Acronym: OneRIF / Clinicaltrials.Gov. NCT02153528

Optimization of the TB treatment regimen cascade

Clinical Trial Protocol
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Sponsor: Damien Foundation
Leopold II-laan 263B-1081 Brussels – Belgium

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Study sites:	Eight selected clinics part of the Damien Foundation Bangladesh TB project in Greater Mymensingh district: Sarishabari, Madhupur, Bhaluka, Fulbaria, Iswarganj, Netrakona, Purbodhola, Kendua
Study drugs:	Standard regimen for TB treatment according to guidelines of the International Union against Tuberculosis and Lung Disease (control arm). The intervention arm will receive a double dose of rifampicin
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Collaborating partners:

This research proposal will be carried out as a collaboration among the Damien Foundation (DF), the Institute of Tropical Medicine (ITM) and the National TB Control Program in Bangladesh. The respective roles and responsibilities of the Sponsor and the partners, in particular ITM to which some of the sponsor responsibilities are delegated, will be defined in an *ad hoc* Memoranda of Understanding.

The Damien Foundation (http://www.damiaanactie.info/damienfoundation/) Brussels Head Office houses the Medical Advisor and Desk Officer for the Bangladesh project. They are involved in the concept stages of the study, its conduct and supervision. They will provide further support throughout the study, mainly by assuring the running budget to the local project where the study will be carried out, besides executing orders of supplies procured outside Bangladesh, both indispensable for the routine services on which the study will be grafted. The laboratory cost at ITM for this study has been accepted for funding by Damien Foundation.

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The Damien Foundation Bangladesh will implement the study in its working area in Greater Mymensingh District, where it is responsible for TB control on behalf of the National TB control Programme. It will also cover all local costs for the study. Besides some 165 outpatient clinics, it runs also 3 dedicated hospitals with 3 reference laboratories within the study area.

Principal Investigator: Dr. Aung Kya Jai Maug, tel. +8801714275898, aung@damienfoundation-bd.com
Country Director: Mr. Bart Rombaut, bart@damienfoundation-bd.com
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The NTP (National TB control Programme) of Bangladesh is responsible for all TB control activities in the country. It is involved in the conduct and supervision of the study.

The Line Director in charge of the study will be the local study coordinator: Dr. Ashaque Hussain, tel +8801912728871; Email: directormbdc@gmail.com

At ITM Antwerp, Dr Armand Van Deun will be the study coordinating Investigator. The Clinical Trials Unit (Céline Schurmans, Yves Claeys, Jozefien Buyze and Raffaella Ravinetto) will ensure the project and GCP management of the study, as well as the clinical data management and statistical aspects. Clinical support will be given to the study coordinating investigator by the Department of Clinical Sciences (Dr. Anja de Weggheleire). The laboratory quality assurance and support with advanced testing will be assured by the mycobacteriology staff (Prof Dr. Bouke de Jong, Head, Mycobacteriology Unit, Department of Biomedical Sciences, +3232476590) who will also be Dr. Van Deun's back-up as study coordinating investigator.

STATEMENT OF COMPLIANCE & CONFIDENTIALITY

By signing this protocol, the Investigator(s) acknowledge(s) and agree(s):

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the Declaration of Helsinki, Good Clinical [Laboratory] Practice (GC[L]P) and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

This document contains information that is privileged and confidential. As such, it may not be disclosed to any other persons than involved research staff and the concerned Ethics Committees, unless specific permission is granted in writing by the Damien Foundation, Belgium, or such disclosure is required by federal, national or other laws or regulations. In any event, persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future study-related information supplied which is regarded as privileged or confidential.

The Sponsor of this study – the Damien Foundation Belgium– will at any time have access to the source documents from which Case Report Form information may have been generated and will be permitted to perform trial-related monitoring and audits. All study material will be maintained according to regulatory requirements and until the Sponsor advises that retention is no longer necessary.

ITM COORDINATING INVESTIGATOR:

Title, Name: Dr. Bouke de Jong Date: _____
Signed:

DF COORDINATING INVESTIGATOR:

Title, Name: Dr. Tine Demeulenaere Date: _____
Signed:

GENERAL DIRECTOR OF THE DAMIEN FOUNDATION BELGIUM:

Title, Name: Mr. Koen Van den Abeele Date: _____
Signed:

PRINCIPAL INVESTIGATOR:

Title, Name: Dr. Aung Kya Jai Maug, Medical Specialist Research, Training and MDR-TB Date: _____
Signed:

Signing this document, I commit to carry out the trial in accordance with the protocol, Good Clinical Practice and applicable ethical and regulatory requirements. I also acknowledge the paragraph relevant to study confidentiality and authorize the Institute of Tropical Medicine, Antwerp, Belgium to record my data on a computerized system containing all the data pertinent to the study.

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SYNOPSIS

HYPOTHESIS	Double dose rifampicin together with earlier monitoring of sputum conversion using vital staining reduces unfavorable outcome of Cat. 1 first-line TB treatment without excess serious toxicity, and allows early switch to specific treatment of MDR-TB without using Cat. 2 retreatment regimen
DESIGN	Open-label randomized clinical trial
STUDY SITE & POPULATION	Damien Foundation Bangladesh TB project in Greater Mymensingh district (8 selected clinics)
DURATION	2.5-3 years
OBJECTIVES AND ENDPOINTS	*Primary objective:* To compare a high-dose rifampicin standard TB regimen of 6 months duration with standard TB regimen in respect to adverse treatment outcome (relapse, failure, death and default) and toxicity, in smear-positive pulmonary TB cases *Secondary objectives:* 1. To assess whether the study regimen also cures low-level rifampicin resistant TB 2. To assess the effectiveness of FDA vital staining screening at two weeks of treatment for early switch of non-responding rifampicin resistant TB to MDR-TB treatment, compared to a clinical evaluation based on fever resolution 3. To assess the negative predictive value of conversion at 2 weeks for relapse 4. To estimate the proportion of acquired rifampicin resistance among failures and relapses 5. To compare the predictive value of auramine with FDA staining at 2 weeks to predict unfavourable outcomes The primary efficacy endpoint is the incidence of adverse treatment outcomes. The primary safety endpoint will be the occurrence of Serious Adverse Events and Grade 3-4 liver toxicity
INCLUSION & EXCLUSION CRITERIA	Inclusion criteria: - smear-positive pulmonary TB - 15 years or older - able and willing to provide written informed consent Exclusion criteria: - MDR-TB diagnosed with resistance on rapid DST for rifampicin prior to start of treatment - extra-pulmonary TB, smear-negative pulmonary TB - patients in need of hospitalization because of very bad general condition or complications at screening - patients with clinically active liver disease at screening - known HIV-positives - known hepatitis B or C infected patients - pregnant women

SCREENING, RECRUITMENT & RANDOMIZATION	Screening by sputum smear microscopy for acid-fast bacilli and chest X-ray if required; referral for rapid rifampicin DST as per TB programme guidelines. 1000 eligible pulmonary TB cases consenting to participate will be block randomized to the intervention or control arm, separately for new and retreatment cases.
TREATMENT	Intervention: 2HREZ/4HR Cat. 1, modified by using double dose rifampicin throughout. This will apply to new as well as retreatment cases Controls: the standard program treatment. This consists for new cases of 6 months of rifampicin and isoniazid, complemented by ethambutol and pyrazinamide during the first 2 months (Cat. 1, 2HREZ/4HR); for retreatment cases, streptomycin is added during the first two months, pyrazinamide is added during the first three, and ethambutol throughout, prolonging to a total of 8 months (Cat. 2, 2SHREZ/1HREZ/5HRE)
FOLLOW-UP	Sputum smears for acid-fast bacilli at 2-3, 5 and 6 months (3-4, 5 and 8 for Cat. 2). Referral of positives for rapid rifampicin DST from 2nd or 3rd month onwards. Additional routine plus vital staining smears after 1 and 2 weeks for the intervention only, with referral for rapid DST if the number of bacilli has not declined by at least one log by 2 weeks; referral for rapid DST in the control arm based on fever still present at 2 weeks. Prompt switch to specific MDR regimen if resistance is detected.
ANALYTICAL METHODS	Analyses are planned at the end of the primary treatment period (interim analysis) and after 1 year post-treatment follow-up (final analysis). *Primary Analysis:* Counts and proportions (and 95% confidence intervals) of patients with any adverse treatment outcome, and by adverse treatment outcome category, will be presented. Odds ratio's (ORs) and 95% CIs will be estimated from a logistic regression model with effects for arm (intervention vs control), clinic, and stratum (new/re-treatment case). Similar analyses will be performed for the primary safety endpoint.
SAFETY	Clinical monitoring at each follow-up visit, with referral to Medical Officers or project hospitals as per standard guidelines. Additionally, both arms will be screened for liver toxicity biochemically at 0, 2, 4 and 8 weeks of treatment. Treatments will be interrupted if transaminase increase to >5 ULN, with referral for hospitalization.

1. INTRODUCTION

1.1 Background

Bangladesh is a country located in South East Asia, of about 144,000 km². With a population estimated at 155 million, it is one of the most densely populated countries in the world. It is among the high-burden countries for tuberculosis (TB) as well as multidrug-resistant TB (MDR-TB), mainly because of the large population. Total TB incidence was estimated at 225/100,000 population in 2011, MDR-TB at 1.4% among new and 29% among retreatment cases.[1] TB mortality is 45/100,000. There is virtually no HIV (0.4/100,000 general population). TB case detection is lagging behind at 45% (all types), and even more for MDR-TB. Of estimated 4000 annual cases only 500 were detected in 2011. This is partly explained by still recent organization of MDR-TB services, but also because of under-detection of suspects, i.e. retreatment cases of whom only 7366 were reported among about 100,000 smear-positives in 2011. In the Damien Foundation (DF) area proposed for this study, the TB and MDR-TB situation and detection look more favorable, possibly because of their more remote, rural character. Detection of TB reaches 60-70%, and MDR-TB was at 0.7%, 0.4%, 0.5% and 2% among new cases as determined by systematic random surveys in 1995, 2001, 2005 and 2010 (the two last ones using molecular methods, which may explain part of the increase).

TB detection in Bangladesh relies mainly on a very well developed and in principle easily accessible network of sputum smear-microscopy laboratories for detection of acid-fast bacilli (AFB, =TB). Culture for detection of the disease is not available and also no longer recommended by the World Health Organization (WHO). Chest radiography is widely available and used for the smear-negative pulmonary and some extra-pulmonary cases. Culture followed by slow drug susceptibility testing (DST) is available for drug resistance surveillance and MDR-TB diagnosis in the Dhaka National TB Reference Laboratory besides a few regional laboratories. The reference laboratories in the study area use also rapid DST on slides to facilitate MDR-TB diagnosis since 2008, followed by slow confirmatory DST. Since 2011 the NTP and partners have started using also automated genetic amplification tests, in principle only for MDR-TB diagnosis. This network of Xpert MTB/RIF is being expanded gradually, and the increased detection of MDR-TB has become visible recently.

Effective TB control is through detection and treatment of active cases. Treatment requires a combination of drugs and long duration (regimens of at least 6 months). The main first-line drugs are rifampicin and isoniazid, and resistance to both defines MDR-TB which proves impossible to cure with these and other first-line drugs (pyrazinamide, ethambutol, streptomycin). MDR-TB treatment is far more difficult and needs second-line drugs. Among the greater variety of these drugs only a few are sufficiently active, i.e the later generation fluoroquinolones (mainly gati- and moxifloxacin) and the 2nd-line injectables (2LI). If MDR-TB develops resistance to these two groups as well, an ultra-resistant form develops called XDR-TB; with only one of both groups resistant this is called pre-XDR-TB. MDR-TB treatments are difficult, toxic, expensive and yield highly unsatisfactory results in almost all NTPs. In the DF area, a short and highly effective MDR-TB treatment was developed and has been used with very good results for already 10 years (see further).

The International Union against Tuberculosis and Lung Disease (Union) and the WHO recommend a cascade of TB treatment regimens each aiming at very high but not 100% effectiveness, while allowing a small percentage of failure and relapse cases to start the next regimen in the cascade. The switch to the next higher [sequentially used and “higher” in complexity] regimen is meant to be made as early as possible. In practice this switch is often made too late, since it depends on careful microscopy to identify suspects in need of rapid drug susceptibility testing, and their efficient referral. Particularly patients with MDR-TB may die, default or acquire resistance to more drugs before being recognized as having MDR-TB and put on an appropriate treatment regimen. For some patients with extensive disease and patients with HIV infection, 6 months of the standard primo-treatment (Cat. 1) is too short, leading more frequently to relapse. The current standard first-line

drug retreatment regimen (Cat. 2) is more toxic but not more effective for MDR-TB than the current Cat. 1, and it has only a marginal advantage for other types of drug-resistant TB at the risk of amplification of resistance.

