



Improving TB management and control through innovative shorter anti-tuberculosis regimens

Daniel Atwine

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**THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITÉ DE MONTPELLIER**

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**IMPROVING TB MANAGEMENT AND
CONTROL THROUGH INNOVATIVE SHORTER
ANTI-TUBERCULOSIS REGIMENS**

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Le 24 novembre 2017

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**UNIVERSITÉ
DE MONTPELLIER**



DEDICATION

*TO ALL PATIENTS BATTLING TUBERCULOSIS,
WHO FOR THE HOPE OF A MUCH SHORTER
AND SAFER TREATMENT, DESPITE BEING TO
THE BENEFIT OF FUTURE PATIENTS,
SELFLESSLY OFFERED TO PARTICIPATE IN
THIS RESEARCH JOURNEY.*

ACRONYMS

3TC	Lamivudine
ABC	Abacavir
ADH	Antidiuretic Hormone
AE	Adverse Event
AFB	Acid-Fast Bacilli
AIDS	Acquired Immuno-Deficiency Syndrome
ALT	Alanine Aminotransferase
ANRS	French National Agency For Research On AIDS And Viral Hepatitis
ART	Antiretroviral Therapy
ARV	Antiretroviral
AST	Aspartate Aminotransferase
ATT	Anti-Tuberculosis Treatment
AUC	Area Under Concentration Versus Time Curve
c	Cobicistat
C ₁₂	Mid-Dose Concentration
CI	Confidence Interval
C _{max}	Maximum Concentration
C _{min} /C ₂₄	Minimum Concentration/24-Hour Concentration
CNS	Central Nervous System
CR	Control Regimen
CXR	Chest X-Ray
CYP	Cytochrome P450
DAIDS	Division Of AIDS
DILI	Drug Induced Liver Injury
DNA	Deoxyribonucleic Acid
DOT	Directly Observed Treatment
DOTS	Directly Observed Treatment Short Course Strategy
DR-TB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Test
DS-TB	Drug Susceptible Tuberculosis
DTG	Dolutegravir
DTH	Delayed-Type Hypersensitivity
DTM	Domiciliary Treatment Monitor
E	Ethambutol
EBA	Early Bactericidal Activity
EFV	Efavirenz
EHRZ	Ethambutol, Isoniazid, Rifampicin And Pyrazinamide
EMA	European Medicines Agency
EMRC	Epicentre Mbarara Research Centre
FDA	Food And Drug Administration
FDC	Fixed Dose Combination
FI	Fusion Inhibitor
FTC	Emtricitabine
GCP	Good Clinical Practice
GM	Geometric Mean

GMR	Geometric Mean Ratio
H	Isoniazid
HBC	High-Burden Country
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IC	Infection Control
ICF	Intensified Case Finding
INSTI	Integrase Strand Transfer Inhibitors
IPT	Isoniazid Preventive Treatment
IQR	Interquartile Range
IRIS	Immune Reconstitution Inflammatory Syndrome
IU/L	International Units Per Litre
LAM	Lipoarabinomannan
LJ	Löwenstein Jensen
LMICs	Low And Middle Income Countries
LPV/r	Ritonavir Boosted Lopinavir
LTBI	Latent Tuberculosis Infection
MAH	Mono-Acetyl Hydrazine
MDR-TB	Multi-Drug Resistant Tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
mITT	Modified Intention To Treat Population
mm	Millimeter
MRC	Medical Research Council
MSF	Médecins sans frontières
MTB	Mycobacterium Tuberculosis Complex
NA	Not Applicable
NALC-NaOH	N-Acetyl-L-Cysteine-Sodium Hydroxide
NAT2	N-Acetyltransferase Type 2
NDA	National Drug Authority
ng/ml	Nanogram Per Milliliter
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
PK	Pharmacokinetics
POC	Point-Of-Care
PPD	Purified Protein Derivative
PTB	Pulmonary Tuberculosis
R	Rifampicin
r	Ritonavir
RCT	Randomized Clinical Trials
RCT	Randomized Clinical Trial
RH	Rifampicin And Isoniazid
RNA	Ribonucleic Acid
RR	Risk Ratio
RR-TB	Rifampicin Resistant Tuberculosis

