Co-Chief Investigator
Signature: Sol Meredith	Date: 16-Feb-2015

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Signature:	Date:
Name:	Dr. Sarah Meredith
Role:	Co-Chief Investigator
Signature:	Date:

SIGNATURE PAGE

Principal Investigator Signature:

The signature below confirms agreement by the individual at the clinical site responsible for signing the clinical trial agreement, that this is the trial protocol, STREAM (Version 6.2) dated February 2015. The trial will be conducted in accordance with this protocol, with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), and the applicable regulatory requirements.

I agree to conduct the trial according to these regulations and guidelines and to appropriately direct and assist the staff under my control, who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Any amendments to this protocol that have a direct influence on the participants in the trial will be approved by the relevant ethics committees, regulatory authorities and the sponsor before implementation.

Principal investigator's name: _____

Signature: _____

Date: _____

This STREAM trial is made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of The International Union Against Tuberculosis and Lung Disease and MRC CTU at UCL and do not necessarily reflect the views of USAID or the United States Government.

GENERAL INFORMATION

This document describes the STREAM trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to site principal investigators in the trial by MRC Clinical Trials Unit at UCL (MRC CTU), London. Clinical problems relating to this trial should be referred to the co-Chief Investigator(s).

Compliance:

The trial will be conducted in accordance with this protocol, with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), and the applicable regulatory requirements.

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ABBREVIATIONS AND GLOSSARY

AE	Adverse Event
AFB	Acid Fast Bacilli
AR	Adverse Reaction
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BDQ	Bedaquiline
ICF	Informed Consent Form
CI	Chief Investigator
CFZ	Clofazimine
CRF	Case Report Form
CTA	Clinical Trials Authorisation
DAIDS	Division of Acquired Immunodeficiency Syndrome
DCF	Data Clarification Form
DOT	Directly Observed Treatment
DST	Drug Susceptibility Test
ECG	Electrocardiogram
EMA	European Medicines Agency
EMB	Ethambutol
EQA	External Quality Assurance
FDA	Fluorescein diacetate staining
US FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GLC	Green Light Committee
HE	Health Economics
HIV	Human Immunodeficiency Virus
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITM	Institute of Tropical Medicine
ITT	Intention To Treat
IUATLD	International Union Against Tuberculosis & Lung Disease
KM	Kanamycin
INH	Isoniazid
LFX	Levofloxacin
LPA	Line Probe Assay
LQAS	Lot Quality Assurance Sampling
M2	Metabolite 2
MDR	Multi-Drug Resistant
MFX	Moxifloxacin
Genotype MTBDRPlus	Rapid test for *M. tuberculosis* Complex and its resistance to Rifampicin and/or Isoniazid
Genotype MTBDR*sl*	Rapid test for *M. tuberculosis* Complex and its resistance to fluoroquinolones and/or second-line injectables/cyclic peptides and/or ethambutol
MIC	Minimal Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units–Variable Number of Tandem Repeats
MRC CTU	Medical Research Council Clinical Trials Unit
NE	Notable Event
NTP	National Tuberculosis Programme
PK	Pharmacokinetics

PI	Principal Investigator
PIS	Patient Information Sheet
PTO	Prothionamide
PZA	Pyrazinamide
QA	Quality Assurance
QT Interval	A measure of time between the start of the Q wave and the end of the T wave in the ECG complex
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using the Fridericia correction
REC	Research Ethics Committee
RMP	Rifampicin
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SSA	Site Specific Assessment
STREAM	The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
The Union	International Union Against Tuberculosis & Lung Disease
TM	Trial Manager
TMG	Trial Management Group
TMT	Trial Management Team
TREAT TB	Technology, Research, Education, and Technical Assistance for Tuberculosis
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
ULN	Upper limit of normal
USAID	United States Agency For International Development
WHO	World Health Organisation
XDR	Extensively Drug Resistant
ZN	Ziehl-Neelsen

Note. In this protocol - time (in weeks) refers to the time from randomisation, e.g. Week 76 refers to 76 weeks from randomisation.

1 SUMMARY

1.1 Abstract and summary of trial design

1.1.1 Type of design

The STREAM study is an international, multi-centre, parallel-group, open-label, randomised, controlled trial.

1.1.2 Disease / patients studied

Patients with multi-drug resistance tuberculosis (MDR-TB) including patients with rifampicin-resistant and isoniazid-sensitive TB.

1.1.3 Trial interventions

Treatments that are evaluated within the STREAM trial include:

Regimen A
The locally-used WHO-approved MDR-TB regimen.

Regimen B
Regimen B is based on the regimen described by Van Deun 2010[1] consisting of clofazimine, ethambutol, moxifloxacin, and pyrazinamide given for 40 weeks, supplemented by isoniazid, kanamycin, and prothionamide in the first 16 weeks (intensive phase).

Regimen C
Regimen C is a 40-week all-oral regimen consisting of bedaquiline, clofazimine, ethambutol, levofloxacin, and pyrazinamide given for 40 weeks supplemented by isoniazid and prothionamide for the first 16 weeks (intensive phase).

Regimen D
Regimen D is a 28-week regimen consisting of bedaquiline, clofazimine, levofloxacin, and pyrazinamide given for 28 weeks supplemented by isoniazid and kanamycin for the first 8 weeks (intensive phase).

1.1.4 Trial Stages

The STREAM trial consists of 2 stages.

Stage 1
Stage 1 of the trial involves one study comparison between two treatment arms: Regimen A and Regimen B (as described in section 1.1.3). During this stage Regimen A acts as the control arm to the investigational treatment arm, Regimen B.

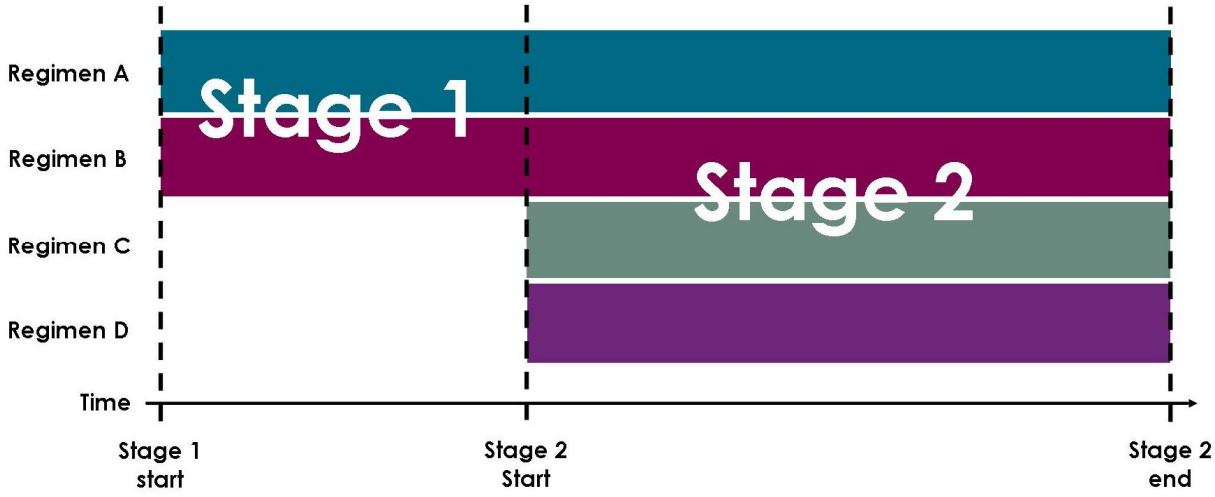
Stage 2
Stage 2 of the trial involves the addition of two further treatment arms: Regimen C and Regimen D. During this stage Regimen B acts as the control arm for two study comparisons with the two new investigational treatment arms, Regimen C and Regimen D, for the primary analyses. Patients will continue to be randomised to Regimen A and a secondary analysis will be undertaken to compare Regimens C and D with Regimen A.

The start of Stage 2
Figure 1 presents an overview of the recruitment stages in the STREAM trial, and illustrates how recruitment to each of the four treatment arms overlap. However, sites may begin Stage

1 and/or Stage 2 at different times, depending upon gaining country-specific approval. Additionally, some sites may only be participating in Stage 1 and others only in Stage 2.

During Stage 1, patients will be randomised to one of two treatment regimens, either Regimen A or Regimen B. For sites participating in both Stage 1 and Stage 2, once Stage 2 begins, patients will be randomised to one of four treatment regimens: Regimen A, Regimen B, Regimen C, or Regimen D. For sites participating in both Stage 1 and Stage 2, or in Stage 2 only, the Stage 2 assessment schedule (described in section 8.1.1) and Stage 2 CRFs will apply to all patients recruited to any treatment arm from then on, including those patients recruited to Regimen A. Sites participating only in Stage 1 will continue to follow the latest version of the Stage 1 protocol.

Figure 1: Trial recruitment stages



1.1.5 Trial objectives

Primary trial objectives of Stage 1 comparison

The primary objectives of Stage 1 of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome at Week 132 on Regimen B is not inferior to that on Regimen A (WHO approved MDR-TB regimen)
2. To compare the proportion of patients who experience grade 3 or greater adverse events, during treatment or follow-up, on Regimen B as compared to Regimen A.

The secondary objectives of Stage 1 of the STREAM trial are listed in section 3.1.2.

Primary trial objectives of Stage 2 comparisons

The primary objectives of Stage 2 of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome at Week 76 on Regimen C is superior to that on Regimen B
2. To assess whether the proportion of patients with a favourable efficacy outcome at Week 76 on Regimen C is not inferior to that on Regimen B
3. To assess whether the proportion of patients with a favourable efficacy outcome at Week 76 on Regimen D is not inferior to that on Regimen B.

Additional secondary objectives, including safety objectives, of Stage 2 of the STREAM trial are listed in section 3.2.2.

1.1.6 Duration of follow-up

All patients in Stage 1 of the study will be followed up to Week 132. The primary analysis will be based on the data accrued to Week 132.
All patients in Stage 2 of the study will be followed up to Week 132. The primary analysis will be based on the data accrued to Week 76; the data accrued to Week 132 will be used in secondary analyses.

1.1.7 Primary outcome measures

Stage 1
The primary efficacy outcome of the Stage 1 comparison is status at the end of follow-up i.e. the proportion of patients with a favourable outcome at Week 132 (as defined in section 11).

The primary safety outcome is the proportion of patients experiencing a grade 3 or greater adverse event during treatment or follow-up.

Stage 2
The primary efficacy outcome of the Stage 2 comparison is status at Week 76 i.e. the proportion of patients with a favourable outcome at Week 76 (as defined in section 11).

1.1.8 Sample Size

Stage 1
A total of at least 400 participants from sites in four or five countries will be randomised to either Regimen A or Regimen B in the ratio 1:2 (i.e. 133 allocated to Regimen A, and 267 allocated to Regimen B).

Stage 2
A total of at least 1155 participants from sites in a number of countries will be randomised to either Regimen A, Regimen B, Regimen C, or Regimen D in a ratio 1:2:2:2 (i.e. 165 allocated to Regimen A, 330 allocated to Regimen B, 330 allocated to Regimen C, and 330 allocated to Regimen D).

Overall sample size
The maximum sample size would be 1555 (400 for Stage 1 and 1155 for Stage 2).

Sites participating in Stage 1 and Stage 2 will transition from Stage 1 to Stage 2 randomisation scheme once the protocol amendment is locally approved. Only data from patients in these sites recruited after this transition will contribute to the analyses of Stage 2 in addition to data from sites only participating in Stage 2.

Recruitment to Stage 1 will end when a total of 400 patients have been recruited to Regimens A and B. Data from all patients recruited to Regimens A or B up to this point will be included in the Stage 1 analysis.

Therefore, the actual overall sample size will depend on the number of patients that will contribute to both the stage 1 and stage 2 analysis.

Figure 2: Trial recruitment stages

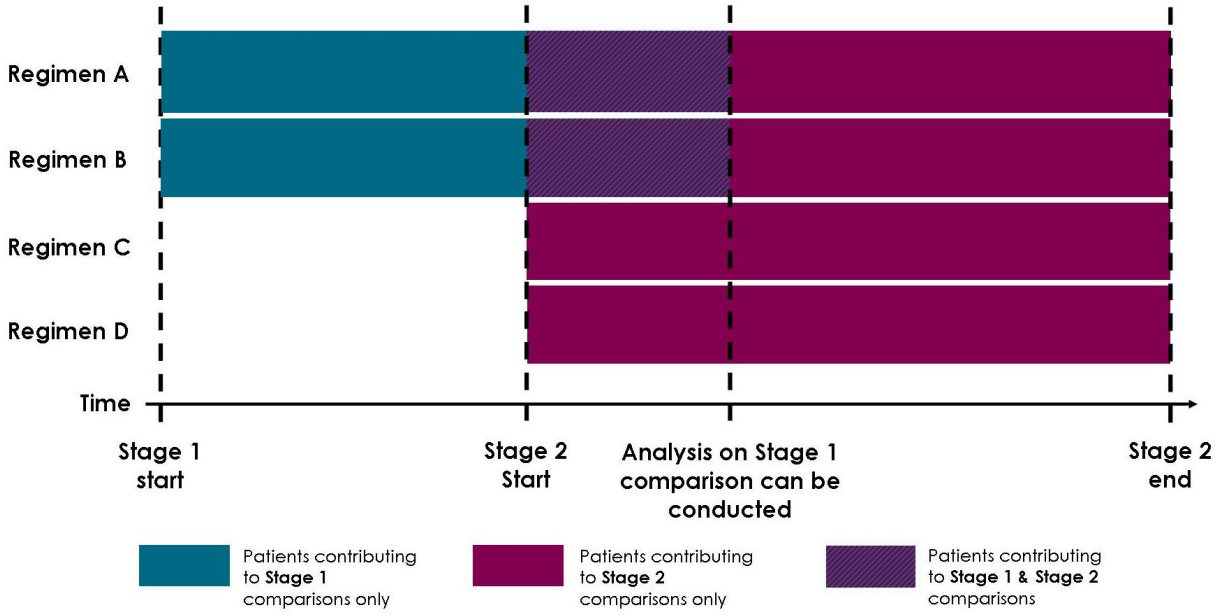
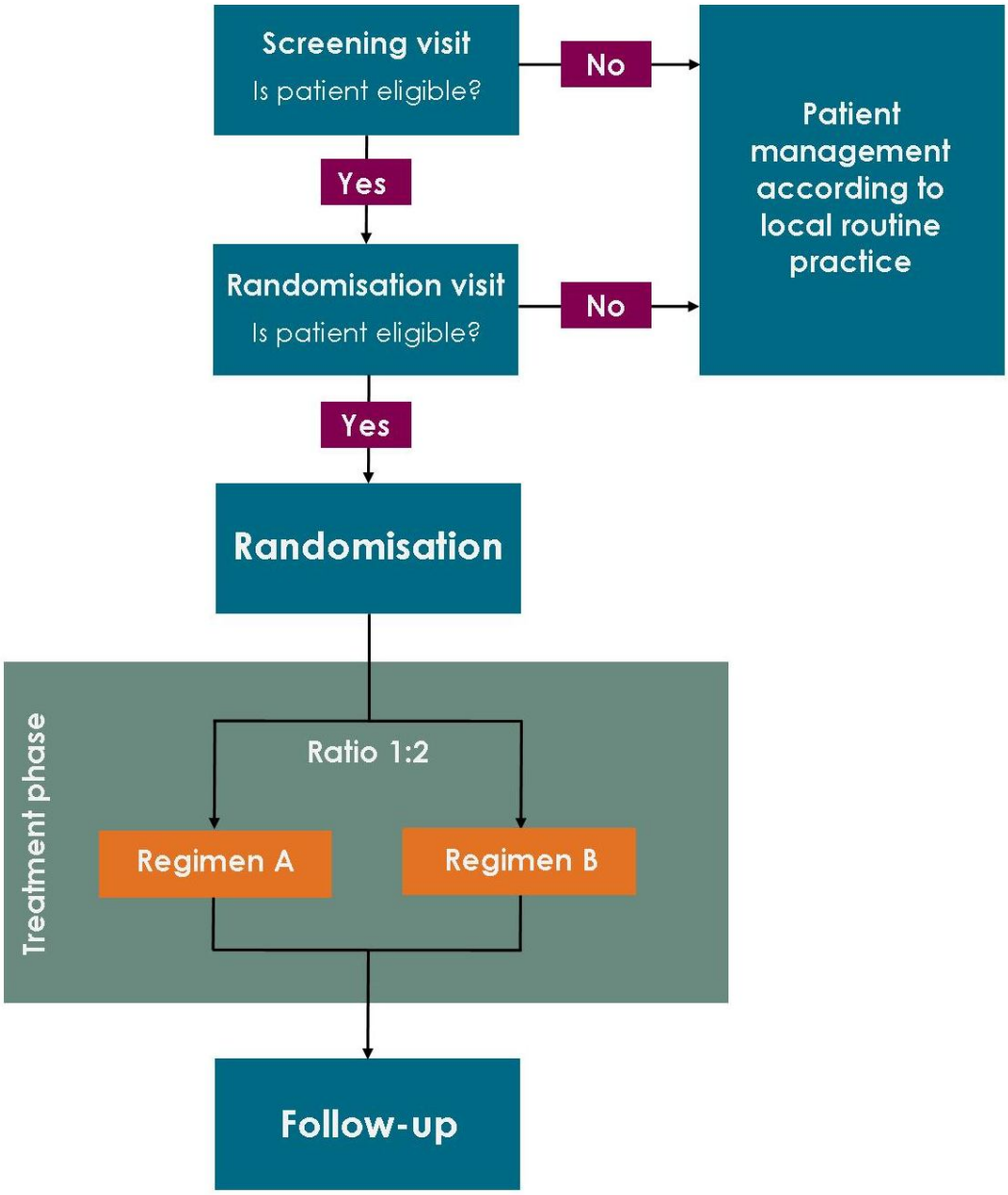
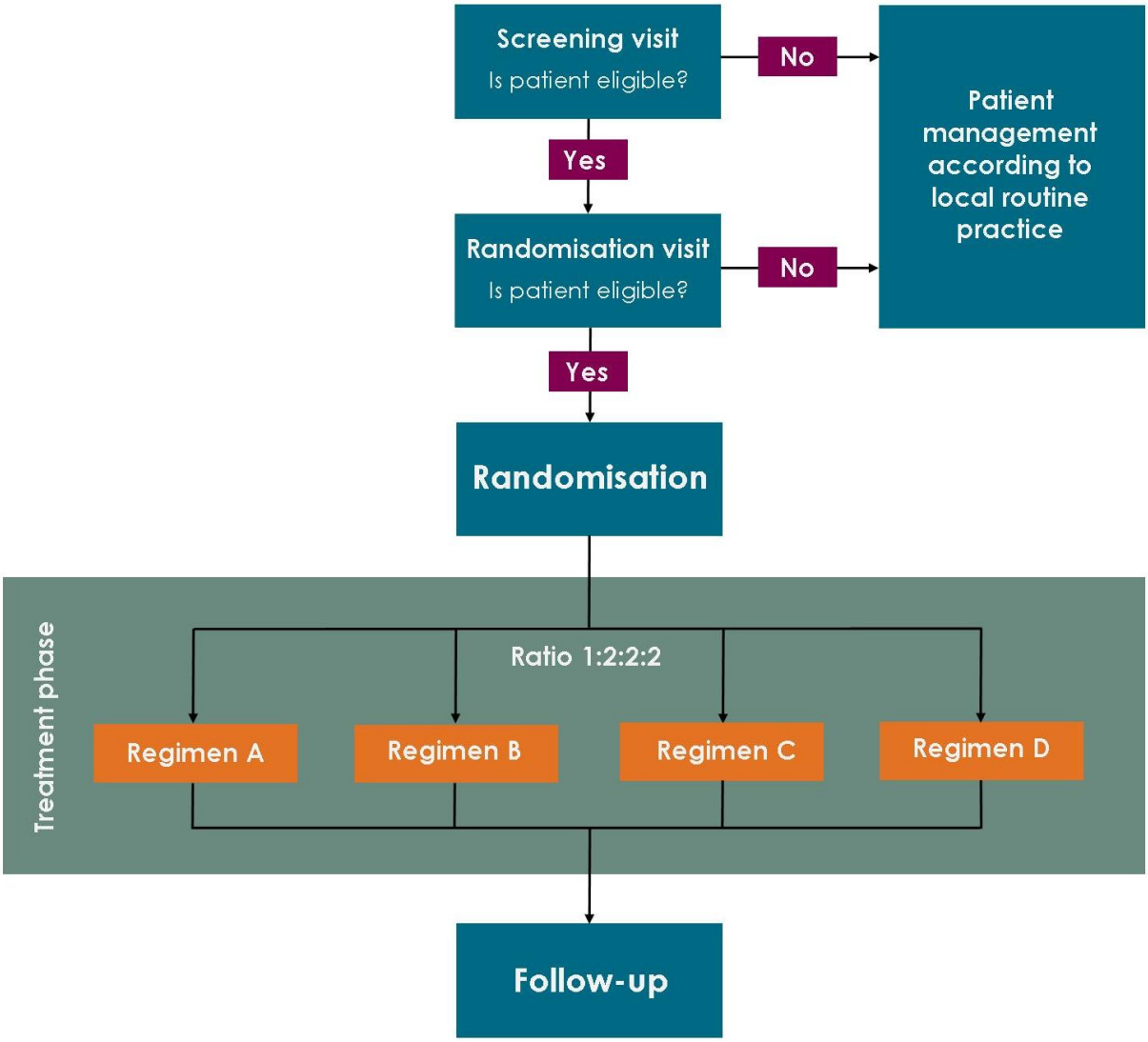


Figure 3: Stage 1 trial entry, randomisation, treatment and follow-up



Note. This figure applies to all sites during Stage 1 (i.e. before commencement of Stage 2), and to those sites only participating in Stage 1.

Figure 4: Stage 2 trial entry, randomisation, treatment and follow-up



Note. This figure applies only to those sites participating in Stage 2 of the STREAM trial once Stage 2 begins.

1.1.9 Blinding

Although the STREAM study is an open-label study, wherever possible it will be conducted masked to treatment allocation. The patient and treating clinician will be aware of treatment allocation, however, all laboratory assessments will be performed blind. See Section 10.1 for further details relating to blinding within the trial.

2 BACKGROUND

2.1 Introduction

Despite the availability of an efficacious and affordable six-month chemotherapy regimen and the definition of an efficient strategy to deliver treatment under direct observation to the majority of TB patients, TB control worldwide is impeded by two major issues: (i) the emergence of multidrug resistance (MDR) and (ii) co-existent HIV infection. The former hampers dramatically the efficacy of widely implemented standard short-course chemotherapy, thus limiting the success of efforts to fight against tuberculosis worldwide[2,3], and since 2002, at least one case of extensively drug-resistant tuberculosis (XDR-TB) has been reported from 45 countries[4]. The current recommended treatment approach for MDR-TB is based largely on expert opinion and there is a lack of good evidence on optimal management.

2.1.1 Relevant studies / trials

World Health Organisation (WHO) guidelines for the treatment for MDR-TB recommend an intensive phase of treatment based on at least four drugs known to be effective and given for a minimum of 20 months[5]. In the most recent WHO TB surveillance report[6], the size of most country cohorts in 2004 was too small to give reliable estimates of treatment outcomes in patients with MDR-TB. Of the nine countries with 100 or more patients, treatment success rates ranged from 73% in the Philippines and 71% in Latvia to 38% in Romania and 25% in Morocco. Results reported by some of the most important projects following these guidelines were disappointing, with cure rates rarely exceeding 80% even in the most favourable sub-group of previously untreated cases.

Further reports of treatment outcomes of patients with MDR-TB are only available from a small number of localised cohort studies, most with limited follow-up. It is likely that these studies represent some of the better rates of treatment outcomes from more well-controlled programmes. Of 238 patients enrolled on treatment for MDR-TB in Taipei from 1992 to 1996, 68 (29%) left treatment prior to its completion[7]. Among 76 MDR-TB patients (74% HIV positive) registered in the Lesotho national TB programme (NTP) between July 2007 and April 2008, 21 (29%) had died with 52 (68%) alive but still on treatment by October 2008[8]. Among 76 patients in a community-based treatment programme in Lima, Peru between August 1996 and February 1999, 17 (22%) died during treatment or in follow-up. Treatment was given for a median of 23 months with a median of six drugs[9]. Among 204 patients assessed retrospectively who began treatment for pulmonary MDR-TB in Latvia between January and December 2000, 135 (66%) patients were cured or completed therapy, 14 (7%) died, 26 (13%) defaulted, and treatment failed in 29 (14%)[10]. A recent meta-analysis reported on average 62% successful outcome and a mortality of 11%[11].

Van Deun et al (2010)[1] reported excellent long-term outcomes in a cohort of over 200 patients in Bangladesh with MDR-TB who were treated with a regimen given for only nine months. Such a regimen, if successful, would represent a considerable advance over current practice. Evaluation of this regimen is the objective of Stage 1 of STREAM.

Bedaquiline is a novel diarylquinoline antibiotic with bactericidal activity. In a phase II trial of patients with MDR-TB time to culture conversion was significantly less in patients receiving bedaquiline compared to those receiving an optimised background regimen only.[12] In December 2012 the US Food and Drug Administration (FDA) approved bedaquiline as part of the treatment regimen for MDR-TB when other agents are unavailable. Stage 2 of STREAM will investigate ways in which Regimen B could be improved either by removing the second-line injectable, which is associated with severe drug toxicity, or by shortening the regimen to 6 months.

2.1.2 Population

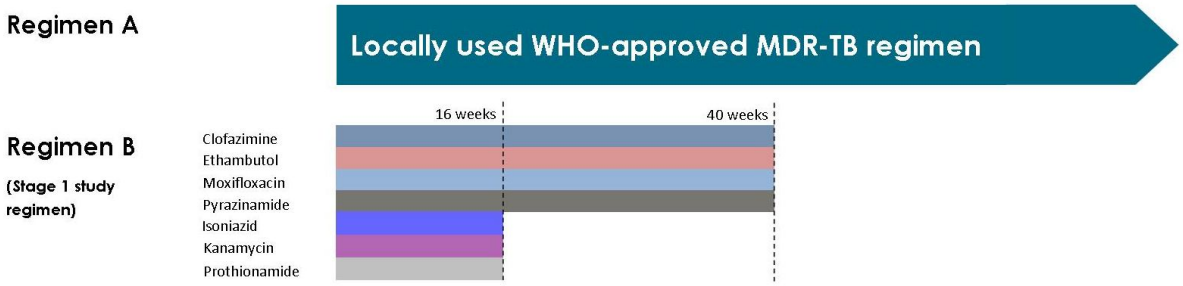
The study population consists of patients diagnosed with MDR-TB who fulfil the inclusion/exclusion criteria outlined in sections 5.1 and 5.2.

2.1.3 Investigational regimens

Stage 1

The investigational regimen in Stage 1 of the STREAM trial is Regimen B. This regimen consists of clofazimine, ethambutol, moxifloxacin, and pyrazinamide given for 40 weeks, supplemented by isoniazid, kanamycin, and prothionamide in the first 16 weeks, as illustrated in Figure 5.

Figure 5: Regimen A & Regimen B



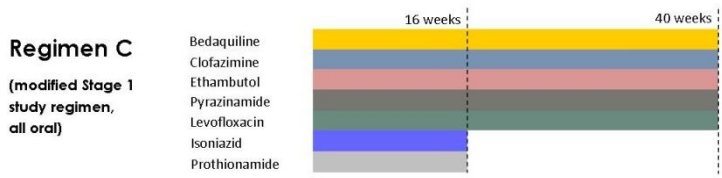
The only change in Regimen B from the regimen described by Van Deun[1] is that gatifloxacin has been replaced by moxifloxacin because gatifloxacin was withdrawn by the original marketing authorisation holder and generic sources investigated did not meet WHO requirements for quality, safety and efficacy.

Stage 2

Stage 2 involves Regimen A and Regimen B as described in Stage 1 above, with the addition of two investigational regimens: Regimen C and Regimen D. These two regimens are modifications of Regimen B, both of which include the newly licensed drug bedaquiline. In this Stage 2 Regimen B becomes the control regimen for Regimen C and Regimen D.

Regimen C is a 40-week, fully-oral regimen consisting of bedaquiline, clofazimine, ethambutol, levofloxacin, and pyrazinamide given for 40 weeks supplemented by isoniazid and prothionamide for the first 16 weeks (intensive phase). This is a modification of Regimen B, as illustrated in Figure 6, in which kanamycin is replaced with bedaquiline, and moxifloxacin is replaced with an alternative fluoroquinolone, levofloxacin, which has a better profile with respect to the potential for QT prolongation.

Figure 6: Regimen C (all oral)



Regimen D is a 28-week regimen consisting of bedaquiline, clofazimine, levofloxacin, and pyrazinamide given for 28 weeks supplemented by isoniazid and kanamycin for the first 8 weeks (intensive phase). This regimen is a modification of Regimen B, as illustrated in Figure 7, in which ethambutol and prothionamide are replaced with bedaquiline, and moxifloxacin is

replaced with an alternative fluoroquinolone, levofloxacin, and the total duration is reduced to 28 weeks. Figure 8 presents the phases of treatments across the four arms of the trial.

Figure 7: Regimen D (shortened)

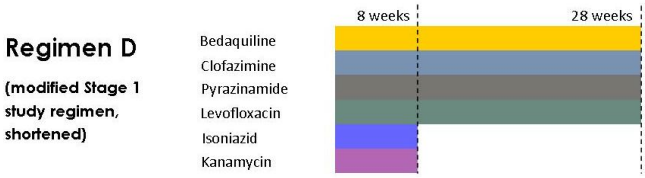
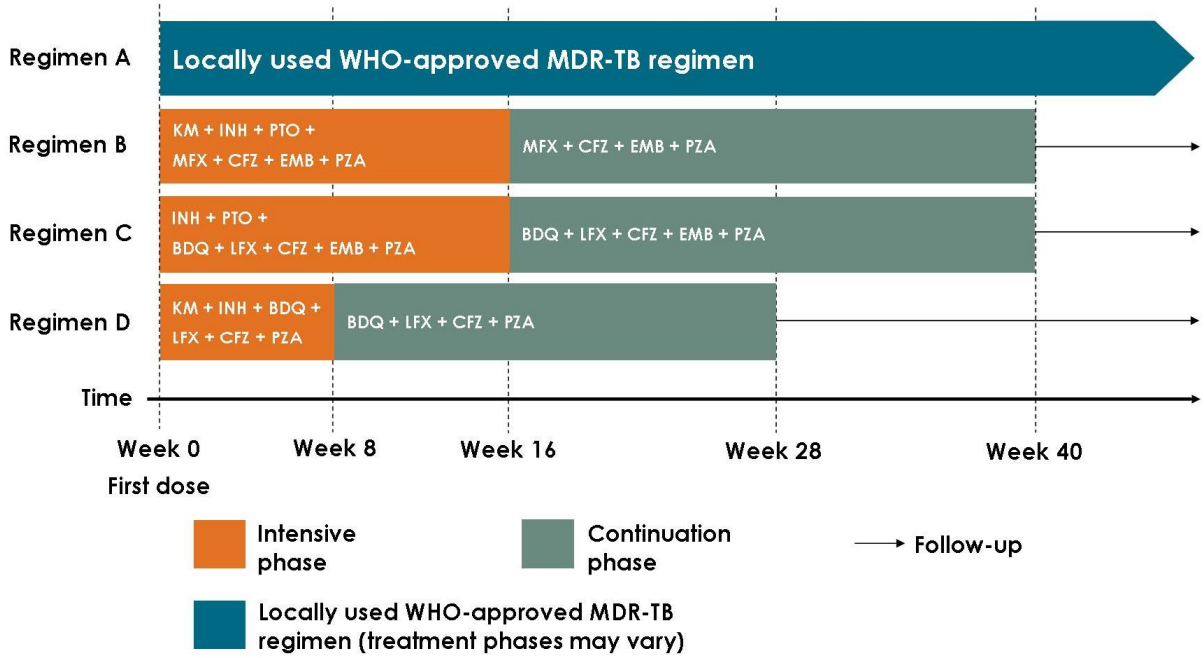


Figure 8: Treatment phases of investigational regimens



2.2 Rationale

Given the urgent need to increase access to treatment for MDR-TB, careful evaluation of treatment strategies is vital to ensure the most effective and feasible approaches are implemented, particularly in low-income settings where most cases of MDR-TB are found. New drugs with novel mechanisms of action for the treatment of MDR-TB are being evaluated. In addition, the maximisation of existing drugs is essential for the protection of new compounds for use in alternative regimens; clinical trials utilising these new compounds in treatment regimens are warranted.

The primary objective of Stage 1 of the STREAM trial is to assess whether Regimen B, which is based on the regimen used in Bangladesh,[1] is non-inferior to Regimen A, the locally-used WHO-approved MDR-TB regimen. Its practical, programme-based study design will also ensure that if the results are favourable they will be generalisable to routine programme settings.

In addition, health system and patient costs associated with implementation will be documented. These will be analysed in association with the clinical outcomes of the trial using the TREAT TB Impact Assessment Framework[13] in order to provide as much information as possible for subsequent policy and practice decision-making.

It was necessary to substitute moxifloxacin for gatifloxacin in Regimen B because the original manufacturer of gatifloxacin withdrew their product from the market due to reports of associated dysglycaemia, and it was not possible to identify a generic source of gatifloxacin that met WHO manufacturing norms and standards for quality, safety and efficacy. If,

therefore, Regimen B is found to be inferior to Regimen A, one possible explanation could be that moxifloxacin is less effective than gatifloxacin. However, moxifloxacin and gatifloxacin have similar bactericidal activity[14] and the trial will therefore test the regimen closest to the standardised regimen developed by Van Deun[1] that is available in routine program setting.

Stage 2 of the STREAM trial will involve the investigation of two alternative regimens, both variations on Regimen B, incorporating the newly available drug bedaquiline. The first of these investigational regimens, Regimen C, involves the removal of the injectable, kanamycin, in order to avoid the associated risks of ototoxicity and renal toxicity. The second investigational regimen, Regimen D, investigates the possibility of treatment being shortened to 28 weeks, with a shorter duration of kanamycin and isoniazid and also whether ethambutol, which is of questionable efficacy, and prothionamide, commonly associated with severe gastric symptoms, can be removed.

The first primary objective in Stage 2 is to assess the superiority of Regimen C over Regimen B; this is a US FDA requirement.

The other primary objectives of Stage 2, of particular relevance to treatment programs, are to assess whether Regimen C is not inferior to Regimen B and to assess whether Regimen D is non-inferior to Regimen B.

As the results of STREAM Stage 1 will not be available until late in enrolment for Stage 2, and due to the urgent public health and clinical need to improve treatment of MDR-TB, Stage 2 will start without randomised controlled trial evidence that Regimen B is non-inferior to Regimen A. Regimen B was selected as the control arm for Stage 2 after careful consideration of the benefits and risks; nonetheless recruitment to Arm A will be continued throughout Stage 2.

2.2.1 Risks and benefits

Regimens B, C and D are substantially shorter than regimens recommended by the WHO guidelines[5] and could therefore increase the risk of treatment failure or relapse, and the acquisition of additional drug resistance. However, this has not been observed in settings where variations of Regimen B have been used. In an updated analysis of over 500 patients in the Bangladesh cohort there was a relapse-free cure rate of 84.4% (95% confidence interval 81.3% - 87.6%),[15] which is at least as good, if not better, than the results achieved in programmes which follow WHO standardised treatment guidelines.

There is a risk that Regimen B will not be shown to be non-inferior to regimen A in Stage 1. The results may be equivocal, in which case the additional data from Stage 2 will help to resolve whether Regimen B is non-inferior or inferior to Regimen A. In the possible if unlikely event that Regimen B is found to be inferior to Regimen A secondary analyses comparing Regimens C and D with Regimen A will provide valuable information as to whether either or both of them are non-inferior to the WHO recommended regimen as well as comparing outcomes in Regimens C and D with Regimen B.

Regimen C and Regimen D may also carry greater risk of failure or relapse compared with Regimen B, due to the removal of kanamycin from Regimen C and the removal of prothionamide and ethambutol from Regimen D. For the same reason there may also be greater risk of acquisition of additional drug resistance in regimens C and D. However, this risk is believed to be small given that in stage 2 of the bedaquiline C208 trial no subject in the bedaquiline arm developed extensively drug-resistant (XDR) or pre-XDR infection with 24 weeks of dosing of bedaquiline compared to six participants in the control arm;[16] this compares with 9% reported from a recent follow-up of over 800 patients treated with the WHO recommended regimen.[17]

Restriction of the second-line injectables and prothionamide to the intensive phase may explain why no acquired resistance to these drugs was observed in the failure or relapse cases in the Van Deun[1] study. Although used with only one second-line drug in the continuation phase, acquired fluoroquinolone resistance did not occur, probably due to the relatively high fluoroquinolone dose used; baseline resistance to ofloxacin rarely resulted in an adverse bacteriological outcome. Moreover, study criteria limiting inclusion to cases with no LPA evidence of resistance to either fluoroquinolones or kanamycin is expected to almost certainly prevent amplification of resistance leading to extensively drug-resistant tuberculosis.