Most ongoing studies on novel TB regimens focus on the new TB drugs, designing regimens for MDR-TB and even primo-treatment composed mostly or entirely of new drugs. Additional studies focus on moxifloxacin, the core drug to treat MDR-TB, replacing isoniazid or ethambutol in Cat. 1 with the aim to shorten treatment or to prevent relapse and failure due to drug resistance to the current core drugs isoniazid and rifampicin. We favor further optimization of the conventional regimens without new drugs, since we still know very little about the potency of the individual new drugs and their adverse events, and even less about the most effective combinations. Defining effective regimens based on several new drugs will be very costly and cannot be expected to be completed within the coming 10 years. Moreover, since complete coverage by rapid and accurate screening for rifampicin resistance is presently not feasible, large-scale use of moxifloxacin for primo-treatment will unavoidably accelerate the emergence of pre-XDR. As a result we are left with a one-chance-only treatment. Harnessing and optimizing the activity of the well-known most potent TB drugs would therefore appear to be a better and safer approach for the near future.

We expect that the standard pulmonary TB treatment regimens recommended by the Union[2] can be optimized by using daily high dose rifampicin during Cat. 1 primo-treatment. For the here proposed pilot study, dosing of rifampicin would be doubled, while standard dose isoniazid, pyrazinamide and ethambutol would be maintained. Management decisions would be made after 2 weeks of intensive phase, based on remaining numbers and decline of TB bacilli on microscopy for AFB [auramine and fluorescein diacetate (FDA) vital staining]. Those with min. 1 log decline in the number of AFB by FDA smear, or already negative, would continue the unaltered regimen. Those not meeting these criteria would be referred for rapid DST by slide DST and/or Xpert MTB/RIF, depending on ease of referral. In case no rifampicin resistance is detected they would continue the optimized Cat. 1 treatment, with further monitoring by smear and rapid DST as per National TB Programme (NTP) indications, i.e. rapid DST in case a smear is positive at 3, 5 or 6 months; those diagnosed with rifampicin resistance at any time and by any technique would be switched immediately to the standard short "Bangladesh" MDR-TB treatment regimen. In the control arm, referral for rapid DST would be based on non-remittance of fever at the end of the second week, otherwise treatment management remains the same. Failures, relapses and other recurrences of the first-line regimen would all undergo rapid DST, and if not found to be rifampicin resistant they would be re-started on the same 6-month Cat. 1. The current Cat. 2 first-line retreatment regimen would no longer be used in the intervention arm.

Intervention and control treatment regimens (Rh: double dose rifampicin)

	New cases
- intervention:	2 HRhZE / 4 HRh
- control:	2 HRZE / 4 HR (Cat. 1 regimen)
	Retreatment cases
- intervention:	2 HRhZE / 4 HRh
- control:	2 SHRZE / 1HRZE / 5 HRE (Cat. 2 regimen)
	MDR patients detected at any time
	4 KHPtoGCEZ / 5 GCEZ (MDR regimen)

The expected benefits from this optimized treatment regimen cascade are:

1. Reduced occurrence of relapse with susceptible bacilli in patients with extensive disease, due to better sterilizing activity while maintaining the same treatment duration. Total absence of relapse with AFB smear at 2 weeks negative or scanty would suggest that shortening of the regimen to 4 months could be tried for fast converters.
2. Prevention of development of MDR- TB and cure of low-level rifampicin-resistant TB (MDR or monoresistant) with this more bactericidal Cat. 1.
3. Very early start of the MDR regimen for high-level rifampicin-resistant disease (MDR or monoresistant), reducing the risk of death and default and preventing accumulation of further or higher-level drug resistance to H, Z or E. This approach better preserves the efficacy of the latter drugs which are all used in the MDR regimen.
4. Reduction of toxicity from injectable drugs and ethambutol during the MDR regimen, by abolishing the Cat. 2 regimen (no streptomycin used prior to kanamycin in the MDR regimen, thus avoiding cumulative vestibular-, renal- and ototoxicity; also ethambutol ocular toxicity is cumulative across courses of treatment). Amplification of first-line drug resistance (S, H, Z and E) will be further avoided by dropping Cat. 2. As streptomycin resistance disappears in the population, streptomycin might eventually remain available as a key drug for MDR (re)treatment, alternative to second-line injectables.
5. Concordance between geno- and phenotypic rifampicin DST would improve if the low-resistance mutations have no impact on high-dose rifampicin treatment. Moreover, new molecular DST formats omitting these now irrelevant mutations could then be simplified and made fully specific for rifampicin resistance. Such tests would identify only mutations highly predictive of bacteriologically poor outcome even with the high-dose regimen. This would at the same time avoid errors of current molecular tests with resistance detection based on the absence of, or poor binding to, wildtype DNA probes: silent mutations, poor reactions and reading errors would no longer interfere with genotypic resistance testing.

Other groups are also studying high-dose rifampicin, yet their aim is to shorten Cat. 1 treatment without increasing relapse. These clinical trials would thus provide complementary information and might show further gains that are possible with increased dosing, e.g. in paucibacillary disease.

1.2 Rationale

Low-level resistance to rifampicin is not rare, and is easily missed by conventional DST. About 15% of all rifampicin resistance in retreatment cases is low level.[3, 4] However, an as yet unpublished survey among our target study population suggests that this proportion may even reach 50% in new cases. Each of these mutations is rare, but a large variety exists. They are detected by absent or reduced signals with wildtype probes by molecular test formats, such as lineprobe assays and the Xpert MTB/RIF assay. This can cause substantial confusion in the interpretation and decision making when conventional DST suggests (false) rifampicin susceptibility. This is particularly problematic in the MGIT system, in which the standard 12 day read-out misses rifampicin resistance in strains with those mutations, while the control tube already meets acceptance criteria for growth. For the same reason the significance of these low-level resistance mutations remains disputed. There is nevertheless evidence that cases caused by such strains can cause outbreaks of MDR and even XDR-TB. Such strains also lead to a high frequency of adverse outcomes in individual cases, comparable to those of the high-level resistance mutations (63% failure/relapse of standard primo-treatment in our reference 2). The MIC of most low-level mutations is around the peak level in serum and tissues achieved with the standard dose of rifampicin. This could explain why more MDR with these mutations seem to be curable with the unmodified Cat. 1 or 2, but often resulting in subsequent relapse.

Historical trials on rifampicin in the 1970's used relatively low doses due to high drug cost and concerns due to raised transaminase, in spite of the recognised proportional increase of activity with higher doses (L. Verbist, personal communication).[5] Increasing the rifampicin dose increases serum concentrations at least proportionally, and a ceiling remains as yet undetermined.[6] According to studies by the group of Gumbo, it is the peak drug serum concentration (Cmax) compared to the bacterial minimal inhibitory concentration (MIC) which determines effectiveness of killing as well as resistant mutant suppression and also determines the duration of the post-antibiotic effect. If the free Cmax/MIC ratio exceeds 175, then no resistance to rifampicin emerges even by high-resistance mutations, and without assistance by companion drugs.[7] Starting from a Cmax/MIC ratio above 17, the resistant population declines. A normal 600 mg rifampicin dose produces a Cmax around 10 mg/L. About 80% of the serum concentration is protein-bound, but the low protein content of epithelial lining fluid and even higher affinity of the lipophilic TB bacteria makes this irrelevant, so that the peak reaches about 20 times the MIC of wildtype strains. This doubles after a 1200 mg dose, or triples after a 1800 mg dose, potentially resulting in permanent cure of tuberculosis caused by a wide range of low-level resistance mutations with MICs 2-8 times above normal.[8, 9] The most frequently described rifampicin resistance *rpoB* mutations, however, have too high MICs to be overcome by higher dosing. [7, 10]

The standard dose 6-month regimen has an overall relapse frequency of about 2% among sputum smear-positive cases with a fully susceptible strain and normal daily dosing. This increases to 6% among patients with extensive disease or HIV co-infection. Relapses increase moderately with intermittent dosing, inversely to dosing frequency and mainly during intensive phase, i.e. from 1.9% (C.I. 1.4-2.7%) with daily to 3.2% (C.I. 2.5-4.1%) with fully thrice-weekly regimens. A thrice-weekly continuation phase following a daily intensive phase does not appreciably increase relapse frequency. Intermittency increases the risk for acquired drug resistance, particularly among HIV-positive patients, but not after the intensive phase.[11, 12] High rifampicin dosing has been shown to produce much faster sterilization of *M. tuberculosis* in mice.[13] In humans, a randomized clinical trial in culture positive patients of a 3-month alternate day regimen with 1200 mg rifampicin and 900 mg isoniazid, supplemented by a normal dose of streptomycin, found culture conversion to be faster by one month. There were only 15% relapses, none of whom had acquired resistance, concentrated among those with radiographically extensive disease. This regimen did not include pyrazinamide, known to allow a reduction of treatment duration. Adverse drug events were not reported.[14]

With daily dosing, only toxic hepatitis occurs at appreciable frequency as a serious rifampicin adverse event. However, with Cat. 1 treatment liver toxicity is more often attributable to pyrazinamide, and is rare except in elderly people with co-morbidities.[15] Only pyrazinamide liver toxicity is clearly dose-related.[13, 16] Isoniazid can very rarely cause rapidly fatal hepatic necrosis, also at normal dosage. Liver toxicity due to rifampicin is usually mild and readily resolved by temporary treatment interruption, without recurrence even at the same dosage. Older published trials do not mention increased risk of liver toxicity with high dose rifampicin.[9, 13] This issue has recently been studied prospectively by three groups, using up to triple rifampicin dosing with intensive toxicity monitoring. Preliminary reports and personal communications on these PanACEA, RAFA and RIFATOX trials indicate that no increased incidence of liver toxicity was seen (Annex 1). The PanACEA study also found that doubling the rifampicin dose to 20 mg/kg led to 3-4 times higher peak- and AUC rifampicin concentrations.
Studies of early bactericidal activity under HRZE extended over 2 weeks, with viable counts of TB bacilli from sputum by culture every few days, have shown that isoniazid produces about 1 log kill over the first 2 days; then rifampicin takes over till the end of the second week, producing a kill of another 1-1.5 log; after that the main kill seems to be due to pyrazinamide, of which the action starts late.[17] This means that susceptibility to isoniazid and/or rifampicin is reflected in the decline of viable bacilli over the first two weeks of treatment, irrespective of the action of other drugs (starting late,

i.e. pyrazinamide, or very low kill, i.e. ethambutol). We have limited data from a study using standard auramine as well as FDA vital staining on serial sputa over the first 2 weeks, showing that in susceptible cases the AFB counts usually decrease by 1 log after 1 week, and by 2 logs after 2 weeks. However, there were exceptions, and the quantification of AFB in direct smears is not that reproducible, so that the exact cut-off or algorithm to screen patients for DST referral needs to be better defined. The challenge will be to balance good sensitivity of screening for serious initial drug resistance and/or extensive disease, versus excessive numbers of patients to be referred for in-depth investigations. To apply earlier screening for drug resistance also to the control group, absence of fever resolution at two weeks (as lead symptom of unsatisfactory clinical improvement) will be used as an alternative referral criterion for rapid DST. This would be the simplest possible screening tool, and may even be more appropriate considering the highly unsatisfactory results of treatment follow-up by means of microscopy in many TB control programmes.
The WHO recommended Xpert MTB/RIF assay as well as lineprobe assays for molecular detection of resistance cover a large variety of mutations in the responsible genes by incorporation of probes covering the relevant stretch of wildtype DNA, plus- in the case of the lineprobe assay- a few 'mutant' probes that confirm the most frequent mutations. A positive signal from the mutant probes will always correctly indicate that this mutation is present. For the wildtype probes a negative signal (absent or low-intensity bands; delayed amplification) is interpreted as resistance. However, this is not satisfactory since poor reactions occur regularly with poor specimens or technique, and a false negative signal (falsely indicating resistance) is possible with "silent mutations", i.e. a change in nucleotide that still codes for the same amino-acid.
Serious confusion regarding the Xpert MTB/RIF assay exists in relation to its use in low rifampicin resistance prevalence settings and for follow-up samples. Doubts regarding false resistant results are widely spread, also because of the earlier WHO recommendation not to trust such results but to confirm with a different test format. This severely reduces the usefulness of this molecular diagnostic tool, that is otherwise the most appropriate rapid DST method for decentralised use and thus early diagnosis. Our studies on the low-level rifampicin resistant strains (reference 3) strongly suggest that the problem lies in reduced sensitivity of the gold standard, conventional DST, and that the "false resistance" detected by molecular methods is significant on the individual and population level. Non-specific reactions or test failures are believed to occur due to the presence of DNA from dead bacilli during treatment. However, the first 6 months of parallel use of Xpert MTB/RIF and the highly accurate slide DST in the Bangladesh project have shown an almost perfect concordance for rifampicin resistance, also for specimens taken at 2-3 months of Cat. 1 treatment. None of the Xpert resistant were found susceptible by slide DST, but slide DST had an incremental yield of about 10% resistant cases. It is very likely that Xpert MTB/RIF assay results at 2 weeks of treatment will be at least as reliable.