SAE	Serious Adverse Event
SGOT	Serum Glutamic Oxaloacetic Transaminase
SR	Study Regimen
TB	Tuberculosis
TDF	Tenofovir Disoproxil Fumarate
T _{max}	Time To Reach Maximum Concentration
TTD	Time-To-Detection
ULN	Upper Limit of Normal
UNAIDS	United Nations Programme On HIV/AIDS
UNCST	Uganda National Council of Science and Technology
USPHS	United States Public Health Service
VL	Viral Load
VOT	Video Observed Treatment
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis
Z	Pyrazinamide
ZDV	Zidovudine

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CHAPTER 1

INTRODUCTION

Tuberculosis (TB) is the most important infectious disease of all time, a global plague, with far reaching devastating effects to its victims, affecting the marginalized persons of lower socioeconomic status and with roots in both the history and present of humanity [1, 2]. The disease is not selective across age groups. In 1993, TB was declared a health emergency by the World Health Organization (WHO). It is one of the world's major infectious diseases with 2-3 billion people infected, 10.4 million new (incident) TB cases worldwide (56% and 34% in males and females respectively). It is most prevalent in poor countries and countries affected by the human immunodeficiency virus (HIV) pandemic, particularly in sub-Saharan Africa and Asia. In about 80% of cases, it manifests itself in a pulmonary form at the origin of its transmission. The disease is curable if treated with appropriate treatment and with optimal adherence. Unfortunately, TB management has two important obstacles: the lack of simple and effective point-of-care diagnostic tests and the length of TB treatment. A long treatment creates problems of adherence and promotes the emergence of the resistant forms of the disease. It is unlikely that new drugs will be available in the near future for shortening TB treatment for drug-susceptible TB so as to avert the poor treatment outcomes which include treatment failure, drug-resistance, drug toxicity, TB recurrence, mortality due to TB, and increases in TB-related costs at household and health-facility level among low and middle income countries with dual epidemic of TB and HIV.

Our work was conducted in Uganda, one of the TB-HIV high-burden countries [3], so as to evaluate the potential of using high rifampicin doses as a strategy towards shortening of drug-susceptible pulmonary TB treatment duration to lower than the current 6 months among adult TB and TB-HIV co-infected patients .

1.1 Microbiology, physiopathology, immunology, risk factors and clinical presentation of tuberculosis

1.1.1 Microbiology

Tuberculosis is caused by a mycobacterium belonging to the tuberculosis complex consisting of *Mycobacterium* (*M*) *tuberculosis*, *M. bovis*, *M. africanum*, *M. canettii* and *M. pinipedii*. *M. tuberculosis* or bacillus of Koch, named after the scientist who discovered it in 1882, is the principal agent responsible for human tuberculosis. *M. bovis* is responsible for about 1% of infections. *M. africanum* infection occurs mainly in West and Central Africa where it accounts for between 20% and 50% of cases of tuberculosis. Mycobacteria are aerobic bacilli, microaerophilic, acid-fast bacilli (AFB), having the general structure of gram positive bacilli and characterized by a thick outer parietal layer rich in mycolic acids. They are characterized by their culture requirement and the slow growth with an average split time of 20 hours, the cultures being positive only after one to several weeks of incubation at 37°C [4]. Secondary identification makes it possible to differentiate mycobacteria from the tuberculosis complex from other non-tuberculous mycobacteria. These atypical mycobacteria from the environment are usually non-pathogenic but can sometimes give clinical manifestations simulating those of TB in immunosuppressed patients or in patients with chronic bronchial conditions [5]. The sequence of the genome of *M. tuberculosis* has recently been identified. It is composed of approximately 4 000 genes and is characterized by the importance of the coding sequences dedicated

to the production of enzymes involved in the synthesis and degradation of lipids [6]. The deciphering of the genome has allowed the development of molecular diagnostic tests for TB and tests for the identification of mutations associated with anti-tuberculosis drug resistance.