Most second-line drugs are associated with unpleasant and sometimes serious toxicities. The removal of prothionamide from Regimen D will avoid the gastric side effects which some patients find intolerable. The reduced duration of kanamycin in the 28-week regimen, Regimen D, and its removal from the fully oral 40-week regimen, Regimen C, will reduce the risk of kanamycin toxicity (renal damage and deafness).

Isoniazid is included in Regimen B, Regimen C, and Regimen D at higher doses than is usual. Similarly the fluoroquinolone doses, moxifloxacin in regimen B and levofloxacin in Regimen C and Regimen D, for patients in the higher weight bands are greater than the standard doses. However, the regimen given in Bangladesh was well tolerated and it is possible that the shorter duration of chemotherapy in Regimen B, Regimen C, and Regimen D may result in fewer severe adverse drug reactions than in Regimen A.

A summary of the safety information on the higher dose of moxifloxacin is provided in Appendix 1. The potential for QT prolongation is of particular concern and regular ECG monitoring is specified in the protocol (see Section 8.3.1).

Bedaquiline given in addition to a standardised background regimen has been shown to greatly reduce the time to culture conversion in MDR-TB (median 83 days compared to 125 days, HR 2.44, 95%CI 1.57-3.80). However, there is less clinical experience with bedaquiline than with the other drugs in the study regimens, and although generally well tolerated, a number of potential risks have been identified from preclinical and clinical studies and the findings from the C208 trial; these are outlined below and will be carefully monitored in the course of this study.

- **Mortality**

In Stage 2 of the bedaquiline C208 trial a difference in the number of deaths was observed between the bedaquiline group (10/79, 12.7%) and the placebo group (3/81, 3.7%), despite better microbiologic outcomes in the former. This imbalance remained in the pooled analysis of C208 Stage 1 and Stage 2. The reason for the increased overall mortality is as yet unclear; the causes of death were varied (the only cause of death reported more than once was death due to TB), and there was a wide range in time to death since last intake of bedaquiline/placebo (2-911 days). In addition, none of the deaths in the bedaquiline arm were considered related to study drug by the investigator. Mortality will be thoroughly evaluated in this study, which includes follow-up to 132 weeks for participants in Stage 2.

- **Liver toxicity**

In the bedaquiline C208 trial, the most frequently observed laboratory abnormalities that were more frequent in the bedaquiline than the placebo arm were increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT). There were two cases (one in a subject in the bedaquiline arm in C208 and one in a subject in the bedaquiline arm in C209) where the transaminase increases were accompanied by increased total bilirubin such that both subjects met laboratory criteria for Hy's law. There were no cases of severe hepatotoxicity attributed to bedaquiline by the investigators in the clinical studies, however in view of the relatively small study populations and the combination with other potentially hepatotoxic drugs in the STREAM regimens, it will be important to monitor this closely.

- **Pancreatic toxicity**

Pancreatic changes were observed in mice and dogs receiving bedaquiline that correlated with increases in amylase and lipase, without trypsin-like immunoreactivity increase. Although there is no clear evidence of an increased risk in the clinical studies, patients with an amylase more than twice the upper limit of normal will be excluded from Stage 2 of the trial and pancreatic function will be monitored closely.

- **Cardiac effects**

Based on the observation of cardiac myocyte degeneration and QT prolongation in dogs that received exposures in excess of the clinical exposure in toxicology studies, cardiac muscle effects are monitored in the current study by creatine kinase-MB and safety ECGs. However, no increases in these laboratory tests suggesting bedaquiline cardiac toxicity were noted in either stage of the bedaquiline C208 trial. QT prolongation has been observed with bedaquiline (a maximal increase in QTcF of 12.2 ms over 24 months in the bedaquiline C209 trial) and in those patients also taking clofazimine (n=17) the mean increase was 32ms.[18] In Stage 2 of STREAM, patients with a QTcF greater than or equal to 450 ms at screening will not be eligible and there will be regular ECG monitoring (see Section 8.3.1). As a consequence of the risk of QT prolongation with bedaquiline, moxifloxacin is contraindicated and has been replaced by levofloxacin (see Appendix 2 for levofloxacin safety summary) in the two bedaquiline-containing experimental regimens: Regimen C and Regimen D.

- **Musculoskeletal effects**

In some animal studies elevations of myoglobin and CK were observed, consistent with the known ability of cationic amphiphilic drugs to cause myopathies, which tend to occur only after prolonged dosing in humans or at high dose administration and are usually reversible after treatment cessation. No myopathies were reported in the clinical studies, but this will be kept under review in STREAM.

- **Reproduction**

Since the effects of bedaquiline on fetal development are unknown, the use of effective contraception is required for both male and female participants (see Section 5.1).

3 OBJECTIVES AND OUTCOMES

3.1 Stage 1 objectives

3.1.1 Stage 1 primary objectives

The primary objectives of the Stage 1 comparison of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen B is not inferior to that on Regimen A (WHO approved MDR-TB), the control regimen for Stage 1, at Week 132, using a 10% margin of non-inferiority.
2. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen B as compared to Regimen A.

3.1.2 Stage 1 secondary objectives

The secondary objectives of the Stage 1 comparison of the STREAM trial are:

1. To determine the proportion of patients with a favourable efficacy outcome on Regimen B in each country setting
2. To compare the economic costs incurred by patients and by the health system during treatment on Regimen B as compared to Regimen A.

3.2 Stage 2 objectives

3.2.1 Stage 2 primary objectives:

The primary objectives of the Stage 2 comparisons of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen C, the fully oral regimen, is superior to that on Regimen B, the control regimen for Stage 2 at Week 76
2. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen C, the fully oral regimen, is not inferior to that on Regimen B at Week 76, using a 10% margin of non-inferiority
3. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen D, the shortened regimen, is not inferior to that on Regimen B at Week 76, using a 10% margin of non-inferiority.

The first primary objective for the Stage 2 comparisons, to assess the superiority of Regimen C over Regimen B, is a requirement of the US FDA; the remaining primary objectives for the Stage 2 comparisons are of programmatic relevance.

In the event that non-inferiority is demonstrated in primary objective 3, Regimen D will be tested for superiority to Regimen B.

3.2.2 Stage 2 secondary objectives:

The secondary objectives of the Stage 2 comparison of the STREAM trial are:

1. To assess whether Regimen C is superior to Regimen B with regards to the proportion of participants with a favourable efficacy outcome at Week 132
2. To assess whether Regimen C is not inferior to Regimen B with regards to the proportion of participants with a favourable efficacy outcome at Week 132
3. To assess whether Regimen C is not inferior to Regimen A with regards to the proportion of participants with a favourable efficacy outcome at Week 132

4. To assess whether Regimen D is not inferior to Regimen B with regards to the proportion of participants with a favourable efficacy outcome at Week 132
5. To assess whether Regimen D is not inferior to Regimen A with regards to the proportion of participants with a favourable efficacy outcome at Week 132
6. To assess whether Regimen B is not inferior to Regimen A with regards to the proportion of participants with a favourable efficacy outcome at Week 132
7. To investigate the safety, including the effect on mortality and tolerability of 40 weeks of bedaquiline in combination with the other drugs of Regimen C
8. To investigate the safety, including the effect on mortality and tolerability of 28 weeks of bedaquiline in combination with the other drugs of Regimen D
9. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen B as compared to Regimen A
10. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen C as compared to Regimen B
11. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen D as compared to Regimen B
12. To evaluate the pharmacokinetics of bedaquiline and M2 in all participants randomised to Regimen C or Regimen D at sites selected for the PK study and assess pharmacokinetic/pharmacodynamics relationships of bedaquiline for safety and efficacy
13. To evaluate the pharmacokinetics of bedaquiline and M2 in a subset of HIV co-infected patients on Regimen C or Regimen D receiving antiretroviral treatment
14. To evaluate the 4β-hydroxycholesterol/cholesterol ratio as a measure of cytochrome P450 3A (CYP3A) activity
15. To compare the economic costs incurred during treatment by patients and by the health system in Regimen C and Regimen D as compared to Regimen B.
16. To compare the proportions of patients having undergone lung surgery (resection or pneumonectomy) by Week 76 and Week 132
17. To compare the development of resistance to background drugs, especially resistance leading to the development of pre-XDR or XDR strains of TB
18. To investigate the development of increased MIC to bedaquiline
19. To investigate the effect of baseline bedaquiline MIC on treatment.

3.3 Outcome measures

3.3.1 Stage 1

The primary efficacy outcome measure of the Stage 1 comparison is the proportion of patients with a favourable outcome at Week 132 as defined in section 11, Statistical Considerations.

For the Stage 1 comparison the primary safety outcome measure is the proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria[19], during treatment and follow-up.

Secondary outcome measures for the Stage 1 comparison include:

- Time to sputum smear conversion
- Time to sputum culture conversion
- Time to unfavourable efficacy outcome
- Time to cessation of clinical symptoms based on PI assessment
- All-cause mortality during treatment or follow-up
- Change of regimen for adverse drug reactions
- Number of serious adverse reactions occurring on treatment and during the follow-up period
- Adherence to treatment.

In selected sites, costs and acceptability of Regimens A and B to stakeholders will be analysed in terms of:

- Costs to the health system
- Household costs
- Patient treatment and support experiences
- Health worker experiences.

3.3.2 Stage 2

The primary efficacy outcome measure of the Stage 2 comparisons is the proportion of patients with a favourable outcome at Week 76 as defined in section 11, Statistical Considerations.

Secondary outcome measures of the Stage 2 comparisons include:

- Time to sputum culture conversion
- Time to sputum smear conversion
- Efficacy status at end of follow-up
- Time to unfavourable efficacy outcome
- The proportion of participants in each category who meet the WHO classification of outcome as applicable at the time of analysis, determined at Week 76 and Week 132
- Time to cessation of clinical symptoms based on PI assessment
- All-cause mortality during treatment or follow-up
- Proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria, during treatment and follow-up
- Change of regimen for adverse drug reactions
- Number of adverse events occurring on treatment and during the follow-up period
- Pharmacokinetic outcomes
- Adherence to treatment.

In selected sites, costs and acceptability of the four regimens to stakeholders will be analysed in terms of:

- Costs to the health system
- Household costs
- Patient treatment and support experiences
- Health worker experiences.

4 SELECTION OF SITES

Country selection is based on background disease burden of TB, MDR-TB, and TB-HIV co-infection. Sites within countries are selected to ensure sufficient numbers of MDR-TB cases to meet recruitment targets. Site suitability, based on the listed criteria in Section 4.1, will be evaluated during pre-trial and feasibility assessments.

4.1 Site inclusion criteria

Participating sites are required to meet the following criteria:

- Experience in treating MDR-TB patients
- Support from the Tuberculosis Control Programme at national or regional level
- A local Principal Investigator (PI) who is a TB specialist and experienced in the treatment of MDR-TB who will oversee the patients throughout the trial, (there may be more than one PI per country)
- Suitable treatment site staff and facilities
- Treatment site staff willing to recruit all eligible patients into the trial (site would ideally function as a single coordinating/recruiting facility and work with satellite sites for treatment and follow-up)
- Acceptable plans for close supervision of patients in treatment and follow-up
- Willing to offer HIV testing to all patients wishing to participate in the trial and routinely available HIV clinical management services (including provision of antiretroviral therapy (ART))
- A network of well-functioning AFB smear microscopy laboratories and a reference laboratory already performing cultures, with a system of quality assurance
- Ability to export sputum culture for testing to ITM, Antwerp, if required
- Ability to get authorisation of importation for the medicines which will be procured and delivered by The Union.
- Agreement to use specified standardised bacteriological methods
- Availability of rapid genotypic line-probe drug susceptibility testing (LPA DST) for rifampicin, second-line injectables and fluoroquinolones of the required quality (or ability to quickly build capacity for this testing)
- Acceptable infection control procedures consistent with WHO guidance.

4.2 Local trial management

The staff members concerned in the management of the study patients at each site will form a Local Management Committee, under the direction of the local Principal Investigator(s). This committee (including a member of the laboratory staff) will meet at regular intervals to discuss the progress of the trial at the site. A brief report of the discussions will be sent to the STREAM Trial Manager.

5 SELECTION OF PATIENTS

Patients will be recruited into the trial from tuberculosis clinics in the catchment area of the main site. The target population is patients with pulmonary TB and evidence of resistance to at least rifampicin[20] (including patients sensitive to isoniazid).

5.1 Patient inclusion criteria

A patient will be eligible for randomisation into the study (Stage 1 or Stage 2) if he/she:

1. Is willing and able to give informed consent to participate in the trial treatment and follow-up (signed or witnessed consent if the patient is illiterate)
2. Is aged 18 years or older
3. Has a positive AFB sputum smear result at screening (at least scanty), unless they are HIV positive in which case a positive GeneXpert result within four weeks prior to screening is sufficient
4. Has evidence of resistance to rifampicin either by line probe assay (Hain Genotype[21]), GeneXpert or culture-based drug susceptibility testing (DST), from a test performed at screening or from a test performed within the four weeks prior to screening
5. Is willing to have an HIV test and, if positive, is willing to be treated with ART in accordance with the national policies but excluding ART contraindicated for use with bedaquiline
6. Is willing to use effective contraception: pre-menopausal women or women whose last menstrual period was within the preceding year, who have not been sterilised must agree to use a barrier method or an intrauterine device unless their partner has had a vasectomy; men who have not had a vasectomy must agree to use condoms. In Stage 2 pre-menopausal women or women whose last menstrual period was within the preceding year, who have not been sterilised must agree to use two methods of contraception, for example a hormonal method and a barrier method.
7. Resides in the area and expected to remain for the duration of the study.

In addition to the criteria above, for Stage 2 only, a patient will be eligible for randomisation to the study provided he/she:

8. Has had a chest X-ray at that is compatible with a diagnosis of pulmonary TB (if such a chest X-ray taken within 4 weeks of randomisation is available, a repeat X-ray is not required)
9. Has normal K_+, Mg_{2+} and corrected Ca_{2+} at screening.

5.2 Patient exclusion criteria

A patient will not be eligible for randomisation into the study (Stage 1 or Stage 2) if he/she:

1. Is infected with a strain of *M. tuberculosis* resistant to a second-line injectables by line probe assay (Hain Genotype[21])
2. Is infected with a strain of *M. tuberculosis* resistant to a fluoroquinolone by line probe assay (Hain Genotype[21])
3. Has tuberculous meningitis or bone and joint tuberculosis
4. Is critically ill, and in the judgment of the investigator, unlikely to survive more than 4 months
5. Is known to be pregnant or breast-feeding
6. Is unable or unwilling to comply with the treatment, assessment, or follow-up schedule
7. Is unable to take oral medication
8. Has AST or ALT more than 5 times the upper limit of normal for Stage 1, and AST or ALT more than 3 times the upper limit of normal for Stage 2
9. Has any condition (social or medical) which in the opinion of the investigator would make study participation unsafe
10. Is taking any medications contraindicated with the medicines in any trial regimen

11. Has a known allergy to any fluoroquinolone antibiotic
12. Is currently taking part in another trial of a medicinal product
13. Has a QT or QTcF interval at screening or immediately prior to randomisation of more than or equal to 500 ms for Stage 1, and more than or equal to 450 ms for Stage 2.

In addition to the criteria above, for Stage 2 only, a patient will not be eligible for randomisation to the study if he/she:

14. Has experienced one or more of the following risk factors for QT prolongation:
    - A confirmed prolongation of the QT or QTcF more than or equal to 450 ms in the screening ECG (retesting to reassess eligibility will be allowed once using an unscheduled visit during the screening phase)
    - Pathological Q-waves (defined as Q-wave more than 40 ms or depth more than 0.4-0.5 mV)
    - Evidence of ventricular pre-excitation (e.g., Wolff Parkinson White syndrome)
    - Electrocardiographic evidence of complete or clinically significant incomplete left bundle branch block or right bundle branch block
    - Evidence of second or third degree heart block
    - Intraventricular conduction delay with QRS duration more than 120 ms
    - Bradycardia as defined by sinus rate less than 50 bpm
    - Personal or family history of Long QT Syndrome
    - Personal history of cardiac disease, symptomatic or asymptomatic arrhythmias, with the exception of sinus arrhythmia
    - Syncope (i.e. cardiac syncope not including syncope due to vasovagal or epileptic causes)
    - Risk factors for Torsades de Pointes (e.g., heart failure, hypokalemia, or hypomagnesemia)
15. Has received treatment for MDR-TB in the 12 weeks prior to screening, other than the maximum permitted treatment specified in Section 5.2.1
16. Has a history of cirrhosis and classified as Child's B or C at screening or a bilirubin more than 1.5 times upper limit of normal.
17. Has an estimated creatinine clearance (CrCl) less than 30 mL/min based on the Cockcraft-Gault equation
18. Is HIV positive and has a CD4 count less than 50 cells/mm^3
19. Has amylase elevation more than two times above the upper limit of normal
20. Has a history of alcohol and/or drug abuse
21. Has had previous treatment with bedaquiline
22. Has taken rifampicin in the seven days prior to randomisation
23. There has been a delay of more than four weeks between the screening consent and randomisation
24. Is an employee or family member of the investigator or study site staff with direct involvement in the proposed study.

Patients found not to be eligible for the STREAM trial due to a laboratory abnormality or previous treatment may be rescreened once. In addition, if a patient has not been randomised within four weeks since screening, then they become ineligible for the trial and may be rescreened once. A patient who fails a second screening is permanently excluded from trial participation. Patients who are rescreened should be reconsented and given a new screening number for the second screening. All patients who are not eligible for the STREAM trial will be managed according to local routine practice.

5.2.1 MDR-TB treatment prior to screening and randomisation

Patients who are AFB smear positive who have received second-line treatment in the 12 weeks prior to screening are permitted to have had up to a maximum of four weeks treatment. However, if they have received more than seven days treatment they are only eligible for the trial if FDA vital staining of their sputum is positive.

To minimise the number of patients recruited without positive baseline cultures, HIV-infected, AFB smear-negative patients are not eligible if they have had MDR-TB treatment in the 12 weeks prior to screening.

MDR-TB treatment should not be initiated or continued between screening and randomisation unless clinically necessary or mandated by local policy, and if used may not exceed three weeks.

5.3 Number and source of patients

Stage 1

A total of at least 400 participants from sites in four to five countries will be randomised to either Regimen A or Regimen B in the ratio 1:2 (i.e. 133 allocated to Regimen A, and 267 allocated to Regimen B) in Stage 1.

Stage 2

A total of at least 1155 participants from a number of countries will be recruited to either Regimen A, Regimen B, Regimen C, or Regimen D in a ratio 1:2:2:2 (i.e. 165 allocated to Regimen A, and 330 allocated to each other regimen).

If recruitment to Regimen A is stopped following IDMC review of Stage 1 data, then the overall sample will be less than 1155 patients. Recruitment to Stage 2 will be completed when 330 patients are randomised to each of arms B, C and D.

Overall sample size

The maximum sample size would be 1555 (400 for Stage 1 and 1155 for Stage 2).

Sites participating in Stage 1 and Stage 2 will transition from Stage 1 to Stage 2 randomisation scheme once the protocol amendment is locally approved. Only data from patients in these sites recruited after this transition will contribute to the analyses of Stage 2 in addition to data from sites only participating in Stage 2.

Recruitment to Stage 1 will end when a total of 400 patients have been recruited to Regimens A and B. Data from all patients recruited to Regimens A or B up to this point will be included in the Stage 1 analysis.

Therefore, the actual overall sample size will depend on the number of patients that will contribute to both the stage 1 and stage 2 analysis.

5.4 Screening procedures

Written informed consent **must** be obtained from the patient before any protocol-specific screening procedures are carried out. Each consenting patient will be assigned a study number which will be used to identify the patient throughout the study.

5.4.1 Screening visit

At the first (screening) visit, the study, including potential risks and benefits of joining the trial, will be explained to prospective participants. This will include a general overview of the trial purpose and procedures as well as the samples to be collected at this visit. Each patient will be asked to sign (or provide a thumb print in the presence of a witness if illiterate) for the

screening procedures and will be given a copy of the signed informed consent form and a patient information sheet to take home.

After giving consent for screening, patients will be assigned a unique study number by entering their name on to the next line of a screening register and evaluated for their eligibility according to the inclusion and exclusion criteria.

Patients who at the time of screening have documented results of second line drug (SLD) resistance tests that provide evidence of resistance to fluoroquinolones or second-line injectables are not eligible for the trial.

The following investigations will be undertaken:

- Sputum sample(s) obtained for:
  - AFB smear
  - Culture
  - Rifampicin resistance testing by LPA or GeneXpert (unless there is a phenotypic result from another reliable source indicating rifampicin resistance from a sputum sample taken no longer than four weeks from date of screening)
  - Line probe assay (Hain Genotype[21]) for second-line injectable and fluoroquinolone, if rifampicin resistant
- Blood samples obtained for:
  - HIV antibodies to be tested in local laboratories (unless there is documentation to show that the patient is already known to be HIV positive)
  - CD4 count and viral load (if patient is HIV positive)
  - Liver function tests (AST and ALT)
  - Pancreatic amylase
  - Serum potassium, calcium and magnesium
  - Creatinine clearance
  - TSH
  - Thyroxine or free thyroxine
- Urine sample for HCG pregnancy test
- 12 lead ECG (one ECG for Stage 1 and triplicate ECGs within 5 minutes for Stage 2).

Inconclusive LPA results at screening should be repeated on a second sample collected from the patient as soon as possible. Confirmation of rifampicin resistance is required before randomisation; patients with inconclusive LPA results for second-line drugs may be included if otherwise eligible.

If a patient is screened successfully and satisfies the criteria to participate in the STREAM trial, the patient should be randomised no more than 4 weeks after screening consent.

If patients are successfully screened, further information and testing is required at the Randomisation visit (see Section 6.1).

5.5 Late identification of drug-resistance or drug-sensitivity

5.5.1 Late identification of drug-resistance

In sites that currently undertake phenotypic second-line drug susceptibility resistance testing (DST), results from pre-treatment samples that provide evidence of fluoroquinolone, or second-line injectable resistance may become available after randomisation. In such cases, the patient's clinical progress should be taken into account before making any changes on the basis of the results, in consultation with the central clinical team. However, if patients are found to have XDR-TB (defined as resistance to fluoroquinolones **and** second-line injectables

from phenotypic DST) these patients should be withdrawn from trial treatment and treated according to national guidelines.

5.5.2 Late identification of rifampicin-sensitivity

Any patients whose initial TB infection is found to be sensitive to rifampicin, after they have been randomised, should be removed from participation in the trial and managed according to the National Tuberculosis Programme (NTP).

6 RANDOMISATION PROCEDURE

6.1 Evaluations at the randomisation visit

Patients will need to be re-assessed for eligibility when returning after their screening visit. The time between the screening consent and randomisation visits should be kept as short as logistically possible, but should be no more than four weeks; those returning after four weeks will have to be re-screened prior to randomisation. Patients who have been on MDR-TB treatment since screening and have had more than three weeks of treatment between the screening consent and randomisation are not eligible for trial participation (see section 5.2.1)

Patients attending the randomisation visit will be given further information about the trial and what would be expected of them in terms of follow-up visits and procedures. If they are still willing to take part, they will be asked to sign an participation informed consent form (or give a thumb print in the presence of a witness if illiterate) and will be given a signed copy to take home together with the Patient Information Sheet. Patients who are ineligible or do not wish to take part will be referred to the NTP for further management.

Once an eligible patient has given consent to participate in the trial, the following will be done:

- Interview to obtain and confirm demographic details, medical history (prior diagnoses and treatment, concomitant disease and medication, smoking history, and current symptoms) and key information on asset ownership to document socio-economic status will be requested at sites participating in the health economic component of the study
- Record contact information
- Record alcohol use
- Clinical examination including height, weight and vital signs (temperature, systolic and diastolic blood pressure (BP) and pulse rate)
- Visual acuity test
- Hearing test (audiometry if available at site, else whisper test)
- Collect two sputum samples, one early morning and one spot (for smear and culture)
- Urinalysis (dipstick)
- A urine pregnancy test (if pre-menopausal woman)
- Serum creatinine, serum potassium, blood glucose, haemoglobin and CD4 count for HIV positive patients
- Posteroanterior (PA) chest X-ray, unless a good quality film is available that has been taken no longer than four weeks prior to randomisation; if possible this should be a digital x-ray
- 12-lead ECG immediately before randomisation and at 4 hours after the first dose of allocated trial treatment.

In addition to the above, in Stage 2 the following investigations will also be undertaken:

- Other tests listed in the serum chemistry panel in section 8.2
- Hepatitis A, B, and C
- Triplicate pre-randomisation 12-lead ECG will be conducted;

If any of the ECGs performed pre-randomisation show a QT or QTcF greater than or equal to 450 ms then the patient will be ineligible for the trial.

At sites participating in sample storage, all patients providing their consent to participate in the study will also be asked to provide their consent for the biostorage of additional specimens for biomarker tests. These samples will be stored for the discovery and validation of TB drug effect biomarkers. Those providing their consent for biostorage of their specimens

will be requested to give blood (at randomisation and at 16 weeks). If human genetic testing is to be performed, specific consent will be sought.

6.2 Allocation of treatment (following randomisation)

In each stage of the trial, patients will be randomised using a web-based randomisation system. Access to the web-based system will be controlled through an authorised username and password. Before treatment allocation the patient's eligibility will need to be confirmed, their site and HIV status, and CD4 count entered into the database. Local laboratory results can be used to determine a patient's HIV status and CD4 count for randomisation, unless central laboratory results are available at the time of randomisation.

Separate randomisation lists for each combination of strata will be prepared in advance, for each site according to whether they are participating in Stage 1 only, Stage 1 and 2, or Stage 2 only, by a statistician independent of the study, using varying block sizes. Should web access not be available at the time of randomisation, a manual alternative using sealed envelopes will be provided.

Stage 1 (before Stage 2 begins, or for sites only participating in Stage 1)

Patients will be randomised to Regimen A or Regimen B. Randomisation will be in a 1:2 ratio in favour of Regimen B to allow more data on efficacy and safety to be collected on this regimen. Randomisation will be stratified by (1) site, (2) HIV status for sites with high TB-HIV co-infection rates.

Stage 2

For sites participating in Stage 1 and Stage 2, and site participating in Stage 2 only, when Stage 2 starts, patients will be randomised to Regimen A, Regimen B, Regimen C, or Regimen D. Randomisation will be to a ratio of 1:2:2:2 in favour of Regimen B, Regimen C, and Regimen D. Randomisation will be stratified by (1) site, (2) HIV status & CD4 count status (i.e. three categories: HIV-negative, HIV-positive with low CD4 count of less than 350 cells/mm^3, or HIV-positive with high CD4 count of more than or equal to 350 cells/mm^3), for all sites.

7 TREATMENT OF PATIENTS

7.1 Introduction

During Stage 1 of the STREAM trial, all patients will be randomised to receive either Regimen A or Regimen B. Once Stage 2 commences, for those sites participating in both stages all newly recruited patients will be randomised to Regimen A, Regimen B, Regimen C, or Regimen D. Some sites may recruit only to Stage 1. Other sites may only participate in Stage 2.

7.2 Trial interventions

7.2.1 Stage 1

The control regimen for the Stage 1 comparison Regimen A, is the locally-used WHO-approved MDR-TB regimen. Country- or site-specific regimens are described in the STREAM Patient Management Guide.

The investigative regimen for the Stage 1 comparison is Regimen B, and consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for 40 weeks, supplemented by kanamycin, isoniazid and prothionamide in the first 16 weeks.

All drugs are given daily (seven days a week), except for kanamycin which is initially given daily and then thrice-weekly from Week 12 onwards in Regimen B.

The intensive phase may be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks, respectively, as described in section 7.3.2.

Table 1: Regimen B doses

Product	Weight group		
	Less than 33 kg	**33 kg to 50 kg**	**More than 50 kg**
Moxifloxacin	400 mg	600 mg	800 mg
Clofazimine	50 mg	100 mg	100 mg
Ethambutol	800 mg	800 mg	1200 mg
Pyrazinamide	1000 mg	1500 mg	2000 mg
Isoniazid	300 mg	400 mg	600 mg
Prothionamide	250 mg	500 mg	750 mg
Kanamycin	15 mg per kilogram body weight (maximum 1g)		

7.2.2 Stage 2

The control regimen for the primary comparisons of Stage 2 is Regimen B, the details for which are given above in section 7.2.1. The investigative regimens for the Stage 2 comparisons are Regimen C and Regimen D. Regimen A (as described in Section 7.2.1) is continued in Stage 2 and data from these patients will be included in secondary analyses at the 132 week endpoint.

Regimen C is an all-oral regimen that is a modification of Regimen B, and consists of bedaquiline, clofazimine, ethambutol, levofloxacin, and pyrazinamide given for 40 weeks, supplemented by isoniazid and prothionamide in the first 16 weeks (intensive phase). All

drugs are given daily (seven days a week), except for bedaquiline which is given daily for the first two weeks and then thrice-weekly from Week 2 onwards. The intensive phase can be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks, respectively, as described in section 7.3.2. If the intensive phase is extended beyond 16 weeks, then the overall treatment time will be extended except for bedaquiline, which will not be given for longer than 40 weeks in total.

Table 2: Regimen C doses, given daily unless otherwise stated

Product	Weight group		
	Less than 33 kg	**33 kg to 50 kg**	**More than 50 kg**
Bedaquiline	400 mg once daily for first 14 days/200 mg thrice weekly thereafter		
Levofloxacin	750 mg	750mg	1000 mg
Clofazimine	50 mg	100 mg	100 mg
Ethambutol	800 mg	800 mg	1200 mg
Pyrazinamide	1000 mg	1500 mg	2000 mg
Isoniazid	300 mg	400 mg	600 mg
Prothionamide	250 mg	500 mg	750 mg

Regimen D is a shortened regimen that is a modification of Regimen B, and consists of bedaquiline, clofazimine, levofloxacin, and pyrazinamide given for 28 weeks, supplemented by kanamycin and isoniazid in the first eight weeks (intensive phase). All drugs are given daily (seven days a week) except for bedaquiline and isoniazid which are given daily for the first two weeks and then thrice-weekly from Week 2 onwards. The intensive phase can be extended from 8 to 12 weeks and 12 to 16 weeks for patients with a smear positive of 2+ or more at 8 or 12 weeks, respectively, as described in section 7.3.2. If the intensive phase is extended, kanamycin will be given then thrice-weekly from Week 8 onwards.

Table 3: Regimen D doses, given daily unless otherwise stated

Product	Weight group				
	Less than 33 kg	**33 kg to less than 40 kg**	**40 kg to 50 kg**	**More than 50 kg to 60 kg**	**More than 60 kg**
Bedaquiline	400 mg once daily for first 14 days/200 mg thrice weekly thereafter				
Levofloxacin	750 mg	750 mg		1000 mg	
Clofazimine	50 mg	100 mg		100 mg	
Pyrazinamide	1000 mg	1500 mg		2000 mg	
Isoniazid	400 mg	500 mg	600 mg	800 mg	900 mg
Kanamycin	15 mg per kilogram body weight (maximum 1g)				

Regimen D uses a higher dose of isoniazid than in the other treatment regimens in the trial. The dose of isoniazid has been kept to a maximum dose of 15 mg/kg (given daily for the first two weeks only). At this dose, isoniazid is likely not to cause excessive adverse effects, the intention being to reach peak serum levels above the median resistance MIC of

around 5 µg/ml, shown by the majority of strains with isoniazid resistance (i.e. the katG mutations) and not only the rarer strains with very low MICs due to inhA mutations

During Stage 2, patients allocated to Regimen B, Regimen C or Regimen D will be prescribed pyridoxine to help minimize the risk of isoniazid-related peripheral neuropathy.

All drugs should be given under directly observed treatment (DOT) by a treatment supervisor. Treatment supervisors may be clinic staff or family members or other members of the community, depending on local circumstances. At the end of the intensive phase of the regimens, drug doses should be adjusted to allow for changes in patient's weight.

7.2.3 Medicines supplies

Supplies for Regimen A will be provided by the participating countries. The sponsor/funders will distribute the drug requirements for Regimen B, Regimen C, and Regimen D. Bedaquiline will be supplied as 100mg oral tablets from commercial stock manufactured for Janssen Products, LP. Details of drug supplies, storage and distribution are provided in the STREAM Pharmacy Plan.

7.2.4 Treatment cards

Following randomisation, the patient and/or a treatment supervisor will be given the relevant Treatment Card and a prescription to take to the pharmacy. The treatment supervisors will be instructed about observing the patient swallowing their oral medication according to intake schedule (directly observed treatment) and recording treatment taken on the treatment card. Treatment Cards should be returned at each visit and a new card issued.

7.3 Treatment procedures

7.3.1 Dispensing and supervision of medicines

Local policy will be followed as to whether the patient will be admitted to hospital during the intensive phase irrespective of the regimen allocated.

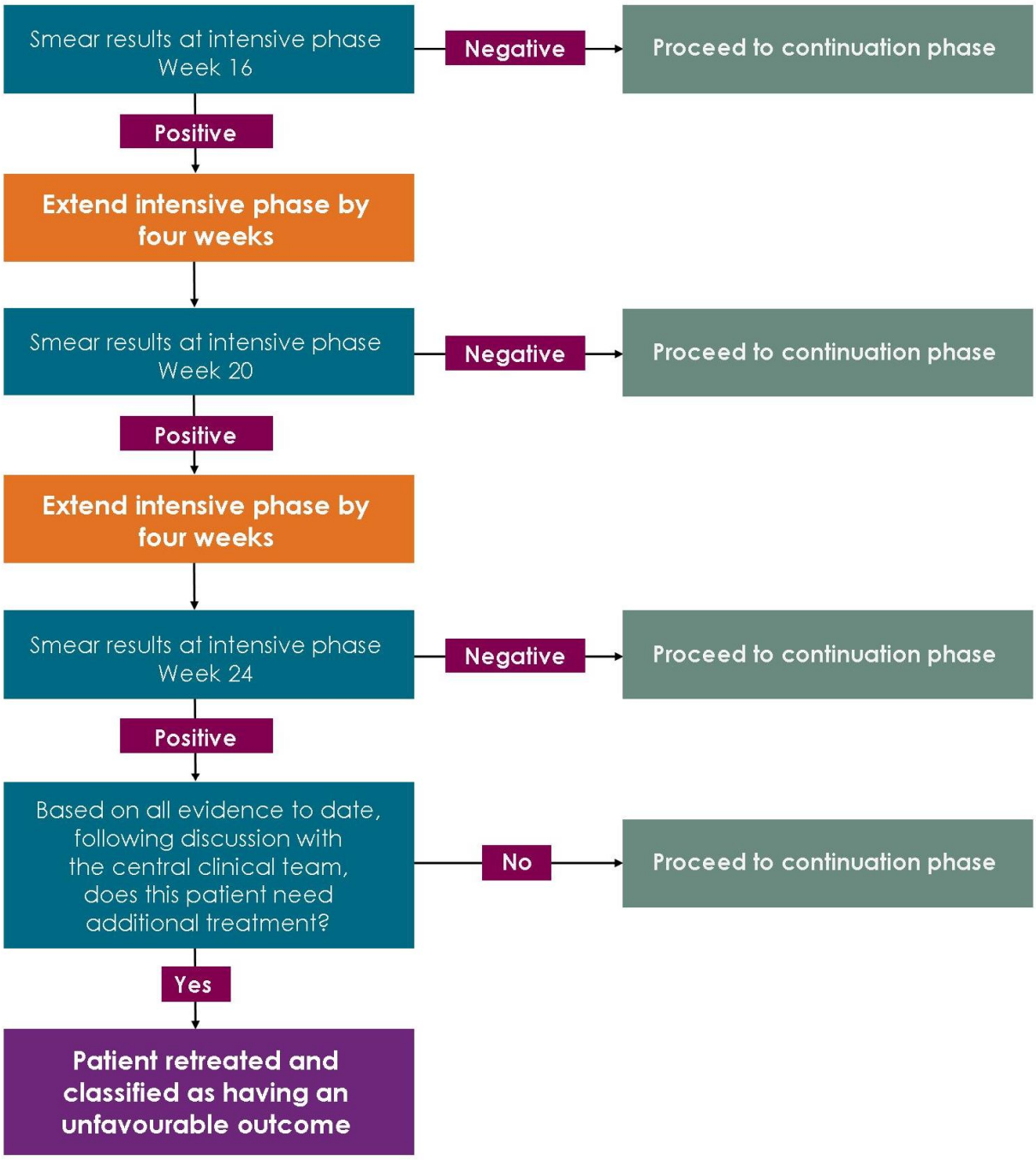
All medicines in Regimen B, Regimen C and Regimen D should be given according to the intake schedule under strict conditions of direct observation of treatment (seen to be swallowed) by a trained treatment supervisor for the whole treatment period. For Regimen A, sites will be strongly encouraged to follow the same standard. Full details of the medicines, regimen, including dosages, for each patient and of the procedure to be followed are also given on each Treatment Card.