2. STUDY DESIGN

2.1 General study design

This study is a randomised clinical trial. Consecutively diagnosed smear-positive pulmonary TB patients, which conform to all of the inclusion and none of the exclusion criteria and provide informed consent, will be randomized to the intervention or control arm.

The control arm will receive the standard pulmonary TB treatment regimens recommended by the International Union against Tuberculosis and Lung Disease (The Union). This consists of:

Treatment:

New cases: 2HRZE/4HR, i.e. 2 months of isoniazid, rifampicin, pyrazinamide and ethambutol intensive phase, followed by 4 months continuation phase isoniazid plus rifampicin) (Cat. 1 regimen, 6 months)

Retreatment cases: 2 months of SHRZE (S= streptomycin) followed by 1 month of HRZE and 5 months HRE (Cat. 2 regimen, 8 months)

Any patients with *MDR TB detected* at any time will be switched to 4KHPtoGCEZ/5GCEZ, i.e. 4 months of kanamycin, isoniazid, prothionamide, gatifloxacin high-dose, clofazimine, ethambutol and pyrazinamide, followed by 5 months of gatifloxacin high-dose, clofazimine, ethambutol and pyrazinamide (MDR regimen, 9 months)

Testing:

Monitoring by smear and rapid DST as per National TB Programme (NTP) indications:

- Smears for AFB [by Ziehl-Neelsen brightfield (ZN) or auramine fluorescence (FM) technique] at diagnosis, after 2, 5 and 6 months (Cat. 1) or after 3, 5 and 8 months (Cat. 2); repeat after month 3, respectively 4, if AFB were seen at month 2 or 3
- Vital staining fluorescein diacetate (FDA) smears for AFB to confirm a positive ZN or FM result during Cat. 1
- rapid DST if fever is still present after 2 weeks, or in case an AFB smear (auramine and/or FDA) is positive during Cat. 1, or from the 3rd month of Cat. 2 onwards. Rapid DST is performed also from any retreatment case (relapse, return after default and other previously treated for at least 1 month), and from previously untreated contacts of MDR-TB

Additionally, patients enrolled in the study (control as well as intervention arm) will be followed-up for relapse 12 months after the end of treatment by clinical examination, sputum smear and slow solid culture.

The intervention consists of the standard care as provided to the control arm with the following modifications:

Treatment:

New cases: Compared to standard regimen dosing of rifampicin is doubled, while standard dose isoniazid, pyrazinamide and ethambutol are maintained, i.e. 2 months of HRhZE followed by 4 months of HRh (6 months)

Retreatment cases: retreatment cases randomized to the intervention arm receive the same care as new cases in the same arm.

Any patients with *MDR TB detected* at any time will be switched to 4 months of KHPtoGCEZ followed by 5 months of GCEZ (MDR regimen)

Testing

In addition to standard monitoring (as described above for the control arm), patients in the intervention arm will have sputum tested by auramine and FDA smear after the first and again after the second week of intensive phase treatment. Management decisions will be made after 2 weeks of intensive phase, based on remaining numbers and decline of AFB on FDA staining, but not based on continuing fever. Those with min. 1 log decline in the number of AFB, or who are already negative, would continue the unaltered regimen. Those not meeting these criteria would be referred for rapid DST by slide DST and/or Xpert MTB/RIF, depending on ease of referral.

This open label, randomised clinical trial is intended as a pilot study on the efficacy and safety of high-dose rifampicin and feasibility and added value of auramine and/or FDA vital staining sputum smear after 2 weeks of intensive treatment phase. If this proof-of-concept study provides substantial indication of benefit without indication of excess toxicity, the data from the study will be used to design a larger scale, cluster-randomized study. The aim of this cluster randomised study would be to provide definite proof of the benefit of the intervention on adverse treatment outcomes and lack of excess toxicity associated with high dose rifampicin. In addition, the cluster-randomized study would provide a more precise assessment of the suppression and prevention of (acquired) resistance endpoints.
An interim analysis is thus planned at the time the last recruited patient finishes treatment, i.e. about 9 months after the end of recruitment. It will focus on assessment of drug toxicity versus suggested benefits of the intervention. This analysis will be primarily performed for the go/no-go decision and design considerations for the cluster-randomized trial. The decision on proceeding to the cluster randomized study will be based on the absence of excess toxicity, a trend toward a reduction of unfavourable outcomes (excluding relapse), and possible favourable effects on initially present low-resistance mutations / mutations acquired during treatment. It will also allow to adapt the design of the larger study particularly regarding the algorithm for resistance screening, and whether or not treatment shortening could be justified with rapid initial conversion.

3. STUDY OBJECTIVES

Primary objective:

To compare a high-dose rifampicin standard TB regimen of 6 months duration with standard TB regimen with respect to adverse treatment outcome (relapse, failure, death and default) and toxicity, in TB cases (new or retreatment, pooled) not shown to have initial rifampicin resistance before one of the standard treatment outcomes applies.

Secondary objectives:

1. To assess whether the study regimen also cures low-level rifampicin resistant TB:
   by comparing and describing adverse treatment outcomes by initial rifampicin mutations (none, low-level, high)
2. To assess the effectiveness of FDA vital staining screening at two weeks of treatment for early switch of non-responding rifampicin resistant TB to MDR-TB treatment, versus clinical evaluation based on fever resolution. The proportions of rifampicin resistant cases started on MDR treatment within the first 2 months of primo-treatment as well as the proportion of initially present resistance never switched to the MDR regimen and not cured will be compared between the two arms.
3. To assess the negative predictive value of conversion at 2 weeks for relapse
4. To estimate the proportion of acquired rifampicin resistance among failures and relapses
5. To compare the predictive value of auramine with FDA staining at 2 weeks to predict unfavourable outcomes

3.1 Primary endpoints

The primary efficacy endpoint is the incidence of adverse treatment outcomes. Following the WHO guidelines from 2010[18], as currently in use in the NTP in Bangladesh, outcomes are defined as follows:

- Cured: A patient whose smear or culture was positive at the beginning of treatment but who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
- Completed: A patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion.

- Relapse: Cured previously from TB or completed treatment for TB and now having bacteriologically positive sputum for TB (at 12 months follow-up or at an earlier time point)
- Default: The patient whose treatment was interrupted for ≥ 2 consecutive months or more.
- Failure: Sputum positive for TB at 5 months or later during treatment. In line with current WHO recommendations, patients detected with MDR-TB or rifampicin resistance before this or another outcome applies and switched to the MDR-TB regimen will be excluded from the outcome analysis.[18] Failure will also be declared if the regimen has to be changed for at least 2 drugs due to adverse events.
- Death: All-cause mortality between case registration and end of TB treatment (related or not to TB or TB treatment)
- Transfer out: A TB patient who transferred out to another recording and reporting unit and whose treatment outcome is unknown.

Analyses will be performed using the NTP smear-based recurrence (failure, relapse) definitions, but as a secondary approach culture-based definitions will be used.

Safety:
The primary safety endpoint will be the occurrence of any of the following:

- Serious Adverse Events
- Grade 3-4 Liver Toxicity following NIH common toxicity criteria (CTC), including transaminase increases to >5-20 ULN (grade 3), or > 20 ULN (grade 4)

3.2 Secondary endpoints

Secondary objectives are assessed with the adverse treatment outcomes defined above (relapse, default, failure and death).
Genetic and laboratory predictors of TB treatment outcome are described in section 5.4, while secondary and tertiary analyses are defined in 8.3.4.

4. SUBJECTS, POPULATION & SELECTION

4.1 Settings, selection & recruitment

The study will take place in eight DF Bangladesh outpatient clinics with an LED TB fluorescence microscopy laboratory (Sarishabari, Madhupur, Bhaluka, Fulbaria, Iswarganj, Netrakona, Purbodhola, Kendua), shown on the map in Annex III. These clinics are located in the Greater Mymensingh district and have been selected because of their proximity to the three DF hospitals with a reference laboratory capable of doing rapid DST and toxicity monitoring. Five of them are also the oldest project clinics, where drug resistance surveillance was started already in 1995. The proposed study would have the additional benefit of adding a fifth drug resistance monitoring data point without extra cost, since *rpoB* sequencing on preserved sputum from all enrolled cases will be performed to provide the denominators for some study endpoints and to define cases with acquired resistance.
Together, these clinics register about 2000 TB cases per year. They have in general advanced disease, but there is practically no HIV and also MDR prevalence is low (1-2% of new cases).

Patients presenting with symptoms suspect for TB as defined by the NTP (mainly cough for at least 3 weeks) will be tested as per standard guidelines, i.e. 2 sputum smears for AFB, which can be repeated at 2 weeks intervals in case the suspicion remains and previous tests are negative. Additionally chest X-Ray is used to diagnose smear-negative pulmonary TB. Other suspects (extra-pulmonary) are referred to Medical Officers for appropriate investigations. Standard AFB-smears will in principle be done using FM technique, which can be temporarily replaced by ZN in case of

equipment breakdown. One sputum smear with at least one AFB detected is sufficient to register a case, or a decision by a Medical Officer based on clinical and other examinations.

Once patients are registered and comply with the exclusion / inclusion criteria that are checked per routine, written informed consent will be asked. Consenting patients will be further assessed for eligibility, and if all in- and exclusion criteria are fulfilled, they will be enrolled and randomized over the 2 treatment arms. Patients refusing will all be put on the standard regimens.

4.2 Inclusion and exclusion criteria

In order to be eligible, study participants **must meet the following criteria**:

- Diagnosed with smear-positive pulmonary TB
- 15 years or older
- Able and willing to provide written informed consent

Potential participants meeting any of the following criteria **will not be enrolled in the study**:

- contacts of MDR-TB patients and other MDR-TB suspects diagnosed with resistance on rapid DST for rifampicin performed prior to start of treatment according to NTP guidelines
- smear-negative pulmonary and extra-pulmonary TB cases
- patients in need of hospitalization because of very bad general condition or complications
- patients with clinically active liver disease, for the study defined as jaundice confirmed by a local Medical Officer (Government)
- any known HIV-positive patient (although none are expected)
- any patient with known hepatitis B or C infection
- pregnant women; in addition, patients in the intervention arm who become pregnant during treatment will be switched to the control arm

4.3 Sample size

The sample size selected is 1000 patients (500 randomized to intervention, 500 to control). The study will be conducted in eight DF Bangladesh outpatient clinics with an LED TB fluorescence microscopy laboratory that register about 2000 TB cases per year of which 65% are smear-positive pulmonary TB (new or retreatment). Enrolment will target 1000 randomised smear-positive pulmonary TB patients. Given an expected 5% refusal rate, it is expected that recruitment will require 9-10 months.

4.4 Randomization

Two randomization lists will be prepared at ITM prior to study start: one for new cases, one for retreatment cases. To ensure (approximate) treatment balance within study sites, the randomization list will be blocked. Treatment allocation will be concealed until the completion of the screening procedures and the final recruitment of the patient using sealed envelopes labelled with the randomization number and containing the treatment group allocation.

4.5 Withdrawal and termination of the study

Reasons for Withdrawal

Subjects may be withdrawn from the study if:

- The subject or legally acceptable representative withdraws the consent
- The Investigator judges that further participation would have negative effects on the subject's health. In this case, the study team will follow the patient up to resolution of such negative event.

Handling of Withdrawals
Patients withdrawn from study participation will continue to receive standard care, as required by their condition. This includes continuation of the best possible TB treatment as long as needed, and also care of any adverse events or complications that may or may not be due to the study procedures. The principal investigator will be responsible to assure that this care is provided, either at DF clinics or hospitals, either by referral to specialists at nearby Medical Colleges or other providers.

Loss to follow-up during treatment is defined by the default outcome definition, i.e. 2 months treatment interruption. As per standard NTP guidelines patients will be contacted by phone or have a home visit performed if they are late for an appointment.
Loss to follow-up for relapse assessment will be considered separately. These patients will be invited to attend 12 months after cure or default, also complemented by phone convocations and home visits as required.