The tuberculosis complex has as its essential reservoir the patients with TB, besides *M. bovis* whose reservoir is animal (domestic or wild bovids). The transmission is human-airborne and all the more important it is related to the density of the bacilli in the air breathed. Patients with "pulmonary caverns" that are very rich in bacilli (100 million bacilli for a cavern about 2 cm in diameter) are the most contagious.

1.1.2 Physiopathology and immunology

After inhalation, the bacilli enter the pulmonary alveolus and are phagocytosed by the alveolar macrophages within which they multiply. Other macrophages and monocytes are attracted, and thus participate in the process of defense against infection. The infectious focus thus constituted is the initial focus. The bacilli and the antigens they release are drained by the macrophages to the lymph node satellite. Within the ganglion, T lymphocytes identify *M. tuberculosis* (MTB) antigens and transform into specific T lymphocytes, resulting in the release of lymphokines and activation of macrophages that inhibit the growth of phagocytic bacilli. At the level of the initial focus an inflammatory tissue is formed, then fibrous scar tissue, in which the macrophages containing bacilli are isolated and die. This initial focus or "chancre inoculation" is then the site of a case-specific necrosis specific to TB. There are then between 1 000 and 10 000 bacilli which gradually lose their viability and have a very slow multiplication. Bacilli called "quiescent" can persist for several years [7]. The same evolution occurs in the lymph node, resulting in the formation of the caseous ganglion which evolves spontaneously, in the majority of cases, towards the fibrous healing then the calcification. It is on average 2 to 3 weeks after infection that the delayed hypersensitivity reaction to humoral mediation and cell-mediated immunity occur. Delayed cell-mediated hypersensitivity is conventionally evidenced by the intradermal injection of tuberculin (inactivated MTB). All these clinical and immunological phenomena observed after the contamination of a healthy subject constitute the primary TB infection. This phase is usually asymptomatic but may, in rare cases, be accompanied by clinical manifestations of hypersensitivity. This patient with non-symptomatic TB infection is considered to have Latent TB infection (LTBI). In 90% of cases (in HIV negative patients), there is no further development with the disappearance of lung lesions, except for the persistence of calcifications [8]. It confers on the infected subject a certain degree of immunity. It translates into tuberculin conversion. The intradermal reaction (IDR) to tuberculin becomes positive 6 to 12 weeks after the infecting contact. This tuberculin conversion is evidence of recent infection and reflects the resulting immunity [7]. The recent identification of genome regions encoding specific proteins of MTB (CFP-10 and EAST-6) has led to the development of novel diagnostic tests for TB infection. These tests are based on the measurement of the release of interferon gamma in the blood of patients after sensitization by these specific antigens [9, 10].

In 10% of the patients, the infection will evolve towards active TB disease, pulmonary or extra-pulmonary, or even disseminated after passage of the bacilli in the blood. Half of the active forms occur in the first year after primary infection. The other half may occur years after the primary infection following reactivation of the dormant or quiescent bacilli in the wake of a decline in immunity e.g following HIV infection, immunosuppressive treatments, malnutrition, elderly, etc) [8]. HIV infection is a major risk factor for progression to TB disease. The HIV-positive patient after primary infection has a 5-10% risk of developing active TB per year, compared with 0.2% in an HIV-negative patient [11]. After 5 years, untreated, pulmonary TB (PTB) spontaneously evolves towards healing in 20-25% of patients; It is fatal in 50-60% of cases and it progresses to a chronic active form in the remaining 20-25% [12]. Below is the figurative summary described by Pai M, et al, to demonstrate the TB spectrum from infection to disease [13] (Figure 1).