The pharmacy staff will maintain drug accountability logs and provide, on a regular basis, a reconciliation report (between products delivered, in stock, dispensed and returned).

7.3.2 Transition from intensive to continuation phase in the regimens

For patients allocated to Regimen B, Regimen C, or Regimen D the following algorithm will be used in Stage 1 and Stage 2 to determine when a patient can proceed from the intensive to the continuation phase.

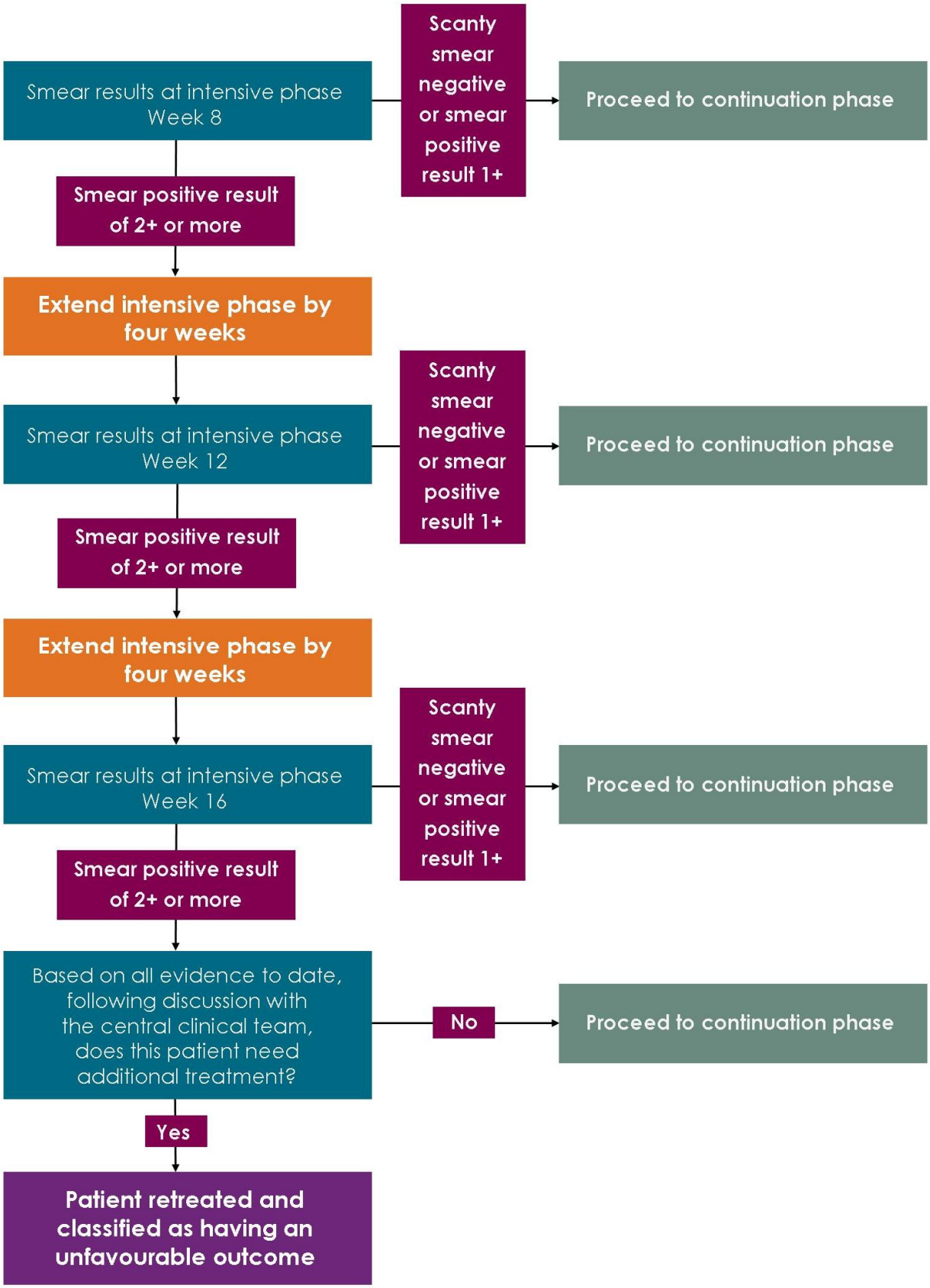
Figure 9: Transition from intensive to continuation phase for patients on Regimen B and Regimen C



Note: smear results based on regular AFB ZN or auramine staining and not FDA vital staining.

Patients randomised to Regimen B or Regimen C will receive 40 weeks of treatment (16 weeks intensive phase plus 24 weeks continuation phase). In the event of positive (at least "scanty" on the IUATLD/WHO scale) AFB smear, the drugs in the intensive phase of these regimens may be extended by 4 weeks twice, allowing a maximum total duration of 48 weeks treatment (except for bedaquiline, which will be given for a maximum duration of 40 weeks regardless of whether the regimen is extended).

Figure 10: Transition from intensive to continuation phase for patients on Regimen D



Patients randomised to Regimen D will receive 28 weeks of treatment (eight weeks intensive phase plus 20 weeks continuation phase). As Regimen D has shorter intensive phase duration than the other treatment regimens, it is expected that more patients would have a smear positive result at the end of the intensive phase due to the shorter time that patients will have been on treatment to that point. Therefore, the less stringent criterion of a smear positive

result of 2+ is sufficient for patients to require an extension of the intense period, i.e. patients can have a positive smear of 1+ and still advance on to the continuation phase, as opposed to Regimen B and Regimen C for which any positive smear result would result in an extension to the intensive phase. In the event of a 2+ or more positive smear, the drugs in the intensive phase of the regimen may be extended by 4 weeks twice, allowing a maximum total duration of 36 weeks treatment.

The procedure for transition from the intensive to the continuation phase in Regimen A will be according to local policy.

7.3.3 Procedure following missed treatment

At the discretion of the investigator, any days missed in either the intensive or the continuation phase may be made up by extending this phase of the regimen by the number of days, with the exception of bedaquiline. Although small amounts of missed bedaquiline may be made up, any patient who has missed 14 consecutive days or more should not be given any further bedaquiline

For managing patients who have had a treatment interruption due to toxicity, refer to Section 8.5.1.

7.3.4 Adherence assessment and counselling

At each visit, patients will be counselled about the importance of taking their medication and the dangers of developing further resistance if they fail to do so.

7.3.5 Pregnancy & breastfeeding

It is possible that some of the drugs in the regimens, if given to a pregnant woman, will harm the unborn child. As pregnant women in this study population have alternative treatment options, they should not enrol in this trial; neither should women who plan to become pregnant during the trial. Women who could become pregnant must use appropriate contraception (as defined in the inclusion criteria in section 5) while on treatment. Women who are pre-menopausal, or whose last menstrual period was less than one year ago, will be asked to have a pregnancy test before taking part to ensure that they are not pregnant. Any woman who finds that she has become pregnant while taking part in the trial should immediately tell her study doctor who will contact a member of the STREAM clinical team to discuss management of the patient.

All pregnancies occurring in a patient or partner of a patient, in the trial, at any point during treatment or follow-up will be followed for outcome even if the pregnancy continues beyond 132 weeks from randomisation in Stage 1 and beyond 132 weeks in Stage 2.

Women taking part in Stage 2 of the trial, and are recruited to Regimen C or Regimen D, should not breastfeed when taking bedaquiline, as the effects to their new-born child are unknown. Women who have a new-born child should consult their physician about the best way to feed their child.

7.4 Non-trial treatment

The following medications are disallowed during administration of study drug:

- The systemic use of moderate and strong CYP3A4 inhibitors (e.g., azole antifungals: ketoconazole, fluconazole, voriconazole, itraconazole, ketolides such as telithromycin; and macrolide antibiotics) for more than 2 weeks
- The systemic use of strong CYP3A4 inducers (e.g., phenytoin, carbamazepine, phenobarbital, St. John's wort [*Hypericum perforatum*], rifamycins, and systemic, multiple dosing of dexamethasone). The examples of potent CYP3A4 inhibitors and inducers do not form a complete list. The investigator should consult the label information, and if necessary contact the appropriate sponsor representative.

Drugs that are known to prolong the QT interval should not be used outside of the trial allocated treatment. The following list includes some examples, but is not comprehensive:

- Antiarrhythmics Class IA, e.g. quinidine, hydroquinidine, disopyramide
- Antiarrhythmics Class III, e.g. amiodarone, sotalol, dofetilide, ibutilide
- Certain neuroleptics, e.g. phenothiazines, pimozide, sertinodole, haloperidol, sultopride
- Tricyclic antidepressive agents
- Certain antimicrobials, e.g. moxifloxacin, gatifloxacin, sparfloxacin, erythromycin IV, pentamidine
- Delaminid
- Certain antimalarials, e.g. halofantrine
- Certain antihistamines, e.g. terfenadine, astemizole, mizolastine
- Others: cisaprid, vincamine IV, bepedril, diphemanil.

7.4.1 Permissible ART

Only the following types of ART are permissible during administration of regimens:

- Triple nucleoside reverse transcriptase inhibitor (NRTI) based regimen, e.g. a regimen made up of zidovudine, lamivudine, and abacavir, or in accordance with local standard of care
- Nevirapine (NVP) based regimen consisting of NVP in combination with any two NRTIs
- Lopinavir/ritonavir (Kaletra™) based regimen consisting of lopinavir/ritonavir (Kaletra™) in combination with any two NRTIs.

For patients on Regimen C or Regimen D alternative ART should not be introduced until more than 4 weeks after the last dose of bedaquiline.

The investigator must assess the risks and benefits of these antiretroviral regimens in the context of co-infection with MDR-TB, acknowledging the following caveats:

a) Triple NRTI is generally not considered optimal chronic ART;
b) Nevaripine based regimens are associated with higher ART failure in subjects having or known to have previously had a viral load more than or equal to 100,000/ mL

8 ASSESSMENTS AND FOLLOW-UP

8.1.1 Assessment schedule

See Table 4 in Section 8.1.1 for details of the assessments required for each visit for Stage 1, and Table 5 in Section 8.1.2 for details of the assessments required for each visit for Stage 2.

The intensive phase of treatment may be extended for late smear conversion or missed treatment (see sections 7.3.2 and 7.3.3). The continuation phase may also be extended for missed treatment.

For selected sites, at randomisation and every 12 weeks thereafter, patients will be interviewed to document the costs e.g. transport and hospitalisation costs, incurred by them in adhering to the regimen. System costs will also be estimated.

Stage 1

In Stage 1, patients will be assessed at screening, randomisation (Week 0), Week 1, Week 2, Week 3, Week 4, and at 4-weekly intervals throughout the study, until the end of follow-up, irrespective of whether on treatment or in the post-treatment follow-up phase.

Sputum for smear and culture will be collected at every visit, except at Week 1, Week 2, and Week 3, when no samples will be collected. At most visits this will be a single specimen during Stage 1, unless otherwise indicated in section 8.1.1. When two samples are required, if a patient does not bring an early morning sample, two spot samples will be collected at the visit.

Stage 2

In Stage 2, patients will be assessed at screening, randomisation (Week 0), Week 1, Week 2, Week 3, Week 4, after which they will be seen 4-weekly until Week 52, after which they will be seen 8-weekly until Week 84, after which they will be seen 12-weekly until Week 132 post randomisation.

A minimum of two sputum specimens should be collected at the screening and randomisation visit (with a third being an early morning sample if possible).

Two sputum specimens will be collected at every subsequent visit for smear and culture, except at Week 1, Week 2, and Week 3, when no samples will be collected. Because early morning samples are preferred, at the conclusion of each visit patients should be given a sputum container for sample collection to be presented at their next visit. The second sample will be taken as a spot sample at the time of clinic attendance. If a patient does not bring an early morning sample, two spot samples will be collected at the visit.

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Table 4: Assessment schedule – for all patients recruited in Stage 1

Observation/ Investigation	Screening	Randomisation	Treatment Phase			Post-Treatment Phase
			Intensive Phase		Continuation Phase	Follow-up
			Weeks 1 - 3	Weeks 4 onwards		
Written informed consent	X	X				
Demographics	X	X				
Medical History	X	X				
Clinical Examination	X	X	X	X	X	X
Clinical assessment (including AEs and concomitant medication during treatment)	X	X	X	X	X	X
Height		X				
Weight		X	X	X	X	X
Simple hearing test		X	If clinically indicated			
HIV antibody test	X					
CD4 (in HIV positive patients)		X	According to national guidelines			
Haemoglobin		X				
AST and ALT	X		X	X		
Serum creatinine		X	X	X		
Serum potassium		X	X	X	If clinically indicated	
Blood glucose		X				
Urinalysis (dipstick)		X	X	X		
Urine: hCG Pregnancy test	X	X	If clinically indicated			
Chest X-ray		X				
ECG (12-Lead)	X	X[4]	X	Weeks 4 & 12	Weeks 24 & 36	
Sputum smear and culture[3]	X[1]	X[2]		X[1]	X[1]	X[1]
Sputum for drug resistance testing	X[7]					
Patient's costs (in selected sites)		X		X	X	X
Blood sample for storage (if patient consents)		X			X[5]	

X indicates assessments required at particular visits.

[1] At least one sample will be collected per visit, except at the final visit of each phase of treatment and at the Week 132 follow-up visit, when two samples will be collected.

[2] At least two samples will be collected at this visit.

[3] All positive strains post-randomisation onwards will be shipped to the reference laboratory for full drug susceptibility testing.

[4] One ECG will be done prior to randomisation, and others at 4 hours after administering the first dose of treatment.

[5] One sample will be collected for storage at 16 weeks, for patients consenting to sample storage.

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[7] Sputum will be collected for LPA sensitivity testing for resistance to rifampicin, fluoroquinolones and second-line injectables. If results for fluoroquinolones and second-line injectables sensitivity are inconclusive, then these tests need to be repeated on a new sputum sample before randomisation.

Table 5: Assessment schedule – for all patients recruited in Stage 2

The following assessment schedule applies to *all* treatment arms in the STREAM trial as soon as Stage 2 begins (for sites participating in Stage 2).

Observation / Investigation	Screening	Randomisation	Treatment Phase			Post-Treatment Phase
			Intensive Phase		Continuation Phase	Follow-up
			Weeks 1 – 3	Weeks 4 onwards		
Written informed consent	X	X				
Demographics	X	X				
Medical History	X	X				
Alcohol Use Questionnaire		X		Week 16	Week 32	Week 52
Clinical Examination	X	X	X	X	X	X
Clinical assessment (including AEs and concomitant medication during treatment)	X	X	X	X	X	X
Height		X				
Weight		X	X	X	X	X
Visual acuity and colour tests		X		Week 12 (and if symptoms)	Week 28 and 40 (and if symptoms)	
Hearing test		X	Week 1 (If clinically indicated)	Week 8 and 16	Week 28 and 40	Weeks 52, 76, 104 and 132
Haemoglobin		X				
HIV antibody test	X					Week 76[16]
CD4 (in HIV positive patients)	X	X	According to national guidelines, at end of BDQ dosing and at end of study			
Viral load (in HIV positive patients)	X	X		X[14]	X[14]	X[14]
Hepatitis A, B and C testing		X				
Urinalysis (dipstick)		X	X	X	X	X
Urine: HCG Pregnancy test	X	X	If clinically indicated, at end of BDQ dosing and at end of study			
Chest X-ray[15]		X				
ECG (12-Lead)[3,4]	X	X	X	X	X	X
Additional Post-Dose ECG (12 Lead)		X	Week 2	Weeks 4, 12 & 16	Weeks 24, 32, & 40	
Sputum smear and culture[2]	X[1]	X[1]		X[1]	X[1]	X[1]
Sputum for drug resistance testing	X[13]					
Patient's costs (in selected sites)		X		X[12]	X[12]	X[12]
Blood sample for storage (if consents)		X			X[5]	
PK samples[7,8]		X[9]	Week 2	Weeks 4, 12 & 16	Weeks 24, 32, & 40	Weeks 44, 76, 120 & 132
Laboratory safety tests[10]	X	X		X	X	X
TSH & thyroxine of free thyroxoine	X					Weeks 40 and 120

X indicates assessments required at particular visits

[1] At screening and randomisation two samples will be collected, with an additional third early morning sample if possible. Two samples will be collected at each subsequent visit, ideally one early morning and one spot sample, or two spot samples if the patient does not provide an early morning sample.

[2] Screening, randomisation, and all positive isolates of MTB post-randomisation from week 8 onwards will be shipped to the reference laboratory for full drug susceptibility testing.

[3] Triplicate ECGs will be conducted prior to randomisation (within 5 minutes), further triplicate ECGs will then be conducted 4 hours after administering treatment at the randomisation visit. Triplicate ECGs will then be collected at each visit until Week 76. In participants who have QTcF increases from baseline, triplicate ECGs will be collected at each visit until it returns to less than a 10ms increase above the baseline value.

[4] For patients on arms C and D, enrolled at sites that have been pre-selected for the PK sub-study , triplicate ECG will also be conducted 4 hours after administering treatment at the week 2, 4, 12, 16, 24, 32 and 40 visits

[5] One sample will be collected for storage at 16 weeks, for patients consenting to sample storage.

[7] The PK samples will be collected predose and post-dose (sample from Week 2 visit). Details of PK sampling are specified in section 8.2.1.

[8] Samples for analysis of the plasma concentration of nevirapine (NVP) and lopinavir (LPV)/ritonavir(RTV) must be taken before intake of ARV and study drug. An additional pre-dose sample will be collected if the antiretroviral treatment regimen of a patient is changed, followed by sampling at time points indicated in the Assessment Schedule.

[9] Sample for analysis of the plasma concentration of nevirapine (NVP) and lopinavir (LPV)/ritonavir (RTV) and 4 β OH-cholesterol 10 See Section 8.2 for blood test details.

[11] Hearing test will be conducted at the first visit of the continuation phase.

[12] Collected every 12 weeks from after randomisation.

[13] Sputum will be collected for drug sensitivity testing for resistance to rifampicin, fluoroquinolones and second-line injectables. If results for fluoroquinolones and second-line injectables sensitivity are inconclusive, then these tests need to be repeated on a new sputum sample before randomisation.

[14] Viral load collected at Week 12, Week 24, Week 40, and Week 76.

[15] A Chest X-ray is required at randomisation that is compatible with a diagnosis of pulmonary TB, however if a a good quality X-ray is available that was taken in the 4 weeks prior to randomisation it does not need to be repeated

[16] HIV test at week 76 (for patients who were found to be HIV negative at screening). For patients found to be HIV positive at this visit a week 76 viral load measurement should also be taken.

8.2 Blood tests for Stage 2

For patients recruited in Stage 2, a blood sample for hepatitis A (IgM), hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb) and hepatitis C virus (HCV) antibody testing will be collected at the randomisation visits

Blood samples for complete blood count (CBC)* and serum chemistry** will be measured at every scheduled evaluation (this excludes visits at weeks 1, 2, and 3) until the end of the trial (as referred to as 'laboratory safety tests' in Table 5.

* The CBC panel includes: RBC, WBC, platelets, Hb level, heamatocrit, MCV and MHC.

** The serum chemistry panel includes: sodium, serum bicarbonate, calcium (uncorrected), calcium (corrected for albumin), serum potassium, magnesium, chloride, blood glucose, blood urea nitrogen, serum creatinine, alkaline phosphatase, pancreatic amylase, human serum albumin, total protein, AST, ALT, total cholesterol, ALP, creatine phosphokinase, gamma-glutamyltransferase, creatine phosphokinase of muscle brain, total direct-indrect bilirubin, triglycerides, lipase, lactate dehydrogenase, uric acid, blood urea nitrogen, CK, CK-MB.

8.2.1 Pharmacokinetic (PK) Evaluations

In Stage 2, the pharmacokinetics of bedaquiline will be assessed in all subjects from Regimen C and Regimen D enrolled at sites that have been pre-selected for the PK sub-study.

To inform on drug-drug interactions of anti-tuberculosis drugs with ARTs, additional PK analysis will be performed in HIV positive patients who are enrolled into the PK sub-study; approximately 60 subjects who are receiving lopinavir/ritonavir (with a maximum of 20 subjects from Regimen D) and approximately 30 subjects who are receiving nevirapine (with a maximum of 10 participants from Regimen D) treatment. The dosing history (doses and dates of doses) of the respective ART up to one month prior to randomisation will be recorded in the CRF.

8.2.2 PK Blood Sampling

Venous blood samples for PK evaluation will be collected from all subjects at sites pre-selected for the PK sub-study at pre-specified time points outlined below and in table 6. Samples will be collected, processed and shipped to the central laboratory as instructed in a laboratory manual provided separately.
All blood samples, with the exception of Baseline, will be quantified for the determination of plasma concentrations of bedaquiline and M2. 4β-hydroxycholesterol will be quantified from PK samples collected at Baseline, and pre-dose samples of Week 4, 12, 24, and 40 for an assessment of the ratio to cholesterol.
Pre-dose blood samples from HIV positive patients will also be quantified for lopinavir/ritonavir or nevirapine.

A pre-dose PK sample will be collected at randomisation and during study visits at Weeks 2, 4, 12, 16, 24, 32, 40, and at each of the follow-up visits at Week 44, 76, 120 and 132. At each of these visits, the pre-dose sample will be collected within 1 hour before the next scheduled bedaquiline intake (i.e. at Week 2 between 23-25 hours and after Week 2 between 47-49 or 71-73 hours after the intake.) A second post-dose sample will be collected at Week 2, 12, 24 and 40 (Week 40 post-dose sample only for subjects in Regimen C.) At each of these visits, the post-dose sample will be collected 4-5 hours after the bedaquiline dose and within 20 minutes of the triplicate ECG (see 8.3.1).

An additional sample should be collected at any visit when BDQ is withheld for suspected toxicity. If the antiretroviral treatment regimen of a patient is changed during the 40-week treatment period for Regimen C or the 28-week treatment period for Regimen D, a pre-dose sample should be collected, followed by collections as described above.

The exact date and time of each PK blood sample and the respective previous 2 doses of bedaquiline will be recorded in the CRF. For participants receiving lopinavir/ritonavir or nevirapine, the last three doses and dates of the ART prior to each PK sample time-point will be recorded in the CRF.

Table 6: PK Sample Collection Timings

Time of sample
Pre-dose randomisation
Pre-dose Week 2
Post-dose Week 2
Pre-dose Week 4
Pre-dose Week 12
Post-dose Week 12
Pre-dose Week 16
Pre-dose Week 24
Post-dose Week 24
Pre-dose Week 32 = Week 32 follow up visit for arm D patients
Pre-dose Week 40
Post-dose Week 40 = Week 40 follow up visit for arm D patients
Week 44
Week 76
Week 120
Week 132

8.2.3 Bioanalysis

The bioanalysis will be performed by a contract research organization (CRO), under the supervision of the Sponsor and/or Janssen. Samples will be analysed using a validated and sensitive liquid chromatography-mass spectrometry/mass spectrometry method.

8.3 Procedures for assessing safety

Throughout this study, patients will be closely monitored for signs and symptoms of drug toxicity. All toxicities leading to the study therapy being temporarily or permanently discontinued and all Grade 3 or greater toxicity effects will require thorough investigation with relevant clinical and laboratory tests, as clinically indicated.

These should be repeated as needed until final resolution or stabilisation of the toxicity; if this is after the end of the study, follow-up will be the responsibility of the treating clinician. All symptoms and laboratory findings will be graded according to severity using DAIDS criteria. Laboratory events will be reported as adverse events only if clinically significant. If the patient has a medical diagnosis at randomisation whose signs or symptoms worsen during the study to a Grade 3 or greater, this is a notable event (NE) that must be reported. Other notable events and SAEs will be reported as they occur to the MRC CTU, as well as to other bodies required to be notified in each country. For details of safety reporting see Section 13.

8.3.1 ECG Monitoring

Several of the drugs used in the STREAM regimens have the potential to prolong QT, therefore ECG monitoring is used in both Stage 1 and Stage 2 to identify and manage patients who are at risk.

In Stage 1, all patients will have a 12-lead ECG immediately prior to randomisation and will be ineligible if the QT or QTcF interval is more than or equal to 500ms. An ECG will be recorded 4 hours after the first dose of trial treatment. Further ECGs will be performed weekly for the first four weeks, and then every four weeks until 52 weeks after randomisation. If a patient has a QT or QTcF of 450ms or more, then a second ECG should be conducted at least 10 minutes after the first ECG.

In Stage 2, triplicate ECGs (three 12-lead ECGs within 5 minutes) will be used throughout the study. At screening, if there is a QT or QTcF measurement of any of the three ECGs of 450 ms or more the patient will not be eligible for randomisation. ECG monitoring for Stage 2 involves triplicate 12-lead ECGs undertaken at baseline (pre and 4 hours post-dose at the randomisation visit), weekly for the first four weeks and at every visit until week 76. All patients whose QTcF at week 76 is higher than the mean of their baseline recordings will continue to have 12-lead ECG monitoring at every visit until the confirmed QTcF is less than 10 ms above the mean baseline value or below 450 ms.

In addition, patients in arms C and D enrolled at sites that have been pre-selected for the PK sub-study, should also have ECGs within 4 hours after administering treatment at the week 2, 4, 12, 16, 24, 32 and 40 visits.

Any QT or QTcF prolongation to more than or equal to 500 ms while on treatment is considered a notable event and should be reported immediately to MRC CTU (See Section 13).

Patients found to have a QT or QTcF more than or equal to 500 ms on a 12-lead ECG at any point during treatment will be further investigated. If confirmed, the investigator should attempt to identify the cause, including checking and correcting abnormal K^{+}, Ca^{+2}, and Mg^{+2}. If the patient is taking any drugs suspected of causing QT prolongation they should be withheld and the tests repeated to try to identify the cause; if in doubt re-introduction of the suspect drug may be used to confirm the cause. Further details are provided in the Patient Management Guide.

8.4 Post-treatment schedule

After completion of treatment the patient will be reminded of the need for follow-up visits by the Principal Investigator, or recruiting clinician, and be informed of the date of their next visit.

During the follow-up visits, the following procedures will be undertaken:

- Clinical investigations (as outlined above) will be carried out
- Patients will be asked about any adverse events that may have occurred after their last visit and any concomitant medications they may have received.

There may be times when the PI requests additional tests for a patient depending on their disease progression at a particular visit.

8.5 Other study considerations

8.5.1 Interruptions to treatment

The treatment regimens or selected drugs may be interrupted at the discretion of the local PI or treating clinician:

- For a serious adverse event
- For a QT or QTcF measurement of more than or equal to 500ms

- If ALT/AST rise to more than or equal to 5 times ULN, or if AST/ALT rise to more than or equal to 3 times ULN in the presence of a total bilirubin rise to more than or equal to 2 times ULN
- If the investigator decides to withhold treatment in the interest of the safety and well-being of the participant.

If treatment is interrupted for a suspected serious drug reaction, attempts should be made to identify the drug concerned. After resolution of the suspected adverse reaction, treatment may be gradually re-introduced until the allocated regimen has been re-instituted. However, there must be no more than a 14 day interruption of bedaquiline, and bedaquiline dose modification is not permitted. After an interruption of bedaquine of less than or equal to 14 days it should be restarted at 200mg thrice weekly. If bedaquiline is stopped for more than 14 consecutive days, then it must not be started again.

In the event that the local PI considers that treatment needs to be modified or changed, he or she should inform the coordinating site by submission of an SAE/NE form and discuss treatment plans with a member of the central clinical team.

8.5.2 Missed visits

For each patient, clinic staff will obtain or confirm contact information. In the event that a patient misses a scheduled appointment, a Home Visitor will try within the week following the missed appointment to establish communication with the patient and/or treatment supervisor through all possible means which they have approved and while protecting their confidentiality (e.g. by telephone if this is possible, writing to the patient and contacts, and/or visiting the patient's home or workplace). All attempts to locate a patient following each missed appointment should be documented in their records. The need to attend all scheduled follow-up visits will be emphasised to all study patients at every visit.

8.5.3 Visit after a missed appointment

Patients who miss their scheduled appointment will be contacted and arrangements made for a new appointment. If patients are not successfully reached by phone/text messaging, a home visit should be made and the outcome recorded on a home visit form.

Patients returning after missed appointments will have procedures for the visit closest to their total time in follow-up performed (e.g. if a patient returns to the clinic at or near to week 16 after missing their visits for weeks 8 and 12, the visit for that day should be recorded as week 16). Subsequent visits will continue as scheduled. However, treatment to be prescribed should be determined by the actual number of days on which a patient has taken their medication and not by the length of time they have been in the study.

8.5.4 Loss to follow-up

If a patient does not return to the clinic before the study is closed, a Final Form will be completed at the time of study closeout, after reasonable effort to contact the patient has been made. The form should indicate that the patient was lost to follow-up. The "loss to follow-up" designation cannot be made for any patient until after the patient's scheduled Week 132 visit.

8.5.5 Follow-up of patients discontinued from treatment

Every effort should be made to follow up all patients for the full duration of the study, including those whose treatment is discontinued or whose treatment is changed, at a reduced

frequency, if necessary, which has been agreed with the STREAM coordinating site (MRC CTU), unless the patient has specifically withdrawn consent for further follow-up. In this event, a final status form should be completed. In patients who have discontinued from the study the vital status should be obtained every six months until Week 132, if necessary by telephone follow up.

If a patient can be contacted and declines further study participation, an investigation into their reasons will be conducted, and the reasons documented. An attempt will be made to have him/her come to the clinic for a final visit, or at least obtain a sputum sample for the assessment of the primary efficacy outcome.

8.5.6 HIV

Patients who are known to be HIV infected or who are found to be HIV infected at trial screening will be recruited into the study and follow the routine study procedures, provided they fulfil all other study eligibility criteria.

Newly-diagnosed HIV positive patients will be given appropriate counselling about the medical consequences of their diagnosis and about the need to take responsible precautions to reduce the risk of infecting others. They will be referred to appropriate medical and social HIV treatment services, and will be given the option of not proceeding to the randomisation stage of the STREAM trial if they wish to re-consider their options.

HIV co-infected patients in the STREAM trial will be managed or co-managed by clinicians with appropriate expertise in HIV medicine. It will be important therefore for the Principal Investigator at each participating site to establish links with the national AIDS programme and/or organisations that provide treatment in their country, and to establish the national criteria for ART eligibility for HIV-infected TB patients. Wherever possible, patients in the STREAM trial who are co-infected with HIV will be managed in a joint treatment clinic to ensure close co-ordination of management of the two conditions, and to ensure that appropriate decisions can be made concerning the management of drug interactions and side-effects.

Guidelines for selection of drugs in ART regimens, use of appropriate opportunistic infection prophylaxis, management of interactions between TB and HIV drugs, management of toxicity, and the timing of initiation of HIV and MDR-TB treatment are provided in the STREAM Patient Management Guide.

8.6 Trial closure

The trial will be considered closed when the last patient has completed their final visit and all follow-up and laboratory reports have been received.

The trial may be terminated early by the Trial Steering Committee (TSC), on the advice of the Independent Data Monitoring Committee (IDMC) (see sections 19.2 and 19.3). In addition, MRC CTU and the sponsor have the right to close this trial and/or a site, at any time, although this should occur only after consultation between involved parties and with the agreement of the TSC.

At trial closure, the local and central Research Ethics Committees/Institutional Review Boards and the regulatory authorities that approved the trial should be informed. It is the responsibility of the sponsor to inform the main REC within 90 days of the ‘end of the trial’ that the trial has closed.

Should a site be closed prematurely, trial materials will be disposed of according to the site agreement with MRC CTU. The Principal Investigator will retain all specified documents, for at least 15 (fifteen) years, until notification is given by MRC CTU for destruction. Patients currently on treatment will be transferred to another STREAM site where available, or referred to the National Tuberculosis Programme for completion of treatment and further management.

8.7 Bacteriology

The following bacteriological tests will be performed at the site microbiology laboratory: smear, culture and diagnostic line probe assays. At each visit, except for Week 1, Week 2, and Week 3, sputum samples will be collected. At most visits during Stage 1 this will be a single specimen unless otherwise indicated in sections 8.1.1, and will be two specimens during Stage 2. All specimens will be tested for AFB smear and culture. Because early morning samples are preferred, at the conclusion of each visit patients should be given a sputum container for sample collection to be presented at their next visit. The second sample will be taken as a spot sample at the time of clinic attendance. When two samples are required, if the patient does not bring an early morning sample, two spot samples will be collected at the visit. If a patient is unable to produce sputum this should be documented on the CRF.

The selected methods and techniques for use by the sites may not be the most sensitive ones, but they are simple and applicable at any site with high reproducibility, thus allowing a high degree of standardisation. Long-term follow-up will compensate for imperfect sensitivity. These methods are:

- hot Ziehl-Neelsen (ZN) or auramine fluorescence technique for all study smears
- FDA vital staining for selected smears in some sites (see 5.2.1)
- decontamination and without neutralisation centrifugation and inoculation for the identification of positive cultures on acidified Ogawa (Kudoh medium) for all study cultures
- Hain Genotype MTBDRPlus line probe assay (LPA) from smear-positive sputum or GeneXpert System (Cepheid automated diagnostic test to identify rifampicin resistant *Mycobacterium tuberculosis*) for screening of suspects. If one of these tests or other DST shows at least resistance to rifampicin, the Hain Genotype MTBDRsl LPA will be performed to exclude fluoroquinolone and second-line second-line injectable resistance.

To increase the probability of having at least one good baseline isolate, the sites should also inoculate the remaining part of the randomization and screening samples using their preferred culture method and medium (e.g. MGIT after neutralization and centrifugation).

All positive isolates will be sent to the designated study reference laboratory, to confirm species identification and susceptibility status. This includes diagnostic strains and recurrence strains, in case of failure or relapse besides isolated positive cultures in-between successive negatives. Strains from recurrences will be tested for DST as well as fingerprinting, to confirm their identity and to compare their resistance pattern with the originally isolated strain. The reference laboratory will store all study strains at -80°C and local laboratories will store at -20°C.

The techniques to be used at the reference laboratory are:

- Slow phenotypic DST using the proportion method on Löwenstein-Jensen medium for first line drugs and agar-based Middlebrook 7H11 medium for second line drugs; for difficult strains, the minimum inhibitory concentration (MIC) and DNA sequencing can be used to arrive at the most correct result
- Fingerprinting; MIRU-VNTR analysis (mycobacterial interspersed repetitive units–variable number of tandem repeats).

A detailed description of the various laboratory tests is found in the STREAM Microbiology Manual.

Results of resistance tests undertaken by the reference laboratory are primarily for analysis purposes and will not be routinely provided to the sites. Treatment changes will not be suggested on the basis of these results unless a patient is not doing well and it is decided that a change of treatment is required.

However, if any patients are found to have XDR-TB (confirmed resistance to fluoroquinolones and second-line injectables from phenotypic DST) from samples collected at baseline, these results will be provided to sites, and the patients should be withdrawn from trial treatment and treated according to national guidelines.

8.8 Health economic assessment:

In sites participating in the health economic component of STREAM, data relevant to the health economic assessments will be collected as explained below:

8.8.1 Health system costs

Health system costs will be obtained through:

- An analysis of health worker time involved in prescribing, monitoring, and supervising the regimens in each country, health worker salary and benefits data from the Ministry of Health and health facility records based on grade of staff rather than named individuals
- Records of drug, consumable and equipment procurements, and cost of hospital non-medical services
- Standard costs of supplies from government purchasing units or other appropriate sources
- An analysis of additional, short-term technical assistance time allocated to implement the regimens. This is distinct from existing or additional staff time required to deliver the regimens
- Salary and benefits data for technical assistance from study implementation financial records.

Costs will be assessed as one-off costs required for establishing the regimens and as costs for recurrent costs for sustaining it.

8.8.2 Patient and household costs

Data on patient and household costs will be collected through interviews with patients at intervals of 12 weeks after initiation of treatment. The interviews will include questions on fees paid to the health system, drugs and laboratory test costs, transport, food and accommodation costs incurred as a result of the treatment process as well as time lost from economic activities due to illness or care-seeking. The STOP-TB costing tool will be adapted for site use.