Termination of Study
If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The EC's will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5. STUDY PROCEDURES

5.1 Study/visit schedule

SCHEDULE OF ASSESSMENTS

	Screening	*Enrollment (V0)*	*Week 1 (V1)*	*Week 2 (V2)*	*Week 4 (V3)*	*Month 2 (V4)*	*Month 3 (V5)*	*Month 4 (V6)*	*Month 5 (V7)*	*Month 6 or 8 (V8)*	*Month +12[4] (V9)*
Medical history		X									
Informed Consent		X									
Clinical examination	X	X		X	X	X	X	X	X	X	X
Weight		X		X	X	X	X	X	X	X	
Temperature		X		X	X	X	X	X	X	X	
Sputum sample for AFB smear (ZN or FM)	X	X[2]	X[2]	X[2]		X	X *(if 2M+)*		X	X	X[5]
Sputum sample for FDA vital staining		X[2]	X[2]	X[2]			X[3]		X[3]	X[3]	
DST referral	**X**[1]			X[2]			X[3]		X[3]	X[3]	
Treatment		X	X	X	X	X	X	X	X	X	
Pregnancy test (urine)		**X**[6]									
Biochemistry (liver tests)		X		X	X	X					
Adverse Event collection				X	X	X	X	X	X	X	
Outcome assessment									X	X	X

[1] Contacts of MDR-TB or retreatment cases only, if smear-positive

[2] - Intervention arm: DST referral only if responding to the 2-weeks screening criteria described in the protocol
- Control arm: DST referral if still fever (temperature >=38°C)

[3] If smear-positive at this time = standard indication of the NTP, controls as well as intervention arm

[4] This is the follow-up visit 12 months after end of treatment

[5] And for culture: this is the only time the specimens will always be used for microscopy and culture

[6] Women of childbearing age only

Controls will be treated as per NTP guidelines, but will additionally be monitored for transaminase increase and toxic hepatitis, as will be those of the intervention arm (see further).
Patients in the intervention arm will receive high-dose rifampicin Cat. 1 regimen and will also be screened earlier for delayed conversion of sputum smears for TB bacilli and rifampicin resistance, as shown in the algorithm in Annex 2. If retreatment is needed for the intervention arm, they will be started or re-started on the high-dose Cat. 1 regimen as long as they are not shown to be rifampicin resistant.
Once rifampicin resistance is detected, patients (intervention, controls or non-consenting) will be put on the MDR regimen.
All surviving patients who can be retrieved will be checked for relapse by smear and culture for TB 12 months after the end of treatment.
The outcome of pregnancy occurring during treatment and the baby's health will be recorded for both treatment arms.

5.2 Obtaining informed consent

The informed consent procedure will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, etc. If a subject (or parent or guardian) is unable to read or write, a signature from a witness to the informed consent discussion will be obtained. Subjects (or parents or guardians) will be informed that participation in the study is completely voluntary and that the participant can withdraw from the study at any time without any negative consequences.

All patients will be asked to give their informed consent to participate in the study, before undergoing any study-specific procedures. The Informed Consent Form (ICF) as well as the informed consent interview will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, the voluntary nature etc., as described in the E6 ICH GCP Guidelines.

The interviews will be conducted in the native language of the patients by a qualified person formally identified by the Investigator. Written information and consent forms in the local language will be provided to the patients or legally authorized representatives for their review. The written information and consent form is currently written in English, but it will be further translated in Bengali, and back to English for the accuracy of proper translation, with certification after verifying the original and back-translated copy.

Individuals potentially eligible for the study (i.e. individuals diagnosed with smear-positive pulmonary TB) will be informed on the modalities of TB treatment and on the possibility to participate in the study by the clinic's TB/Leprosy Control Assistants (TLCA) routinely in charge of diagnosis and treatment. Additional information on the eligibility criteria will be obtained (e.g. age) if missing in the patient file. Once eligibility has been confirmed, TLCAs will ask if the potential participant is ready to decide whether or not to join the trial. Patients must be given enough time to ask questions and to take a free decision (including a time frame of a few days to discuss their participation with family members). If the individual is willing to participate, he/she will be given the ICF and the content will be read by the TLCA. Once checked the ICF is understood, the TLCA will invite the participant to sign the form, add his/her name (or thumb-printing whenever they are illiterate) and countersign it.

If the patient is minor of age/not emancipated, the consent must be given by a parent or legal guardian according to national law; however, in this case, the investigator is responsible to check that the patient him/herself is also freely willing to take part in the study by means of giving informed and written assent.

If a patient (or guardian, if applicable) is unable to read or write, an independent witness must be present during the informed consent interview and the signature of the independent witness will be obtained, in addition to the patients' thumbprint. For adult individuals incapable of providing

informed consent for other reasons (e.g. mentally disabled), consent is obtained from a legally authorized representative in accordance with applicable law.

5.3 Specific procedures and activities

- Screening visit:
    - Clinical symptoms and signs of TB to select suspects for AFB-smear; refer to Medical Officer if not a suspect
    - Spot sputum collected for AFB if responding to TB suspect criteria; request for a second early-morning sputum next day
        - If AFB positive and patient does not respond to MDR suspect criteria: proceed with V0 procedures (same day if possible)
        - If positive and patient responds to MDR suspect criteria (MDR contact or retreatment case): referral for DST, treatment depending on rapid result
            - MDR regimen if rifampicin resistant; not eligible for the study
            - if susceptible or no result: eligible, continue with V0
- Enrolment visit V0:
    - Always clinical symptoms and signs, medical history, weight, temperature, patient information on standard TB treatment and DOT arrangements
        - Medical history: previous TB treatment; concomitant disease such as diabetes and HIV
    - Signs and symptoms: check for vital signs, severe anemia, dyspnea, chest pain, suspect of TB complications: refer if severely ill or complications suspected. Responding to exclusion criteria: register as non-study and start standard TB treatment or refer (active liver disease, patients to be hospitalized…)
    - No exclusion: explain study, ask informed consent
        - Refused: register as non-study and start standard TB treatment
        - Accepted: perform study specific procedures
          Perform urinary pregnancy test for all women of childbearing age not aware that they are pregnant. If the test is positive, do not include in the study but register as non-study patient and start on standard TB treatment.
          Pregnancy excluded: liver test, register as study patient and randomise to intervention or control arm
            - **Intervention only**: quantified auramine and FDA smears; start high-dose Cat. 1
            - Control arm: start Cat. 1 or Cat. 2 depending on history
- Visit after one week V1: **intervention patients only**
    - Give treatment, record attendance
    - Sputum for quantified AFB smears (auramine and FDA)
    - Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after two weeks V2: Intervention + control arm
    - Clinical check, weight, temperature: improving signs and symptoms of TB?
    - Give treatment, record attendance
    - Liver test
    - **Intervention arm only**: Sputum for quantified AFB smears (auramine and FDA), apply algorithm
        - refer for rapid DST if indicated by algorithm; switch to MDR if rifampicin resistant
        - otherwise continue high-dose treatment
    - **Control arm only**:

        - refer for rapid DST if still fever (temperature >=38°C); switch to MDR if rifampicin resistant
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after four weeks V3: intervention + control arm
    - Clinical check, weight, temperature: improved signs and symptoms of TB?
    - Give treatment, record attendance
    - Liver test
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after 2 months V4: intervention + control arm
    - Clinical check, weight, temperature: signs and symptoms of TB absent?
    - Give treatment, record attendance
    - Cat. 1: sputum smear for AFB
        - Refer for rapid DST if AFB-positive (auramine and FDA) and clinically poor evolution; switch to MDR treatment if rifampicin resistant
    - Liver test
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after 3 months V5: intervention + control arm
    - Clinical check, weight, temperature: signs and symptoms of TB absent?
    - Give treatment, record attendance
    - Sputum smear for AFB: all Cat. 2 and Cat. 1 patients positive at month 2
        - Refer for rapid DST if AFB-positive (auramine and FDA); switch to MDR treatment if rifampicin resistant
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after 4 months V6: intervention + control arm
    - Clinical check, weight, temperature: signs and symptoms of TB absent?
    - Give treatment, record attendance
    - Sputum smear for AFB: Cat. 2 positive at month 3 only
        - Refer for rapid DST if AFB-positive; switch to MDR treatment if rifampicin resistant
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after 5 months V7: intervention + control arm
    - Clinical check, weight, temperature: signs and symptoms of TB absent?
    - Give treatment, record attendance
    - Sputum smear for AFB: all patients
        - Declare failure if positive and refer for rapid DST if AFB-positive
        - Switch to MDR treatment if rifampicin resistant
        - Rifampicin sensitive:
            - Control arm: start Cat. 2
            - Intervention: re-start Cat. 1
    - Enquire, register adverse effects. Stop all drugs and refer in case of hepatitis suspicion (feeling sick, anorexia, vomiting, jaundice)
- Visit after 6 months (or 8 months for Cat. 2) V8: intervention + control arm
    - Clinical check, weight, temperature: signs and symptoms of TB absent?
    - Stop treatment, record attendance
    - Sputum smear for AFB: all patients
        - Declare cured if negative and inform about signs of relapse
        - Declare failure if positive and refer for rapid DST if AFB-positive

- Switch to MDR treatment if rifampicin resistant
- Rifampicin sensitive:
    - Control arm: re-register, start Cat. 2
    - Intervention: re-register, re-start Cat. 1

- Enquire, register adverse effects
- Make appointment for check-up after 12 months

- Visit 12 months after end of treatment V9 (intervention + control arm **cured or defaulted**)
    - Reminders, home visit if not attending
    - History from relatives / neighbours if not met (retreated or died of TB?)
    - Clinical check: signs and symptoms of TB absent?
    - If attending at the clinic only: weight, temperature
    - Sputum smear and culture for AFB
        - If negative: further passive follow-up
        - If positive: declare relapse and refer for rapid DST
            - Switch to MDR treatment if rifampicin resistant
            - Rifampicin sensitive:
                - Control arm: re-register, start Cat. 2
                - Intervention: re-register, re-start Cat. 1

All enrolled patients will be monitored intensively for drug toxicity (clinical; liver enzymes during the first 2 months). Treatment will be interrupted in case of a serious adverse event, possibly due to the TB drugs, and patients referred to the nearest project hospital. In case of toxic hepatitis, after full recovery drugs will be re-introduced in hospital one by one, aiming at re-starting the same regimen since this toxicity is often transient and probably not rifampicin dose-related.

Unscheduled visits can take place at any time during the patients participation in the study. During these visits, all study procedures will be performed if needed, and data will be collected.

5.4 Laboratory procedures

All analysis of clinical trial samples will be carried out in compliance with Good Clinical Laboratory Practice (GCLP). A Lab Analytical Plan will be available, in line with WHO-GCLP

FDA vital staining at 1 and 2 weeks will be used as part of the screening to identify intervention arm patients to be referred for rapid DST, and promptly switched to the MDR regimen if rifampicin resistant. Also an auramine AFB smear will be performed for comparison with FDA screening. Further follow-up will be standard and the same in the study and control cohort, by auramine AFB-smear at 2/3 months, 5 months and at end of treatment, when patients may again be referred for DST. Patients found positive on smear will be declared failure as per NTP definitions and registered for retreatment. Also relapse will be declared based on smears, as per NTP definitions. All failure and relapse cases will have sputum referred for conventional culture and DST, besides the rapid screening for rifampicin resistance. If not found resistant to this drug, such recurrence patients belonging to the intervention arm will not be re-started on the standard Cat. 2 but on the study regimen.

The initial, ethanol preserved pre-treatment specimen from all enrolled cases will be forwarded to ITM, Antwerp, for DNA sequencing of the *rpoB* gene. The *katG* and *inhA* genes will only be sequenced if an *rpoB* mutation is detected. All strains from cases diagnosed with rifampicin resistance at any time (2 weeks; failure; relapse or default), and all failure or relapse recurrence strains will also be forwarded to ITM, Antwerp. ITM will perform full DST and species identification, besides DNA sequencing of the *rpoB* genes. This will allow to compare the distribution of resistance-conferring mutations in the two study arms, and to identify possible acquired rifampicin resistance comparing

with the pre-treatment sequence. Spoligotyping on both pre-treatment sputum and recurrence isolate will be performed for confirmation of strain identity of all recurrences, to avoid confusion with re-infection, and to confirm presumed acquired resistance.
Cured patients will be informed about the possibility and symptoms of relapse, the need to report if symptoms recur, and given an appointment for a follow-up check after 12 months. All patients still in the area (cured or defaulted) will have evaluation of clinical condition and sputum for AFB smear and TB culture once, 12 months after treatment was stopped, performing a home visit if required. Further follow-up for relapse will be passive.