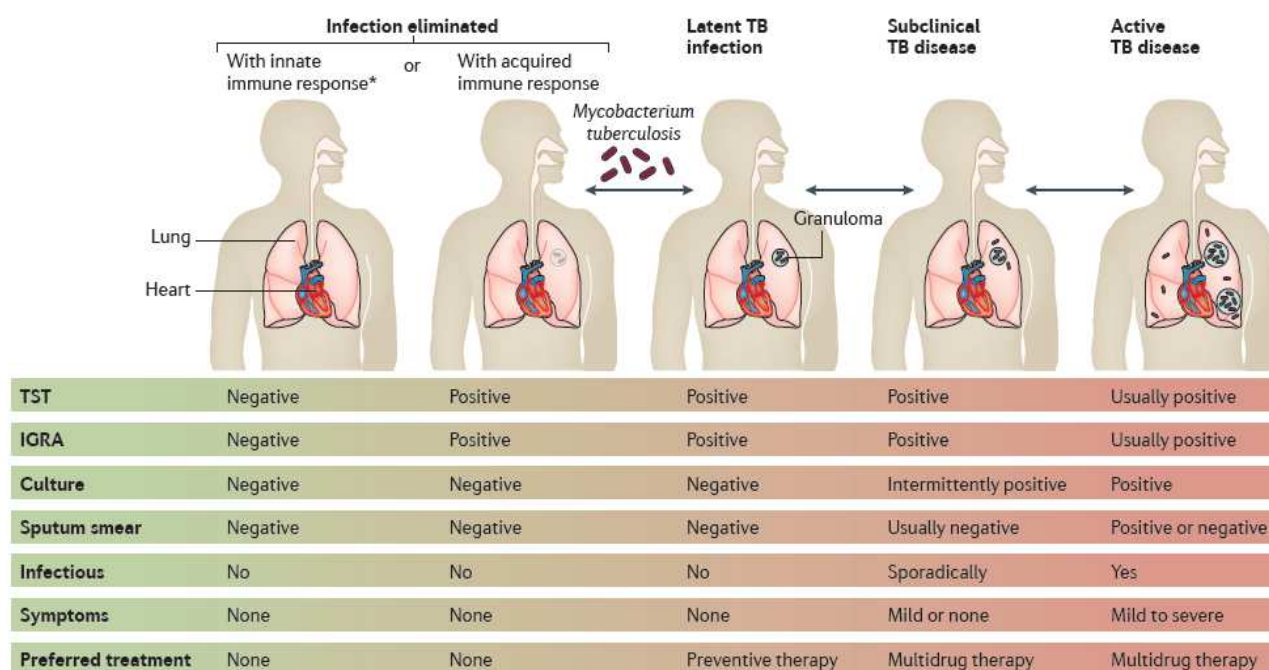


Figure 1. TB spectrum from *Mycobacterium tuberculosis* infection to active (pulmonary) TB disease, from: Pai M, et al. 2016 [13]

1.1.3 Risk-factors of tuberculosis

A review involving the 22 TB high-burden countries summarizes the common risk-factors of TB infection and disease[14]. This review notes that the progression from exposure to the TB bacilli to the development of active disease is a two-stage process governed by both exogenous and endogenous risk factors (see Figure 2). Exogenous factors play a key role in accentuating the progression from exposure to infection among which the bacillary load in the sputum and the proximity of an individual to an infectious TB case are key factors. Similarly endogenous factors lead in progression from infection to active TB disease. Along with well-established risk-factors (such as HIV, malnutrition, and young age), emerging variables such as diabetes, indoor air pollution, alcohol, use of immunosuppressive drugs, and tobacco smoke, play a significant role at both the individual and population level. Socioeconomic and behavioral factors are also shown to increase the susceptibility to infection. Specific groups

such as health care workers and indigenous population are also at an increased risk of TB infection and disease [14].