8.8.3 Socio-economic status

The socioeconomic status of patients will be assessed through asking patients about asset ownership. The assets will be determined based on existing poverty analyses or similar sources (demographic and Health Surveys, population income surveys or census data) for the country or region within the country. These questions about asset ownership will be included in the demographic assessment at randomisation and again every 12 weeks after the initiation of treatment.

9 DISCONTINUATION FROM TREATMENT

In consenting to trial participation, patients are consenting to study treatment, follow-up and data collection. If a patient wishes to discontinue their allocated study treatment they should not be withdrawn from follow-up unless they expressly request it. Patients should be told about the importance of remaining on follow-up, or failing this, of allowing routine follow-up data to be used for study purposes.

The treating clinician will be discouraged from changing or restarting treatment without evidence of treatment failure or recurrence of MDR-TB. As soon as the treating clinician has any indication of a treatment failure, recurrence, or serious toxicity, they should contact the STREAM central clinical team to discuss whether treatment should be modified. Guidelines for retreatment, in the STREAM patient management guide, will be used to inform the decision which will be made on a case by case basis using all the available bacteriological and clinical data. If the decision is made not to retreat, then the case should be reassessed as further data accumulates with further discussions with the STREAM central clinical team as necessary.

9.1 Discontinuation of allocated regimen

The Investigator must make every reasonable effort to keep each patient on their allocated regimen and in follow-up for the whole duration of the study. However, if it is necessary to discontinue or change a patient's allocated regimen, every reasonable effort will be made to ensure the patient continues to be followed-up.

The following are justifiable reasons for the Investigator to discontinue or modify a patient's allocated treatment:

1. Unacceptable toxicity

2. Patient refuses to take study drugs
3. Serious violation of the study protocol (including persistent patient attendance failure, non-adherence to treatment and persistent non-compliance)
4. The Investigator decides to discontinue a patient's treatment for clinical reasons not related to the regimens
5. Evidence of treatment failure based on consistently positive bacteriology usually accompanied by signs and symptoms of disease
6. Pregnancy; women who become pregnant will stop their allocated trial treatment, and be treated according to local practice
7. A confirmed QT or QTcF more than or equal to 500 ms on 12-lead ECG in patients for whom no other cause can be identified
8. Confirmed resistance to fluoroquinolones and second-line injectables from phenotypic DST.

If it is local practice to do so, results from local drug susceptibility tests showing a patient's isolate is resistant to trial medications may be used to justify a change of treatment for patients, although this is discouraged if the patient is doing well clinically. Drug susceptibility test results alone should not be used to justify a change or discontinuation of treatment for patients in the regimens, although patients found to have resistance to **both** fluoroquinolones **and** second-line injectables from phenotypic DST (confirmed XDR-TB) should be withdrawn from trial treatment and treated according to national guidelines (see section 5.5.1). If these results are obtained from a local laboratory, a patient can remain on trial medication at the discression of the treating clinician if the patient appears to be doing well on treatment. However, if results from the central laboratory confirm XDR-TB, then the patient should be removed from the trial and treated according to national guidelines.

Should drug unavailability occur, local supply of drug(s) through the National Tuberculosis Programme or local market will be considered (after ensuring acceptable quality) while regular study supplies are replenished. In instances where drug(s) remain unavailable for any period of time the central clinical team will be consulted to advise on appropriate management.

Any change or discontinuation of treatment should be discussed with the STREAM central clinical team before a decision is made, unless in response to a medical emergency.

9.2 Salvage regimens

Salvage regimens will be provided for trial patients who require retreatment provided adequate follow up can be maintained. Trial patients who initiate a salvage regimen close to the end of their trial follow-up period may require treatment and follow up beyond this time point. In addition, where appropriate the Sponsor may initiate salvage regimens for patients who have completed their 132 week Study period.

The composition of the salvage regimen will be based on treatment history and DST results and be selected by the site clinicians in consultation with the central clinical team. Salvage regimens will also take into account local guidelines and availability of medicines. Patients who are unwilling or unable to be followed during their salvage regimen treatment, including those whose planned intensive phase will not be completed prior to site closure, will be referred to the National Tuberculosis Programme for management. Upon site closure, patients still undergoing salvage regimen treatment will be referred to the National Tuberculosis Programme to ensure treatment follow up and completion.

Procurement and provision of the salvage regimen for trial patients will be supported by the Sponsor as needed and site-specific approaches to ensuring availability of medicines will be developed and documented. Bedaquiline provided as IMP for the trial will be limited to 24 weeks in a salvage regimen and only utilized where trial patients initiate their salvage regimen

within their 132 Study period. Outside of the Patient's 132 week Study period, and where available, the Sponsor may, at their discretion, use bedaquiline in a salvage regimen from an alternative source. The safety and appropriateness of the salvage regimen is the responsibility of the investigator.

9.3 Patient transfers

For patients moving out of the area, every effort should be made to continue to follow them if at all possible; this could include follow-up at another participating site.

9.4 Early stopping of follow-up

If patients explicitly state that they do not wish to contribute further data to the study, MRC CTU should be informed in writing of the patient's decision and a final form should be completed. Such patients who discontinue the study may not be re-randomised and will not be replaced.

10 DATA MANAGEMENT

Data will be recorded on paper case report forms (CRFs) and entered into a database either at each local site or at a central location. At each visit, details of clinical findings, procedures, tests and results will be recorded in the patient's case notes and on the appropriate CRF. The CRF top copy will be sent for data entry, and the duplicate retained in the patient's Trial Folder. Entries made in the CRF must be either verifiable against source documents, or have been directly entered into the CRF, in which case the entry in the CRF will be considered as the source data. Instances where data may be entered directly in a CRF will be agreed by the Sponsor and documented. The Investigator Site File and all source data should be retained until notification is given by the sponsor for destruction.

Instructions on data capture, cleaning and subsequent storage can be found in the STREAM Data Management Plan.

10.1 Blinding of data

The co-chief investigators will be kept masked to allocation where possible, except in instances where the knowledge is needed for the review of adverse events. However, one of the co-chief investigator's, Professor Andrew Nunn, will be kept entirely blinded to treatment allocation throughout the trial. The trial statisticians and data manager in the central team will not be blinded to the allocation. However, before database lock the only data summarised by treatment allocation will be within reports to the IDMC, which will not be distributed outside of the IDMC. The Trial Statisticans will be responsible for preparing the IDMC reports, and will be the only persons outside the IDMC who will have access to these reports. Should any modifications to the trial design be required, the unblinded statisticians will be excluded from the discussion.

11 STATISTICAL CONSIDERATIONS

11.1 Analysis population definitions

The analysis populations for Stage 2 only include patients from Regimen B and Regimen A that are randomised after the start of Stage 2.

Intention-to-treat (ITT)
All randomised patients will be included in the ITT analysis population.

Safety population
All randomised patients who have taken at least one dose of trial treatment will be included in the safety analysis population.

Modified intention-to-treat (mITT)
The mITT population is defined as all randomised patients that have a positive culture for *M. tuberculosis* on acidified Ogawa (Kudoh medium) at screening or randomisation, with the exception of patients with isolates taken before randomisation that are subsequently found to be susceptible to rifampicin, and patients with isolates taken before randomisation that are subsequently found to be resistant to both fluoroquinolones and second-line injectables (i.e. XDR-TB) on phenotypic DST. Results from the central reference laboratory will take priority over any results from local laboratories where available.

Per protocol (PP)
The PP population will be the same as the mITT population with the exclusion of patients not completing a protocol-adherent course of treatment (see Section 11.2.2), other than for treatment failure or death. Treatment failure is defined as failure to attain and maintain culture negativity until the end of allocated treatment.

11.2 Outcome measures

Only culture results obtained using acidified Ogawa (Kudoh medium) will be used in analysis.

11.2.1 Primary efficacy outcome

The primary outcome for the Stage 1 comparison between Regimen B and Regimen A is efficacy status at 132 weeks after randomisation, and at 76 weeks after randomisation for the two Stage 2 comparisons between Regimen C and, Regimen B, and between Regimen D and Regimen B. Efficacy status for each study comparison is determined as follows:

Favourable
A patient's outcome will be classified as **favourable** if their last two culture results are negative unless they have previously been classified as unfavourable. These two cultures must be taken on separate visits; the latest of which being no more than six weeks earlier than Week 132 (for the Stage 1 comparison) or Week 76 (for the Stage 2 comparisons), the time-points of interest for primary outcomes in the Stage 1 comparison and Stage 2 comparisons , respectively.

For the purpose of the primary analysis in the Stage 1 and Stage 2 comparisons, the Week 132 and Week 76 windows are defined as no more than six weeks prior to, or six weeks after 132 or 76 weeks respectively from randomisation.

Unfavourable
A patient's outcome will be classified as **unfavourable** if:

1. They are discontinued from their allocated study treatment and subsequently restarted on a different MDR-TB regimen
2. Treatment is extended beyond the scheduled end of treatment for any reason other than making up of days when no treatment was given (missed treatment) for a maximum of eight weeks
3. They are restarted on any MDR-TB treatment after the scheduled end of treatment, but before 132 weeks after randomisation for Stage 1 and before 76 weeks after randomisation for Stage 2.
4. They change their allocated study treatment for any reason other than the replacement of a single drug
5. Bedaquiline is started where the allocated regimen did not originally contain that drug (Regimen A or B).
6. A drug from the class of nitroimidazoles is started
7. They die at any point during treatment or follow-up
8. At least one of their last two culture results, from specimens taken on separate occasions, is positive
9. They do not have a culture result at Week 76 or thereafter for the Stage 1 comparison, or within the Week 76 window for the Stage 2 comparison
10. The failure or recurrence specimen at or before the Week 76 window was a different strain to their randomisation specimen, i.e. re-infection (the Stage 2 comparison only).

Starting a single drug other than bedaquiline (in Regimen A or B) or from the class of nitroimidazoles (in any regimen) is not considered to be a substantial change to the regimen and therefore does not result in an unfavourable outcome, providing none of the other criteria above are met. However, in Stage 2, a sensitivity analysis will be conducted where a patient's outcome will also be classified as unfavourable if the patient starts a second-line injectable in Regimen C

For analysis of the Stage 1 comparison, re-infections with a different strain are classified as not assessable.

Only data before or within the Week 76 window will be used for the determination of the primary efficacy outcome for Stage 2, where patient follow-up continues beyond Week 76.

An extension of the intensive phase of treatment in any study arm does not constitute an unfavourable outcome, as long as the extension is in accordance with either the algorithms described in figures 6 and 7 in section 7.3.2 for patients on the Regimen B, Regimen C, or Regimen D, or the locally-used WHO-approved MDR-TB regimen for patients on Regimen A. Similarly, the discontinuation of drugs that are not replaced does not constitute an unfavourable outcome.

During Stage 1, a patient who has a culture result within the Week 76, but not within the Week 132 window having not otherwise been classified as unfavourable (based on the definitions above) will be regarded as **not assessable** and will be excluded from the primary analysis provided their last two cultures, from specimens taken on separate occasions, are negative; one of these cultures should be from a sample taken at week 76. Any patient who does not have a culture result within the Week 132 window and does not fulfil these criteria will be classified as **unfavourable**. These definitions apply to both Regimen A and Regimen B in Stage 1 of the trial.

11.2.2 Definition of a protocol-adherent course of treatment

Patients will be excluded from the per-protocol analysis if they do not complete a protocol-adherent course of treatment, other than for treatment failure or death.

A patient will have completed a protocol-adherent course of treatment when they have taken 80% of doses within 120% of the minimum duration in both the intensive phase and in the whole treatment period. For this purpose, a dose is defined as all the study medications at the correct dose for that particular day.

Stage 1
For Regimen B, **with or** without an extension of the intensive phase, a patient will have completed a protocol-adherent course of treatment if they have taken:

- 90 doses (80% of 16 weeks) within 134 days (120% of 16 weeks) in the intensive phase, and
- 224 doses (80% of 40 weeks) within 336 days (120% of 40 weeks) over the whole treatment period (i.e. the combined intensive and continuation phases) regardless of treatment extensions.

The same algorithm will apply for Regimen A, the control regimen for Stage 1, and the exact number of doses and days depends on the duration of the intensive and continuation phases of Regimen A.

Stage 2
For Regimen B and Regimen C, with or without an extension of the intensive phase, a patient will have completed a protocol-adherent course of treatment if they have taken:

- 90 doses (80% of 16 weeks) within 134 days (120% of 16 weeks) in the intensive phase, and
- 224 doses (80% of 40 weeks) within 336 days (120% of 40 weeks) over the whole treatment period (i.e. the combined intensive and continuation phases) regardless of treatment extensions.

For Regimen D, with or without an extension of the intensive phase, a patient will have completed a protocol-adherent course of treatment if they have taken:

- 45 doses (80% of 8 weeks) within 67 days (120% of 8 weeks) in the intensive phase, and
- 157 doses (80% of 28 weeks) within 235 days (120% of 28 weeks) over the whole treatment period (i.e. the combined intensive and continuation phases) regardless of treatment extensions.

11.3 Sample size: Stage 1

A meta-analysis of treatment outcome in patients with MDR-TB found an overall favourable outcome of 64% (95% CI 59-68) in patients given individualised treatment and 54% (95% CI 43-68) in patients given standardised treatment[11]. A reasonable estimate of the efficacy of Regimen A in the STREAM trial would therefore be 70%.

Based on the experience with Regimen B, a reasonable estimate of its efficacy in the STREAM trial would be between 75% and 85%. The lower estimate is used for the sample size calculations below.

11.3.1 Power to demonstrate non-inferiority in the primary efficacy outcome

Based on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 2 gives the total number of patients required to demonstrate non-inferiority under the specified scenarios using a margin of non-inferiority of 10%, assuming that Regimen B is actually 5% better. These totals allow for 20% of patients being classified as not assessable in a per-protocol analysis and are based on a one-sided level of significance of 2.5%.

Table 6: Power to demonstrate non-inferiority in the primary efficacy outcome

Power	Percentage favourable outcomes in Regimen A	Difference in percentage favourable outcomes in Regimen B compared to Regimen A		
		0%	5%	10%
80%	60%	1060	464	255
	65%	1005	435	238
	70%	928	398	214
90%	60%	1419	620	340
	65%	1345	583	318
	70%	1242	533	287

Therefore, 398 patients would be required (rounding to 400 gives: 267 on Regimen B and 133 on Regimen A) to demonstrate non-inferiority with 80% power assuming 70% favourable outcomes in Regimen A and 75% in Regimen B and 20% not assessable. A larger difference in response rates of 10% would require fewer patients and could also be demonstrated with greater than 90% power with a total recruitment of approximately 400 patients.

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden and duration and the expected increase in adherence in reducing a treatment regimen from 104 weeks (as with Regimen A), to 40 weeks (as with Regimen B).

If the difference in response rates in favour of Regimen B is more than 10% it may be possible to demonstrate superiority of that regimen over the control for the Stage 1 comparison, Regimen A.

At least 400 patients will need to be recruited across all countries to give sufficient power to demonstrate non-inferiority. Patients will be randomised to Regimen B and Regimen A in the ratio 2:1.

11.3.2 Power to demonstrate non-inferiority in the primary safety outcome

Assuming a sample size of 400 on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 3 gives the power available to demonstrate non-inferiority in the primary safety outcome under different proportions of grade 3 or 4 events on Regimen A and Regimen B. These calculations assume a margin of non-inferiority of 10% and a one-sided level of significance of 2.5%. All randomised patients who have received at least one dose of study medication will be included in the safety analysis.

Table 7: Power to demonstrate non-inferiority in the primary safety outcome

Proportion grade 3 or 4 on Regimen A	Assuming same proportion in Regimen A and Regimen B	Assuming an absolute 5% lower proportion on Regimen B than Regimen A
10%	88%	99%
15%	75%	99%
20%	65%	96%
25%	58%	93%
30%	53%	89%
35%	50%	86%
40%	48%	83%

11.4 Sample size: Stage 2

11.4.1 Power to demonstrate non-inferiority in the primary efficacy outcome (primary objectives 2 and 3 relating to Regimens B, C and D)

Based on a 2:2:2 allocation ratio between Regimen B, Regimen C, and Regimen D, Table 8 gives the total number of patients required to demonstrate non-inferiority of Regimen C to Regimen B, and Regimen D to Regimen B (primary objectives 2 and 3). These totals allow for 10% of patients being excluded in a per-protocol analysis and are based on a margin of non-inferiority of 10% and one-sided level of significance of 2.5%.

Unlike in Stage 1, it is assumed that the proportion of favourable outcomes is the same in each of the three arms in Stage 2.

The primary endpoint for Stage 2 is at 76 weeks rather than 132 weeks for Stage 1. A patient in Stage 1 that is lost to follow-up between 76 and 132 weeks is classified as not assessable in the primary analysis for Stage 1, but such a patient in Stage 2 will have reached an outcome in the primary analysis for Stage 2. Therefore, not assessables are not defined in Stage 2, so it is assumed that the number of exclusions from the per-protocol analysis in Stage 2 would be 10% rather than the 20% in Stage 1.

Table 8: Power to demonstrate non-inferiority in the primary efficacy outcome (Regimens B, C and D only)

Power	Proportion favourable outcomes in each arm	Total evaluable patients	Total sample size accounting for 10% exclusions.
80%	65%	1074	1194
	70%	990	1100
	75%	**885**	**984**
	80%	756	840
90%	65%	1437	1597
	70%	1326	1474
	75%	1185	1317
	80%	1011	1124

Assuming a favourable efficacy outcome at week 76 in Regimen B of 75% (consistent with Stage 1), using a non-inferiority margin of 10% and a one-sided significance level of 2.5%, 295 patients will be required in each of the three trial arms to demonstrate non-inferiority of either Regimen C or Regimen D with 80% power.

To account for the 10% of patients excluded from the PP analysis population, a total of 990 patients will be required across the three arms: Regimen B, Regimen C and Regimen D. The number of patients excluded from the mITT analysis population will be fewer than the per-protocol population and therefore this sample size will be adequate also for the analysis using this population.

In case the number of exclusions from the PP population exceeds the anticipated number, additional patients may be randomized in order to have 295 evaluable patients in each of the three arms available for the primary efficacy evaluation for the Stage 2 comparisons.

As the 2 comparisons, between Regimen C with Regimen B, and Regimen D with Regimen B, support different objectives, no adjustment is made for multiplicity.

11.4.2 Power to demonstrate superiority in the primary efficacy outcome (primary objective 1)

For the purposes of the superiority comparison of Regimen C compared to Regimen B (primary objective 1) it is assumed that the proportion of favourable outcomes is increased by 10% from 75% (Regimen B) to 85% (Regimen C).

A total of 504 evaluable patients would be required to demonstrate superiority with 80% power using a two-sided significance level of 5%.

The planned total of 660 patients in both Regimen C and Regimen B, giving 594 evaluable patients (excluding at most 10% from the mITT analysis population) gives power of 86% to demonstrate superiority using a two-sided significance level of 5% for the primary analysis.

11.4.3 Power for secondary analyses involving Regimen A in Stage 2

In addition to the 330 patients randomised in Stage 2 to each of Regimens B,C and D, 165 patients will also be concurrently randomised to Regimen A such that the allocation ratio is 1:2:2:2 (A:B:C:D).

Secondary objectives of Stage 2 are to assess whether each of Regimens B, C and D are not inferior to Regimen A at Week 132. Consistent with the power calculations for Stage 1, if 70% of outcomes are favourable on Regimen A and 75% of outcomes are favourable on Regimen B with 20% not assessable at Week 132, these numbers on Stage 2 give 87% power to demonstrate non-inferiority of Regimen B compared to Regimen A using a one-sided significance level of 2.5% in each case. If 75% of outcomes are also favourable on Regimens C and D (consistent with Stage 2 assumptions), then there will also be 87% power to demonstrate non-inferiority of C or D compared to Regimen A.

11.4.4 Sample size

Stage 1

A total of at least 400 participants from sites in four or five countries will be randomised to either Regimen A or Regimen B in the ratio 1:2 (i.e. 133 allocated to Regimen A, and 267 allocated to Regimen B).

Stage 2

A total of at least 1155 participants from sites in a number of countries will be randomised to either Regimen A, Regimen B, Regimen C, or Regimen D in a ratio 1:2:2:2 (i.e. 165 allocated to Regimen A, 330 allocated to Regimen B, 330 allocated to Regimen C, and 330 allocated to Regimen D).

Overall sample size

The maximum sample size would be 1555 (400 for Stage 1 and 1155 for Stage 2).

Sites participating in Stage 1 and Stage 2 will transition from Stage 1 to Stage 2 randomisation scheme once the protocol amendment is locally approved. Only data from patients in these sites recruited after this transition will contribute to the analyses of Stage 2 in addition to data from sites only participating in Stage 2.

Recruitment to Stage 1 will end when a total of 400 patients have been recruited to Regimens A and B. Data from all patients recruited to Regimens A or B up to this point will be included in the Stage 1 analysis.

Therefore, the actual overall sample size will depend on the number of patients that will contribute to both the stage 1 and stage 2 analysis.

11.5 Interim monitoring and analyses

There will be no formal interim analyses of the data (with the exception of that mentioned in Section 11.7), but the Independent Data Monitoring Committee (IDMC) will review efficacy and safety data every six months after commencement of recruitment or as required, including an early assessment of QT data after three months from the start of Stage 1, and an early assessment of safety data after three months from the start of Stage 2 (unless recruitment is low at three months, in which case this initial assessment would be delayed). The IDMC will give particular attention to the QT and QTcF data at these times and at other times as necessary, with technical assistance provided by a cardiologist to enable them to interpret the results and their implication on the study. The IDMC will also consider failure rates, and give attention to mortality data; reviewing numbers and cause of failure/death by treatment arm.

It is not the intention to stop the trial in Stage 2 based on differences in efficacy between any of the treatment arms unless this is regarded as a safety issue. Recommendations to stop the trial prematurely will be based only on safety considerations; therefore no type I error correction will be done for the primary analysis.

Further details of the role and function of the IDMC is given in Section 19 and in the STREAM IDMC charter.

Interim plasma concentration data (including an evaluation of patients receiving bedaquiline and LPV/rtv) will be reviewed during the first 3 IDMC meetings and on an ad-hoc basis thereafter if deemed necessary by the IDMC/Sponsor.

11.6 Preliminary analysis plan

In sites participating in only Stage 1, patients will only contribute data to the analysis of that stage. In sites participating in both Stage 1 and Stage 2, patients recruited to Regimen A and Regimen B once recruitment to Regimen C and Regimen D has begun at that site, can be used in the analyses of both Stage 1 and Stage 2.

All patients included in the analysis will be analysed in the treatment group to which they were originally assigned.

Detailed analysis plans for the primary and/or final analyses of Stage 1 and Stage 2 will be developed and approved prior to database lock for the relevant analysis of the respective stages. These will include details of a number of sensitivity analyses.

Results concerning time to sputum conversion will be shared with the TREAT-TB transmission modelling team in order that the longer term impacts of reducing treatment times may be assessed.

In Stage 2, data from patients on Regimen A will be included in the efficacy and safety analyses at 132 weeks, but not the primary Week 76 efficacy analyses except for descriptive analyses. The Week 132 analyses comparing Regimens C and D with Regimen B, and comparing Regimens B, C and D with Regimen A will mirror the Stage 1 analyses described above and will be described fully in the statistical analysis plan.

Stage 1

In general, the primary efficacy analysis will be based on both per protocol and modified intention-to-treat (mITT) populations.

For the primary analysis, the difference in proportion of favourable outcomes between Regimen A and Regimen B with 95% confidence intervals will be estimated. The analysis will be stratified by the randomisation stratification factors. For the non-inferiority comparisons, the analyses will be repeated on a per protocol sub-population. Non-inferiority will be shown if the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes between Regimen A and B is less than the 10% margin of non-inferiority; this must be shown in both the mITT and the PP populations.

The proportion of unfavourable outcomes with 95% confidence intervals will be estimated for each country. The primary safety outcome is the occurrence of a Grade 3 or greater adverse events. This analysis will be repeated in subgroups according to HIV infection status and drug resistance patterns.

Stage 2

The first primary objective (the superiority of Regimen C over Regimen B) provides evidence for the added effect of bedaquiline and the second and third objectives are regimen comparisons.

The first primary objective in Stage 2 is to assess the superiority of regimen C over regimen B; this is a regulatory requirement for the approval of bedaquiline.

The second and third objectives are regimen comparisons; they do not provide evidence of the added effect of bedaquiline to the regimen due to the unknown added effect of kanamycin (Regimen C) or prothionamide (Regimen D) to the regimen in the control arm (Regimen B).

For programmatic adoption, non-inferiority might be sufficient provided that there are advantages in terms of toxicity and ease of delivery. There are therefore three primary objectives for Stage 2 (see Section 3.2.1).

For the primary analysis, the difference in favourable outcome rate between Regimen B and each of Regimen C or Regimen D with corresponding two-sided 95% confidence intervals and p-values will be estimated using a stratified analysis of the risk difference from each stratum using Cochran-Mantel-Haenszel weights.[22] For either Regimen C or Regimen D, non-inferiority will be shown if the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes is less than the 10% margin of non-inferiority; this must be shown in both the mITT and PP populations. The analysis will be stratified only by HIV status with three strata: HIV negative, HIV positive with CD4 count less than 350 cells/mm^3, and HIV positive with CD4 count more than or equal to 350 cells/mm^3. For the superiority comparison of Regimen C compared to Regimen B (primary objective 1), only the mITT analysis will be primary. For the non-inferiority comparisons (primary objective 2 and 3), both the mITT and PP analyses are considered primary. Non-inferiority must be shown in both the mITT and the PP populations for the either Regimen C or Regimen D to be declared non-inferior.

Secondary outcomes at Week 132 will be analysed in a similar way, with the outcomes defined in the same way as the primary outcomes described in 11.1.1, with the time point of interest being at Week 132.

Mortality rates will be calculated for the individual treatment arms and treatment group differences in mortality rates will be calculated together with a 95% confidence interval. In addition the number of deaths, per-patient years of exposure will be calculated by treatment arm.

11.7 IDMC review of Stage 1 data to consider impact of results of Stage 1 on the trial

An additional IDMC meeting will be scheduled to occur immediately after the results of Stage 1 are available. The IDMC will be asked to review the entirety of the results from Stage 1 including all efficacy and safety endpoints as well as available data from Regimen A and Regimen B from Stage 2 and data from other relevant studies and external sources. The IDMC will be asked to consider recommending to the TSC termination of recruitment to Regimen A if there is sufficient evidence to show that Regimen B is safe and non-inferior to Regimen A.

If Regimen B is shown to have inferior efficacy to Regimen A or has an inferior safety profile that is considered clinically significant, the IDMC will be asked to consider making an appropriate recommendation for Stage 2 based on all available data and external evidence as appropriate.

11.8 Pharmacokinetic Analysis

Individual plasma concentrations will be listed and descriptive statistics will be generated for all quantified drugs, metabolites, and 4β-hydroxycholesterol at each respective sampling time. Pharmacokinetic parameters will be derived based on the individual plasma concentration-time data including C_{trough}: the predose plasma concentration; C_{last}: the last measurable plasma concentration; t_{last}: the time of the last measurable plasma concentration; and AUC_{τ}: the area under the plasma concentration-time curve from time of administration to the end of the dosing interval. A population PK model will be used to provide individual estimates of PK parameters.

The drug-drug interaction between bedaquiline and ARTs will be assessed by descriptive statistics and graphical analysis of plasma concentration-time data and pharmacokinetic parameters.

Pharmacokinetic/pharmacodynamic relationships between bedaquiline concentrations and efficacy and safety parameters will be investigated.

12 TRIAL MONITORING

Before the trial can be initiated, the prerequisites for conducting the trial must be clarified and the organisational preparations made with the sites. MRC CTU must be informed immediately of any change in the personnel involved in the conduct of the study.

The purposes of trial monitoring are to verify that:

- The rights and well-being of human participants are protected
- The reported trial data are accurate, complete, and verifiable from source documents
- The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with the principles of GCP, and with the applicable regulatory requirement

12.1 Risk assessment

A risk assessment was carried out during the feasibility assessment for this trial and is updated and reviewed approximately annually as the trial progresses and ahead of Stage 2 of the trial. The outcome of this assessment and its components are detailed in a separate document; the STREAM Monitoring Plan, which reflects issues identified in the study risk assessment and periodic reviews.

12.2 Monitoring plan

A detailed monitoring plan has been developed to reflect specific needs of the trial as determined by the risk assessment. This plan specifies the responsibilities and qualifications of monitors, central monitoring procedures, and the site monitoring visit procedures. Site visits by MRC CTU (or delegated collaborators) will be made in accordance with MRC CTU SOPs to assure the quality and accuracy of data collected and entered in the database, to determine that the applicable regulatory requirements are met and that rights and well-being of trial participants are protected.

On-site monitoring visits will be made at a frequency determined by the risk assessment and pre-defined triggers, including 'for-cause' monitoring as detailed in the monitoring plan. These visits will be made by the Trial Manager, Data Manager and/or other members of MRC CTU Trial Team, and/or delegated collaborators.

12.3 Clinical site monitoring

12.3.1 Direct access to data

Participating investigators must agree to allow trial-related monitoring and audits, ethics committee review and regulatory inspections by providing direct access to source data/documents, including electronic records, as required. Patients' consent for this is obtained as part of the trial consent process.

During the trial the MRC CTU TM is responsible for monitoring data quality in accordance with MRC CTU SOPs. Before the study start, the Local Trial Coordinator will be advised of the anticipated frequency of the monitoring visits and will receive reasonable notification before each monitoring visit. Responsibilities of the monitors are outlined in the Monitoring plan.

During the course of this trial, the TM will maintain contact with the study sites on a regular basis. This will include a training/initiation visit prior to participant randomisation; a monitoring visit soon after screening/randomisation begins and further visits as detailed in the monitoring plan. The monitor will meet with the investigators on a regular basis during the study to provide feedback on study conduct. Closeout visits will be conducted after trial

participation is completed. The sites will be contacted in advance to schedule each visit. All participant records, CRFs, and other source documents for the patients recruited in this study will, where possible, be made available for review by the monitor(s). A site-visit log will be maintained at each study site to record all STREAM-related site visits made by authorised individuals.

12.3.2 Quality assurance (QA) procedures

QA procedures at MRC CTU include a systematic review of the trial protocol by the Protocol Review Committee (PRC), the preparation of a Risk Assessment and Quality Management documents. A review of these documents is undertaken by the MRC CTU Research Governance Committee (RGC) and Quality Management Advisory Group (QMAG) which form the QA function of MRC CTU. Internal audits of the Trial Master File will be conducted as directed by the RGC. Audits of sites may be conducted by or on behalf of the sponsor. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Participant privacy, must however, be respected. The Investigator and staff are responsible for being present and available for consultation during routinely scheduled site audit visits. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

Good Clinical Practice (GCP) training, and where appropriate Good Laboratory Practice (GLP)[23] training will be provided for all staff involved in the trial; this will form part of the capacity strengthening component of the trial.

12.3.3 Microbiology laboratory quality control

Details of the arrangements for laboratory quality control (QC) are found in the STREAM Laboratory Manual.

ITM Antwerp will assess and prepare all laboratories before start of the trial, and assure quality of the sites' microscopy and cultures throughout the trial and GeneXpert MTB/RIF and/or LPA during the screening. Test performance will be periodically monitored and reported.

The following QC procedures will be used:

- Microscopy: internal control of newly prepared lots of staining solutions at the sites, together with random checking of smears performed at the presumed end of the intensive phase.
- Cultures: monitoring of false negative and contamination rates.
- LPA DST: a water blank in each run, to check for cross-contamination; strip-inbuilt controls for QC of amplification and colour reaction. EQA will be performed by sending panels composed of bacilli suspensions with known resistance patterns (Proficiency Testing).
- GeneXpert MTB/RIF: monitoring of errors plus proficiency testing panels will be used for the sites. There will be QC of phenotypic DST, DNA sequencing of resistance-conferring mutations performed at the central laboratory in Antwerp.

Details of QC will be provided in the laboratory manual.

12.4 Central monitoring

Central monitoring of data at MRC CTU will be conducted by CRF review, with appropriate and range and consistency checks programmed into the database. MRC CTU will raise any

concerns they may have about the data captured by use of query forms sent to the site, as detailed in the Data Management Plan.

The Trial Master File will be stored at MRC CTU and will be maintained by the TM throughout the trial. All trial specific documents will be centrally tracked and copies obtained from the sites for all communication with regulatory bodies. Details about maintaining trial files and any other monitoring that will be carried out centrally are in the Monitoring Plan, other study documentation and plans as appropriate.

13 SAFETY REPORTING

GCP requires that both investigators and sponsor follow specific procedures when notifying and reporting adverse events/reactions in clinical trials. These procedures are described in this section of the protocol. Section 13.1 lists definitions, section 13.2 gives details of the institution/investigator responsibilities and section 13.3 provides information on MRC CTU responsibilities.

AEs are collected from the time the informed consent form is signed at screening until follow-up is completed or the participant withdraws from the trial. If a patient is found to be ineligible for the trial at screening, or chooses not to be randomised, no further AE data should be collected from that point.

13.1 Definitions

The definitions of the EU Directive 2001/20/EC Article 2 based on GCP apply in this protocol. These definitions are given in Table 9.

Table 9: Safety reporting definitions

Term	Definition
Adverse Event (AE)*	Any untoward medical occurrence in a patient or clinical trial participant 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) (see 13.2.1 (d) for causality definition)	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 • Other medically important condition, including suspected transmission of any infectious agent via administration of a medicinal product; • Is a combination of the above (to be specified).

13.1.1 Clarifications and exceptions

*In addition, events from the point that the participant gives informed consent to screening until randomisation are also defined as AEs for patients in stage 2.

**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 that has not worsened (including elective procedures) do not constitute an SAE; nor do hospital admissions for social and not medical reasons. 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.

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

13.1.2 Trial specific exceptions to expedited SAE notification and reporting

Data on disease relapse or progression are collected as part of the primary outcome of the trial and are not considered to be SAEs, unless fatal. QT prolongation does not require grading since the actual QT and QTcF values are collected as part of routine monitoring, but QT or QTcF prolongation to more than or equal to 500 ms should be recorded as a notable event as specified in 13.1.3. Planned hospitalisations for the initial phase of trial treatment are also not considered as SAEs as noted in section 13.1.1 above.

13.1.3 Additional notable events

The following notable events should also be identified and reported to the MRC CTU within the same time frame as an SAE (unless they also meet the criteria for an SAE, in which case they should be reported as such):

- Pregnancy in a patient or partner of a patient while on protocol treatment
- QT or QTcF measurement more than or equal to 500 ms while on treatment
- ALT/AST more than or equal to ten times ULN, or ALT/AST more than or equal to three times ULN in the presence of total bilirubin more than or equal to 2 times ULN
- Creatinine kinase more than or equal to 10 times ULN
- Pancreatic amylase more than or equal to 2 times ULN
- The occurrence of any grade 3 or higher adverse event
- Any toxicity that leads to a change of allocated treatment
- A clinically significant dysrhythmia, such as an episode of ventricular tachycardia, with three or more irregular beats in a row.
- An overdose of trial medication.

13.2 Institution/investigator responsibilities

The severity (i.e. intensity) of all AEs/ARs (serious and non-serious) in this trial should be graded using the DAIDS criteria.

13.2.1 Investigator assessment

(a) Seriousness

When an AE/AR occurs the investigator responsible for the care of the patient must first assess whether the event is serious using the definition given in Table 9. If the event is serious and not exempt from expedited reporting, then an SAE/NE form must be completed and the MRC CTU notified.

(b) Causality

The Investigator must assess the causality of all serious events/reactions in relation to each trial drug that the patient has received using the definitions in Table 10. There are 5 categories: unrelated, unlikely, possibly, probably and definitely related to trial treatment. If the causality assessment is "unrelated" or "unlikely to be related" to trial treatment the event is classified as an unrelated SAE. If the causality is assessed as possible, probable or definitely related then the event is classified as a Serious Adverse Reaction (SAR).

(c) Expectedness

If the event is a SAR the Investigator must assess the expectedness of the event, with the exception of events thought to be caused by bedaquiline, which will be assessed for expectedness at the coordinating site. The definition of an unexpected adverse reaction (UAR) is given in Table 9. A list of expected toxicities associated with the drugs being used in this trial is provided in the STREAM Patient Management Guide. If a SAR is assessed as being unexpected it is a Suspected Unexpected Serious Adverse Reaction, or SUSAR.

(d) Notification

Investigators should notify the MRC CTU of all SAEs and other notable events as defined above, within one working day of them becoming aware of the event.