Preservation of all smear-positive diagnostic- and recurrence sputa and referral for *rpoB* sequencing at ITM will continue for patients registered after study enrolment has stopped, to provide a better estimate for the prevalence of low-resistance mutations, and how these strains are affected by standard treatment. Additionally, the clinics will check reduction of bacillary load by auramine and FDA smears after 1 and 2 weeks also for these patients, not belonging to the study population and otherwise treated following routine programme guidelines, including normal dose rifampicin. This will provide further information needed for better understanding of the intervention benefits.

Sputum AFB smears will be performed at the 8 participating centres, which have already a long experience with auramine smears; continuous external quality assessment has been standard practice since many years.
The FDA technique will be introduced three months prior to the start of the study, and reliability will be assured before the results are used for the study.
Serum transaminase will be performed at the DF Bangladesh reference laboratory using a biochemistry automate and kits, according to Good Clinical Laboratory Practice.
All molecular bacteriology will be done at the accredited ITM Antwerp lab on forwarded ethanol-preserved specimens or strains. Primary culture will be performed at the DF Bangladesh Netrakona reference laboratory with continuous quality monitoring. All isolates found rifampicin-resistant on rapid DST (Xpert, slide DST), and all failure and relapse isolates, will be periodically sent to ITM lab for DST on LJ for confirmation, completion of resistance profile and strain identification in case of recurrence.

Serum transaminase
Only ALT will be performed for screening, as the more sensitive test; AST will be performed additionally in case a rise of ALT is found.
Serum collected in the clinics will be kept refrigerated for maximum 3 days with batched transport by courier to one of the DF hospital laboratories, where it will be tested the day of arrival.
The test will be performed on an ERBA Chem V5.3 analyser using IFCC (International Federation of Clinical Chemistry) standard kinetic technique kits with internal quality controls. The local SOP followed is part of the study technical manual.
Test reports will be collected weekly by the field supervisors and transferred to the clinics for registration. An abnormal result will be promptly communicated by the lab to the clinic staff by phone.

Microscopy
Two sputum samples will be collected from suspects: one spot and one morning sputum (unless the first is already positive), and one morning sputum for treatment monitoring at each occasion. For positive suspects, the morning sputum will be examined by repeat auramine and FDA staining after standing overnight with spontaneous liquefaction and homogenization. Patients of the intervention arm will be told to expectorate the 1 and 2 week follow-up morning specimen the day before coming to the clinic, so that it is already homogenised.

Auramine and FDA techniques will be applied according to the respective SOPs, which are part of the study technical manual. All positive results will be expressed as number of AFB per field (eventually a decimal).

Decontamination and culture

Standard procedures will be used: decontamination by the Petroff method, centrifugation at 3000g and inoculation of 2 tubes LJ, with incubation for 8 weeks before declaring negative. Any number of colonies will be registered as a positive result, counting on strain differentiation to avoid errors due to cross-contamination or misidentification of samples.

The local SOP followed is part of the study technical manual.

DST and species identification

After sub-culture at ITM, LJ DST will be performed using the proportion method on LJ, for H (0.2, 1.0 and 5.0 µg/ml), R (40µg/ml), E (2 µg/ml), S (4 µg/ml), and para-nitrobenzoic acid (PNB, 500 µg/ml).[19] PNB-resistant strains will be further identified by means of 16S sequence analysis. If confirmed non-TB, they will be excluded from analysis.

The ITM SOPs followed are part of the study technical manual.

Rapid DST (Xpert and/or slide DST) will be performed at the Netrakona and other DF regional reference labs according to the respective SOPs, which are part of the study technical manual.

Xpert MTB/RIF testing will be performed as per manufacturer's instructions. A rifampicin resistant result will prompt to more extensive rapid DST using the slide technique.

A rifampicin resistant result by any test will be considered as correct and used to start MDR treatment promptly; only if there is serious doubt regarding correct patient identification the same test will be repeated on another sample for confirmation. However, for analysis the ITM result will override local results if different.

Sequencing of the *rpoB, katG* and *inhA* genes

DNA from the alcohol-preserved sputa or strains is extracted using the Boom method; amplification by PCR of the *rpoB* gene primarily will target all known resistance conferring sequences using extended primers, with a repeat PCR targeting only the shorter fragment of core region I in case the large-fragment PCR fails.[20] The genes conferring most resistance to isoniazid, i.e. *katG* and *inhA* will be sequenced only for sputa with an *rpoB* mutation already detected. DNA sequencing (ABI 3700 DNA capillary system) and comparison with the wildtype (H37Rv) sequence (ClustalX) will be performed for all amplified products.

The ITM SOPs followed are part of the study technical manual.

Fingerprinting techniques

DNA fingerprinting from sputum is best done by spoligotyping according to standard techniques, in view of the limited amount of material available.[21] We have a large experience in comparing paired samples and isolates from the same patients, showing that spoligotyping is equally discriminating as 15-loci MIRU-VNTR for this application.

The ITM SOP followed is part of the study technical manual.

6. STUDY INVESTIGATIONAL PRODUCT

6.1 Purchasing, preparation and administration

Rifampicin is part of the national treatment protocol in Bangladesh. The ITM's SOP CTUPRO217 will be followed in the purchase, to ensure the quality -including bio-equivalence- of the investigational product.

Rifampicin has been selected from the following WHO Prequalified manufacturer: Rifampicine - Eremfat® from Fatol Arzneimittel (registered in Germany).

The routine treatment will be purchased by the National TB Programme, in collaboration with and according to the principles of the WHO/GDF. Also these products will be prequalified by WHO, or registered in a country with stringent regulatory authorities.

6.2 Subject compliance monitoring

All TB treatment will be delivered directly observed (DOT). The DF project assures DOT already routinely for all patients, using all possible means: hospitalization for the very weak / complicated / severely drug resistant cases; daily attendance at the DF clinic if living nearby; a treatment supervisor from among Ministry of Health field staff or other responsible person. Many patients are supervised near their home by a network of Village Doctors which have been specifically trained and are regularly supervised for this task by the DF staff.[22]

In all cases any dose taken under supervision will be appropriately marked on the routine treatment card, these marks are different from those used for exceptional unsupervised intake (i.e. on request by a traveling patient, …).

6.3 Prior and concomitant therapy

Rifampicin activates cytochrome P450 liver enzymes, accelerating the metabolism of many common drugs also at normal dosage (corticosteroids, warfarin, oral contraceptives..). This may need a dose adjustment of these drugs or other precautions (i.e. change to other contraceptive methods). Anti-retroviral drugs need to be changed when rifampicin is started, but no patient on ARV is expected in this population. Patients taking concomitant drugs that need dose re-adjustment or other adaptations will be referred to a Medical Officer.

Conversely the metabolism or activity of rifampicin is not known to be affected by concomitant drugs.

All the concomitant medications will be recorded in the study CRF.

6.4 Packaging

The commercial formulation of rifampicin procured for the study will be used.

6.5 Reception, storage, dispensing and return

All TB drugs are stored at the air-conditioned DF hospital pharmacies under appropriate conditions. Quarterly supplies are sent to the study clinics, where they are kept at ambient temperature in closed cupboards.

7. SAFETY ASSESSMENT

7.1 Adverse events

Safety and tolerability of the treatments will be evaluated by recording Adverse Events (AE's) and grading laboratory and vital signs evaluations.

Definition of an Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

At each visit, the staff will ascertain the occurrence of any adverse events since the last visit. Once treatment has started and for its entire duration, clinic staff will enquire about the more common possible TB drug side effects: vomiting and anorexia, headache, tingling sensation in the extremities, insomnia, joint pain, rashes; in case streptomycin is administered also ringing in the ears, ataxia, hearing loss. Clinical examination for drug side effects will include checking the colour of the sclerae to detect jaundice (toxic hepatitis or hemolytic anemia); or joint swelling, muscle weakness etc. as

indicated by the reported symptoms. Patients will be referred to a Medical Officer or hospital in case of more severe symptoms or signs or for further investigations. In mild cases symptomatic treatment will be given.
Clinic staff will promptly record any AE on the patient file treatment follow-up sheet; for hospitalised patients this will be done by the Medical Officer on the hospitalization sheets. Liver test results will be systematically added to these documents.
In case of a SAE the clinic staff will refer the patient to the DF hospital; if jaundice is apparent or liver damage is suspected in any other way (including transaminase rise >5 ULN) all treatment will be stopped immediately and the patient referred for hospitalization. It is standard management to restart drugs one by one, and almost always there is no recurrence. In case of recurrence, these patients will not be withdrawn but one or more drugs need to be replaced. If 2 drugs are replaced, the outcome of treatment will be considered as adverse. This will be described in detail in the technical guide.

The AEs and related treatments will be recorded in the patient file. An event is defined as an SAE when it meets one of the pre-defined outcomes.

Examples of an AE include:

1. Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
2. New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
3. Signs, symptoms, or the clinical sequelae of a suspected interaction.
4. Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

Examples of an AE do NOT include a/an:

1. Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
2. Anticipated day-to-day fluctuations of pre-existing chronic disease(s) or condition(s) present or detected at the start of the study that do not worsen.
3. Hospital admission for DOT (e.g. intensive phase of MDR treatment) or rapid DST
4. Pregnancy occurring during treatment, in absence of pregnancy complications

Serious Adverse Events
A Serious Adverse Event (SAE) is any adverse event/experience occurring at any study drug dose that results in any of the following outcomes:

- Death;
- Life threatening (subject at immediate risk of death);
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant disability or incapacity;
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

NOTE (1): In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is an SAE. When in doubt as to whether "hospitalization" occurred or was necessary, the event should be considered an SAE. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE, nor hospitalization for non-medical reasons (e.g., the patient stays at the hospital overnight because (s)he lives too far and/or there is not transport).

NOTE (2): All hospitalisations for treatment of TB will not be considered an SAE, unless if due to complications arisen after giving the TB treatment.

NOTE (3): *The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.*

NOTE (4): *The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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*.

Severity, relationship of event to study drug and outcome

All SAE's will be assessed by the clinic's trained TLCA (or visiting supervisor or Medical Officer) using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity:

1. ***Mild:*** *events require minimal or no treatment and do not interfere with the subject's daily activities.*
2. ***Moderate:*** *events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.*
3. ***Severe:*** *events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.*
4. ***Life-threatening:*** *Subject at risk for death at the time of the event*

Assessment of causality

The investigator is obliged to assess the relationship between investigational product and the occurrence of each SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the IMP will be considered and investigated. The investigator will also consult the drug information and the DSMB as needed in the determination of his/her assessment.

The relationship of an adverse event to study drug is to be assessed according to the following definitions and can only be done by the study physician:

1. **Definitely unrelated**: Reserved for those events which cannot be even remotely related to study participation (e.g. injury caused by a third party).

2. **Unlikely**: There is no reasonable temporal association between the study drug and the AE and the event could have been produced by the subject's clinical state or other modes of therapy administered to the subject.

3. **Possible**: The suspected AE may or may not follow a reasonable temporal sequence from study drug administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the subject's clinical state or by other modes of therapy concomitantly administered to the subject.

4. **Likely**: The suspected adverse event follows a reasonable temporal sequence from study drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

5. **Definitely related**: Reserved for those events which have no uncertainty in their relationship to test drug administration: this means that a re-challenge was positive.

The outcome of each SAE must be assessed according to the following classification:

Completely recovered :	The patient has fully recovered with no observable residual effects
Not yet completely recovered :	Improvement in the patient's condition has occurred, but the patient still has some residual effects
Deterioration :	The patient's overall condition has worsened
Permanent damage :	The AE has resulted in a permanent impairment
Death :	The patient died due to the AE
Ongoing :	The AE has not resolved and remains the same as at onset
Unknown :	The outcome of the AE is not known because the patient did not return for follow-up (lost to follow-up)

All SAEs will be compiled by the clinic supervisor on the paper report form for the corresponding visit, and completed by the Medical Officer in charge of the hospitalization as appropriate. The Investigator will systematically check and validate the paper records on SAE and other points during his supervision visits to clinics and hospitals, and correct / complete as required before entering the data in the electronic CRF. SAE will be registered as soon as treatment has started and up to its end.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to the pharmacovigilance. However, it is very important that the investigator always makes an assessment of causality for every event prior to transmission of the report to the pharmacovigilance. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE-report form accordingly.

All SAE's whether or not deemed drug related or expected must be reported immediately or within 24 hours (one working day) using the SAE Notification Form by telefax or email to:

Clinical Trials Unit
Institute of Tropical Medicine
Nationalestraat 155
B-2000 Antwerp – Belgium
Fax: +32 3 247 66 47
Email: pharmacovigilance@itg.be

The title of the e-mail/fax should mention the name of the study code, the patient code and the wording "SAE – Initial/follow up report".

Line listings of all reported SAE's will be sent at least quarterly to the DSMB, and on a yearly basis to the IRB of the Union, ITM and the EC of UZA. They will also be sent yearly to the study coordinator in Bangladesh to submit these to the respective Authorities and/or ECs.