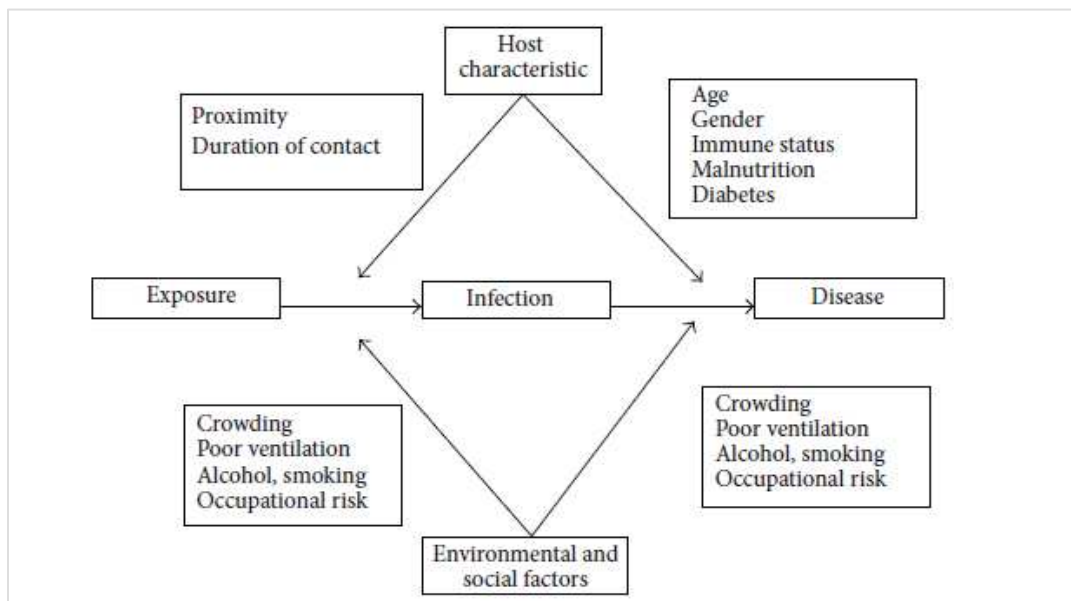


Figure 2. Risk-factors of TB infection and disease, from Narasimhan P, et al. 2013 [14]

1.1.4 Clinical features of tuberculosis

The clinical features of TB vary from classical to specific based on the part of the body affected. The disease can also be asymptomatic [15]. The classical clinical features associated with active pulmonary TB (commonest type of TB in 2/3 of patients) are: cough, hemoptysis, chest pain, and constitutional signs (weight loss/anorexia, fever, night sweats, and fatigue). Chest pain in patients with TB can also result from acute TB pericarditis. Pericardial TB can lead to cardiac tamponade or constriction. Elderly individuals with TB may not display typical signs and symptoms of active TB disease, because they may not mount a good immune response. Active TB disease in this age group may manifest as non-resolving pneumonitis. Extra-pulmonary TB symptoms and biological signs may be nonspecific or they can include leukocytosis, anemia, and hyponatremia due to the release of ADH (antidiuretic hormone)-like hormone from affected lung tissue. Patients with TB meningitis may present with a headache that has been either intermittent or persistent for 2-3 weeks. Subtle mental status changes may progress to coma over a period of days to weeks. Fever may be low grade or absent. In patients with skeletal TB, the most commonly affected skeletal site is the spine (Pott disease); symptoms include back-pain or stiffness. Lower-extremity paralysis occurs in up to half of patients with undiagnosed Pott disease. TB arthritis usually involve only one joint. Although any joint may be involved, the hips and knees are affected most commonly, followed by the ankle, elbow, wrist, and shoulder. Pain may precede radiographic changes by weeks to months. For genitourinary TB, symptoms may include flank pain, dysuria, and frequent urination. In men, genital TB may manifest as a painful scrotal mass, prostatitis, orchitis, or epididymitis. In women, genital TB may mimic pelvic inflammatory disease. TB is the cause of approximately 10% of sterility cases in women worldwide and of approximately 1% in industrialized countries [15].