Table 10: Definitions of causality

Relationship	Description	Event Type
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

Notification procedure:

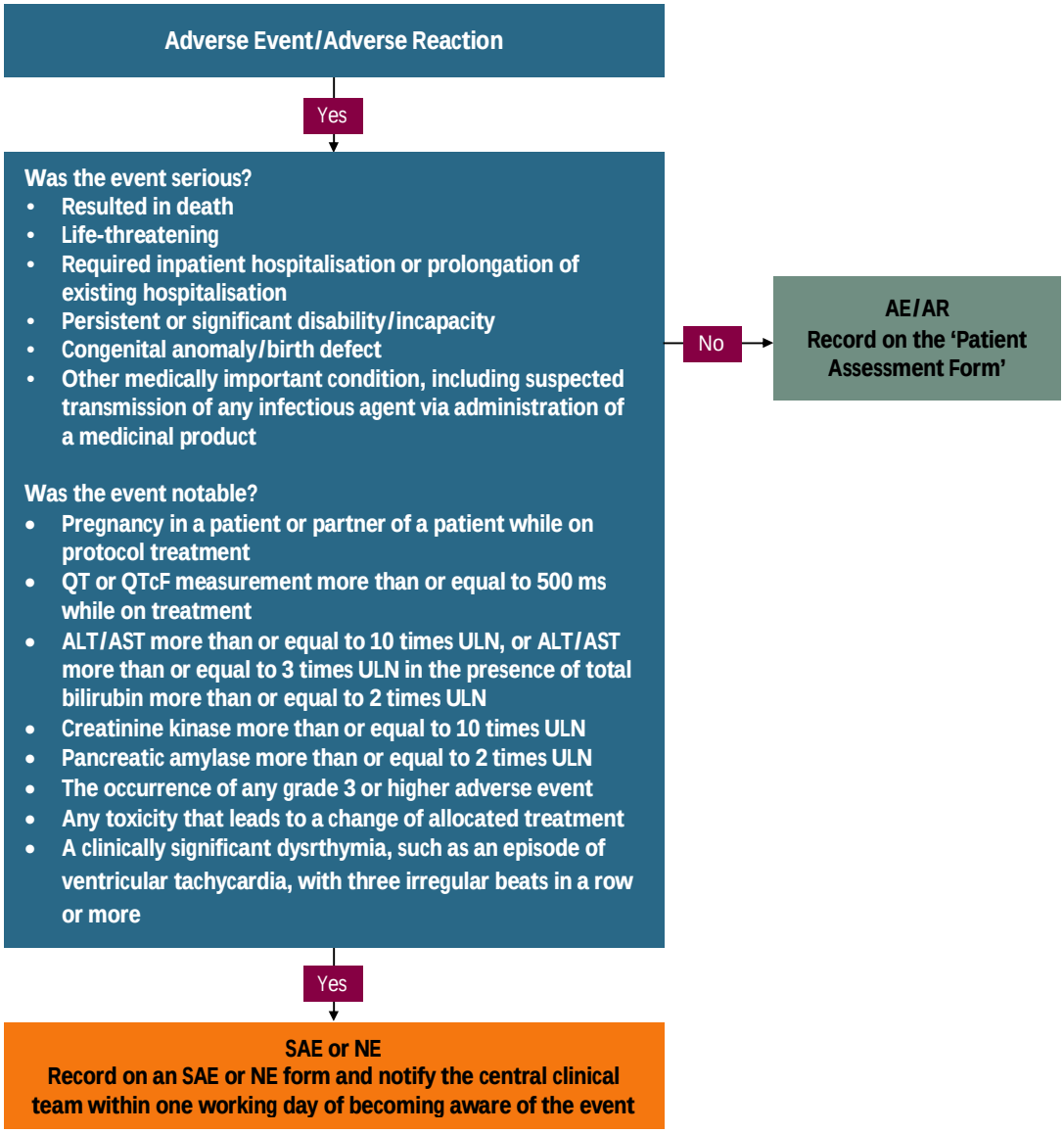
1. The SAE/NE form must be completed by the Investigator (consultant named on the signature list and delegation of responsibilities log who is responsible for the patient's care), with due care being paid to the grading, causality and expectedness of the event as outlined above. In the absence of the responsible investigator the form should be completed and signed by a member of the site trial team delegated to do so. The responsible investigator should subsequently check the form, make changes as appropriate, sign and then re-send to the MRC CTU as soon as possible. The initial report shall be followed by detailed, written reports as appropriate.

2. Send the SAE/NE form by fax or email to the MRC CTU within one working day.
   Fax Number: + 44 (0) 20 7670 4829 Email: streamdata@ctu.mrc.ac.uk

3. Follow-up: Patients must be followed-up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of protocol treatment if necessary. Follow-up information should be noted on a further SAE/NE form by ticking the box marked 'follow-up' and emailing or faxing it to the MRC CTU as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The patient must be identified by study number, date of birth and initials only. The patient's name should not be used on any correspondence.

4. Staff at the investigator site must notify the research ethics committee of the event (as per the institutions standard local procedure).

Figure 11: Safety reporting flowchart



13.3 MRC CTU responsibilities

Medically qualified staff at the MRC CTU or the Co-CI's medically qualified delegate will review all SAE reports received. This may involve discussions with the STREAM central clinical team as outlined in the Patient Management Guide. The causality assessment given by the local clinical investigator cannot be overruled and in the case of disagreement, both opinions will be provided in any subsequent reports. The investigator's assessment of expectedness may be modified by the medical reviewer.

The MRC CTU is undertaking the duties of trial management and is responsible for providing the Sponsor's research ethics committee and the regulatory authorities that have approved the trial with the safety reports that they require.

The MRC CTU will provide the Independent Data Monitoring Committee (see section 19) with aggregated reports of SAEs for their review and will keep all investigators informed of any safety issues that arise during the course of the trial. After receipt and review of these reports, MRC CTU will also notify the trial sponsor.

SAE/NE NOTIFICATION

Within one working day of becoming aware of an SAE/NE,
please fax a completed SAE/NE form to the MRC CTU on:

Fax: +44 (0) 20 7670 4829

Email: mrcctu.streamdata@ucl.ac.uk

13.4 Product quality complaint handling

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labelling, or packaging, i.e. any dissatisfaction relative to the identity, quality, durability, and reliability of a product, including its labelling or package integrity. PQCs may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from clinical studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies worldwide.

13.4.1 Procedures

All initial PQCs must be reported to the MRC CTU by the site staff as soon as possible after being made aware of the event.

If the defect is combined with an SAE, the site staff must report the PQC to the MRC CTU according to the SAE reporting timelines (see Section 13.2). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

14 ETHICAL CONSIDERATIONS AND APPROVAL

14.1 Ethical considerations

The study will abide by the principles of the Declaration of Helsinki.

14.1.1 Research Ethics Committee (REC) review and approval

Before initiating the study at any given site, the study must be approved in writing by the local REC and/or Institutional Review Board (IRB), where appropriate, as well as the Ethics Advisory Group of The Union. The study will be conducted in accordance with all conditions of approval by the REC. The local Principal Investigator will forward the approval letter to MRC CTU.

Before starting the trial, the protocol, patient information sheet, informed consent form, study specific patient cards and any local advertising materials must be reviewed by the MRC CTU Protocol Review Committee; and be approved by the Trial Steering Committee (TSC) and the appropriate Ethics Committee in all participating countries.

It is the local Principal Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information becomes available that might affect the patient's willingness to continue in the trial. The Principal Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented, where appropriate.

The sponsor and Investigators must ensure that the study is carried out in accordance with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), the Declaration of Helsinki and applicable regulations in each country.

14.1.2 Informed consent

No patient may be screened for or randomised into this study until the investigator has obtained his/her informed consent. Informed consent encompasses all oral or written information given to the participant about the study and the study materials. All such information will be in a language which is understandable to him/her. The information will not include any language in which the participant is made to waive any of their rights or which releases (or appears to release) the investigator, the investigator's institution or the MRC CTU, from liability for negligence.

Consent for study participation will be based on a template Patient Information Sheet (PIS) which will be provided to all participating sites. The information contained in the PIS will be translated into the relevant local languages and back-translated to ensure consistency of content. Literate patients will be asked to read the form and illiterate patients will have the contents explained to them by a counsellor, in the presence of a witness who will be present during the whole consent process. Patients will have the opportunity to discuss the PIS with the medical officer/treatment supervisor. They will be assured that their decision to participate in the trial or not will not affect the quality of care they will receive. Once this person is satisfied that the patient has understood the PIS and the informed consent form, the patient will be asked to give consent.

The patient will sign (or thumbprint) and the investigator or designee will also sign the informed consent form. If the patient is illiterate, their witness will also sign the form. One copy of the signed informed consent form will be offered to the participant, a second copy will be filed in the patient's medical notes (where available) and the original signed informed consent form will be kept in his/her study file. The investigator is also responsible for

developing tools that may help in explaining the study to patients, these materials will also be submitted to MRC CTU at least one week before submission to the local REC.

14.1.3 Randomisation

Prior to the start of any trial procedures, the randomisation process will be explained as part of the patient information sheet at the patient's randomisation visit. Patients will be given a chance to ask any questions they may have before they consent to taking part in the trial.

14.1.4 Patient confidentiality

The confidentiality of all patients participating in this study will be protected to the fullest extent possible. All patient information will be kept secure and will be available only to the treatment staff and representatives of the sponsors, regulators, and ethics committees.

Study patients should not be identified by name on any case report form, email or on any other documentation sent to MRC CTU and will not be reported by name in any report, presentation or publication resulting from data collected in this study. Patients' data/specimens will be identified by study number or hospital number only.

The trial will comply with the principles of the UK Data Protection Act or the equivalent regulation/legislation of the country of the participating site.

14.1.5 Additional trial requirements

Patients may be required to provide additional samples or may be required to come to the clinic for more visits if clinically indicated.

14.1.6 Record retention

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical trial and the quality of the data produced to be evaluated and verified.

All essential documents (according to GCP and MRC CTU SOPs) required to be held by the Investigator must be stored in such a way that ensures that they are readily available, upon request, to the local Regulatory Agency, the sponsor, or for US FDA or EMA inspection, for the minimum period required by national legislation or for longer if needed by MRC CTU. Records must not be destroyed without prior written approval from MRC CTU.

The medical files of trial participants shall be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

At the end of the trial, photocopies of pertinent study documentation (such as REC correspondence, etc.) will be kept by MRC CTU. CRFs will remain in the patient's study file at the participating sites. The signed original informed consent documents for each participant and originals of other study documentation (e.g. drug inventory forms, participant clinic records, original laboratory reports, etc.) will be retained by the local PI for a minimum of 15 years (as specified in MRC CTU working instructions on archiving). If those years have passed with no request for the data, the sites may request permission in writing from MRC CTU to destroy the records. No records may be destroyed without written permission from MRC CTU.

14.1.7 Audit

The investigator may be subject to a field audit by The Union, MRC CTU, or a health authority inspection to validate the participation of study patients, to verify the data reported on the Case Report Forms and to confirm the compliance of the conduct of the trial with applicable regulations and requirements and the protocol. This audit could occur while the study is in progress, several years after the study is completed, or when the data are under review. All of the patients' records and other study documentation must be filed and accessible on short notice (3-5 days) during the study and subsequent retention period.

14.2 Protocol deviations

No waivers will be given by MRC CTU on behalf of the sponsor for patients who do not fulfil the eligibility criteria for this trial. No deviations from, or changes to, the protocol should be initiated without prior written REC/IRB, regulatory authority approval/favourable opinion and approval from MRC CTU on behalf of the sponsor.

The reporting procedures and how to handle deviations are detailed in the MRC CTU SOPs and trial-specific Working Practice Documents for protocol deviations.

14.3 Ethical approval

The Union's Ethics Advisory Group has given a favourable opinion for the trial and has indicated in broad terms that the trial concept is consistent with ethical requirements. The final protocol will be submitted to the Ethics Advisory Group for assessment. Each participating site will need to submit the protocol to their relevant Ethical Review Committees and/or Institutional Review Boards. All substantial amendments to this protocol will have to be submitted for approval.

A copy of local REC/IRB approval of the protocol and of the participant information sheet (PIS) and informed consent form (ICF) on local headed paper and any other written information given to the participant should be forwarded to MRC CTU before patients are randomised into the trial. Each patient's consent to participate in the trial should be obtained after a full explanation has been given of the treatment options, including the conventional and generally accepted methods of treatment.

The right of the patient to refuse to participate in the trial without giving reasons must be respected. After the patient has entered the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any Stage, after discussions with the STREAM central clinical team, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the patient will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the patient must remain free to withdraw at any time from the protocol treatment and trial follow-up without giving reasons and without prejudicing his/her further treatment.

15 REGULATORY ISSUES

All Investigators will be expected to obtain, in writing, approval to participate from their local Regulatory Authority. Copies of the approval (or non-approval) must be submitted to MRC CTU no later than 5 working days from receipt of the same.

A special authorisation of importation for the medicines to be used in the study should be obtained, by the responsible person at each site, from the National Drug Regulatory Authorities (NDRA) and provided to The Union.

16 INDEMNITY

The sponsor of the trial is The International Union Against Tuberculosis and Lung Disease (The Union North America). Global insurance coverage for the trial was obtained by the sponsor. Country-specific insurance policies for the trial were also obtained by the sponsor where required.

The local Principal Investigator/Investigator's institution is liable for negligent harm, for each of the participating sites unless alternative provisions have been made by the sponsor. All personnel involved in the trial will be expected to be indemnified by their employing authority; in exceptional circumstances, the country-specific policies may also cover local investigator/practitioner liability.

17 ANCILLARY STUDIES

Ancillary studies may be conducted at selected sites participating in STREAM provided they have been approved by the Trial Steering Committee to ensure no negative impact on the main STREAM trial. Each ancillary study will have its own protocol and informed consent form and be approved by the relevant ethical and regulatory committees.

18 FINANCE

The trial is sponsored by The International Union Against Tuberculosis and Lung Disease (The Union North America). The primary funder of the trial is the United States Agency for International Development (USAID), with additional funding from the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement
and Janssen Research & Development, LLC. This trial will be managed and coordinated by the Medical Research Council Clinical Trials Unit at UCL (MRC CTU).

Each participating site will be supported according to the submissions of their budgetary requirements.

Reimbursements will be made according to sub-agreements signed between the MRC and the sponsor, with the participating sites.

19 TRIAL COMMITTEES

19.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will consist of representatives from different disciplines involved in the day to day running and management of the trial. It will include the Co-Chief Investigators, a member with clinical expertise in MDR-TB, members of the MRC CTU involved in the running of STREAM, namely the Trial Manager, the Data Manager, Project Manager and Trial Statistician. The group will also include representatives from The International Union Against Tuberculosis and Lung Disease (The Union North America), including the Trial Pharmacist and others involved in the trial operations. Trial staff from ITM in Antwerp the Impact Assessment team from the Liverpool School of Tropical Medicine and the MRC CTU Data Management Systems will attend TMG meetings periodically upon request. The TMG will have access to clinical expertise in cardiac arrhythmias and hepatology for review of safety. Input to the TMG from relevant operational staff of Janssen Pharmaceuticals may be requested intermittently, on an as needed basis. The TMG will convene approximately monthly and it will report to the TSC on progress and issues.

19.2 Trial Steering Committee (TSC)

A TSC with an independent chair and a majority of independent voting members will be responsible for the oversight of the trial and provide advice to the investigators. No important decision should be made in the absence of a Co-Chief Investigator. Additional observers, including other investigators, a sponsor representative, and funder representatives, may be in attendance at the TSC meetings; they may provide additional input as requested. A STREAM TSC charter describes the membership and responsibilities of the TSC which include:

- providing expert oversight of the trial
- making decisions as to the future continuation (or otherwise) of the trial
- monitoring recruitment rates and encourage the TMG to develop strategies to deal with any recruitment problems
- reviewing regular reports of the trial from the trials unit (sent on behalf of the Trial Management Group (TMG))
- assessing the impact and relevance of any accumulating external evidence
- approving any amendments to the protocol, where appropriate
- approving any proposals by the TMG concerning any change to the design of the trial, including additional sub-studies
- overseeing the timely reporting of trial results
- approving the statistical analysis plan
- approving the publication policy
- approving the main trial manuscript
- approving any abstracts and presentations of any results *during* the running of the trial
- Approving external or early internal requests for release of data or subsets of data or samples including clinical data and stored biological samples.

19.3 Independent Data Monitoring Committee (IDMC)

The IDMC will review safety and efficacy data regularly. The list of members and their responsibilities is included in the STREAM IDMC charter. The IDMC could, in exceptional circumstances, recommend termination of the study or termination of one of the treatment regimens due to unacceptable levels of drug toxicity, or mortality; the trial should not be modified on account of differences in efficacy between treatment arms unless there is a concern for patient safety (except the possibility of the IDMC recommendation of termination of recruitment to Regimen A, see section 11.7). The IDMC will be asked to give advice to the

TSC on whether the accumulated data from the trial, together with results from other relevant trials, justifies continuing recruitment of further patients. They may recommend modification or closure of the study in a country or sub-group of patients, such as those who are HIV-infected.

The IDMC will convene approximately 6 monthly but may meet more frequently if it becomes necessary to do so. A charter describes in full the responsibilities of the IDMC and the format of their meetings and members will be required to sign this before the first meeting.

20 PUBLICATION

20.1 Publication

The definition of publication for this purpose is any public representation of the data emerging from this trial. Results of this study will be submitted for publication in a peer reviewed journal. This will be the analysis of the primary objectives of the study and this manuscript together with subsequent manuscripts, including any single site data, will require the review and agreement of the TSC prior to submission.

Details for producing manuscripts, abstracts, press releases and any other publications including guidelines for authorship are outlined in the STREAM Publication Policy.

20.2 Dissemination of results to trial participants

Study results will be shared with participants through mechanisms and materials reviewed and approved by The Union's Ethics Advisory Group and other relevant stakeholders.

21 APPENDICES

Appendix 1: High-dose moxifloxacin safety summary

1. Rationale

Gatifloxacin (400mg daily for patients less than 33kg, 600mg for those 33-50kg, and 800mg if more than 50kg) was considered to be a critical component of the success of the regimen developed by van Deun et al; the ofloxacin-containing regimens tested were associated with inferior outcomes[1] . Because an internationally acceptable, quality-assured supply of gatifloxacin is not available, it was necessary to substitute a different fluoroquinolone, and moxifloxacin was judged to be the best alternative. Gatifloxacin and moxifloxacin have similar bactericidal activity at the same dose[1] and based on pharmacokinetic modelling there is reason to believe that the higher than standard doses are needed to prevent secondary fluoroquinolone resistance[24].

The standard dose of moxifloxacin is 400mg daily without any weight adjustment. In the STREAM study, as in the regimen developed by van Deun et al, 400mg will be used for patients less than 33kg, 600mg for those 33-50kg, and 800mg if more than 50kg. The main concern about the substitution of moxifloxacin for gatifloxacin is the potential for cardiac toxicity.

2. Cardiac safety of moxifloxacin at the standard dose

Moxifloxacin is an 8-methoxy quinolone, a member of the widely used fluoroquinolone family of anti-bacterial agents, which are some of the most frequently prescribed antibiotics in the world. Fluoroquinolones, in particular moxifloxacin, are known to prolong the QT interval, which occurs when drugs prevent the outward flow of potassium through cardiac voltage-gated potassium channels[25]. This causes a delay in cardiac repolarisation and may increase the risk of torsades de pointes (TdP), a life threatening ventricular tachycardia. However, despite this propensity and its extensive use, there are very few reported cases of TdP induced by moxifloxacin[26].

QT prolongation is defined as a QT interval above the upper limit of normal: 450 ms for men and 470 ms for women[27, 28]. However, the best indicator that a drug has the potential to induce arrhythmias is if it causes QTc (QT interval corrected for heart rate) prolongation to greater than 500ms[28].

The QTc increase following moxifloxacin has been well documented. Florian et al. reported an average increase of 10-14 ms following a single 400 mg dose across several investigations[29]. Tsikouris et al. acquired similar results after conducting an open label cross-over study in 13 healthy participants, including moxifloxacin at 400 mg, revealing an average QTc increase of 11 ms at 2-hours post dose[30].

Based on all the clinical trial data for moxifloxacin at the standard dose, ventricular tacharrhymias are estimated to occur in less than 1/1,000 and torsades de pointes and cardiac arrest in less than 1/10,000[31]. The case reports of TdP potentially related to moxifloxacin have occurred in elderly patients with pre-existing heart conditions[32,33,34].

Rubinstein's 2002 review reported that there were no cases of cardiovascular morbidity attributable to QTc prolongation recorded in 6000 patients involved in moxifloxacin phase II-IV clinical trials, though there were four cases of arrhythmias (three non-specified) and one case of TdP in one elderly female patient with pre-existing risk factors including

hypokalaemia, coronary artery disease, digoxin treatment and a pacemaker. They concluded that the fluoroquinolones in question (including moxifloxacin) were safe but should be closely monitored in patients with pre-existing conditions or those taking concomitant medication[34].

The trial data includes a randomised trial comparing the cardiac safety of moxifloxacin 400mg and levofloxacin 500mg in 387 elderly patients with community acquired pneumonia over 70% of whom had pre-existing cardiac disease; no difference in cardiac safety was detected[35].

3. Higher doses of moxifloxacin

Investigations using higher doses of moxifloxacin have been conducted although there is considerably less experience than with the standard dose. Démolis et al. conducted a placebo-controlled crossover study in 18 healthy volunteers in which both 400mg and 800mg prolonged the QT interval compared to placebo, but there was little difference between the two doses: the 400mg and 800mg doses increased QTc by 4.0% ± 5.1% and 4.5% ± 3.8%, respectively[36]. At two hours post dose, mean QTc intervals were recorded as 394 ±33 ms (400mg) and 396 ±28 ms (800mg) compared with the placebo mean of 379 ± 24ms. 800mg doses of moxifloxacin were also used in a 4-sequence cross-over study in 48 healthy patients across a spectrum of ages was conducted by Noel et al.[37]. Mean corrected QTc (Bazett) was recorded at 425-430 ms post-dose, with the peak between 2-4 hours; 6/47 patients (12.8%) had QTc intervals above the normal limits. All adverse events (six following moxifloxacin treatment) were described as mild, brief and spontaneously resolving.

In a trial of moxifloxacin-based treatments for *H. pylori* a total of 94 patients with a mean age of 50 received 800 mg moxifloxacin daily in conjunction with amoxicillin and esomeprazole for 10 days, 102 for 7 days and 98 for 5 days (294 in total) without any cardiac adverse events[38]; no ECG monitoring was undertaken.

Stass et al. conducted a study of moxifloxacin at doses ranging from 50mg – 600 mg in 7 healthy participants[39]. The study drug was well tolerated at all doses, with no clinically relevant changes in electrocardiogram data and only mild adverse events with no deaths or drop-outs.

In addition, there is one case report of a patient with miliary TB whose treatment included 800 mg moxifloxacin[40]. Results confirmed that the peak plasma concentration was between 2-4 hours with a mean QTc of 442.

4. Safety monitoring in STREAM

The available literature suggests that the difference in the effects of moxifloxacin on the QT interval at doses between 400 and 800mg are unlikely to be substantial, while the benefits in relation to prevention of acquired resistance are likely to be integral to the regimen.
The safety measures to be undertaken in STREAM are robust and designed to monitor the possible effects of moxifloxacin at peak concentration and to detect any possible cumulative effects. In Stage 1 any patient with a QT or QTc above 500 ms prior to treatment will be excluded from the trial. In Stage 2 any patient with a QT or QTc above 450 ms prior to treatment will be excluded from the trial.

In Stage 1, all patients will be monitored with a 12-lead ECG at 4 hours post the initial dose to capture the peak QTc increase, with further ECGs at weeks 1-4, and then every 4 weeks until Week 52. In Stage 2, the 4-weekly 12-lead ECGs will continue until Week 76;

As described in Section 8.31, patients found to have a QT or QTc more than or equal to 500 ms on a 12-lead ECG at any point during treatment will be further investigated to confirm the finding and identify the cause. If moxifloxacin is found to be the cause, and no other cause can be identified and eliminated, moxifloxacin at the standard dose (400 mg) should be tried. If standard dose moxifloxacin also causes persistent QT or QTc more than 500 ms moxifloxacin will be discontinued and levofloxacin tried instead. Concomitant medications will be closely monitored throughout the trial, in particular anti-retroviral therapy; however, the recent findings from the SMART trial would suggest that their effects are likely to be small[41].

Although ECG monitoring of this intensity would not be feasible in routine practice, it is being implemented here both to protect the patients in the trial and to determine the safety of the regimen. The current data suggests that TdP with moxifloxacin is a rare event. The STREAM protocol is designed to closely monitor patients and those at greatest risk of cardiac toxicity will be excluded. The potential risks of the regimens, Regimen B, Regimen C and Regimen D, should be balanced against both the risks of MDR-TB for which outcomes are poor and mortality is high (11% of patients in a systematic review of 33 studies of MDR-TB treatment died during treatment)[11], as well as the widely documented and serious adverse effects related to alternative MDR-TB treatment regimens.

Appendix 2: Levofloxacin Safety Summary

Stage 1 of STREAM (The evaluation of a standardized treatment regimen of anti-tuberculosis drugs for patients with MDR-TB is an ongoing trial to evaluate the "Bangladesh regimen" compared to the WHO standard of care. In Stage 1 patients receive moxifloxacin (MFX) adjusted for weight instead of gatifloxacin which was used in the observational trial in Bangladesh.[1] This change was necessitated by the withdrawal of gatifloxacin by the marketing authorization holder. The weight adjusted MFX dosing in Stage 1 of STREAM was used to provide maximal fluoroquinolone activity which was felt by many experts to be critical to the reported success of the "Bangladesh regimen". In Stage 2 of STREAM, levofloxacin will be used in the bedaquiline containing arms (C and D) instead of MFX to minimize the QT prolongation associated with bedaquiline. Levofloxacin (LFX) will be dosed at 750mg/day in subjects weighing $\leq$ 50 kg and 1,000 mg in subjects weighing > 50kg. In Arm B MFX will be dosed at 400mg/day in subjects weighing < 33 kg, 600 mg/day in subjects weighing $\geq$ 33 kg to $\leq$ 50 kg, and 800 mg in subjects weighing > 50kg. This document summarizes information about the relative in vitro potencies against *Mycobacteria tuberculosis* of each fluoroquinolone. In addition, pertinant articles from the limited clinical literature that directly compares the use of both agents for the treatment of MDR TB is discussed.

Table 11 shows the MIC values obtained by Dr. Ji's group for ofloxacin (OFX), LFX, clinafloxacin (CFX) and MFX against banked clinical isolates of MTB. The MIC_{50} and MIC_{90} were 0.50 and 1.0 ug/ml, respectively, for both LFX and MFX. Other investigators have reported that MFX was more potent in vitro than LFX.[42] However, lower MICs for MFX in comparison to LFX were not predictive of superior efficacy based upon pharmacokinetics/pharmacodynamics principles.

Table 11: MIC values for fluoroquinolones

Compound	MIC range	MIC_{50}	MIC_{90}	Reference
OFX	0.50-2.0	1.0	1.0	Ji et al. 1995[43]
LFX	0.25-1.0	0.50	1.0	Ji et al. 1995[43]
MFX	0.12-0.50	0.50	1.0	Ji et al. 1998[44]
CFX	0.12-1.0	0.50	1.0	Ji et al, 1998[44]

A recent evaluation of MDR-TB like treatment regimens given for 6 or 7 months in the mouse model of infection (see Table 12) reported similar relapses at 5 months, but fewer relapses with MFX compared to LFX at months 6 and 7.

Table 12: Relapse* after Treatment Completion [45]

	% (Proportion) of Mice Relapsing after Treatment for:		
Regimen	**5 mo**	**6 mo**	**7 mo**
2 mo RHZ + RH	23 (7/30)	0 (0/30)	not done
2 mo MEtZA + MEtZ	97 (28/29)	59 (17/29)	20 (6/30)
2 mo LEtZA + LEtZ	100 (26/26)	79 (23/29)	38 (11/29)

Definition of abbreviations: A = amikacin; Et = ethionamide; H = isoniazid; L = levofloxacin; M = moxiflocacin; R = rifampin; Z = pyrazinamide.

* Relapse was defined by a positive culture upon plating the entire lung homogenate harvested 3 mo after completing the indicated duration of treatment.

- Using an extended Early Bactericidal Activity study design (eEBA)[14] described the early and extended bactericidal activity (eEBA) of LFX, gatifloxacin and MFX in patients with drug sensitive pulmonary tuberculosis. In this randomized, open-label trial, 40 adults

with newly diagnosed smear-positive DS TB (10 subjects/arm) were assigned to receive: isoniazid (INH) 300 mg, LFX 1000 mg, gatifloxacin 400 mg, or MFX 400 mg daily for 7 days. Sputum for quantitative culture was collected for 2 days before and daily during 7 days of monotherapy. Bactericidal activity was estimated by measuring the decline in bacilli during the first 2 days (EBA 0–2) and last 5 days of monotherapy (extended EBA, EBA 2–7). The EBA 0–2 days of INH (0.67 log10 cfu/ml/day) was significantly greater than that of MFX and gatifloxacin (0.33 and 0.35 log10 cfu/ml/day, respectively), but was not significantly greater than LFX 1000 mg daily (0.45 log10 cfu/ml/day) (*P*=0.14). Bactericidal activity between days 2 and 7 was similar for all three fluoroquinolones. The authors concluded that MFX, gatifloxacin, and high-dose LFX have excellent EBA, only slightly less than for INH, and greater extended EBA and that these drugs warrant further study in the treatment of drug-susceptible TB.

- In a recent retrospective analysis from China, the efficacy of MFX and LFX was explored in the treatment of multidrug-resistant tuberculosis (MDR-TB).[46] 158 patients with MDR-TB receiving either MFX- or levofloxacin-containing regimens were described. Clinical data from patients were subjected to univariate analysis, stratification and multiple logistic regression to compare the roles of moxifloxacin and levofloxacin in multidrug regimens. In total, *72 patients received 400 mg of moxifloxacin once daily* and *86 patients received 509.9 ± 79.4 mg (mean ± standard deviation) of levofloxacin once daily* together with similar active agents for similar durations. The time to sputum culture conversion were similar. Adverse reactions occurred at comparable rates. *The success rates for the MFX group were 65.3% (overall), 77.1% (ofloxacin-susceptible cases) and 54.1% (ofloxacin-resistant cases) in comparison with 55.8%, 60.4% and 50.0%, respectively, for the LFX group.* No demographic, clinical, bacteriological or treatment characteristics were independent predictors of favorable outcome. Fourteen patients from the MFX group and twelve patients from the levofloxacin group had bacteriological relapse after treatment cessation. The authors concluded that compared with levofloxacin, MFX did not show superior efficacy when incorporated into multidrug regimens used for the treatment of MDR-TB.

- Koh et al.[47] described the effectiveness of LFX and MFX compared in terms of culture conversion after 3 months of treatment for MDR-TB. In this prospective multicenter randomized open label trial, 182 patients with MDR-TB (sensitive to LFX and MXF) received either LFX (750 mg/day; 90 patients) or MXF (400 mg/day; 92 patients) with a background drug regimen. The primary outcome was the proportion of patients who achieved sputum culture conversion at 3 months of treatment. Secondary outcomes were the proportions of adverse drug reactions. At 3 months of treatment, 68 (88.3%) of the 77 patients in the LFX group and 67 (90.5%) of the 74 in the MXF group showed conversion to negative sputum cultures (odds ratio for LFX compared with MXF, 0.78; 95% confidence interval, 0.27-2.20). Adverse drug reactions were reported in six patients (7.7%) in the LFX group and four (5.2%) in the MXF group (P = 0.75). The authors concluded that the choice of LFX or MXF for treatment of patients with MDR-TB did not affect sputum culture conversion at 3 months of treatment.

Another group of investigators from Korea reported results from a retrospective review of the use of LFX and MFX in the treatment of MDR TB.[48] The outcomes of 171 patients are

described. Detailed information on dosing and duration are not provided. One hundred and twenty-three of the 171 received LFX and 97 (78.9%) were classified as treatment successes compared to 40 treatment successes (83.3%) in the 48 who received MFX.
In conclusion, many authorities believe that MFX is more potent than LFX in vitro but that superior potency does translate into a better efficacy either in animal models or the treatment of MDR-TB in patients. In the mouse, there is a trend for fewer relapses when MFX is used as part of an MDR TB like regimen compared to LFX. However, an EBA study and several recent small clinical reports describe acceptable and generally similar clinical outcomes when either LFX or MFX was used as part of an MDR TB regimen. This data supports the use of levofloxacin adjusted for weight in Arms C and D of the STREAM trial.

Based the necessity of using LFX with bedaquiline and the design of the ongoing STREAM trial which utilizes weight based dosing for MFX we assert that the weight based dosing of LFX proposed in Stage 2 of STREAM is justified. This is supported by the limited clinical data and expert opinion.[49]

22 REFERENCES

1. Van Deun A. 9-month standardised MDR-TB regimen in Bangaldesh: an update 43rd World Conference on Lung Health; 2012; 43rd World Conference on Lung Health, Kuala Lumpur; 2012.

2. Laserson KF, Wells CD. Reaching the targets for tuberculosis control: the impact of HIV. Bulletin of the World Health Organization. 2007; **85**(5): 377-81;discussion 82-6.

3. Zignol M, Hosseini MS, Wright A, Weezenbeek CL, Nunn P, Watt CJ, et al. Global Incidence of Multidrug-Resistant Tuberculosis. Journal of Infectious Diseases. 2006 Aug 15; **194**(4): 479-85.

4. Wells CD, Cegielski JP, Nelson LJ, Laserson KF, Holtz TH, Finlay A, et al. HIV Infection and Multidrug-Resistant Tuberculosis—The Perfect Storm. Journal of Infectious Diseases. 2007 Aug 15; **196 Suppl 1**: S86-107.

5. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis - 2011 update: World Health Organization; 2011.

6. World Health Organization. Global tuberculosis control - epidemiology, strategy, financing; WHO Report 2009.

7. Chiang C-Y, Enarson DA, Yu M-C, Bai K-J, Huang R-M, Hsu C-J, et al. Outcome of pulmonary multidrug-resistant tuberculosis: a 6-yr follow-up study. European Respiratory Journal. 2006; **28**(5): 980-5.

8. Seung KJ, Omatayo DB, Keshavjee S, Furin JJ, Farmer PE, Satti H. Early Outcomes of MDR-TB Treatment in a High HIV-Prevalence Setting in Southern Africa. PLoS ONE. 2009; **4**(9): e7186.

9. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, Alcantara F, et al. Community-Based Therapy for Multidrug-Resistant Tuberculosis in Lima, Peru. New England Journal of Medicine. 2003 Jan 9; **348**(2): 119-28.

10. Leimane V, Riekstina V, Holtz TH, Zarovska E, Skripconoka V, Thorpe LE, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. The Lancet. 2005; **365**(9456): 318-26.

11. Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. The Lancet Infectious Diseases. 2009; **9**(3): 153-61.

12. Diacon A, Donald P, Pym A, Grobusch M, Patientia R, Mahanyele R, et al. Randomized pilot trial of 8 weeks of bedaquiline (TMC207) for MDR-TB: long-term outcome, tolerability and effect on emergence of drug resistance. Antimicrobial Agents and Chemotherapy. 2012: AAC. 06126-11.

13. Mann G, Squire SB, Bissell K, Eliseev P, Du Toit E, Hesseling A, et al. Beyond accuracy: creating a comprehensive evidence base for TB diagnostic tools. The International Journal of Tuberculosis and Lung Disease. 2010; **14**(12): 1518-24.

14. Johnson JL, Hadad DJ, Boom WH, Daley CL, Peloquin CA, Eisenach KD, et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006; **10**(6): 605-12.

15. Aung KJ, Van Deun A, Declercq E, Sarker MR, Das PK, Hossain MA, et al. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2014; **18**(10): 1180-7.

16. Diacon AH PA, Grobusch MP, de los Rios JM, Gotuzzo E, Vasilyeva I, Leimane V, Dannemann B, on behalf of the TMC207-C208 study group. . Final 120-week results of a Phase II randomised, double-blind, placebo-controlled study of 24-weeks bedaquiline treatment for MDR-TB (C208). 44th World Conference on Lung Health; 2013; Paris; 2013.

17. Cegielski JP, Dalton T, Yagui M, Wattanaamornkiet W, Volchenkov GV, Via LE, et al. Extensive Drug Resistance Acquired During Treatment of Multidrug-Resistant Tuberculosis. Clin Infect Dis. 2014: ciu572.