The Principal Investigator is responsible to report the SAE to the relevant Institutional Ethics Committees and the Bangladesh Regulatory Authorities immediately or according to the deadline established by each Ethics Committee.

7.2 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) with at least 3 independent members will be established for the purpose of providing independent advice on the quality of the data produced and the safety of the treatment tested, so contributing to safeguarding the interests of the trial subjects. Based on the review of the (safety) data, the DSMB can advise the sponsor to modify, halt, discontinue, or continue the trial.
Two types of interim analyses are performed during the trial. The DSMB will meet quarterly after the start of enrolment of the first patients and assess toxicity data to safeguard the study participants safety. The formal interim analysis of the toxicity and efficacy to aid the design of a follow-up study will only be performed when treatment has completed for all patients as full information is needed for the complete treatment period.

A DSMB charter, where the relevant terms of reference including any stopping rules if deemed appropriate are clearly defined, as well as the composition and independency of the Board, will be approved and signed for approval by each member of the DSMB.

8. STATISTICAL METHODS

Statistical analysis of the study will be performed by the biostatistician at the CTU according to a statistical analysis plan (SAP) prepared before the initial data analysis. Analyses will be performed at the following time-points (in addition to safety analyses performed by the independent DSMB):
1) Assessment of referral for rapid DST plus its outcome based on the auramine and/or FDA decline for the patients randomized in the intervention arm versus persisting fever, for the patients randomized in the control arm, 2 weeks after the first 500 patients (intervention + controls) are enrolled. With this analysis we want to assess whether any of those can really be used as an early indicator of serious rifampicin resistance, and also assess the impact of improvement of staining techniques that were made after the first ITM monitoring visits. The results in terms of resistance missed / correctly suspected will be analysed based on the initial genetic rifampicin resistance data which will by that time also be available for part of these patients.
In addition we would like to add the stratification as additional variable. Firstly, we will compare the recruitment at start (end of 2014) against the recruitment in 2015 to assess whether improvement has been made. Secondly we will compare the recruitment of new versus retreatment cases.
2) Assessment of drug toxicity and initial treatment response will be performed at the time the last recruited patient finishes treatment. The data will be compiled at 9 months after the end of recruitment to ensure all treatment related data, including 8 months of treatment for retreatment cases in the control arm, are available. This analysis will be primarily performed for the go/no-go decision and design considerations for the cluster-randomized trial. The decision on proceeding to the cluster randomized study will be based on the absence of excess toxicity, a trend toward a reduction of unfavourable outcomes (excluding relapse), possible favourable effects on low-resistance mutations.

3) A final analysis of the study endpoints will be performed one year after end of treatment of the last enrolled patients, i.e. about 2.5 years after enrolment start.

8.1 Study hypotheses

This study is a pilot study to estimate the differences in the incidences in adverse treatment outcomes and excess toxicity. Consequently, the focus will be in describing event rates in each study arm and estimating the differences in event rates using odds-ratio's together with 95% confidence intervals (CIs) and not on formal hypothesis testing. P-values for comparisons of event rates (adverse treatment outcomes and safety endpoints) between intervention and control arms will be provided, but will need to be interpreted with care.

8.2 Variables of interest

All variables of interests are event rates as defined in Section 3.1. In each analysis, if a patient has two events for the analysis of interest (e.g. relapse followed by death), the first endpoint will be taken into account.

8.3 Statistical methods

8.3.1 Analysis populations

We will analyse the efficacy data both using Intention-to-Treat and Per-Protocol approaches, with Intention-to-Treat as primary approach. In the Intention-to-Treat analysis, all patients will be analysed according to their randomized allocation, even in case they receive another treatment regimen, show protocol violations prior to or during the study, or are lost-to follow. Patients lost-to-follow-up before the 12 month post treatment follow-up or who do not provide a sputum smear are considered to be failures in this analysis, unless they are known to have moved out of the study region. In the per-protocol analysis only patients who receive study drug as planned, have complete follow-up (till 2.5 years or development of a study endpoint) or have reached a study endpoint at an earlier time-point, and follow the protocol as planned are included.
Safety analysis will be performed using an all-patients-treated approach, including all patients who received at least a single dose, and according to the treatment regimen actually received.

8.3.2 Baseline characteristics

Patients in each treatment group, and in each hospital, will be described with respect to baseline characteristics. The clinical importance of any imbalance will be noted though statistical tests of significance will not be undertaken.

8.3.3 Primary analysis

Counts and proportions (end 95% CI) of patients with any adverse treatment outcome, and by adverse treatment outcome category, will be presented. The confidence intervals for incidences will be estimated using Wilson's score method. Odds ratio's (ORs) and 95% CIs for the OR will be estimated from a logistic regression model with effects for arm (intervention vs control), hospital, and stratum (new/re-treatment case). The p-value for the test of no difference among arms will be presented. If the number of events is low a logistic regression model with an effect for hospital may be used instead of the full model proposed.
Similar analysis will be performed for the primary safety endpoint.

8.3.4 Secondary and tertiary analysis

Secondary Objectives

1. *To assess whether the study regimen also cures low-level rifampicin resistant TB*
   Adverse treatment outcomes will be described and compared among treatment groups in subgroups defined by initial rifampicin resistance mutations (performed in all patients) detected:
   - none

- low-level
- high

2. *To assess the effectiveness of standard auramine versus FDA vital staining screening at two weeks of treatment for early switch of non-responding rifampicin resistant TB to MDR-TB treatment*

   The proportion of initial rifampicin resistant case detected by FDA (i.e. the sensitivity of FDA) will be estimated together with 95% CI. The same analysis will be performed for auramine or ZN AFB smears and compared with FDA. Also the number of referrals needed with both techniques will be compared.

3. *To assess the negative predictive value of conversion at 2 weeks for relapse*

   The Negative Predictive value (and 95% CI) of conversion in the intervention arm will be estimated as the % of relapses among those with a minimum 1 log decline in the number of AFB, or who are already negative or only scanty positive on AFB smear (auramine or FDA) at week 2.

4. *To estimate the proportion of acquired rifampicin resistance among failures and relapses*

   Proportions will be estimated together with 95% confidence intervals, using the number of failure / relapse cases without mutation detected at diagnosis as the denominator and comparing intervention and control arms.

8.3.5 Subgroup analyses

In addition to the main results, pooling new and retreatment cases, all data will be described for new and retreatment TB cases separately.

8.3.6 Multiplicity and Missing Data

As this is a pilot estimation study with a single primary efficacy and safety endpoint, no multiplicity adjustments are needed. An interim analysis will be performed on the end-of treatment data, but this analysis will be done after recruitment is completed and will not influence for continuation of the study. The DSMB will only review safety data and will not stop the study for efficacy advantages.
The handling of missing data will be specified in the SAP. In the intention-to-treat analysis, patients who are lost-to-follow-up will be counted as treatment failures, unless they are known to have moved outside of the area.

8.4 Sample size and power

The sample size of two times 500 patients is a trade-off between precision and exposing an excessively high number of patients to potentially more toxic treatment. Power has not been taken into account, but if the results are favourable large scale cluster-randomized study will be designed to bring definitive proof.
The registered incidence of clinically apparent hepatitis from any cause during TB treatment is 1.5% for the study population. With the proposed sample size, an increase by at least 2% compared to the control arm will attain statistical significance (p=0.047).
With the standard (=control) regimens and definitions, about 2% failures and 2% relapses are currently notified by the DF project, but only 50% of those are culture-positive, and about half of the culture positive failures and relapses are rifampicin-resistant. Overall cure rate (new and retreatment cases together) is close to 90%, with 4% default and 4% deaths. Based on the data from 2010 about 20 cases with rifampicin resistance may be enrolled, 10 in each arm. We estimate that the more effective study regimen plus early detection of serious drug resistance rolled over to MDR-TB

treatment will prevent drug resistant failure and relapse, reduce rifampicin susceptible relapse to an estimated two third, and reduce death and default because of unrecognized rifampicin resistance to 80% of the control arm. This total estimated reduction of unfavourable outcome from 10% to 7% remains non-significant with the targeted enrolment of two times 500 cases. With 80% power and 95% confidence level, 2 x 1421 patients would have to be enrolled, which is not feasible and also difficult to justify. However, a slightly larger reduction of unfavourable outcome from 10% to 6.5% comparing two arms of 500 cases each would already result in a significant p-value. Additional evidence will be obtained studying *rpoB* mutations, initially (all smear-positives) and at time of failure or relapse (only in the few such cases expected). Absence of low-level resistance mutations only among patients with unfavourable outcome on the high-dose arm, while they were present among new cases in both arms, will be interpreted as additional evidence of effectiveness of the increased dosing.

9. MONITORING AND QUALITY ASSURANCE

This study will be monitored in accordance with regulations applicable to clinical trials, including ICH-GCP and WHO-GCLP, and sponsor-specific SOPs. The PI and involved site research staff will allocate adequate time and resources for such monitoring activities. The investigator will also ensure that the monitor or QA reviewer is given access to all the above noted study-related documents and study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) and has adequate space and resources to conduct monitoring and source data verification.

The task of the Monitor, who is appointed by the sponsor, is to verify the conduct of the study through frequent contacts by phone and in person with the Principal Investigator and site staff, in accordance with applicable regulations, Good Clinical Practice requirements and study-specific Standard Operating Procedures. The objectives and specific tasks of the Monitor are described in the ICH Guidelines E6. The monitoring visits will enable the Monitor to maintain current, personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct of the study with the Investigator.

The monitoring and Source Data Verification will be detailed in a specific monitoring plan. Each site will be visited by the clinical monitor before the study is started (“pre-study visit”), to assess the site suitability for the study. In addition, it will be visited at the beginning of the study (“site initiation visit”) and at least 3 times during the conduct of the trial, plus a “close-out visit” (or “end of trial visit”) after the last patient has completed the follow up and the database has been locked.

The remote support (by e-mail, Skype) to the Investigator in-between site visits will be an important part of the monitor’s work, as well as the coordination of the TMG meetings.

After completion of the trial, a final study report will be prepared, signed and dated by the coordinating investigators. The report will be submitted to the relevant EC bodies, the DSMB and regulatory authorities before any publication.

The Principal Investigator must ensure that source documents are maintained for each patient in the study, consisting in case and visit notes (hospital or clinic medical records) containing the Investigator’s copy of the signed informed consent, demographic and medical information, laboratory data, and the results of any other tests or assessments, according to applicable GCP requirements. All information in the CRF must be traceable to these source documents in the patient’s file. The investigator must give the monitor, who is bound by a confidentiality agreement to protect patients’ confidentiality, access to all relevant source documents to confirm their consistency with the CRF entries. We refer to the relevant SOP for further details. Further adaptations to the specific context can be made after the pre-study visit.

The Principal Investigators - by signing this protocol - declare that he/she will permit trial-related monitoring, external audits, Independent Ethic Committee review, and regulatory inspections, providing direct access to source data/documents as well as any other trial related documentation.

The Principal Investigators agree to conduct the present study in full agreement with the principles of the "Declaration of Helsinki" as amended in Fortaleza, Brazil in 2013 (see Appendix 3) and any subsequent versions.

10. DATA MANAGEMENT

The DF labs and clinics participating in the study will maintain the routine NTP and DF treatment records and register, and will follow routine NTP and DF reporting formats and frequency. These records already include a unique TB treatment identifier for each patient and patient treatment episode, which will also be used for the study-specific records and reports. The patient's name will not appear on any document communicated to researchers outside the DF project. A standard CRF will be created and given to sites to complete study relevant data.

Choice of laptops, clinical trial specific software and enforcing internet capacity falls under the responsibility of the CTU Data Manager and Coordinating Investigator to ensure a validated and GCP compliant data flow (with audit trail, data cleaning system and source document verification). In case internet capacity and data management on the field allows it, the MACRO (Infermed©) is the first choice software, a CFR21Part11 in built query system, audit trail, electronic signature and thus these data can be considered to be trustworthy, reliable and equivalent to paper records. This system will be validated. Alternatively validation will be done on paper CRF.

The CRF will be sent to the ITM on regular basis. This will be described in detail in the data management plan by the CTU Data Manager. The electronic data will be protected with password control.

The Data Management SOP of the CTU will be used to follow the general Data Management Process. The Data Management Plan will be made by CTU Data Manager and contains all details on the study specific data flow, timelines and roles and responsibilities which will vary if the first choice of an electronic CRF (MACRO) can be used, and whether or not offline or online.

The participants will be identified by a study specific participant number and/or code in any database. The name and any other identifying detail will not be included in any study data electronic file.