Physical examination findings associated with TB also depend on the organs involved. Patients with pulmonary TB have abnormal breath sounds, especially over the upper lobes or involved areas. Rales or bronchial breath signs may be noted, indicating lung consolidation. Signs of extra-pulmonary TB differ according to the tissues involved. They may include the following: confusion, coma, neurologic deficit, chorioretinitis, lymphadenopathy, and cutaneous lesions. Lymphadenopathy in TB occurs as painless swelling of 1 or more lymph nodes. Lymphadenopathy is usually bilateral and typically involves the anterior and posterior cervical chain or supraclavicular nodes. The absence of any significant physical findings does not exclude active TB. Classical symptoms are often absent in high-risk patients, particularly those who are immunocompromised or elderly. Up to 20% of patients with active TB may be asymptomatic. Therefore, further investigations are essential when chest radiographic findings are suggestive of TB [15]. It is important to note that atypical presentation of TB occurs in HIV infected people.

1.2 Epidemiology of tuberculosis

1.2.1 Global TB burden

TB is one of the world's major infectious diseases with 2-3 billion infected people, 10.4 million new (incident) TB cases worldwide (56% and 34% in males and females respectively). Sub-Saharan Africa and Asia bear the largest incidence rates (See Figure 3). Six countries accounting for 60% of the new cases are: India, Indonesia, China, Nigeria, Pakistan and South Africa. Global progress depends on major advances in TB prevention and care in these countries[16]. In 2005, the World Health Organization (WHO) Regional Committee for Africa comprising health ministers from 46 Member States declared tuberculosis an emergency in the African region [17]. About 1.4 million TB deaths, and an additional 0.4 million deaths resulting from TB disease among people living with HIV have been reported (See Figure 4). Despite a fall in TB deaths by 22% between 2000 and 2015, TB has remained one of the top 10 causes of death worldwide. With the global rate of decline in TB incidence remaining at only 1.5% from 2014 to 2015, a rate far below the needed 4–5% annual decline by 2020 set to ensure attainment of the first milestones of the End TB Strategy [16], there is need for new advances in TB prevention and management [16].

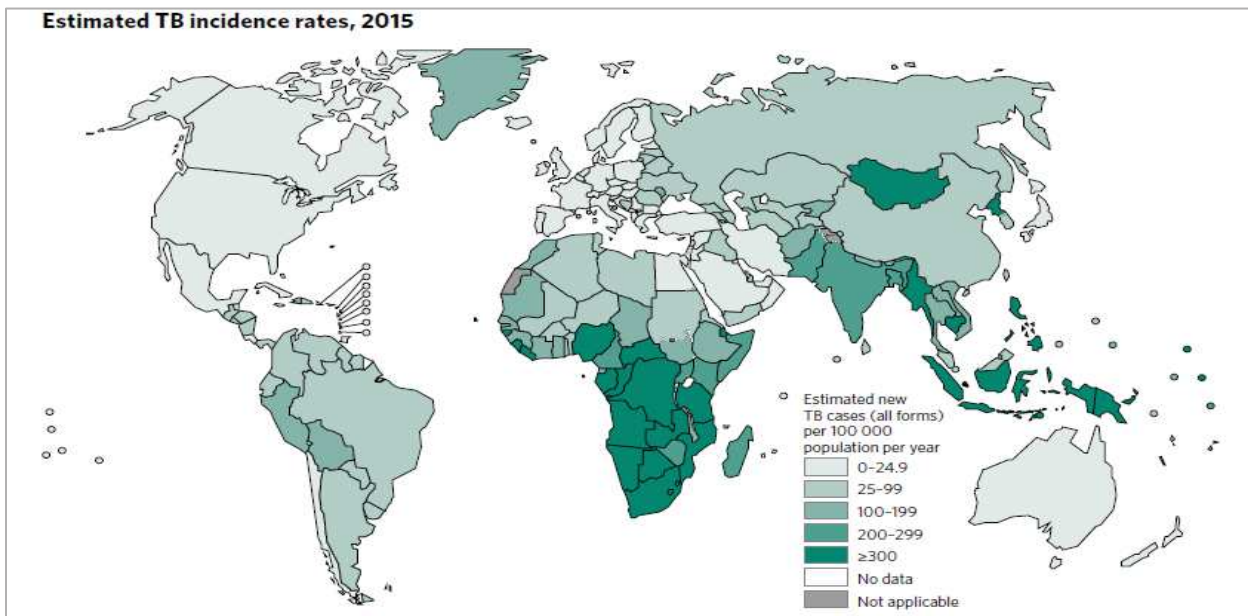


Figure 3. Estimated incidence rates, 2015, from WHO TB report 2016.
http://www.who.int/tb/publications/global_report/en/ [16]