18. Fox GJ, Menzies D. A Review of the Evidence for Using Bedaquiline (TMC207) to Treat Multi-Drug Resistant Tuberculosis. Infectious Diseases and Therapy. 2013; **2**(2): 123-44.

19. DAIDS RSC. Division of AIDS table for grading the severity of adult and pediatric adverse events. Version 1.0, December 2004 (Clarification August 2009) ed. DAIDS Regulatory Support Centre; 2009.

20. Mukinda FK, Theron D, van der Spuy GD, Jacobson KR, Roscher M, Streicher EM, et al. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2012; **16**(2): 196-202.

21. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType(R) MTBDRsl Assay for fluoroquinolone, amikacin/capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and in clinical specimens. Journal of Clinical Microbiology. 2009; **47**(6): 1767-72.

22. Mohamed K, Embleton A, Cuffe RL. Adjusting for covariates in non-inferiority studies with margins defined as risk differences. Pharmaceutical statistics. 2011; **10**(5): 461-6.

23. World Health Organization. Handbook: Good Laboratory Practice (GLP). 2009.

24. Gumbo T et al. Selection of a Moxifloxacin Dose That Suppresses Drug Resistance in Mycobacterium tuberculosis, by Use of an In Vitro Pharmacodynamic Infection Model and Mathematical Modeling. The Journal of Infectious Diseases. 2004; **190**(1642-1651).

25. Falagas M, Rafailidis P, and Rosmarakis E. Arrhythmias associated with fluoroquinolone therapy. International Journal of Antimicrobial Agents. 2007; **29**(374-379).

26. Altin T et al. Torsades de pointes associated with moxifloxacin: A rare but potentially fatal adverse event (case report). The Canadian Journal of Cardiology. 2007; **23**(11): 907-8.

27. Haddad P et al. Antipsychotic-Related QTc Prolongation, Torsade de Pointes and Sudden Death. Drugs. 2002; **62**(11): 1649-71.

28. Committee for Proprietary Medicinal Products (CPMP). The Assessment of the Potential for QT Interval Prolongation by Non-Cardiovascular Medicinal Products; London 17 December 1997.

29. Florian J et al. Population Pharmacokinetic and Concentration-QTc Models for Moxifloxacin: Pooled Analysis of 20 Thorough Qt Studies. The Journal of Clinical Pharmacology. 2011.

30. Tsikouris J et al. Effects of Three Fluoroquinolones on QT Analysis After Standard Treatment Courses. ANE. 2006; **11**: 52-6.

31. Bayer plc. Avelox (moxifloxacin) Summary of Product Characteristics. Last updated 03/12/2010.

32. Sherzi Sea. Moxifloxacin-induced torsade de pointes (Case Report). 2008; **15**: 71-3.

33. Dale K M et al. Moxifloxacin and Torsade de Pointes (Case Report). The Annals of Pharmacotherapy. 2007; **41**: 336-40.

34. Rubenstein E and Camm J. Cardiotoxicity of fluoroquinolones. Journal of Antimicrobial Chemotherapy. 2002; **49**: 593-6.

35. Morganroth J et al. A Randomized Trial Comparing the Cardiac Rhythm Safety of Moxifloxacin vs Levofloxacin in Elderly Patients Hospitalized With Community-Acquired Pneumonia. CHEST. 2005; **128**: 3398-406.

36. Demolis J-L et al. Effect of a single oral dose of moxifloxacin (400mg and 800mg) on ventricular repolarization in healthy subjects. Clincal Pharmacology & Therapeutics. 2000; **8**: 658-66.

37. Noel G J et al. Effects of three fluoroquinolones on QT interval in healthy adults after single doses. Clinical Pharmacology & Therapeutics. 2003; **73**: 292-303.

38. Sacco F et al. Efficacy of four different moxifloxacin-based triple therapies for first-line H. pylori treatment. Digestive and Liver Disese. 2010; **42**: 110-4.

39. Stass H et al. Pharmacokinetics, Safety and Tolerability of Ascending Single Doses of Moxifloxacin a New 8-Methoxy Quinolone, Administered to Healthy Subjects. Antimicrobal Agents and Chemotherapy. 1998; **42**(8): 2060–5.

40. Alffenaar J et al. Plasma and Cerebrospinal Fluid Pharmacokinetcis of Moxifloxacin in a Patient with Tuberculosis Meningitis (Letter). Antimicrobal Agents and Chemotherapy. 2008; **52**: 2293-5.

41. Soliman E Z et al. Boosted protease inhibitors and the electrocardiographic measures of QT and PR durations. AIDS. 2011; **25**(3): 367-77.

42. Angeby KA, Jureen P, Giske CG, Chryssanthou E, Sturegard E, Nordvall M, et al. Wild-type MIC distributions of four fluoroquinolones active against Mycobacterium tuberculosis in relation to current critical concentrations and available pharmacokinetic and pharmacodynamic data. The Journal of antimicrobial chemotherapy. 2010; **65**(5): 946-52.

43. Ji B, Lounis N, Truffot-Pernot C, Grosset J. In vitro and in vivo activities of levofloxacin against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1995; **39**(6): 1341-4.

44. Ji B, Lounis N, Maslo C, Truffot-Pernot C, Bonnafous P, Grosset J. In vitro and in vivo activities of moxifloxacin and clinafloxacin against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1998; **42**(8): 2066-9.

45. Ahmad Z, Tyagi S, Minkowski A, Peloquin CA, Grosset JH, Nuermberger EL. Contribution of moxifloxacin or levofloxacin in second-line regimens with or without continuation of pyrazinamide in murine tuberculosis. American Journal of Respiratory and Critical Care Medicine. 2013; **188**(1): 97-102.

46. Jiang RH, Xu HB, Li L. Comparative roles of moxifloxacin and levofloxacin in the treatment of pulmonary multidrug-resistant tuberculosis: a retrospective study. Int J Antimicrob Agents. 2013; **42**(1): 36-41.

47. Koh WJ, Lee SH, Kang YA, Lee CH, Choi JC, Lee JH, et al. Comparison of levofloxacin versus moxifloxacin for multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2013; **188**(7): 858-64.

48. Lee J, Lee CH, Kim DK, Yoon HI, Kim JY, Lee SM, et al. Retrospective comparison of levofloxacin and moxifloxacin on multidrug-resistant tuberculosis treatment outcomes. Korean J Intern Med. 2011; **26**(2): 153-9.

49. Yew W-W, Nuermberger E. High-dose fluoroquinolones in short-course regimens for treatment of MDR-TB: the way forward?[Editorial]. Int J Tuberc Lung Dis. 2013; **17**(7): 853-4.

STREAM

The evaluation of a standard treatment regimen of anti-tuberculosis drugs for patients with MDR-TB

An international, multi-centre, open-label, parallel-group, randomised, controlled trial

STAGE 1 STATISTICAL ANALYSIS PLAN

v.1.0 | MARCH 2015

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GENERAL INFORMATION

This document describes and substantiates the statistical principles and methods used for the analysis of data from Stage 1 of the STREAM trial. This document is designed to support the STREAM protocol. This Statistical Analysis Plan (SAP) supersedes version 0.1 of the SAP. Every care was taken in the drafting of this SAP, but corrections or amendments may be necessary. The final version of the SAP will be signed off before database lock for final Stage 1 analysis.

The STREAM trial consists of two stages. Stage 1 involves the comparison of two treatment regimens: Regimen A and Regimen B. Stage 2 involves two additional regimens, Regimen C and Regimen D, and makes two comparisons between Regimen B and Regimen C, and Regimen B and Regimen D for the analysis of the primary endpoint. All treatment regimens are described in detail in the STREAM protocol, Section 2.1.3. Stage 1 and Stage 2 of the STREAM trial each have SAPs listed below. Each SAP has differences, but the fundamental statistical principles will be consistent across all SAPs.

Document	**Description**
Stage 1 SAP	All analyses relating to stage 1
Core Stage 2 SAP	Core analyses for stage 2 relating to analyses after the Week 76 database lock
Extensive Stage 2 SAP	Expanded analyses for stage 2 relating to analyses after the Week 76 database lock
Core Stage 2 Week 132 SAP	Core analyses for stage 2 relating to further analyses conducted after the final (Week 132) database lock
Extensive Stage 2 Week 132 SAP	Expanded analyses for stage 2 relating to further analyses conducted after the final (Week 132) database lock

Compliance:

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, in accordance with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), and the applicable regulatory requirements in the participating countries.

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ABBREVIATIONS AND GLOSSARY

AE	Adverse Event
AFB	Acid Fast Bacilli
AR	Adverse Reaction
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BDQ	Bedaquiline
ICF	Informed Consent Form
CI	Chief Investigator
CFZ	Clofazimine
CRF	Case Report Form
CTA	Clinical Trials Authorisation
DAIDS	Division of Acquired Immunodeficiency Syndrome
DCF	Data Clarification Form
DOT	Directly Observed Treatment
DST	Drug Susceptibility Test
ECG	Electrocardiogram
EMA	European Medicines Agency
EMB	Ethambutol
EQA	External Quality Assurance
FDA	Fluorescein diacetate staining
US FDA	United States Food and Drugs Administration
GCP	Good Clinical Practice
GLC	Green Light Committee
HE	Health Economics
HIV	Human Immunodeficiency Virus
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITM	Institute of Tropical Medicine
ITT	Intention To Treat
KM	Kanamycin
INH	Isoniazid
LFX	Levofloxacin
LPA	Line Probe Assay
LQAS	Lot Quality Assurance Sampling
M2	Metabolite 2
MDR	Multi-Drug Resistant
MFX	Moxifloxacin
Genotype MTBDRPlus	Rapid test for *M. tuberculosis* Complex and its resistance to Rifampicin and/or Isoniazid
Genotype MTBDR*sl*	Rapid test for *M. tuberculosis* Complex and its resistance to fluoroquinolones and/or second-line injectables/cyclic peptides and/or ethambutol
MIC	Minimal Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units–Variable Number of Tandem Repeats
MRC CTU	Medical Research Council Clinical Trials Unit
NE	Notable Event
NTP	National Tuberculosis Programme
PK	Pharmacokinetics
PI	Principal Investigator
PIS	Patient Information Sheet
PTO	Prothionamide

PZA	Pyrazinamide
QA	Quality Assurance
QT Interval	A measure of time between the start of the Q wave and the end of the T wave in the ECG complex
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using the Fridericia correction
REC	Research Ethics Committee
RMP	Rifampicin
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SSA	Site Specific Assessment
STREAM	The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TM	Trial Manager
TMG	Trial Management Group
TMT	Trial Management Team
TREAT TB	Technology, Research, Education, and Technical Assistance for Tuberculosis
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
ULN	Upper limit of normal
The Union	International Union Against Tuberculosis & Lung Disease
USAID	United States Agency For International Development
WHO	World Health Organisation
XDR	Extensively Drug Resistant
ZN	Ziehl-Neelsen

Note. In this statistical analysis plan, time (in weeks) refers to the time from randomisation, e.g. Week 132 refers to 132 weeks from randomisation.

1 TRIAL OVERVIEW

1.1 Study design

The STREAM study is an international, multi-centre, parallel-group, open-label, randomised, controlled trial.

Patients with multidrug-resistant tuberculosis (MDR-TB) are studied in the STREAM trial.

In Stage 1 of the STREAM trial, the comparison being made is between Regimen A and Regimen B.

Regimen A: The locally-used WHO-approved MDR-TB regimen forms the control treatment regimen.

Regimen B: Regimen B is the study regimen, and is based on the regimen described by Van Deun 2010[1] (updated results[2]) consisting of clofazimine, ethambutol, moxifloxacin, and pyrazinamide given for 40 weeks, supplemented by isoniazid, kanamycin, and prothionamide for the first 16 weeks.

All patients in Stage 1 of the study will be followed up to Week 132.

Under versions of the protocol prior to version 6.0 which describe only Stage 1 of the trial, patients are allocated to either Regimens A or Regimen B. Stage 2 of the trial includes two additional arms, Regimens C and D and is implemented in protocol version 6.0 and subsequent versions which also include minor changes to the eligibility criteria, visit schedule and components of the composite primary outcome. If the total sample size for Stage 1 is not reached before Stage 2 is initiated, then the analysis of Stage 1 will include some patients also randomised to Regimens A or Regimen B under the Stage 2 protocol (see section 4.1). A sensitivity analysis will be conducted to repeat the primary analysis under the definition of the primary outcome as described in version 5.2, the last version of the protocol prior to Stage 2 (see section 9.3.1).

1.2 Trial objectives

The primary objectives of Stage 1 of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen B is not inferior to that on Regimen A (WHO approved MDR-TB), the control regimen for Stage 1, at Week 132, using a 10% margin of non-inferiority
2. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen B as compared to Regimen A.

The secondary objectives of Stage 1 of the STREAM trial are:

1. To determine the proportion of patients with a favourable efficacy outcome on the Regimen B in each country setting
2. To compare the economic costs incurred by patients and by the health system during treatment on Regimen B as compared to Regimen A.

1.3 Patient eligibility criteria

A patient will be eligible for randomisation into the study (Stage 1 or Stage 2) if he/she:

1. Is willing and able to give informed consent to participate in the trial treatment and follow-up (signed or witnessed consent if the patient is illiterate)

2. Is aged 18 years or older
3. Has a positive AFB sputum smear result at screening (at least scanty), unless they are HIV positive in which case a positive GeneXpert result within four weeks prior to screening is sufficient
4. Has evidence of resistance to rifampicin either by line probe assay (Hain Genotype), GeneXpert or culture-based drug susceptibility testing (DST), from a test performed at screening or from a test performed within the four weeks prior to screening
5. Is willing to have an HIV test and, if positive, is willing to be treated with ART in accordance with the national policies but excluding ART contraindicated for use with bedaquiline
6. Is willing to use effective contraception: pre-menopausal women or women whose last menstrual period was within the preceding year, who have not been sterilised must agree to use a barrier method or an intrauterine device unless their partner has had a vasectomy; men who have not had a vasectomy must agree to use condoms. In Stage 2 pre-menopausal women or women whose last menstrual period was within the preceding year, who have not been sterilised must agree to use two methods of contraception, for example a hormonal method and a barrier method.
7. Resides in the area and expected to remain for the duration of the study.

In addition to the criteria above, for Stage 2 only, a patient will be eligible for randomisation to the study provided he/she:

8. Has had a chest X-ray at that is compatible with a diagnosis of pulmonary TB (if such a chest X-ray taken within 4 weeks of randomisation is available, a repeat X-ray is not required)
9. Has normal K_{+}, Mg_{2+} and corrected Ca_{2+} at screening.

A patient will not be eligible for randomisation into the study (Stage 1 or Stage 2) if he/she:

1. Is infected with a strain of *M. tuberculosis* resistant to a second-line injectables by line probe assay (Hain Genotype)
2. Is infected with a strain of *M. tuberculosis* resistant to a fluoroquinolone by line probe assay (Hain Genotype)
3. Has tuberculous meningitis or bone and joint tuberculosis
4. Is critically ill, and in the judgment of the investigator, unlikely to survive more than 4 months
5. Is known to be pregnant or breast-feeding
6. Is unable or unwilling to comply with the treatment, assessment, or follow-up schedule
7. Is unable to take oral medication
8. Has AST or ALT more than 5 times the upper limit of normal for Stage 1, and AST or ALT more than 3 times the upper limit of normal for Stage 2
9. Has any condition (social or medical) which in the opinion of the investigator would make study participation unsafe
10. Is taking any medications contraindicated with the medicines in any trial regimen
11. Has a known allergy to any fluoroquinolone antibiotic
12. Is currently taking part in another trial of a medicinal product
13. Has a QT or QTcF interval at screening or immediately prior to randomisation of more than or equal to 500 ms for Stage 1, and more than or equal to 450 ms for Stage 2.

In addition to the criteria above, for Stage 2 only, a patient will not be eligible for randomisation to the study if he/she:

14. Has experienced one or more of the following risk factors for QT prolongation:
    - A confirmed prolongation of the QT or QTcF more than or equal to 450 ms in the screening ECG (retesting to reassess eligibility will be allowed once using an unscheduled visit during the screening phase)

- Pathological Q-waves (defined as Q-wave more than 40 ms or depth more than 0.4-0.5 mV)
- Evidence of ventricular pre-excitation (e.g., Wolff Parkinson White syndrome)
- Electrocardiographic evidence of complete or clinically significant incomplete left bundle branch block or right bundle branch block
- Evidence of second or third degree heart block
- Intraventricular conduction delay with QRS duration more than 120 ms
- Bradycardia as defined by sinus rate less than 50 bpm
- Personal or family history of Long QT Syndrome
- Personal history of cardiac disease, symptomatic or asymptomatic arrhythmias, with the exception of sinus arrhythmia
- Syncope (i.e. cardiac syncope not including syncope due to vasovagal or epileptic causes)
- Risk factors for Torsades de Pointes (e.g., heart failure, hypokalemia, or hypomagnesemia)

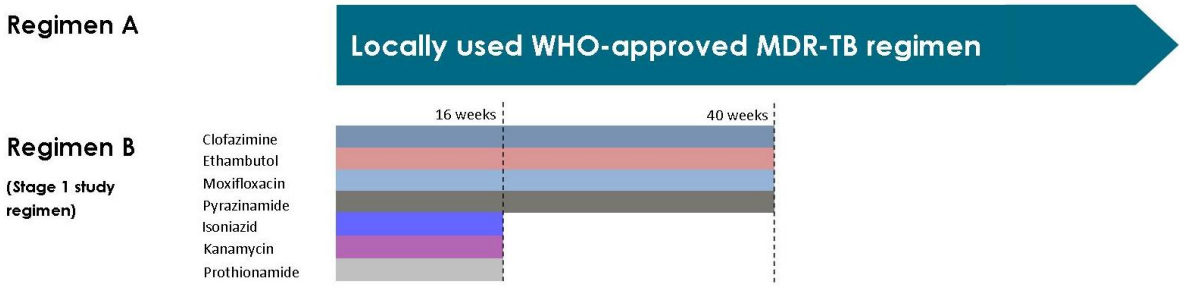
15. Has received treatment for MDR-TB in the 12 weeks prior to screening, other than the maximum permitted treatment specified in Section 5.2.1
16. Has a history of cirrhosis and classified as Child's B or C at screening or a bilirubin more than 1.5 times upper limit of normal.
17. Has an estimated creatinine clearance (CrCl) less than 30 mL/min based on the Cockcraft-Gault equation
18. Is HIV positive and has a CD4 count less than 50 cells/mm^3
19. Has amylase elevation more than two times above the upper limit of normal
20. Has a history of alcohol and/or drug abuse
21. Has had previous treatment with bedaquiline
22. Has taken rifampicin in the seven days prior to randomisation
23. There has been a delay of more than four weeks between the screening consent and randomisation
24. Is an employee or family member of the investigator or study site staff with direct involvement in the proposed study.

1.4 Study interventions

The control regimen, Regimen A, is the locally-used WHO-approved MDR-TB regimen. Country- or site-specific regimens are described in the STREAM Patient Management Guide.

The investigative regimen is Regimen B, and consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for 40 weeks, supplemented by kanamycin, isoniazid and prothionamide in the first 16 weeks (intensive phase).

Figure 1: Regimen A & Regimen B



In Regimen B, all drugs are given daily (seven days a week), except for kanamycin which is initially given daily and then thrice-weekly from Week 12 onwards.

The intensive phase may be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks, respectively, as described below.

Table 1: Regimen B doses

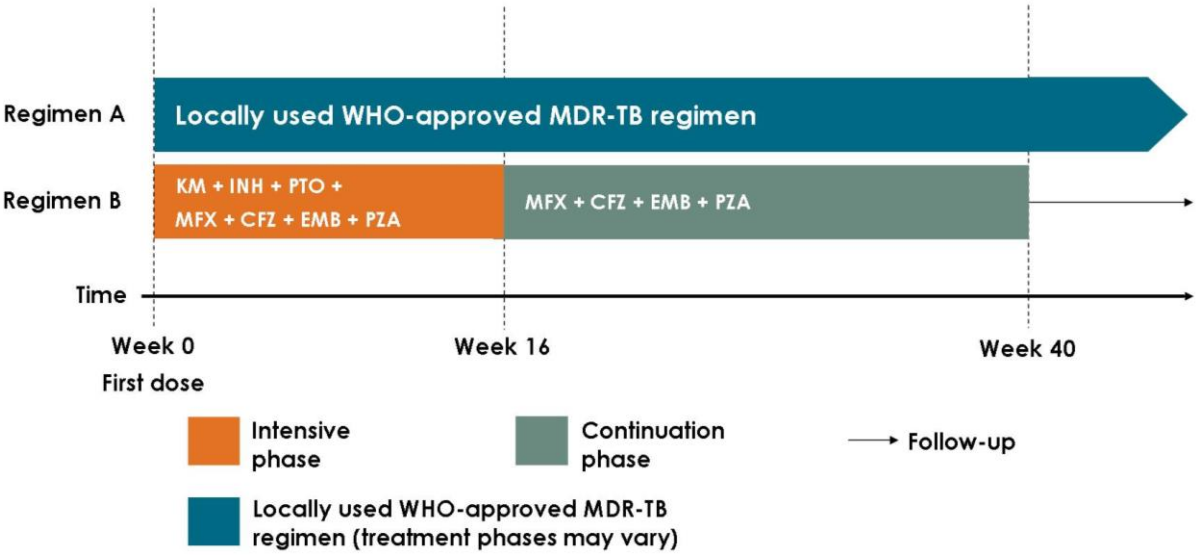
Product	Weight group		
	Less than 33 kg	33 kg to 50 kg	More than 50 kg
Moxifloxacin	400 mg	600 mg	800 mg
Clofazimine	50 mg	100 mg	100 mg
Ethambutol	800 mg	800 mg	1200 mg
Pyrazinamide	1000 mg	1500 mg	2000 mg
Isoniazid	300 mg	400 mg	600 mg
Prothionamide	250 mg	500 mg	750 mg
Kanamycin	15 mg per kilogram body weight (maximum 1g)		

Patients randomised to Regimen B will receive 40 weeks of treatment (16 weeks intensive phase plus 24 weeks continuation phase), as shown in Figure 1.

1.5 Treatment phases

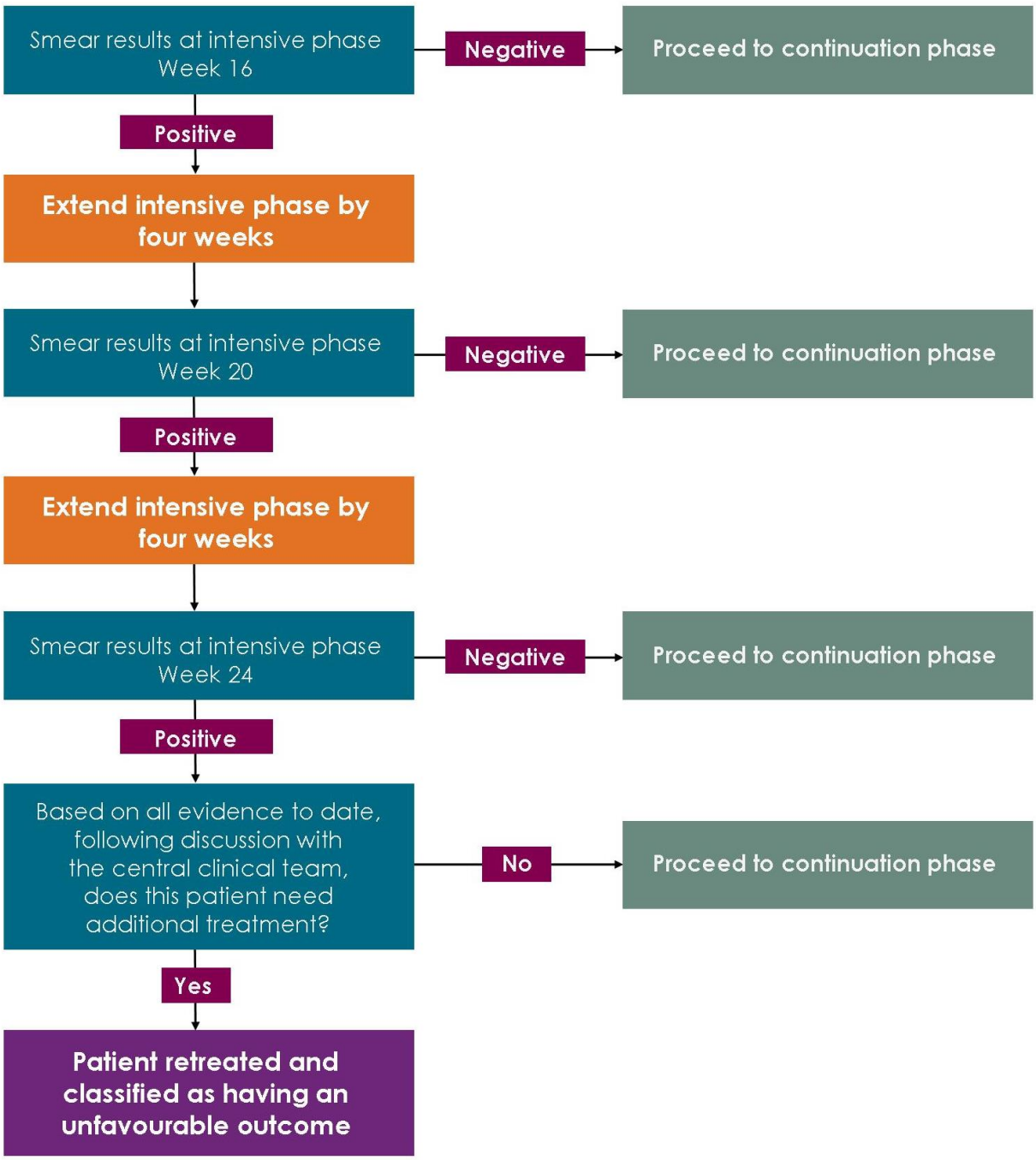
The study regimen, Regimen B, consists of 2 phases; an intense phase followed by a continuation phase, as shown in Figure. 1.

Figure 2: Treatment phases



For patients randomised to Regimen B the following algorithm will be used to determine when a patient can proceed from the intensive to the continuation phase.

Figure 3: Transition from intensive to continuation phase for patients on Regimen B



Patients randomised to Regimen B will be prescribed 40 weeks of treatment (16 weeks intensive phase and 24 weeks continuation phase). In the event of a positive (at least "scanty" on the IUATLD/WHO scale) AFB smear at Week 16, the drugs in the intensive phase of this regimens may be extended by 4 weeks, if the smear is still positive at 20 weeks the intensive phase may be extended by a further 4 weeks allowing a maximum intensive phase of 24 weeks, and hence a maximum total duration of 48 weeks treatment.

1.6 Randomisation procedure

Patients will be randomised to Regimen A or Regimen B. Randomisation will be in a 1:2 ratio in favour of Regimen B to allow more data on efficacy and safety to be collected on this regimen. Randomisation will be stratified by (1) site, (2) HIV status for sites with high TB-HIV co-infection rates.

Separate randomisation lists for each combination of strata will be prepared in advance by a statistician independent of the study, using varying block sizes. Should web access not be available at the time of randomisation, a manual alternative using sealed envelopes will be provided.

Patients will be randomised using a web-based randomisation system. Access to the web-based system will be controlled through an authorised username and password. Before treatment allocation the patient's eligibility will need to be confirmed, and their site, HIV status, and CD4 count entered into the database.

2 SAMPLE SIZE

2.1 Power to demonstrate non-inferiority in the primary efficacy outcome

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden and duration and the expected increase in adherence in reducing a treatment regimen from 104 weeks (as with Regimen A), to 40 weeks (as with regimen B).

A meta-analysis of treatment outcome in patients with MDR-TB found an overall favourable outcome of 64% (95% CI 59-68) in patients given individualised treatment and 54% (95% CI 43-68) in patients given standardised treatment[3]. A reasonable estimate of the efficacy of regimen A in the STREAM trial would therefore be 70%.

Based on the experience with regimen B[1], a reasonable estimate of its efficacy in the STREAM trial would be between 75% and 85%. The lower estimate is used for the sample size calculations below.

Based on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 2 gives the total number of patients required to demonstrate non-inferiority under the specified scenarios using a margin of non-inferiority of 10%. These totals allow for 20% of patients being classified as not assessable in a per-protocol analysis and are based on a one-sided level of significance of 2.5%.

Table 2: Power to demonstrate non-inferiority in the primary efficacy outcome

Power	Percentage favourable outcomes in Regimen A	Difference in percentage favourable outcomes in Regimen B compared to Regimen A		
		0%	5%	10%
80%	60%	1060	464	255
	65%	1005	435	238
	70%	928	398	214
90%	60%	1419	620	340
	65%	1345	583	318
	70%	1242	533	287

Therefore, 398 patients would be required (rounding to 400 gives: 267 on Regimen B and 133 on Regimen A) to demonstrate non-inferiority with 80% power assuming 70% favourable outcomes in Regimen A and 75% in Regimen B and 20% not assessable. A larger difference in response rates of 10% would require fewer patients and could also be demonstrated with greater than 90% power with a total enrolment of approximately 400 patients.

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden, duration, and resource utilisation, and the expected increase in adherence in reducing a treatment regimen from 104 weeks (as with Regimen A), to 40 weeks (as with Regimen B).

If the difference in response rates in favour of Regimen B is more than 10% it may be possible to demonstrate superiority of that regimen over the control for stage 1, Regimen A.

At least 400 patients will need to be enrolled across all countries to give sufficient power to demonstrate non-inferiority. Patients will be randomised to Regimen B and Regimen A in the ratio 2:1.

2.2 Power to demonstrate non-inferiority in the primary safety outcome

Assuming a sample size of 400 on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 3 gives the power available to demonstrate non-inferiority in the primary safety outcome under different proportions of grade 3 or 4 events on Regimen A and Regimen B. These calculations assume a margin of non-inferiority of 10% and a one-sided level of significance of 2.5%. All randomised patients who have received at least one dose of study medication will be included in the safety analysis.

Table 3: Power to demonstrate non-inferiority in the primary safety outcome

Proportion grade 3 or 4 on Regimen A	Assuming same proportion in Regimen A and Regimen B	Assuming an absolute 5% lower proportion on Regimen B than Regimen A
10%	88%	99%
15%	75%	99%
20%	65%	96%
25%	58%	93%
30%	53%	89%
35%	50%	86%
40%	48%	83%

3 PRIMARY OUTCOMES

3.1 Primary analysis Week 132 window

The Week 132 window is defined as the time period from six weeks before 132 weeks since randomisation with no upper bound, i.e. from Week 126 with no upper bound.

3.2 Primary efficacy outcome

The primary efficacy outcome measure is the proportion of patients with a favourable outcome (as defined below) at Week 132.

Only culture results obtained using acidified Ogawa (Kudoh medium) will be used in the primary efficacy analysis.

A positive culture on Ogawa is defined as at least one colony and a negative culture is defined as absence of growth (no colonies).

Favourable

A patient's outcome will be classified as **favourable** if their last two culture results are negative unless they have previously been classified as unfavourable. These two cultures must be taken on separate visits (on different days); the latest of which being within the Week 132 window.

Patients that don't have a culture result within the Week 132 window because they were unable to produce sputum, will be classified as favourable if their last two cultures before the Week 132 window are negative and they have not previously been classified as unfavourable; such patients will be identified separately in tables.

Unfavourable

A patient's outcome will be classified as **unfavourable** if:

1. They are discontinued from their allocated study treatment and subsequently restarted on a different MDR-TB regimen
2. Treatment is extended beyond the scheduled end of treatment for any reason other than making up of days when no treatment was given (missed treatment) for a maximum of eight weeks
3. They are restarted on any MDR-TB treatment after the scheduled end of treatment, but before 132 weeks after randomisation
4. They change their allocated study treatment for any reason other than (1) the replacement of a single drug or (2) for patients allocated to Regimen A when the change is as a result of changes in local guidelines and not related to any change in the patient's circumstances or condition.
5. Bedaquiline is started where the allocated regimen did not originally contain that drug (Regimen A or Regimen B).
6. A drug from the class of nitroimidazoles is started
7. They die at any point during treatment or follow-up
8. At least one of their last two culture results, from specimens taken on separate occasions, is positive
9. They do not have a culture result at the end of the Week 76 window or thereafter

Starting a single drug other than bedaquiline or from the class of nitroimidazoles is not considered to be a substantial change to the regimen and therefore does not result in an unfavourable outcome, providing none of the other criteria above are met.

Changes of treatment in patients allocated to Regimen A that result from a change in local guidelines not related in any way to any change in the patient's circumstances or condition will not be classified as unfavourable. A sensitivity analysis will be conducted where these changes *are* classified as unfavourable. However, this sensitivity analysis can only result in an increase in unfavourable outcomes on Regimen A, thereby increasing the chance of demonstrating the non-inferiority of Regimen B and therefore the primary analysis described here is more conservative.

All re-infections with a different strain are classified as **not assessable**.

An extension of the intensive phase of treatment in any study arm does not constitute an unfavourable outcome, as long as the extension is in accordance with the algorithm described in Figure 3 of Section 1.5 for patients on Regimen B. Similarly, the discontinuation of drugs that are not replaced does not constitute an unfavourable outcome.

A patient who has a culture result within the Week 76 window, but not within the Week 132 window, having not otherwise been classified as unfavourable (based on the definitions above) will be regarded as **not assessable** and will be excluded from the primary analysis provided their last two cultures, from specimens taken on separate occasions, are negative. Any patient who does not have a culture result within the Week 132 window and does not fulfil these criteria will be classified as **unfavourable**. These definitions apply to both Regimen A and Regimen B.

3.3 Primary safety outcome

The primary safety outcome measure is the proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria[4], at any time during treatment and follow-up.

4 ANALYSIS POPULATIONS

4.1 Stage 1 analysis population

All patients randomised in Stage 1 and Stage 2 of the STREAM trial to Regimen A and Regimen B up to the Stage 1 randomisation end date will be included in the Stage 1 analysis population.

The Stage 1 randomisation end date is defined as no earlier than the date when 133 patients are randomised to Regimen A and 267 patients randomised to Regimen B. If, during the period of recruitment, it is expected that the proportion of patients not assessable for the primary analysis is likely to exceed the anticipated 20%, then the closure of Stage 1 randomisation may be delayed to allow more than 400 patients to be randomised in Stage 1. This will be at the discretion of the Trial Steering Committee, who will remain blind to aggregated data by treatment arm at all times before database lock.

4.2 Intention-to-treat (ITT)

All randomised patients will be included in the ITT analysis population.

4.3 Safety population

All randomised patients that have taken at least one dose of treatment will be included in the safety analysis population.

4.4 Modified intention-to-treat (mITT)

The mITT population is defined as all randomised patients that have a positive culture for *M. tuberculosis* on acidified Ogawa (Kudoh medium) at screening or randomisation, with the

exception of patients with isolates taken before randomisation that are subsequently found to be susceptible to rifampicin, and patients with isolates taken before randomisation that are subsequently found to be resistant to both fluoroquinolones and second-line injectables (i.e. XDR-TB) on phenotypic DST. Results from the central reference laboratory will take priority over any results from local laboratories where available.

4.5 Per protocol (PP)

The PP population will be the same as the mITT population with the exclusion of patients not completing a protocol-adherent course of treatment, other than for treatment failure or death. Treatment failure is defined as failure to attain and maintain culture negativity until the end of allocated treatment.

4.5.1 Definition of a protocol-adherent course of treatment

Patients will be excluded from the per-protocol analysis if they do not complete a protocol-adherent course of treatment, other than for treatment failure or death.

A patient will have completed a protocol-adherent course of treatment when they have taken 80% of doses within 120% of the minimum duration in both the intensive phase and in the whole treatment period. For this purpose, a dose is defined as all the study medications at the correct dose for that particular day.

For Regimen B, **with or without** an extension of the intensive phase, a patient will have completed a protocol-adherent course of treatment if they have taken:

- 90 doses (80% of 16 weeks) within 134 days (120% of 16 weeks) in the intensive phase, and
- 224 doses (80% of 40 weeks) within 336 days (120% of 40 weeks) over the whole treatment period (i.e. the combined intensive and continuation phases) regardless of treatment extensions.

The same algorithm will apply for Regimen A, the control regimen; the exact number of doses and days depends on the duration of the intensive and continuation phases of Regimen A.

5 GENERAL ANALYSIS PRINCIPLES

5.1 Analysis populations

The analyses of the primary outcomes will be based on both the mITT and the PP populations. All patients included in the analysis will be analysed in the treatment group to which they were originally assigned. Further sensitivity analyses are planned (see Section 9 Sensitivity Analyses).