The data collection and data cleaning process will refer to the source document used in routine. An audit trail will track any changes made to the study data (CRF), queries raised during the data review process will be stored in the patient file. All details on data collection, -entry, -review, -transfer and achieving will be documented in detail in the data management plan before the start of the study.

11. ETHICAL ISSUES

11.1 Ethical and regulatory review

This study will be submitted for formal review and approval to the Institutional Review Board of the ITM, the EC of the University Hospital in Antwerp, the Ethical Advisory Group (EAG) of the International Union against Tuberculosis and Lung Disease, and the National Research Ethics Committee of the Bangladesh Medical Research Council. No participants will be enrolled or subject related activities performed before written approval from these bodies is obtained.
The study will be carried out according to the principles stated in the Declaration of Helsinki, all applicable regulations and according to the most recent GCP guidelines.

11.2 Protocol amendments

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the appropriate steps before being implemented. Any substantial change must be approved by all the bodies and EC's that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns (in which case the immediate implementation can be necessary for the sake of subject's protection). In case modifications to the protocol or amendment are requested by any local EC/CA during the review process, these must be discussed and agreed upon with the Sponsor prior to any resubmission incorporating those changes.

11.3 Informed consent

No subject may be admitted into the study until the Investigator or designee has obtained the written informed consent form.

All patients will be asked to give their informed consent to participate in the study, before undergoing any study-specific procedures. The Informed Consent Form (ICF) as well as the informed consent interview will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, the voluntary nature etc., as described in the E6 ICH GCP Guidelines. The informed consent procedure is further described in paragraph 5.2.

Additional to the study-consent, specific consent for the long-term storage of sputum samples is also requested from the potential participants.

11.4 Confidentiality

All patients' data will be coded in the CRF and database by means of a unique subject assigned patient study number. The documents which can identify the patients, e.g. the ICF's, laboratory print-outs and medical record, will only be accessible to the relevant study staff and to study monitors, auditors and inspectors only under confidentiality agreements.
Patient integrity and safety are safeguarded by on-site monitoring and by an independent data safety monitoring board, which can steer the study as it deems necessary or strongly suggest the sponsor to prematurely terminate the trial if necessary.

11.5 Risks and benefits

We believe that all patients who receive the intervention will benefit: by reduced risk of relapse for those with more extensive disease; and/or by reduced adverse outcome due to initial or acquired resistance to rifampicin, lessening the need for a more toxic 2nd line regimen. Also patients switched to MDR-treatment will fare better, with less risk of hearing loss, due to removal of Cat. 2 regimen with reduced cumulative injectable exposure, as well as reduced failure/relapse due to pre-existing pyrazinamide, ethambutol and higher level isoniazid resistance.

There is no reason why the study would hold an increased risk for the DF staff. To the contrary, faster conversion of all smear-positives and earlier detection and treatment of MDR cases will reduce their occupational risk of contracting TB, whether drug susceptible or resistant.

11.6 Assurance of clinical care

Patients who decline participation will be treated and followed according to standard NTP guidelines.

Clinical staff of the participating DF TB centers will receive refresher training regarding early symptoms and signs of hepatitis; since these clinics are located within Government peripheral hospitals with extensive staffing, including nine medical doctors, wards and an emergency department, urgent referrals will always be possible. However, Damien Foundation Bangladesh will assure proper care of patients experiencing adverse drug events, in its own hospitals and with its

own Medical Officers, or if needed by referral to the Medical Colleges in its project area. Such referrals will be paid by the project.
The DF project has a long tradition of accurate recording and reporting, with strict implementation of guidelines, and its past mass treatment studies have been successfully completed and published. For this study the project will be prepared and monitored for compliance with Good Clinical Practice and Good Clinical Laboratory Practice. The ITM Clinical Trial Unit will also set up a dedicated database with audit trail, and assure proper record keeping. Patient confidentiality will be guaranteed by the use of anonymous unique patient identifiers on study specific clinical record forms, reports and laboratory database listings that will be used by the researchers. The TB Supra-National Reference Laboratory at ITM, Antwerp, has closely collaborated with the DF project for 20 years already. It will assure proper training and supervision of the local bacteriology, and perform the more advanced tests such as DNA sequencing, MICs and spoligotyping.

11.7 Study Insurance

The Sponsor will obtain a study-specific no-fault insurance to cover harm/damage patients might incur as a result of participation in the study. A copy of the insurance certificate or contract will also be submitted to the applicable EC's for formal review and approval.

12 DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY

The protocol, CRF, SOPs and other related study documents are developed together by the study partners, approved by the Sponsor and provided to the Investigators and his/her appointed staff in confidence. None of this material may be disclosed to any party not directly involved with the study, without written permission from the Sponsor.

Communication and publication of the study results will be carried out jointly by the Sponsor and the study partners, with a key-role played by the Coordinating Investigator(s), the CTU biostatistician and the DF Principal Investigator, with involvement also of DF Brussels. A more detailed publication plan will be agreed before completion of the study.

13. ARCHIVING

The sponsor and Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be verified. The relevant essential documents are those documents which individually and collectively permit to assess the conduct of the trial, the quality of the data produced and the compliance with GCP standards and applicable regulatory requirements. The Investigator's File (IF) should at least contain all the (essential) documents as listed in the ITM's procedure "Set up and maintenance of the Investigator File". A copy of all source data and Case Report Forms must remain on site at all times.

After completion of the study, the IF and any study source data or databases not contained in the IF will remain available at the Damien Foundation Dhaka central office for at least 20 years for internal audits and/or inspections of regulatory authorities, unless differently requested by national authorities.
The Sponsor should be informed prior to destruction of the files.
Sputum samples preserved in alcohol will be stored at ITM Mycobacteriology Laboratory and may be used for future studies, as stated in the ICF.

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15. LIST OF ABBREVIATIONS

AFB	acid-fast bacilli
AUC	area under the curve
C	clofazimine
C.I.	confidence interval
C max	peak drug serum concentration
CA	competent authorities
Cat. 1	first-line standard regimen for new cases
Cat. 2	first-line standard regimen for previously treated cases
(e-)CRF	(electronic) case record form
CTU	clinical trial unit
DF	Damien Foundation
DNA	desoxyribonucleic acid
DSMB	data safety and monitoring board
DST	drug susceptibility testing
E	ethambutol
EC	ethics committee
FDA	fluorescein diacetate
G	gatifloxacin
GCP	good clinical practice
GCLP	good clinical laboratory practice
H	isoniazid
HIV	human immune-deficiency virus
(I)CF	(informed) consent form
IF	Investigator file
IRB	institutional review board
ITM	Institute of Tropical Medicine, Antwerp
FM	fluorescence microscopy
K	Kanamycin
LED	light-emitting diode
LJ	Löwenstein-Jensen medium
MDR-TB	multidrug-resistant TB
MGIT	Mycobacterium growth indicator tube
MIC	minimum inhibitory concentration
NTP	national TB control programme
PCR	polymerase chain reaction
PI	principal investigator
PNB	para-nitro benzoic acid
Pto	Prothionamide
QA	quality assurance
QC	quality control
R	Rifampicin
S	Streptomycin
(S)AE	(serious) adverse event
SOP	standard operating procedure
TB	Tuberculosis
UZA	University Hospital Antwerp
WHO	World Health Organization

XDR-TB	extensively drug resistant TB
Z	Pyrazinamide
ZN	Ziehl-Neelsen

16. ANNEXES

Annex I Statements regarding lack of excessive toxicity high-dose rifampicin

1. PanACEA Consortium Study

Primary source: Conference on Retroviruses and Opportunistic Infections
Source reference:
Boeree M, et al "Paper #148LB: What Is the "right" dose of rifampin?" 20th conference on retroviruses and opportunistic infections" *CROI* 2013: Abstract 128.

Paper #148LB

What Is the "Right" Dose of Rifampin?

Martin Boeree*[1], A Diacon[2], R Dawson[3], A Venter[2], J du Bois[2], K Narunsky[3], M Hoelscher[4], S Gillespie[5], P Phillips[6], R Aarnoutse[1], and PanACEA Consortium
[1]Radboud Univ Nijmegen Med Ctr, The Netherlands; [2]Univ of Stellenbosch, Cape Town, South Africa; [3]Univ of Cape Town, South Africa; [4]Med Ctr of the Univ of Munich, Germany; [5]Univ of St Andrews, UK; and [6]MRC Clin Trial Unit, London, UK

Background: In 1971 the dose of 10 mg/kg rifampin (RIF) was arbitrarily chosen without a maximum tolerated dose (MTD) study. Current murine and human data show that an increase in dose of rifampin may significantly shorten treatment duration. The primary aim of this study was to assess the MTD of RIF.

Methods: We performed a 14-day study in adult smear-positive TB patients. Consecutive groups of 8 and 15 patients received 10 and 20, 25, 30, and 35 mg/kg RIF (days 1-7), complemented with standard doses of isoniazid, pyrazinamide, and ethambutol (8-14). RIF plasma levels were sampled at days 7 and 14. Colony forming units (CFU) of *Mycobacterium tuberculosis* on solid medium and time to culture positivity (TTP) in liquid medium were assessed at baseline and days 1 to 7, 12, and 14. After each dose increase, adverse events were reviewed by a committee and dose increments were approved.

Results: In the 5 dose groups (N = 68) we recorded 163 adverse events, 110 were possibly related (102) or related (8) to RIF: 88 grade 1, 19 grade 2, and 3 grade 3. All 8 events related to RIF were grade 1.Grade 3 events included a case of transient hyperkalaemia (20 mg/kg) and 2 cases of elevated liver enzymes (30 and 35 mg/kg; onset days 21 and 17). The fall of log CFU over 14 days was dose-related and bi-phasic with a slightly steeper fall over the first than the second week in some groups (0.18, 0.15, 0.16, 0.25 log CFU/mL/day in the 10, 20, 25, and 30 mg/kg groups). The highest fall was seen with 35 mg/kg: 0.25 (95% confidence interval [CI] 0.31 - 0.18). The increase in log TTP was similarly dose-related (0.02, 0.03, 0.03, 0.04 log TTP/day in the 10, 20, 25, and 30 mg/kg groups). The maximum increase over 14 days was seen with 35 mg/kg: 0.04 (95%CI 0.04 - 0.05). Mean RIF AUC_{0-24} at day 7 was 26.3, 112.6, 134.5, and 189.4 h*mg/L in the 10, 20, 25, and 30 mg/kg arms, showing an initial non-linear increase followed by a more proportional increase in exposure. Mean C_{max} values were 7.4, 21.6, 25.1, and 33.1 mg/L in the 10, 20, 25, and 30 mg/kg groups.
Conclusions: RIF up to 35 mg/kg was safe and well tolerated with the highest activity seen with 35 mg/kg indicating that the current accepted treatment dose of RIF may be too low. RIF exposure increases with dose without an apparent ceiling effect. Based on the result of this trial we will evaluate 35 mg RIF/kg administered for 12 weeks in a multiple arm, multiple stage design within the PanACEA consortium.

2. RIFATOX Study

The results were first reported at a late breaker session at the 2013 World Conference of the IUATLD in Paris. The abstract of this communication is inserted below.

A multicentre randomised controlled clinical trial to evaluate the toxicity of high dose rifampicin in the treatment of pulmonary tuberculosis (RIFATOX)

Authors: A Jindani1, B Shrestha2, I Westermann de Patiño3, R Alvarez de Fernandes3, T Gonzales3, D Atwine4, M Bonnet5, N Patel1,
G Borgulya1 and D Mitchison1. 1Infection and Immunity Research Centre, Division of Clinical Sciences, St George's, University of London. 2German
Nepal Tuberculosis Project, Kathmandu, Nepal. 3Centers Broncopulmonares, Cruz Roja, Santa Cruz, Bolivia. 4 Epicentre, c/o MSFCH Geneve, Switzerland,
5MSF-Epicentre, Mbarara, Uganda

Background
In vitro and animal studies suggest that higher doses of rifampicin result in increased bactericidal activity against M.tuberculosis. The aim of this trial is to assess whether an increase in the daily dose of rifampicin from 10 mg/Kg to 15 and 20 mg/Kg, for the first four months of the standard six month regimen, will result in an increase in severe (grade 3 or 4) adverse events.
Methods
HIV negative patients with newly diagnosed, microscopy positive pulmonary tuberculosis in Kathmandu, Nepal, Santa Cruz, Bolivia and Mbarara, Uganda, were invited to participate provided they fulfilled all the inclusion criteria. All received the WHO recommended 6 month regimen. They were randomly allocated to either the standard treatment (Control Regimen, CR) or a higher dose of rifampicin, of either 15mg/kg (Regimen 1, R1), or 20mg/kg (Regimen 2, R2) for the first 4 months. Liver function tests were carried out 2, 4, 8, 12 and 16 weeks. Sputum was cultured for M.tuberculosis pretreatment and after 8 weeks of treatment.
Results
A total of 300 patients were enrolled. Results from 250, who have completed 4 months of treatment, are presented with 84 in the CR, 84 in R1 and 82 in R2. The baseline gender distribution is 68% male and median age 29 years. There were 34 reported adverse events of which only 1 was hepatic (R1) and was classified as severe. There were 38 late screening failures and withdrawals. Of 220 patients for whom pretreatment and 8 week culture results are available, culture negativity rates at 8 weeks are 80.5% (CR), 85% (R1) and 85.5% (R2) respectively.
Conclusion
Rifampicin at 15mg/kg and 20mg/kg did not result in any increase in adverse events. A linear regression shows a trend for higher ALT levels with increasing doses of rifampicin suggesting caution with further dose increases.

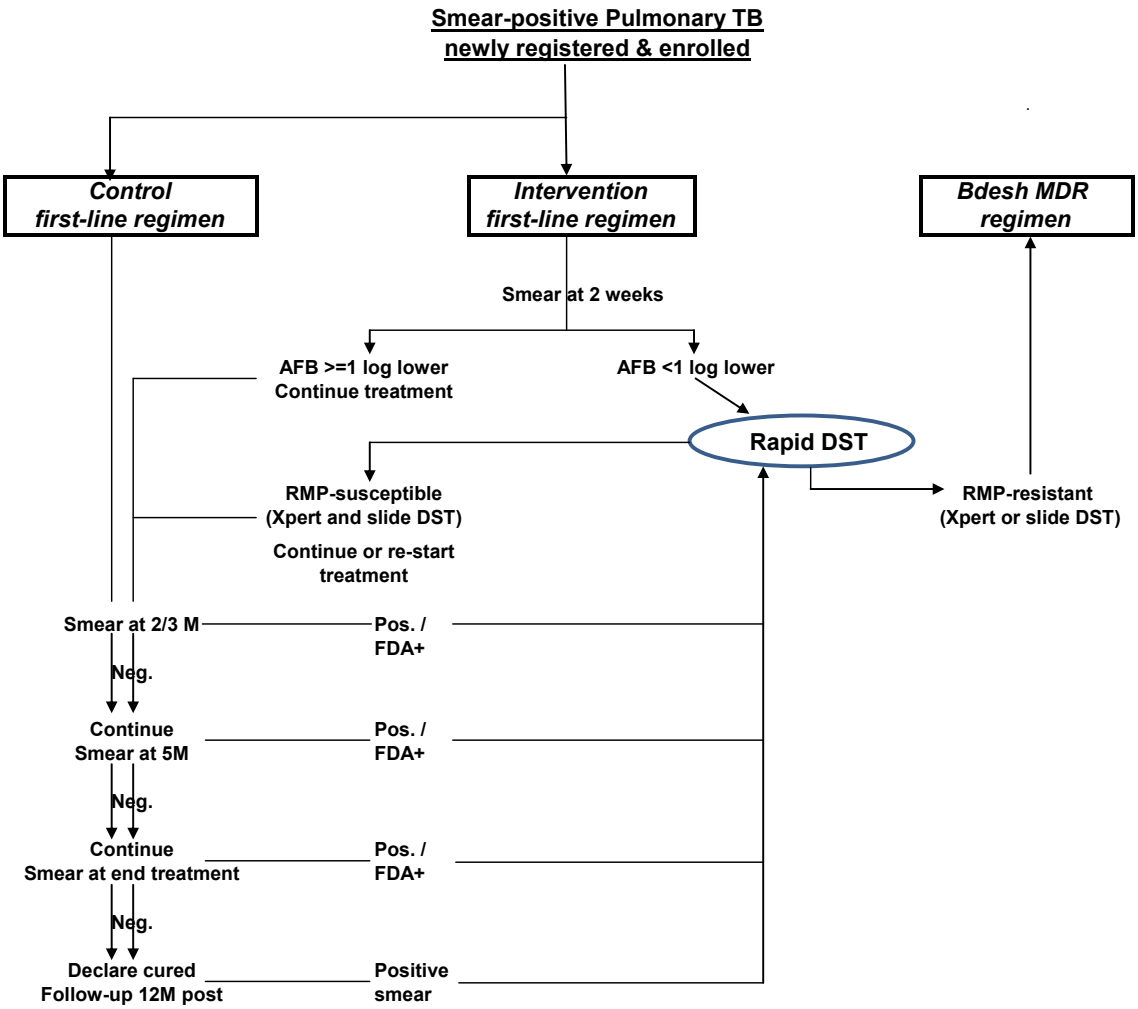
3. RAFA Study

There are no preliminary reports on RAFA study as yet. However, I was assured by researchers implicated that no excess toxicity over the controls has been documented: email Dr. Corinne Merle, London School, here below

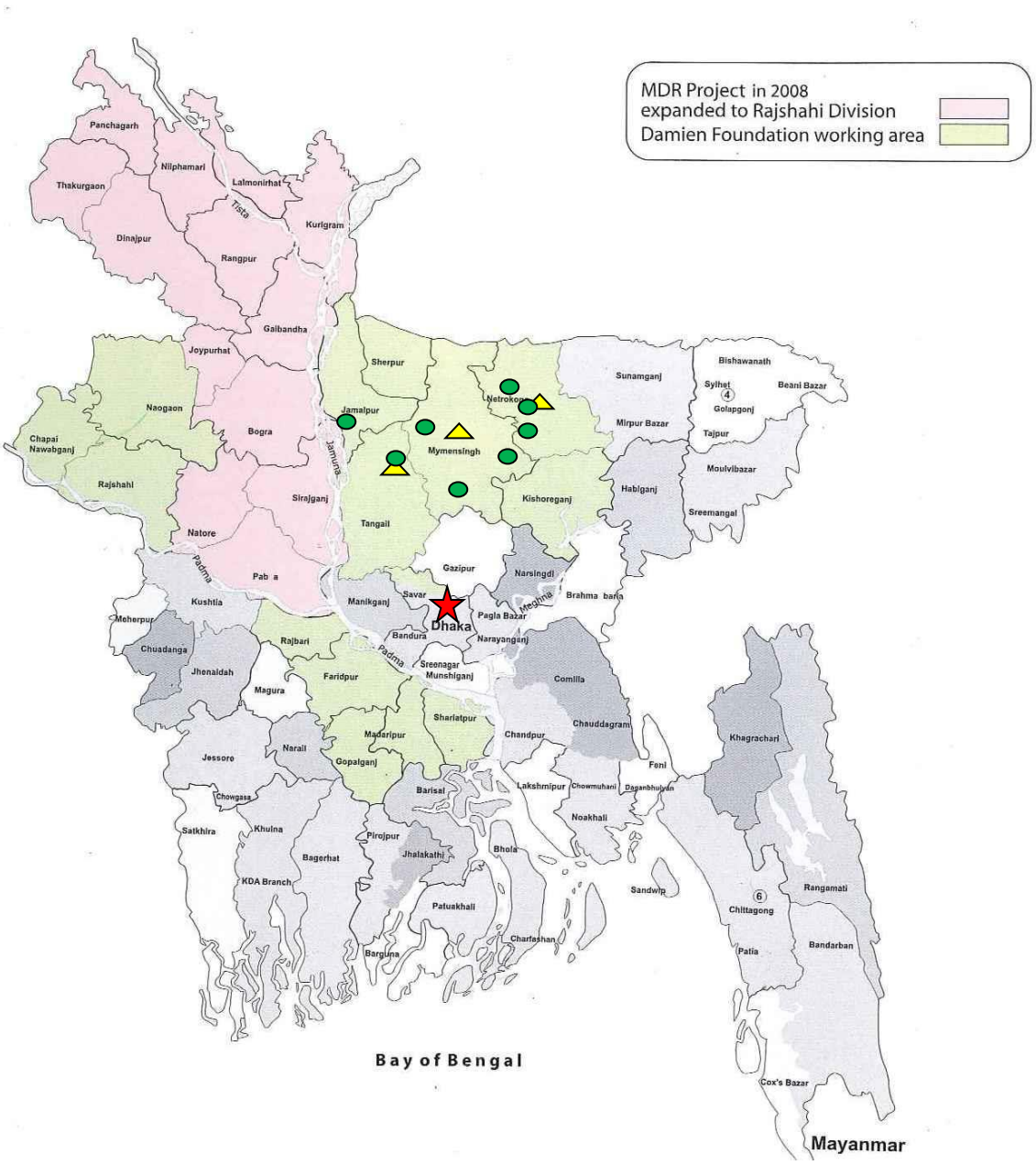
From: Corinne Merle [mailto:Corinne.Merle@lshtm.ac.uk]
Sent: donderdag 29 augustus 2013 20:33
To: Armand Van Deun
Subject: Re: high RIF

Dear Armand,
Yes I confirm that we don't have any hepatic issue so far in the RAFA project (i.e. we have some ALAT grade 1 increase but it is balanced when compared to the standard treatment). I am not in London currently but will be back the 10th of September and can give you a more detailed figure (i.e. exact number of patients in the high dose arm and % of ALAT increase events with corresponding grading) if it can be useful.
In this study the population of patients are TB/HIV patients ARV naive with CD4 count>50 CD4. The dose we are using in the high dose rifampicin arm is 15mg/Kg for the 2 months initiation phase only (because ARV are initiated at week 8 and it would get too complex for the dosage of Efavirenz.).
I hope it can help,
Kind regards
Corinne

Annex II **Algorithm for treatment monitoring, switch to the MDR-regimen and follow-up, for first-line control and intervention regimens**



Annex III **Map of Bangladesh with the DF project areas and study sites**



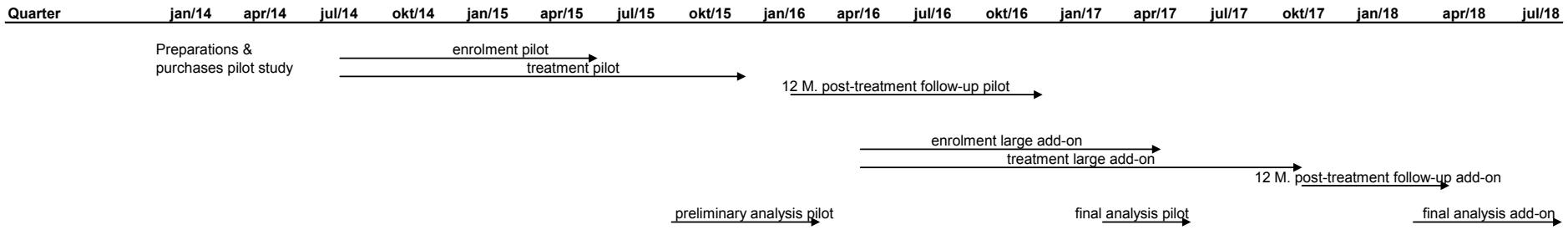
★ DF Dhaka Head Office

△ DF Hospital and reference lab

● Study clinics

Annex IV Study timeline

TB cascade study timeline: both the pilot and the larger add-on study are shown, although this proposal only concerns the pilot



Quarter	jan/14	apr/14	jul/14	okt/14	jan/15	apr/15	jul/15	okt/15	jan/16	apr/16	jul/16	okt/16	jan/17	apr/17	jul/17	okt/17	jan/18	apr/18	jul/18
Insurance	Purchase																		
Rifampicin	Purchase																		
Database			Install	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	enter	
Training GCP/GCLP			do						do		do								
CTU visits			do		do		do			do		do		do		do			
Sequencing at ITM			do	do	do	do	do	do	do	do	do	do	do	do	do	do	do		
Lab visits		do			do				do				do				do		

Annex V **Helsinki Declaration**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee.

After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents

giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for

ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

Annex VI **Summary of amendments to OneRIF Protocol version 3.0**

- Throughout the protocol the NCT code is added and the protocol version number and date were adapted.
- On pages 2, 3 and 5 Dr. Bouke de Jong is added as back-up of the ITM study coordinating investigator Dr. Armand Van Deun.
- On pages 2, 3 and 5 Dr. Tine Demeulenaere is added as back-up of the DF study coordinating investigator Dr. Etienne Declercq.
- On page 3 the list of CTU members involved in the study is updated.
- On page 8 the instructions on how to write the synopsis are deleted.
- On pages 16 and 17, section 3.1 primary endpoints, the outcome definitions are updated
- On page 17 the section 3.2 secondary endpoints is updated.
- At the top of page 23 a sentence is deleted.
- On pages 25 and26 the sections of microscopy and DST and species identification are updated.
- On page 31 an interim statistical analysis is added.
- Reference 18 is updated on page 39.