5.2 Treatment and follow-up phase definitions

For the purpose of analysis, the screening, treatment, and follow-up phases for an individual patient will be defined as follows:

- **Screening phase**
  - Start: date of screening consent
  - End: day before randomisation
- **Treatment phase**
  - Start: date of randomisation.
  - End: date of last dose of any TB treatment defined as last dose of any TB treatment (including retreatment for relapse), plus 7 days.
- **Follow-up phase**
  - Start: the day after the end of the treatment phase.
  - End: date of the last patient contact (scheduled or unscheduled, or other contact e.g. phone call).

The treatment phase includes any extension of treatment or retreatment, and so the Allocated Treatment phase is defined as follows:

- **Allocated Treatment phase**
  - Start: date of randomisation.
  - End: date of last dose of trial treatment defined as last dose of allocated regimen or last dose before the addition of a new drug, whichever happens sooner, plus 7 days.

5.3 Visit window definitions

During Stage 1 (under protocol versions prior to version 6.0), patients will be assessed at screening, randomisation (Week 0), Week 1, Week 2, Week 3, Week 4, and at 4-weekly intervals throughout the study, until the end of follow-up, irrespective of whether on treatment or in the post-treatment follow-up phase.

During Stage 2 (under version 6.0 and later versions of the protocol), patients will be assessed at screening, randomisation (Week 0), Week 1, Week 2, Week 3, Week 4, after which they will be seen 4-weekly until Week 52, after which they will be seen 8-weekly until Week 84, after which they will be seen 12-weekly until Week 132 post randomisation.

For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of randomisation. For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of randomisation. When referring to a visit hereon, this implies within the defined visit window as specified below.

Visit	target date, days after randomisation +1	Analysis window
Screening / Baseline	1	Date of screening consent - 1
Week 4	29	2-42
Week 8	57	43-70
Week 12	84	71-98
Week 16	113	99-126
...		
Week a	b = 1+ (a x 7)	(b-14) – (b+13)
...		
Week 120	841	827-854
Week 124	869	855-882
Week 128	N/A (included within 132 week analysis window)	
Week 132	925	833-no upper bound

Any visit, scheduled or unscheduled, that falls into the analysis window will be assigned to that visit for the purpose of analysis. If two visits fall within the same interval, the one closest to the target date will be used for analyses by visit, so that there is only one unique visit for each patient and analysis time-point.

There are additional study visits at Weeks 1, 2 and 3 only for ECG monitoring. For the analysis of ECG data only, there will be additional visit windows: Week 1 (2-11), Week 2 (12-18), Week 3 (19-25) and the Week 4 visit window will be modified to (26-42).

5.4 Definition of a culture result

A culture result will be called positive for *M. tuberculosis* if the culture tests positive for the presence of microorganisms, at least one colony, and the microorganisms present are then identified as being *M. tuberculosis*. However, if an identification test is not carried out for a particular culture, then for analysis purposes a culture will still be considered positive for *M. tuberculosis* if the culture tests positive for the presence of microorganisms and if that culture result is obtained seven days or more since the start date of sputum processing and incubation of the inoculated Ogawa. If the culture result is obtained less than seven days since the start date of sputum processing and incubation of the inoculated Ogawa, the culture result will not be considered as positive for *M. tuberculosis*, and the culture result will be considered missing in the analysis.

If more than one culture result is available from sputum collected on the same day, this will be regarded as a single culture result for the purposes of all analyses with the following overall result:

i. **Positive**, if at least one of the culture results is positive
ii. **Negative**, if at least one of the culture results is negative and none of the culture results are positive
iii. **Contaminated** if at least one of the culture results is contaminated and none of the culture results are positive or negative.
iv. **Missing**, if no culture result is available.

5.5 Definition of a smear result

A smear result will be called positive if it is graded as 'scanty' or 'rare AFB' or at least 1+.

If more than one smear result is available from sputum collected on the same day, this will be regarded as a single smear result for the purposes of all analyses with the following overall result:

i. **Positive**, if at least one of the smear results is positive
ii. **Negative**, if at least one of the smear results is negative and none of the smear results are positive
iii. **Missing**, if no smear result is available.

5.6 Reference laboratory bacteriology

A number of clinical isolates will be sent from the STREAM sites to a reference laboratory at the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Drug sensitivity results from the reference laboratory will be used in all analyses in preference to those obtained from local site laboratories where available.

5.7 Adverse events

For all analyses of adverse events, only those occurring after randomisation will be included.

6 ANALYSIS OF PRIMARY OUTCOMES

6.1 Primary efficacy analyses

6.1.1 Modelling technique used in analysis

For the primary efficacy analysis the difference in proportions of favourable outcome between two specified trial regimens with corresponding 95% confidence intervals and p-values will be estimated using a stratified analysis of the risk difference from each stratum using Cochran-Mantel-Haenszel weights.[5] The analysis will be stratified only by HIV status: HIV negative and HIV positive. Where there is a difference between data used for stratification and correct data (if randomisation was inadvertently done on incorrect data), the correct data will be used for adjustment in the analysis.

6.1.2 Primary efficacy analysis: non-inferiority of Regimen B

Non-inferiority will be demonstrated if the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes between Regimens A and B is less than the 10% margin of non-inferiority in both the mITT and PP populations.

6.1.3 Superiority of Regimen B

If Regimen B is declared non-inferior to Regimen A, then superiority of Regimen B compared to Regimen A will be assessed.

If the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes between Regimens A and Regimen B is less than zero, then superiority of Regimen B compared to Regimen A will be declared. For this analysis, the mITT population will be primary and the PP population will be one of several secondary analyses.

6.2 Tabulation of primary endpoint classification

Since the primary endpoint is a composite of various components, the actual reason (component) for outcome will also be tabulated by treatment arm.

Patients will be classified by the first event that made the patient unfavourable (see section 3.3) and further sub-classified by their microbiological outcome at the time that this outcome occurred (see section 7.1 below) and further sub-classified by whether the patients subsequently died before or during the Week 76 window. For example, a patient that has their treatment regimen changed during the treatment phase but subsequently has a positive culture during the Week 76 window will be classified as having had their regimen changed and further sub-classified by whether they had achieved culture conversion when their regimen was changed.

6.3 Subgroup analyses

This primary efficacy analysis will be repeated in subgroups according to HIV infection status, baseline drug resistance patterns (i.e. resistance to pyrazinamide, a fluoroquinolone, a second-line injectable, and isoniazid), BMI (<18, 18-<20, 20-<25, ≥25), cavitation (presence, absence), study centre, age (<45, 45-<65, ≥65), sex, smoking history (current smoker, ex-smoker and never smoked) and ethnicity.

In addition, to evaluate any effect of the minor differences in the protocol after the initiation of Stage 2, the primary efficacy analysis will be repeated in the subgroup of patients enrolled under protocol 5.2 and prior versions, and in the subgroup of patients enrolled under protocol 6.0 (Stage 2) and later versions.

6.4 Primary safety analysis

The primary safety outcome is the occurrence of a Grade 3 or greater adverse events.

The difference in proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria, during the treatment and follow-up phases, between Regimen

B and Regimen A with corresponding two-sided 95% confidence intervals and p-values will be estimated (see Section 6.1).

This analysis will be conducted on the whole study period, and separately for each phase (Treatment, Follow-up and Allocated treatment).

7 ANALYSIS OF SECONDARY OUTCOMES

7.1 Microbiological outcome

Sputum culture negative status is defined as two consecutive negative cultures from sputa collected on different days without an intervening positive. Culture negative status is lost when a culture result is positive, but can subsequently be re-achieved if two consecutive cultures from sputa collected on different days are negative without an intervening positive

7.1.1 Microbiological outcome at unfavourable outcome

The microbiological outcome at unfavourable outcome is defined using culture results up to and including the date of the first event that made their primary efficacy outcome unfavourable (the 'unfavourable outcome event'). It is defined as follows:

- **Culture negative.** Culture negative status was satisfied at the date of the unfavourable outcome event.
- **Never culture converted**. The patient never achieved culture negative status at any time during the study prior to the unfavourable outcome event.
- **Culture positive**. Culture negative status was achieved at some point during the study, but was not satisfied at the date of the unfavourable outcome event. Culture positive will be further classified as **Culture positive: Reinfection** if it has been shown that the M. tuberculosis strain of the positive culture is different to baseline; and **Culture positive: Relapse** otherwise.

Patients will be classified by the first event that made the patient unfavourable and further sub-classified by their microbiological outcome at unfavourable outcome and further sub-classified by whether the patients subsequently died (see Section 6.2).

7.1.2 Microbiological outcome at Week 132

The microbiological outcome at Week 132 will be defined as follows:

- **Culture negative at Week 132**. Culture negative status was satisfied when last seen with a negative culture within the Week 132 window.
- **Culture negative: did not complete follow-up.** There were no culture results during the Week 132 window and culture negative status was satisfied when the patient was last seen.
- **Never culture converted**. The patient never achieved culture negative status at any time during the study up to Week 132.
- **Culture positive**. Culture negative status was achieved at some point during the study, but was not satisfied when the patient was last seen (at least one of the last two non-missing culture results was positive). Culture positive will be further classified as **Culture positive: Reinfection** if it has been shown that the M. tuberculosis strain of the positive culture is different to baseline; and **Culture positive: Relapse** otherwise.

Microbiological outcome at Week 132 will be tabulated by regimen. Patients that die will be classified as above based on their available culture results when last seen, but classified separately from patients that did not die.

7.2 Efficacy outcomes

Secondary efficacy outcomes will be analysed on both the mITT and PP analysis populations.

7.2.1 Time to sputum smear and culture conversion

Time to sputum smear conversion is defined as the time from randomisation to the first of two consecutive negative sputum results, collected on separate days. All patients in the respective analysis population will be included in this analysis, except those with no positive smear result at screening or randomisation. Patients that never achieve smear conversion will be censored at the date of collection of sputum that yielded their last smear result.

Time to sputum culture conversion is defined as the time from randomisation to the first of two consecutive negative culture results, collected on separate days. Patients that never achieve culture conversion will be censored at the date of collection of sputum that yielded their last culture result.

Median time to sputum smear and culture conversion will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

The equality of survivor functions for time to sputum conversion for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

Even when Kaplan-Meier curves of time to culture conversion have been shown to diverge in the presence of an effective drug (such as bedaquiline), they tend to converge later in follow-up potentially violating the assumption of proportional hazards. In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

The analyses above of time to sputum smear conversion and time to sputum culture conversion will be repeated with the alternative definition as time from randomisation to the first negative culture or smear result respectively (without the need for a second negative culture or smear to confirm).

7.2.2 Time to unfavourable efficacy outcome

Time to unfavourable efficacy outcome is defined as the time from randomisation to the first event that results in the definition of an unfavourable efficacy outcome for that patient (as defined in Section 3.2). Patients that do not culture convert during the treatment and follow-up phases (i.e. fail to have 2 consecutive culture negative results), and have not otherwise been called unfavourable, will be called unfavourable at the date of the last visit when a culture positive result was obtained.

Patients classified as favourable or not assessable will be censored in this analysis at the date of collection of sputum that yielded their last negative culture result.

Median time to unfavourable efficacy outcome will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

The equality of survivor functions for time to unfavourable efficacy outcome for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

7.2.3 Time to cessation of clinical symptoms based on PI assessment

Time to cessation of clinical symptoms is defined as the time from randomisation to the first of two consecutive visits where cessation of **all three** of the TB symptoms: a productive cough, fever, and night sweats, as reported by the patient. Patients with none of the TB symptoms at screening and none of the TB symptoms at baseline with be excluded from this analysis. This definition matches the definition of time to culture conversion as the first of two consecutive symptom-free months.

Median time to cessation of clinical symptoms will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

For patients who do not cease clinical symptoms, cessation of clinical symptoms will be censored at the patients' last visit.

The equality of survivor functions for time to cessation of clinical symptoms for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

7.3 Safety outcomes

Safety outcomes will be analysed using the safety analysis population.

7.3.1 Placement of events by study phases

Adverse events are placed in study phases (see section 6.1 for definitions) based on the start date. If the start date of an event falls between (or on) the start and stop date of a phase, the AE is attributed to that phase.

In case of partial start dates, the following approach is used:

- **Missing day only**: The event is placed in all phases that overlap the given month and year for the event, excluding any phases that start after the end date of the AE (if specified).
- **Missing day and month only**: The event is placed in all phases that overlap the given year for the event, excluding any phases that start after the end date of the AE (if specified).
- **Missing start date:** The event is placed in the treatment phase, unless the end date of the AE is specified and is before randomisation, in which case the event is placed in the screening phase.

7.3.2 All-cause mortality during treatment or follow-up

All-cause mortality is defined as a patient who has died from any-cause (both TB- or non-TB-related) while in the trial either during treatment or during follow-up.

The number of patients who die during treatment and follow-up will be tabulated by treatment arm.

Survival analysis will be conducted for time to death.
A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, with no stratification.

For patients that do not die, time will be censored at their final visit.

The equality of survivor functions for time to death for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

7.3.3 Change of regimen for adverse events

A change of regimen for an adverse event is defined as when a patient's regimen is modified in any way (including stopping a drug, changing the dose of a drug or starting a new drug) with the main reason being an adverse event.

The difference in proportion of patients who have a change of regimen for adverse events between Regimen B and Regimen A will be calculated.

7.3.4 Proportion of patients experience treatment-related grade 3 or greater adverse events occurring on treatment and during the follow-up period

The proportion of patients with treatment-related grade 3 or greater adverse events that occur on treatment and during the follow-up period is defined as the number of grade 3 or greater adverse events considered to be possibly, probably or definitely related to treatment.

The difference in proportion of treatment-related adverse events between Regimen B and Regimen A will be calculated.

7.3.5 Adherence to treatment

Adherence to treatment is defined as either **adherent**; if a patient has taken at least 80% of doses within 120% of the time (as defined above), or **non-adherent**; if a patient has not met these conditions.

The difference in proportion of those who have been adherent to treatment between Regimen B and Regimen A will be calculated.

7.4 Acceptability outcomes

In selected sites, acceptability of Regimen A and B to stakeholders will be analysed in terms of:

- Costs to the health system
- Household costs
- Patient treatment and support experiences
- Health worker experiences.

The analyses of health and household costs and patient and health worker experiences will be described in a separate document.

8 DATA SUMMARIES

8.1 Recruitment and baseline characteristics

8.1.1 Recruitment, screening, & eligibility

The number of patients screened, randomised and treated will be tabulated by centre and treatment arm. The number of patients who failed screening, and the reasons for ineligibility will be presented by randomised group.

8.1.2 Exclusions from analysis

The number of patients excluded from the mITT and PP analysis populations will be tabulated by treatment arm and by reason for exclusion.

8.1.3 Baseline characteristics

All eligible patients randomised will be included in tables of baseline comparisons by treatment group. Characteristics will include sex, age, ethnicity, height, weight, BMI, and laboratory parameters such as, HIV status, CD4 count (if applicable), smoking status (current smoker, ex-smoker, never smoked) smear and culture status, and drug susceptibility status for a number of TB drug types. The baseline characteristics table will be repeated for each of the ITT, safety, PP and mITT populations.

8.2 Efficacy and adherence

Each analysis will be repeated using the mITT and PP analysis populations.

8.2.1 Sputum smear and culture

Sputum smear and culture results (positive or negative) will be tabulated by visit and treatment arm.

8.2.2 Adherence

Adherence will be summarised by treatment arm as the percentage of each of the intensive and continuation phase doses completed and overall across both phases.

8.2.3 Drug resistance

Drug resistance at screening or baseline will be tabulated by treatment arm, with separate tables for genotypic and phenotypic DSTs. Acquired resistance to any drugs will also be described and tabulated by treatment arm using the last available DST result for each drug for each patient.

In addition, acquired resistance to any drugs will also be described and tabulated by treatment arm using any available post-randomisation DST result only from the reference laboratory at the Institute of Tropical Medicine (ITM) in Antwerp (i.e. ignoring any results from local site laboratories) for each drug for each patient.

In a further analysis, acquired resistance to any drugs will also be described and tabulated by treatment arm using any available post-randomisation DST result (i.e. classifying as resistant if any result is resistant from ITM or local site laboratories) for each drug for each patient.

Acquired resistance for each definition will also be tabulated by category of primary endpoint and microbiological outcome to determine any cases of acquired resistance that didn't result in an unfavourable outcome.

8.3 Retention and description of follow-up

8.3.1 Description of follow-up and populations

Completion of treatment and completion of scheduled follow-up will be summarised by treatment group including reasons for failure to complete treatment or follow-up. This analysis will be using the ITT, PP, safety, and mITT analysis populations.

8.4 Safety outcomes

Safety outcomes will be analysed using the safety analysis population.

8.4.1 Electro-cardiology

Both mean (and SE) QT, QTcF and heart rate (HR) by visit and treatment arm, and mean (and SE) QT, QTcF and HR change from baseline by visit (within visit window) and treatment arm will be tabulated.

QT and QTcF will be categorised (<450, 450-479, 480-499, ≥500) and tabulated by visit and treatment arm, and highest post-randomisation value overall by treatment arm. Change from baseline of QT and QTcF will also be categorised (<30, 30-59, ≥60) and tabulated by treatment arm, and highest post-randomisation value overall by treatment arm.

These tables will be done for the whole study period and repeated for the treatment phase only.

Time to first QTcF over 450ms and first QTcF over 500ms and QTcF increase from baseline by 30ms and by 60ms analyses will be conducted. Number of each of these events (i.e. whether a threshold was exceed or not) will be tabulated by treatment arm. Hazard ratios with

corresponding two-sided 95% confidence intervals will be estimated using a Cox Proportional Hazards model will be used, with no stratification.

The outcomes will be censored at the patients' last visit.

The equality of survivor functions for time to QTcF over 450ms and over 500ms and QTcF increase from baseline by 30ms and by 60ms for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, with no stratification.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

QTcF will be estimated by visit and by treatment arm using a linear mixed model, adjusted for the stratification factors. Estimates with 95% confidence intervals will be plotted by visit and treatment arm. This will be repeated for change in QTcF from baseline.

It is likely that treatment and dose changes will impact on QTcF and so this analysis will be repeated ignoring any results after discontinuation or change of dose of any drug.

All of the electro-cardiology analysis will be repeated separately by HIV status and by sex. An interaction between HIV status or sex and QTcF will be tested by including an interaction term in the linear mixed models for QTcF and change in QTcF from baseline.

8.4.2 Liver function

ALT, and AST will be categorised (<1xULN; 1-<3xULN, 3-<5xUlLN; 5ULN-<10ULN; ≥10ULN) and tabulated by visit and treatment arm.

Mean ALT, and AST will be presented by visit and treatment arm. The number of patients experiencing more than or equal to five times above the upper normal limit will be tabulated by arm.

8.4.3 Hearing impairment

The number (and proportion) of patients reporting experiencing clinically significant hearing loss (unilateral or bilateral) during the combined treatment and follow-up period will be tabulated by treatment arm.

8.4.4 Weight gain

Patient weight will be tabulated by treatment arm and visit in addition to change from baseline weight by visit and treatment arm.

8.4.5 SAE/NE

SAE and NE data will be tabulated as follows:

i. Event grade by treatment arm, with details of type of SAE/NE listed with frequencies for each event grade
ii. Event relatedness to study drugs by treatment arm
iii. Number of patients experiencing Grade 3 or higher adverse events by treatment arm
iv. Number of Grade 3 or higher adverse events by treatment arm.

9 SENSITIVITY ANALYSES

9.1 Additional adjusted and unadjusted primary efficacy analyses

All primary efficacy analyses will be repeated:

1. Unadjusted for any covariates.
2. Adjusted for randomisation stratification factors HIV status and centre. Small strata with fewer than 10 patients will be combined within geographical regions.
3. Adjusted for randomisation stratification factors and any additional important covariates such as cavitation at baseline or baseline bacillary load.

9.2 Additional analysis populations for primary efficacy analysis

In addition to the mITT and PP analysis populations, the primary efficacy analyses will be repeated for the (1) ITT analysis population, (2) the safety analysis population, and (3) the mITT analysis population excluding patients that didn't start treatment.

9.3 Reclassification of primary efficacy endpoint

9.3.1 Classification using pre-Stage 2 primary outcome definitions

A sensitivity analysis will be conducted to repeat the primary analysis under the definition of the primary outcome as described in version 5.2, the last version of the protocol prior to Stage 2.

9.3.2 Classification including treatment changes due to changes in local guidelines as unfavourable

A sensitivity analysis will be conducted where any treatment changes due changes in local guidelines are classified as unfavourable (rather than not assessable). However, this sensitivity will only result in more unfavourable outcomes on Regimen A (if any), thereby increasing the chance of demonstrating the non-inferiority of Regimen B.

10 DATA SHARING

Results concerning time to sputum culture conversion will be shared with the TREAT-TB transmission modelling team in order that the longer term impacts of reducing treatment times may be assessed. Any data sharing will follow the MRC CTU SOP 61 on Data Sharing.

11 REFERENCES

1. Van Deun A, Maug AKJ, Salim MAH, et al. Short, Highly Effective, and Inexpensive Standardized Treatment of Multidrug-resistant Tuberculosis. *Am J Respir Crit Care Med* 2010; **182**(5): 684-92.

2. Aung KJ, Van Deun A, Declercq E, et al. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tuberc Lung Dis* 2014; **18**(10): 1180-7.

3. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *The Lancet Infectious Diseases* 2009; **9**(3): 153-61.

4. DAIDS RSC. Division of AIDS table for grading the severity of adult and pediatric adverse events. Version 1.0, December 2004 (Clarification August 2009) ed. DAIDS Regulatory Support Centre; 2009.

5. Mohamed K, Embleton A, Cuffe RL. Adjusting for covariates in non-inferiority studies with margins defined as risk differences. *Pharmaceutical statistics* 2011; **10**(5): 461-6.

STREAM

The evaluation of a standard treatment regimen of anti-tuberculosis drugs for patients with MDR-TB

An international, multi-centre, open-label, parallel-group, randomised, controlled trial

STAGE 1 STATISTICAL ANALYSIS PLAN

v.1.1 | June 2017

Authorised by:

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GENERAL INFORMATION

This document describes and substantiates the statistical principles and methods used for the analysis of data from Stage 1 of the STREAM trial. This document is designed to support the STREAM protocol. This Statistical Analysis Plan (SAP) supersedes version 0.1 of the SAP. Every care was taken in the drafting of this SAP, but corrections or amendments may be necessary. The final version of the SAP will be signed off before database lock for final Stage 1 analysis.

The STREAM trial consists of two stages. Stage 1 involves the comparison of two treatment regimens: Regimen A and Regimen B. Stage 2 involves two additional regimens, Regimen C and Regimen D, and makes two comparisons between Regimen B and Regimen C, and Regimen B and Regimen D for the analysis of the primary endpoint. All treatment regimens are described in detail in the STREAM protocol, Section 2.1.3. Stage 1 and Stage 2 of the STREAM trial each have SAPs listed below. Each SAP has differences, but the fundamental statistical principles will be consistent across all SAPs.

Document	**Description**
Stage 1 SAP	All analyses relating to stage 1
Core Stage 2 SAP	Core analyses for stage 2 relating to analyses after the Week 76 database lock
Extensive Stage 2 SAP	Expanded analyses for stage 2 relating to analyses after the Week 76 database lock
Core Stage 2 Week 132 SAP	Core analyses for stage 2 relating to further analyses conducted after the final (Week 132) database lock
Extensive Stage 2 Week 132 SAP	Expanded analyses for stage 2 relating to further analyses conducted after the final (Week 132) database lock

Compliance:

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, in accordance with the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP), and the applicable regulatory requirements in the participating countries.

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LIST OF CHANGES

Changes from v1.0 to v1.1

Section	Change	Reason for change
1.2 and 1.4	Clarification that Regimen A is in accordance with 2011 WHO guidelines.	In line with text in the protocol. WHO guidelines were amended in 2016.
1.3	Patient eligibility criteria are removed and reference made to relevant section of the trial protocol.	Eligibility criteria were slightly amended in v7.0 of the protocol.
3.1	Definition of the Week 76 analysis window for the primary analysis is added.	This was previously unclear. Now in line with SAP for Stage 2.
3.2	Inclusion of culture media other than Ogawa for primary analysis.	At a small number of patient visits, Ogawa results were unavailable and other culture media had to be used.
3.2	Definition of unfavourable expanded.	This change is to bring it in line with protocol v7.0. Previous wording was ambiguous.
3.2	Patients unable to produce sputum at Week 132 can be favourable rather than not assessable.	This change is to ensure the text is consistent with the definition of favourable.
4.1	Only patients from Stage 1 are included in the Stage 1 analysis population	Previous text allowed for the possibility of an overlap between Stage 1 and Stage 2. Now that Stage 2 has started, no overlap occurred.
4.4	Inclusion of other culture media and Week 4 culture result for defining the MITT population.	As above, other culture media have been included to limit the inclusions from the MITT population where the Ogawa result is not available. In addition, cultures up to Week 4 are allowed to increase the number of patients in the analysis populations.
5.3	Removal of text referring to the visit schedule in Stage 2.	No overlap occurred between Stage 1 and Stage 2, so no reference to Stage 2 analyses or visit schedules is required.
5.8	Addition of new section specifying that the definition of treatment extensions and restarts is based on data from the treatment log.	This detail of how treatment extensions and restarts are defined was previously missing.
6.2	Addition of text 'or not' in '…further sub-classified by	Clarification

	whether or not the patients subsequently died before or during the Week 76 window'.	
6.3	Addition of weight band and smear grade at baseline for subgroup analyses	Additional subgroup analyses of interest.
6.4	Addition of 'Using the methods described in Section 6.1.1'	Clarification.
7.1.2	Addition of 'No sputum produced at Week 132' category.	Allows for distinguishing between favourable outcomes based on negative cultures only and those based on no sputum produced at Week 132.
7.3.3	Addition of '(including changes for QT prolongation)'	Clarification
8.4.1	Replacement of linear mixed effects model with simple analysis presenting mean and SD. Addition of ECG subgroup analyses by weight band and choice of fluoroquinolone in control arm.	Presentation of raw means and SD was considered more appropriate than a linear mixed effect models for this secondary outcome. Further subgroup analyses are of clinical interest to understand differences in QT prolongation.
8.4.5	Change from 'SAE and NE' to 'AE'	Clarification

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ABBREVIATIONS AND GLOSSARY

AE	Adverse Event
AFB	Acid Fast Bacilli
AR	Adverse Reaction
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BDQ	Bedaquiline
ICF	Informed Consent Form
CI	Chief Investigator
CFZ	Clofazimine
CRF	Case Report Form
CTA	Clinical Trials Authorisation
DAIDS	Division of Acquired Immunodeficiency Syndrome
DCF	Data Clarification Form
DOT	Directly Observed Treatment
DST	Drug Susceptibility Test
ECG	Electrocardiogram
EMA	European Medicines Agency
EMB	Ethambutol
EQA	External Quality Assurance
FDA	Fluorescein diacetate staining
US FDA	United States Food and Drugs Administration
GCP	Good Clinical Practice
GLC	Green Light Committee
HE	Health Economics
HIV	Human Immunodeficiency Virus
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
ISRCTN	International Standard Randomised Controlled Trial Number
ITM	Institute of Tropical Medicine
ITT	Intention To Treat
KM	Kanamycin
INH	Isoniazid
LFX	Levofloxacin
LPA	Line Probe Assay
LQAS	Lot Quality Assurance Sampling
M2	Metabolite 2
MDR	Multi-Drug Resistant
MFX	Moxifloxacin
Genotype MTBDRPlus	Rapid test for *M. tuberculosis* Complex and its resistance to Rifampicin and/or Isoniazid
Genotype MTBDR*sl*	Rapid test for *M. tuberculosis* Complex and its resistance to fluoroquinolones and/or second-line injectables/cyclic peptides and/or ethambutol
MIC	Minimal Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units–Variable Number of Tandem Repeats
MRC CTU	Medical Research Council Clinical Trials Unit
NE	Notable Event
NTP	National Tuberculosis Programme
PK	Pharmacokinetics
PI	Principal Investigator
PIS	Patient Information Sheet
PTO	Prothionamide

PZA	Pyrazinamide
QA	Quality Assurance
QT Interval	A measure of time between the start of the Q wave and the end of the T wave in the ECG complex
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using the Fridericia correction
REC	Research Ethics Committee
RMP	Rifampicin
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SSA	Site Specific Assessment
STREAM	The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TM	Trial Manager
TMG	Trial Management Group
TMT	Trial Management Team
TREAT TB	Technology, Research, Education, and Technical Assistance for Tuberculosis
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
ULN	Upper limit of normal
The Union	International Union Against Tuberculosis & Lung Disease
USAID	United States Agency For International Development
WHO	World Health Organisation
XDR	Extensively Drug Resistant
ZN	Ziehl-Neelsen

Note. In this statistical analysis plan, time (in weeks) refers to the time from randomisation, e.g. Week 132 refers to 132 weeks from randomisation.

1 TRIAL OVERVIEW

1.1 Study design

The STREAM study is an international, multi-centre, parallel-group, open-label, randomised, controlled trial.

Patients with multidrug-resistant tuberculosis (MDR-TB) are studied in the STREAM trial.

In Stage 1 of the STREAM trial, the comparison being made is between Regimen A and Regimen B.

Regimen A: The locally-used WHO-approved MDR-TB regimen forms the control treatment regimen.

Regimen B: Regimen B is the study regimen, and is based on the regimen described by Van Deun 2010[1] (updated results[2]) consisting of clofazimine, ethambutol, moxifloxacin, and pyrazinamide given for 40 weeks, supplemented by isoniazid, kanamycin, and prothionamide for the first 16 weeks.

All patients in Stage 1 of the study will be followed up to Week 132.

Under versions of the protocol prior to version 6.0 which describe only Stage 1 of the trial, patients are allocated to either Regimens A or Regimen B. Stage 2 of the trial includes two additional arms, Regimens C and D and is implemented in protocol version 6.0 and subsequent versions which also include minor changes to the eligibility criteria, visit schedule and components of the composite primary outcome.

A sensitivity analysis will be conducted to repeat the primary analysis under the definition of the primary outcome as described in version 5.2, the last version of the protocol prior to Stage 2 (see section 9.3.1).

1.2 Trial objectives

The primary objectives of Stage 1 of the STREAM trial are:

1. To assess whether the proportion of patients with a favourable efficacy outcome on Regimen B is not inferior to that on Regimen A (WHO 2011 long MDR-TB regimen), the control regimen for Stage 1, at Week 132, using a 10% margin of non-inferiority
2. To compare the proportion of patients who experience grade 3 or greater adverse events during treatment or follow-up in Regimen B as compared to Regimen A.

The secondary objectives of Stage 1 of the STREAM trial are:

1. To determine the proportion of patients with a favourable efficacy outcome on the Regimen B in each country setting
2. To compare the economic costs incurred by patients and by the health system during treatment on Regimen B as compared to Regimen A.

1.3 Patient eligibility criteria

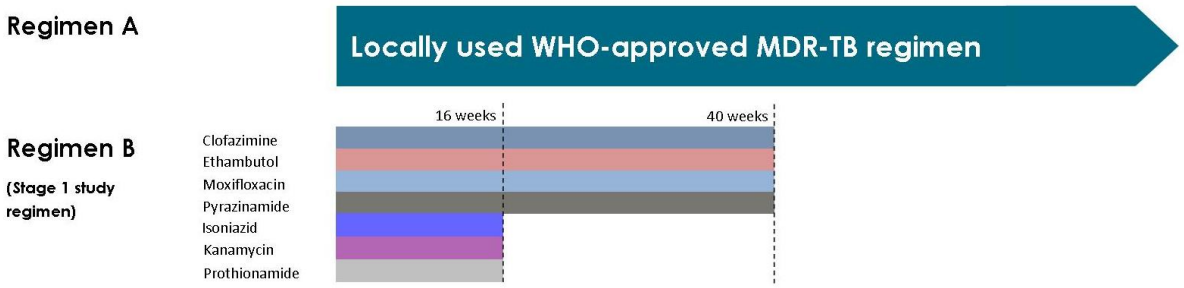
Patient eligibility criteria are listed in section 5 of the trial protocol.

1.4 Study interventions

The control regimen, Regimen A, is the locally-used WHO-approved MDR-TB regimen in accordance with 2011 WHO MDR-TB treatment guidelines. Country- or site-specific regimens are described in the STREAM Patient Management Guide.

The investigative regimen is Regimen B, and consists of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for 40 weeks, supplemented by kanamycin, isoniazid and prothionamide in the first 16 weeks (intensive phase).

Figure 1: Regimen A & Regimen B



In Regimen B, all drugs are given daily (seven days a week), except for kanamycin which is initially given daily and then thrice-weekly from Week 12 onwards.

The intensive phase may be extended from 16 to 20 or 24 weeks for patients whose smear has not converted by 16 or 20 weeks, respectively, as described below.

Table 1: Regimen B doses

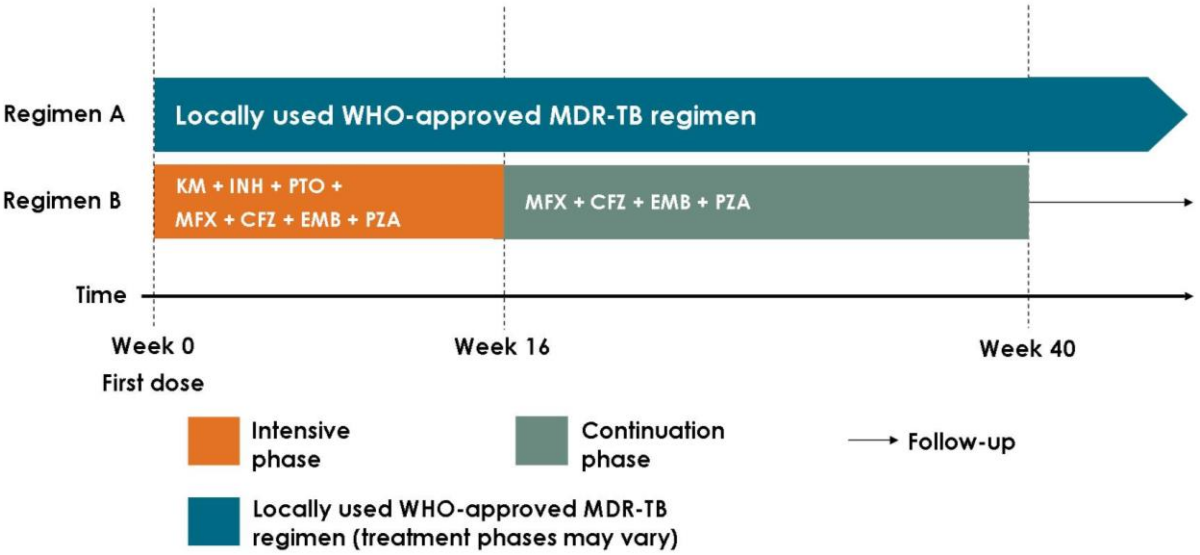
Product	Weight group		
	Less than 33 kg	33 kg to 50 kg	More than 50 kg
Moxifloxacin	400 mg	600 mg	800 mg
Clofazimine	50 mg	100 mg	100 mg
Ethambutol	800 mg	800 mg	1200 mg
Pyrazinamide	1000 mg	1500 mg	2000 mg
Isoniazid	300 mg	400 mg	600 mg
Prothionamide	250 mg	500 mg	750 mg
Kanamycin	15 mg per kilogram body weight (maximum 1g)		

Patients randomised to Regimen B will receive 40 weeks of treatment (16 weeks intensive phase plus 24 weeks continuation phase), as shown in Figure 1.

1.5 Treatment phases

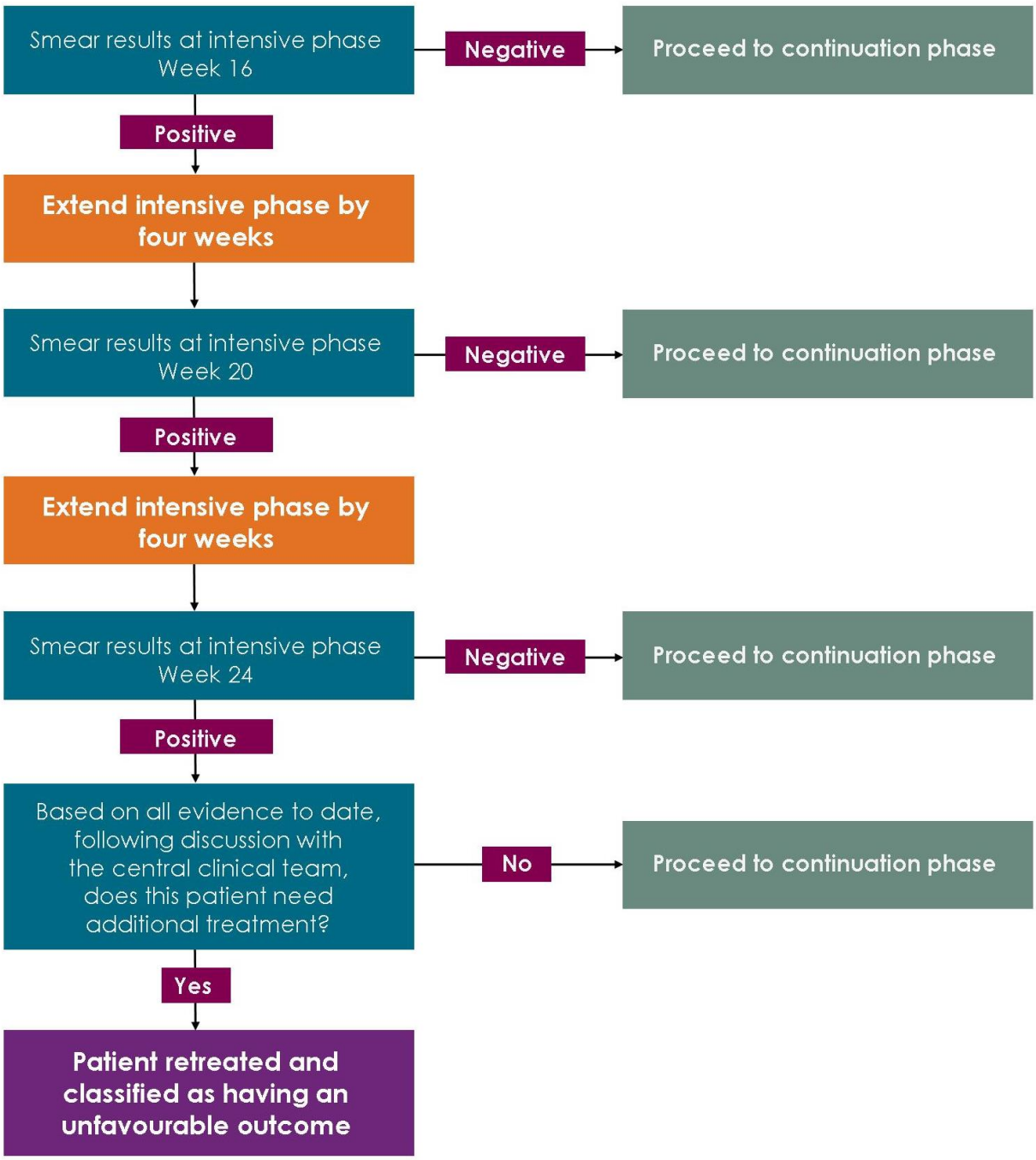
The study regimen, Regimen B, consists of 2 phases; an intense phase followed by a continuation phase, as shown in Figure. 1.

Figure 2: Treatment phases



For patients randomised to Regimen B the following algorithm will be used to determine when a patient can proceed from the intensive to the continuation phase.

Figure 3: Transition from intensive to continuation phase for patients on Regimen B



Patients randomised to Regimen B will be prescribed 40 weeks of treatment (16 weeks intensive phase and 24 weeks continuation phase). In the event of a positive (at least "scanty" on the IUATLD/WHO scale) AFB smear at Week 16, the drugs in the intensive phase of this regimens may be extended by 4 weeks, if the smear is still positive at 20 weeks the intensive phase may be extended by a further 4 weeks allowing a maximum intensive phase of 24 weeks, and hence a maximum total duration of 48 weeks treatment.

1.6 Randomisation procedure

Patients will be randomised to Regimen A or Regimen B. Randomisation will be in a 1:2 ratio in favour of Regimen B to allow more data on efficacy and safety to be collected on this regimen. Randomisation will be stratified by (1) site, (2) HIV status for sites with high TB-HIV co-infection rates.

Separate randomisation lists for each combination of strata will be prepared in advance by a statistician independent of the study, using varying block sizes. Should web access not be available at the time of randomisation, a manual alternative using sealed envelopes will be provided.

Patients will be randomised using a web-based randomisation system. Access to the web-based system will be controlled through an authorised username and password. Before treatment allocation the patient's eligibility will need to be confirmed, and their site, HIV status, and CD4 count entered into the database.

2 SAMPLE SIZE

2.1 Power to demonstrate non-inferiority in the primary efficacy outcome

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden and duration and the expected increase in adherence in reducing a treatment regimen from 104 weeks (as with Regimen A), to 40 weeks (as with regimen B).

A meta-analysis of treatment outcome in patients with MDR-TB found an overall favourable outcome of 64% (95% CI 59-68) in patients given individualised treatment and 54% (95% CI 43-68) in patients given standardised treatment[3]. A reasonable estimate of the efficacy of regimen A in the STREAM trial would therefore be 70%.

Based on the experience with regimen B[1], a reasonable estimate of its efficacy in the STREAM trial would be between 75% and 85%. The lower estimate is used for the sample size calculations below.

Based on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 2 gives the total number of patients required to demonstrate non-inferiority under the specified scenarios using a margin of non-inferiority of 10%. These totals allow for 20% of patients being classified as not assessable in a per-protocol analysis and are based on a one-sided level of significance of 2.5%.

Table 2: Power to demonstrate non-inferiority in the primary efficacy outcome

Power	Percentage favourable outcomes in Regimen A	Difference in percentage favourable outcomes in Regimen B compared to Regimen A		
		0%	5%	10%
80%	60%	1060	464	255
	65%	1005	435	238
	70%	928	398	214
90%	60%	1419	620	340
	65%	1345	583	318
	70%	1242	533	287

Therefore, 398 patients would be required (rounding to 400 gives: 267 on Regimen B and 133 on Regimen A) to demonstrate non-inferiority with 80% power assuming 70% favourable outcomes in Regimen A and 75% in Regimen B and 20% not assessable. A larger difference in response rates of 10% would require fewer patients and could also be demonstrated with greater than 90% power with a total enrolment of approximately 400 patients.

A 10% margin of non-inferiority is considered an acceptable reduction in efficacy given the considerably reduced pill burden, duration, and resource utilisation, and the expected increase in adherence in reducing a treatment regimen from 104 weeks (as with Regimen A), to 40 weeks (as with Regimen B).

If the difference in response rates in favour of Regimen B is more than 10% it may be possible to demonstrate superiority of that regimen over the control for stage 1, Regimen A.

At least 400 patients will need to be enrolled across all countries to give sufficient power to demonstrate non-inferiority. Patients will be randomised to Regimen B and Regimen A in the ratio 2:1.

2.2 Power to demonstrate non-inferiority in the primary safety outcome

Assuming a sample size of 400 on a 2:1 allocation ratio in favour of Regimen B to Regimen A, Table 3 gives the power available to demonstrate non-inferiority in the primary safety outcome under different proportions of grade 3 or 4 events on Regimen A and Regimen B. These calculations assume a margin of non-inferiority of 10% and a one-sided level of significance of 2.5%. All randomised patients who have received at least one dose of study medication will be included in the safety analysis.

Table 3: Power to demonstrate non-inferiority in the primary safety outcome

Proportion grade 3 or 4 on Regimen A	Assuming same proportion in Regimen A and Regimen B	Assuming an absolute 5% lower proportion on Regimen B than Regimen A
10%	88%	99%
15%	75%	99%
20%	65%	96%
25%	58%	93%
30%	53%	89%
35%	50%	86%
40%	48%	83%

3 PRIMARY OUTCOMES

3.1 Primary analysis Week 132 window

The Week 132 window is defined as the time period from six weeks before 132 weeks since randomisation with no upper bound, i.e. from Week 126 with no upper bound.

For the purposes of defining the primary efficacy outcome, the Week 76 analysis window is defined as the time period from six weeks before 76 weeks since randomisation to six weeks after 76 weeks since randomisation, i.e. from Week 70 to Week 82. This definition is used for consistency with STREAM Stage 2, but any tabulations of secondary outcomes by visit will use the visit windows as defined in section 5.3 below.

3.2 Primary efficacy outcome

The primary efficacy outcome measure is the proportion of patients with a favourable outcome (as defined below) at Week 132.

Culture results obtained using acidified Ogawa (Kudoh medium) will be used in the primary efficacy analysis, although results from other culture media will be used if the Ogawa result is missing.

A positive culture on Ogawa is defined as at least one colony and a negative culture is defined as absence of growth (no colonies).

Favourable

A patient's outcome will be classified as **favourable** if their last two culture results are negative unless they have previously been classified as unfavourable. These two cultures must be taken on separate visits (on different days); the latest of which being within the Week 132 window.

Patients that don't have a culture result within the Week 132 window because they were unable to produce sputum, will be classified as favourable if their last two cultures before the Week 132 window are negative and they have not previously been classified as unfavourable; such patients will be identified separately in tables (see section 6.2).

Unfavourable

A patient's outcome will be classified as **unfavourable** if:

1. They are discontinued from their allocated study treatment and subsequently restarted on a different MDR-TB regimen
2. Treatment is extended beyond the scheduled end of treatment for any reason other than making up of days when no treatment was given (missed treatment) for a maximum of eight weeks. A maximum of 14 days of extra treatment (irrespective of reason) is acceptable before it is classified as treatment extension. In addition, if the intensive phase of treatment has been extended for delayed sputum conversion (maximum 8-week extension permitted) the scheduled end of treatment will also be extended by the same amount, in accordance with Section 7.3.2 of the protocol.
3. They are restarted on any MDR-TB treatment after the scheduled end of treatment, but before 132 weeks after randomisation.
4. They change their allocated study treatment for any reason other than (1) the replacement of a single drug or (2) for patients allocated to Regimen A when the change is as a result of changes in local guidelines and not related to any change in the patient's circumstances or condition.
5. Bedaquiline is started where the allocated regimen did not originally contain that drug (Regimen A or Regimen B).

6. A drug from the class of nitroimidazoles is started
7. They die at any point during treatment or follow-up
8. At least one of their last two culture results, from specimens taken on separate occasions, is positive
9. They do not have a culture result within the Week 76 window or thereafter

Providing none of the other criteria above are met, starting a single drug is not considered to be a substantial change to the regimen and therefore does not result in an unfavourable outcome, with the exception of adding bedaquiline or a drug from the class of nitroimidazoles.

An extension of the intensive phase of treatment in any study arm does not constitute an unfavourable outcome, as long as the extension is in accordance with either the algorithms described in section 7.3.2 for patients on Regimen B, or the locally-used WHO 2011 long MDR-TB regimen for patients on Regimen A. Similarly, the discontinuation of drugs that are not replaced does not constitute an unfavourable outcome.

Changes of treatment in patients allocated to Regimen A that result from a change in local guidelines not related in any way to any change in the patient's circumstances or condition will not be classified as unfavourable. A sensitivity analysis will be conducted where these changes *are* classified as unfavourable. However, this sensitivity analysis can only result in an increase in unfavourable outcomes on Regimen A, thereby increasing the chance of demonstrating the non-inferiority of Regimen B and therefore the primary analysis described here is more conservative.

All re-infections with a different strain are classified as **not assessable**.

A patient who has a culture result within the Week 76 window or thereafter, but not within the Week 132 window, having not otherwise been classified as unfavourable (based on the definitions above) will be regarded as **not assessable** and will be excluded from the primary analysis provided their last two cultures, from specimens taken on separate occasions, are negative. Such patients that don't have a culture result within the Week 132 window because they were unable to produce sputum will be instead classified as **favourable**. Any patient who does not have a culture result within the Week 132 window and does not fulfil these criteria will be classified as **unfavourable**. These definitions apply to both Regimen A and Regimen B.

3.3 Primary safety outcome

The primary safety outcome measure is the proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria[4], at any time during treatment and follow-up.

4 ANALYSIS POPULATIONS

4.1 Stage 1 analysis population

Only patients randomised in Stage 1 of the STREAM trial will be included in the Stage 1 analysis population.

4.2 Intention-to-treat (ITT)

All randomised patients will be included in the ITT analysis population.

4.3 Safety population

All randomised patients that have taken at least one dose of treatment will be included in the safety analysis population.

4.4 Modified intention-to-treat (mITT)

The mITT population is defined as all randomised patients that have a positive culture for *M. tuberculosis* on acidified Ogawa (Kudoh medium) or other culture media if the Ogawa result is not available, at screening or randomisation or up to Week 4, with the exception of patients with isolates taken before randomisation that are subsequently found to be susceptible to rifampicin, and patients with isolates taken before randomisation that are subsequently found to be resistant to both fluoroquinolones and second-line injectables (i.e. XDR-TB) on phenotypic DST. Results from the central reference laboratory will take priority over any results from local laboratories where available.

4.5 Per protocol (PP)

The PP population will be the same as the mITT population with the exclusion of patients not completing a protocol-adherent course of treatment, other than for treatment failure or death. Treatment failure is defined as failure to attain and maintain culture negativity until the end of allocated treatment.

4.5.1 Definition of a protocol-adherent course of treatment

Patients will be excluded from the per-protocol analysis if they do not complete a protocol-adherent course of treatment, other than for treatment failure or death.

A patient will have completed a protocol-adherent course of treatment when they have taken 80% of doses within 120% of the minimum duration in both the intensive phase and in the whole treatment period. For this purpose, a dose is defined as all the study medications at the correct dose for that particular day.

For Regimen B, **with or without** an extension of the intensive phase, a patient will have completed a protocol-adherent course of treatment if they have taken:

- 90 doses (80% of 16 weeks) within 134 days (120% of 16 weeks) in the intensive phase, and
- 224 doses (80% of 40 weeks) within 336 days (120% of 40 weeks) over the whole treatment period (i.e. the combined intensive and continuation phases) regardless of treatment extensions.

The same algorithm will apply for Regimen A, the control regimen; the exact number of doses and days depends on the duration of the intensive and continuation phases of Regimen A.

5 GENERAL ANALYSIS PRINCIPLES

5.1 Analysis populations

The analyses of the primary outcomes will be based on both the mITT and the PP populations. All patients included in the analysis will be analysed in the treatment group to which they were originally assigned. Further sensitivity analyses are planned (see Section 9 Sensitivity Analyses).

5.2 Treatment and follow-up phase definitions

For the purpose of analysis, the screening, treatment, and follow-up phases for an individual patient will be defined as follows:

- **Screening phase**
  - Start: date of screening consent
  - End: day before randomisation
- **Treatment phase**
  - Start: date of randomisation.
  - End: date of last dose of any TB treatment defined as last dose of any TB treatment (including retreatment for relapse), plus 7 days.
- **Follow-up phase**
  - Start: the day after the end of the treatment phase.
  - End: date of the last patient contact (scheduled or unscheduled, or other contact e.g. phone call).

The treatment phase includes any extension of treatment or retreatment, and so the Allocated Treatment phase is defined as follows:

- **Allocated Treatment phase**
  - Start: date of randomisation.
  - End: date of last dose of trial treatment defined as last dose of allocated regimen or last dose before the addition of a new drug, whichever happens sooner, plus 7 days.

5.3 Visit window definitions

During Stage 1, patients will be assessed at screening, randomisation (Week 0), Week 1, Week 2, Week 3, Week 4, and at 4-weekly intervals throughout the study, until the end of follow-up, irrespective of whether on treatment or in the post-treatment follow-up phase.

For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of randomisation. For the purpose of analysis, each scheduled visit will have a window before and after the target date, calculated from date of randomisation. When referring to a visit hereon, this implies within the defined visit window as specified below.

Visit	target date, days after randomisation +1	Analysis window
Screening / Baseline	1	Date of screening consent - 1
Week 4	29	2-42
Week 8	57	43-70
Week 12	84	71-98
Week 16	113	99-126
...		
Week a	b = 1+ (a x 7)	(b-14) – (b+13)
...		
Week 120	841	827-854
Week 124	869	855-882
Week 128	N/A (included within 132 week analysis window)	
Week 132	925	833-no upper bound

Any visit, scheduled or unscheduled, that falls into the analysis window will be assigned to that visit for the purpose of analysis. If two visits fall within the same interval, the one closest to the target date will be used for analyses by visit, so that there is only one unique visit for each patient and analysis time-point.

There are additional study visits at Weeks 1, 2 and 3 only for ECG monitoring. For the analysis of ECG data only, there will be additional visit windows: Week 1 (2-11), Week 2 (12-18), Week 3 (19-25) and the Week 4 visit window will be modified to (26-42).

5.4 Definition of a culture result

A culture result will be called positive for *M. tuberculosis* if the culture tests positive for the presence of microorganisms, at least one colony, and the microorganisms present are then identified as being *M. tuberculosis*. However, if an identification test is not carried out for a particular culture, then for analysis purposes a culture will still be considered positive for *M. tuberculosis* if the culture tests positive for the presence of microorganisms and if that culture result is obtained seven days or more since the start date of sputum processing and incubation of the inoculated Ogawa. If the culture result is obtained less than seven days since the start date of sputum processing and incubation of the inoculated Ogawa, the culture result will not be considered as positive for *M. tuberculosis*, and the culture result will be considered missing in the analysis.

If more than one culture result is available from sputum collected on the same day, this will be regarded as a single culture result for the purposes of all analyses with the following overall result:

i. **Positive**, if at least one of the culture results is positive
ii. **Negative**, if at least one of the culture results is negative and none of the culture results are positive
iii. **Contaminated** if at least one of the culture results is contaminated and none of the culture results are positive or negative.
iv. **Missing**, if no culture result is available.

5.5 Definition of a smear result

A smear result will be called positive if it is graded as 'scanty' or 'rare AFB' or at least 1+.

If more than one smear result is available from sputum collected on the same day, this will be regarded as a single smear result for the purposes of all analyses with the following overall result:

i. **Positive**, if at least one of the smear results is positive
ii. **Negative**, if at least one of the smear results is negative and none of the smear results are positive
iii. **Missing**, if no smear result is available.

5.6 Reference laboratory bacteriology

A number of clinical isolates will be sent from the STREAM sites to a reference laboratory at the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Drug sensitivity results from the reference laboratory will be used in all analyses in preference to those obtained from local site laboratories where available.

5.7 Adverse events

For all analyses of adverse events, only those occurring after randomisation will be included.

5.8 Defining treatment extensions and restarts

For the purposes of classifying the primary outcome, only data from the treatment logs (CRF 18) will be used to determine treatment extensions, changes or restarts.

6 ANALYSIS OF PRIMARY OUTCOMES

6.1 Primary efficacy analyses

6.1.1 Modelling technique used in analysis

For the primary efficacy analysis the difference in proportions of favourable outcome between two specified trial regimens with corresponding 95% confidence intervals and p-values will be estimated using a stratified analysis of the risk difference from each stratum using Cochran-Mantel-Haenszel weights.[5] The analysis will be stratified only by HIV status: HIV negative and HIV positive. Where there is a difference between data used for stratification and correct data (if randomisation was inadvertently done on incorrect data), the correct data will be used for adjustment in the analysis.

6.1.2 Primary efficacy analysis: non-inferiority of Regimen B

Non-inferiority will be demonstrated if the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes between Regimens A and B is less than the 10% margin of non-inferiority in both the mITT and PP populations.

6.1.3 Superiority of Regimen B

If Regimen B is declared non-inferior to Regimen A, then superiority of Regimen B compared to Regimen A will be assessed.

If the upper bound of the 95% confidence interval of the difference in proportion of favourable outcomes between Regimens A and Regimen B is less than zero, then superiority of Regimen B compared to Regimen A will be declared. For this analysis, the mITT population will be primary and the PP population will be one of several secondary analyses.

6.2 Tabulation of primary endpoint classification

Since the primary endpoint is a composite of various components, the actual reason (component) for outcome will also be tabulated by treatment arm.

Patients will be classified by the first event that made the patient unfavourable (see section 3.3) and further sub-classified by their microbiological outcome at the time that this outcome occurred (see section 7.1 below) and further sub-classified by whether or not the patients subsequently died before or during the Week 76 window. For example, a patient that has their treatment regimen changed during the treatment phase but subsequently has a positive culture during the Week 76 window will be classified as having had their regimen changed and further sub-classified by whether they had achieved culture conversion when their regimen was changed.

6.3 Subgroup analyses

This primary efficacy analysis will be repeated in subgroups according to HIV infection status, baseline drug resistance patterns (i.e. resistance to pyrazinamide, a fluoroquinolone, a second-line injectable, and isoniazid), BMI (<18, 18-<20, 20-<25, ≥25), cavitation (presence, absence), study centre, age (<45, 45-<65, ≥65), sex, smoking history (current smoker, ex-smoker and never smoked), weight band, smear grade at baseline, and ethnicity.

In addition, to evaluate any effect of the minor differences in the protocol after the initiation of Stage 2, the primary efficacy analysis will be repeated in the subgroup of patients enrolled under protocol 5.2 and prior versions, and in the subgroup of patients enrolled under protocol 6.0 (Stage 2) and later versions.

6.4 Primary safety analysis

The primary safety outcome is the occurrence of a Grade 3 or greater adverse events.

The difference in proportion of patients experiencing a grade 3 or greater adverse event, as defined by the DAIDS criteria, during the treatment and follow-up phases, between Regimen B and Regimen A with corresponding two-sided 95% confidence intervals and p-values will be estimated (using the methods described in Section 6.1.1).

This analysis will be conducted on the whole study period, and separately for each phase (Treatment, Follow-up and Allocated treatment).

7 ANALYSIS OF SECONDARY OUTCOMES

7.1 Microbiological outcome

Sputum culture negative status is defined as two consecutive negative cultures from sputa collected on different days without an intervening positive. Culture negative status is lost when a culture result is positive, but can subsequently be re-achieved if two consecutive cultures from sputa collected on different days are negative without an intervening positive

7.1.1 Microbiological outcome at unfavourable outcome

The microbiological outcome at unfavourable outcome is defined using culture results up to and including the date of the first event that made their primary efficacy outcome unfavourable (the 'unfavourable outcome event'). It is defined as follows:

- **Culture negative.** Culture negative status was satisfied at the date of the unfavourable outcome event.
- **Never culture converted**. The patient never achieved culture negative status at any time during the study prior to the unfavourable outcome event.
- **Culture positive**. Culture negative status was achieved at some point during the study, but was not satisfied at the date of the unfavourable outcome event. Culture positive will be further classified as **Culture positive: Reinfection** if it has been shown that the M. tuberculosis strain of the positive culture is different to baseline; and **Culture positive: Relapse** otherwise.

Patients will be classified by the first event that made the patient unfavourable and further sub-classified by their microbiological outcome at unfavourable outcome and further sub-classified by whether the patients subsequently died (see Section 6.2).

7.1.2 Microbiological outcome at Week 132

The microbiological outcome at Week 132 will be defined as follows:

- **Culture negative at Week 132**. Culture negative status was satisfied when last seen with a negative culture within the Week 132 window.
- **No sputum produced at Week 132**. Culture negative status was satisfied when last seen but there were no culture results during the Week 132 window because they were unable to produce sputum.
- **Culture negative: did not complete follow-up.** There were no culture results during the Week 132 window (and this was not because no sputum was produced) and culture negative status was satisfied when the patient was last seen.
- **Never culture converted**. The patient never achieved culture negative status at any time during the study up to Week 132.

- **Culture positive**. Culture negative status was achieved at some point during the study, but was not satisfied when the patient was last seen (at least one of the last two non-missing culture results was positive). Culture positive will be further classified as **Culture positive: Reinfection** if it has been shown that the M. tuberculosis strain of the positive culture is different to baseline; and **Culture positive: Relapse** otherwise.

Microbiological outcome at Week 132 will be tabulated by regimen. Patients that die will be classified as above based on their available culture results when last seen, but classified separately from patients that did not die.

7.2 Efficacy outcomes

Secondary efficacy outcomes will be analysed on both the mITT and PP analysis populations.

7.2.1 Time to sputum smear and culture conversion

Time to sputum smear conversion is defined as the time from randomisation to the first of two consecutive negative sputum results, collected on separate days. All patients in the respective analysis population will be included in this analysis, except those with no positive smear result at screening or randomisation. Patients that never achieve smear conversion will be censored at the date of collection of sputum that yielded their last smear result.

Time to sputum culture conversion is defined as the time from randomisation to the first of two consecutive negative culture results, collected on separate days. Patients that never achieve culture conversion will be censored at the date of collection of sputum that yielded their last culture result.

Median time to sputum smear and culture conversion will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

The equality of survivor functions for time to sputum conversion for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

Even when Kaplan-Meier curves of time to culture conversion have been shown to diverge in the presence of an effective drug (such as bedaquiline), they tend to converge later in follow-up potentially violating the assumption of proportional hazards. In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

The analyses above of time to sputum smear conversion and time to sputum culture conversion will be repeated with the alternative definition as time from randomisation to the first negative culture or smear result respectively (without the need for a second negative culture or smear to confirm).

7.2.2 Time to unfavourable efficacy outcome

Time to unfavourable efficacy outcome is defined as the time from randomisation to the first event that results in the definition of an unfavourable efficacy outcome for that patient (as defined in Section 3.2). Patients that do not culture convert during the treatment and follow-up phases (i.e. fail to have 2 consecutive culture negative results), and have not otherwise been called unfavourable, will be called unfavourable at the date of the last visit when a culture positive result was obtained.

Patients classified as favourable or not assessable will be censored in this analysis at the date of collection of sputum that yielded their last negative culture result.

Median time to unfavourable efficacy outcome will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

The equality of survivor functions for time to unfavourable efficacy outcome for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

7.2.3 Time to cessation of clinical symptoms based on PI assessment

Time to cessation of clinical symptoms is defined as the time from randomisation to the first of two consecutive visits where cessation of **all three** of the TB symptoms: a productive cough, fever, and night sweats, as reported by the patient. Patients with none of the TB symptoms at screening and none of the TB symptoms at baseline with be excluded from this analysis. This definition matches the definition of time to culture conversion as the first of two consecutive symptom-free months.

Median time to cessation of clinical symptoms will be calculated for Regimen A and Regimen B.

A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, adjusted for the stratification factors.

For patients who do not cease clinical symptoms, cessation of clinical symptoms will be censored at the patients' last visit.

The equality of survivor functions for time to cessation of clinical symptoms for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

7.3 Safety outcomes

Safety outcomes will be analysed using the safety analysis population.

7.3.1 Placement of events by study phases

Adverse events are placed in study phases (see section 6.1 for definitions) based on the start date. If the start date of an event falls between (or on) the start and stop date of a phase, the AE is attributed to that phase.

In case of partial start dates, the following approach is used:

- **Missing day only**: The event is placed in all phases that overlap the given month and year for the event, excluding any phases that start after the end date of the AE (if specified).
- **Missing day and month only**: The event is placed in all phases that overlap the given year for the event, excluding any phases that start after the end date of the AE (if specified).
- **Missing start date:** The event is placed in the treatment phase, unless the end date of the AE is specified and is before randomisation, in which case the event is placed in the screening phase.

7.3.2 All-cause mortality during treatment or follow-up

All-cause mortality is defined as a patient who has died from any-cause (both TB- or non-TB-related) while in the trial either during treatment or during follow-up.

The number of patients who die during treatment and follow-up will be tabulated by treatment arm.

Survival analysis will be conducted for time to death.
A hazard ratio with corresponding two-sided 95% confidence intervals and p-value will be estimated using a Cox Proportional Hazards model will be used, with no stratification.

For patients that do not die, time will be censored at their final visit.

The equality of survivor functions for time to death for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, stratified by the randomisation stratification factors.

7.3.3 Change of regimen for adverse events

A change of regimen for an adverse event is defined as when a patient's regimen is modified in any way (including stopping a drug, changing the dose of a drug or starting a new drug) with the main reason being an adverse event (including changes for QT prolongation).

The difference in proportion of patients who have a change of regimen for adverse events between Regimen B and Regimen A will be calculated with 95% confidence intervals.

7.3.4 Proportion of patients experience treatment-related grade 3 or greater adverse events occurring on treatment and during the follow-up period

The proportion of patients with treatment-related grade 3 or greater adverse events that occur on treatment and during the follow-up period is defined as the number of grade 3 or greater adverse events considered to be possibly, probably or definitely related to treatment.

The difference in proportion of treatment-related adverse events between Regimen B and Regimen A will be calculated.

7.3.5 Adherence to treatment

Adherence to treatment is defined as either **adherent**; if a patient has taken at least 80% of doses within 120% of the time (as defined above), or **non-adherent**; if a patient has not met these conditions.

The difference in proportion of those who have been adherent to treatment between Regimen B and Regimen A will be calculated.

7.4 Acceptability outcomes

In selected sites, acceptability of Regimen A and B to stakeholders will be analysed in terms of:

- Costs to the health system
- Household costs
- Patient treatment and support experiences
- Health worker experiences.

The analyses of health and household costs and patient and health worker experiences will be described in a separate document.

8 DATA SUMMARIES

8.1 Recruitment and baseline characteristics

8.1.1 Recruitment, screening, & eligibility

The number of patients screened, randomised and treated will be tabulated by centre and treatment arm. The number of patients who failed screening, and the reasons for ineligibility will be presented by randomised group.

8.1.2 Exclusions from analysis

The number of patients excluded from the mITT and PP analysis populations will be tabulated by treatment arm and by reason for exclusion.

8.1.3 Baseline characteristics

All eligible patients randomised will be included in tables of baseline comparisons by treatment group. Characteristics will include sex, age, ethnicity, height, weight, BMI, and laboratory parameters such as, HIV status, CD4 count (if applicable), smoking status (current smoker, ex-smoker, never smoked) smear and culture status, and drug susceptibility status for a number of TB drug types. The baseline characteristics table will be repeated for each of the ITT, safety, PP and mITT populations.

8.2 Efficacy and adherence

Each analysis will be repeated using the mITT and PP analysis populations.

8.2.1 Sputum smear and culture

Sputum smear and culture results (positive or negative) will be tabulated by visit and treatment arm.

8.2.2 Adherence

Adherence will be summarised by treatment arm as the percentage of each of the intensive and continuation phase doses completed and overall across both phases.

8.2.3 Drug resistance

Drug resistance at screening or baseline will be tabulated by treatment arm, with separate tables for genotypic and phenotypic DSTs. Acquired resistance to any drugs will also be described and tabulated by treatment arm using the last available DST result for each drug for each patient.

In addition, acquired resistance to any drugs will also be described and tabulated by treatment arm using any available post-randomisation DST result only from the reference laboratory at the Institute of Tropical Medicine (ITM) in Antwerp (i.e. ignoring any results from local site laboratories) for each drug for each patient.

In a further analysis, acquired resistance to any drugs will also be described and tabulated by treatment arm using any available post-randomisation DST result (i.e. classifying as resistant if any result is resistant from ITM or local site laboratories) for each drug for each patient.

Acquired resistance for each definition will also be tabulated by category of primary endpoint and microbiological outcome to determine any cases of acquired resistance that didn't result in an unfavourable outcome.

8.3 Retention and description of follow-up

8.3.1 Description of follow-up and populations

Completion of treatment and completion of scheduled follow-up will be summarised by treatment group including reasons for failure to complete treatment or follow-up. This analysis will be using the ITT, PP, safety, and mITT analysis populations.

8.4 Safety outcomes

Safety outcomes will be analysed using the safety analysis population.

8.4.1 Electro-cardiology

Both mean (and SE) QT, QTcF and heart rate (HR) by visit and treatment arm, and mean (and SE) QT, QTcF and HR change from baseline by visit (within visit window) and treatment arm will be tabulated.

QT and QTcF will be categorised (<450, 450-479, 480-499, ≥500) and tabulated by visit and treatment arm, and highest post-randomisation value overall by treatment arm. Change from baseline of QT and QTcF will also be categorised (<30, 30-59, ≥60) and tabulated by treatment arm, and highest post-randomisation value overall by treatment arm.

These tables will be done for the whole study period and repeated for the treatment phase only.

Time to first QTcF over 450ms and first QTcF over 500ms and QTcF increase from baseline by 30ms and by 60ms analyses will be conducted. Number of each of these events (i.e. whether a threshold was exceed or not) will be tabulated by treatment arm. Hazard ratios with corresponding two-sided 95% confidence intervals will be estimated using a Cox Proportional Hazards model will be used, with no stratification.

The outcomes will be censored at the patients' last visit.

The equality of survivor functions for time to QTcF over 450ms and over 500ms and QTcF increase from baseline by 30ms and by 60ms for Regimen A and Regimen B will be compared using a (Wilcoxon) Log rank test, with no stratification.

The assumption of proportional hazards will be tested using the proportional hazards test based on the Schoenfeld residuals after fitting the Cox Proportional Hazards model.

In the case where there is adequate evidence that the proportional hazard assumptions are violated at the 5% level (i.e. $p<0.05$), methods where proportional hazards is not a necessary assumption will be used, such as restricted mean survival time.

QTcF will be summarised by visit and by treatment arm using means and standard deviations. Mean and +/- 1 SD will be plotted by visit and treatment arm. This will be repeated for change in QTcF from baseline.

It is likely that treatment and dose changes will impact on QTcF and so this analysis will be repeated ignoring any results after discontinuation or change of dose of any drug.

All of the electro-cardiology analysis will be repeated separately by HIV status, by sex, by weight band, and by choice of fluoroquinolone in the control arm (levofloxacin or moxifloxacin). An interaction between covariates and QTcF will be tested by including an interaction term in the linear mixed models for QTcF and change in QTcF from baseline.

8.4.2 Liver function

ALT, and AST will be categorised (<1xULN; 1-<3xULN, 3-<5xUlLN; 5ULN-<10ULN; ≥10ULN) and tabulated by visit and treatment arm.

Mean ALT, and AST will be presented by visit and treatment arm. The number of patients experiencing more than or equal to five times above the upper normal limit will be tabulated by arm.

8.4.3 Hearing impairment

The number (and proportion) of patients reporting experiencing clinically significant hearing loss (unilateral or bilateral) during the combined treatment and follow-up period will be tabulated by treatment arm.

8.4.4 Weight gain

Patient weight will be tabulated by treatment arm and visit in addition to change from baseline weight by visit and treatment arm.

8.4.5 Adverse Events

AE data will be tabulated as follows:

i. Event grade by treatment arm, with details of type of AE listed with frequencies for each event grade
ii. Event relatedness to study drugs by treatment arm
iii. Number of patients experiencing Grade 3 or higher adverse events by treatment arm
iv. Number of Grade 3 or higher adverse events by treatment arm.

9 SENSITIVITY ANALYSES

9.1 Additional adjusted and unadjusted primary efficacy analyses

All primary efficacy analyses will be repeated:

1. Unadjusted for any covariates.
2. Adjusted for randomisation stratification factors HIV status and centre. Small strata with fewer than 10 patients will be combined within geographical regions.
3. Adjusted for randomisation stratification factors and any additional important covariates such as cavitation at baseline or baseline bacillary load.

9.2 Additional analysis populations for primary efficacy analysis

In addition to the mITT and PP analysis populations, the primary efficacy analyses will be repeated for the (1) ITT analysis population, (2) the safety analysis population, and (3) the mITT analysis population excluding patients that didn't start treatment.

9.3 Reclassification of primary efficacy endpoint

9.3.1 Classification using pre-Stage 2 primary outcome definitions

A sensitivity analysis will be conducted to repeat the primary analysis under the definition of the primary outcome as described in version 5.2, the last version of the protocol prior to Stage 2.

9.3.2 Classification including treatment changes due to changes in local guidelines as unfavourable

A sensitivity analysis will be conducted where any treatment changes due changes in local guidelines are classified as unfavourable (rather than not assessable). However, this sensitivity will only result in more unfavourable outcomes on Regimen A (if any), thereby increasing the chance of demonstrating the non-inferiority of Regimen B.

10 DATA SHARING

Results concerning time to sputum culture conversion will be shared with the TREAT-TB transmission modelling team in order that the longer term impacts of reducing treatment times may be assessed. Any data sharing will follow the MRC CTU SOP 61 on Data Sharing.

11 REFERENCES

1. Van Deun A, Maug AKJ, Salim MAH, et al. Short, Highly Effective, and Inexpensive Standardized Treatment of Multidrug-resistant Tuberculosis. *Am J Respir Crit Care Med* 2010; **182**(5): 684-92.

2. Aung KJ, Van Deun A, Declercq E, et al. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tuberc Lung Dis* 2014; **18**(10): 1180-7.

3. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *The Lancet Infectious Diseases* 2009; **9**(3): 153-61.

4. DAIDS RSC. Division of AIDS table for grading the severity of adult and pediatric adverse events. Version 1.0, December 2004 (Clarification August 2009) ed. DAIDS Regulatory Support Centre; 2009.

5. Mohamed K, Embleton A, Cuffe RL. Adjusting for covariates in non-inferiority studies with margins defined as risk differences. *Pharmaceutical statistics* 2011; **10**(5): 461-6.