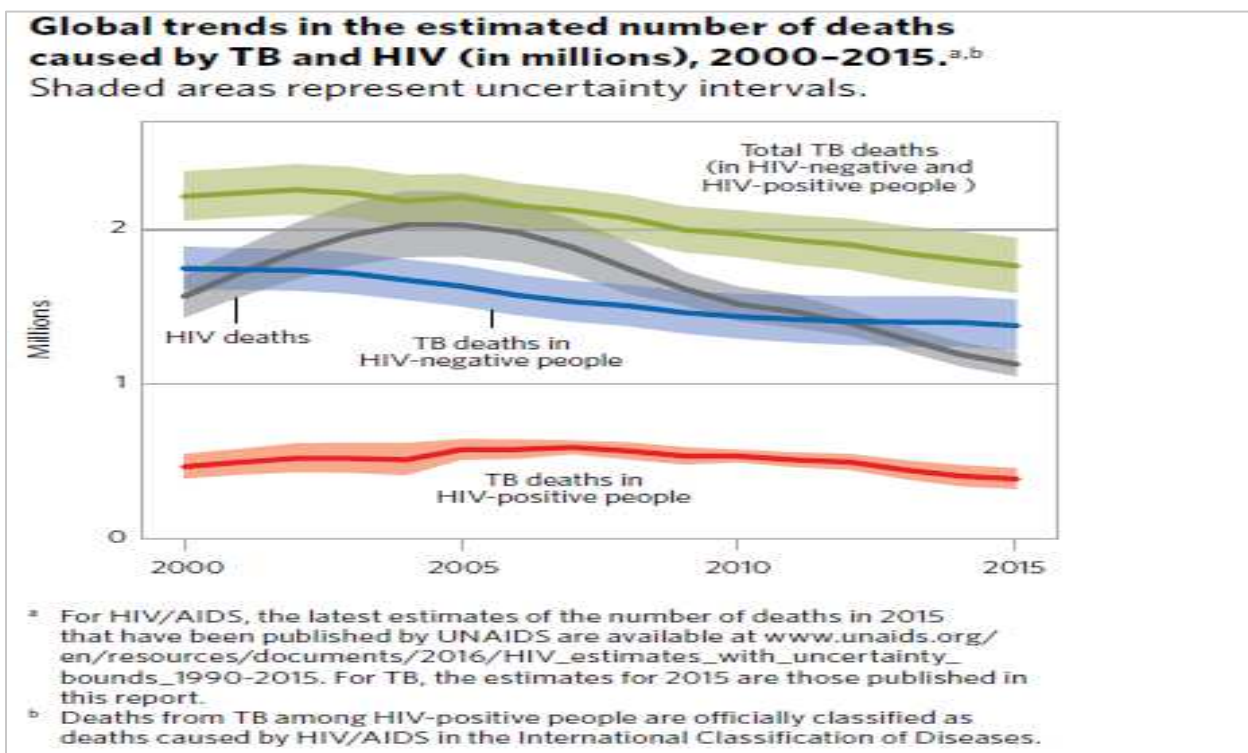


Figure 4. Global trends in the estimated number of deaths caused by TB and HIV (in millions), 2000-2015, from WHO TB report, 2016. http://www.who.int/tb/publications/global_report/en/ [16]

Since 2015, WHO has classified countries into 3 groups: 1) high-burden TB countries; 2) high-burden TB/HIV countries; 3) high-burden multi-drug resistant TB (MDR-TB) countries (See Figure 5).

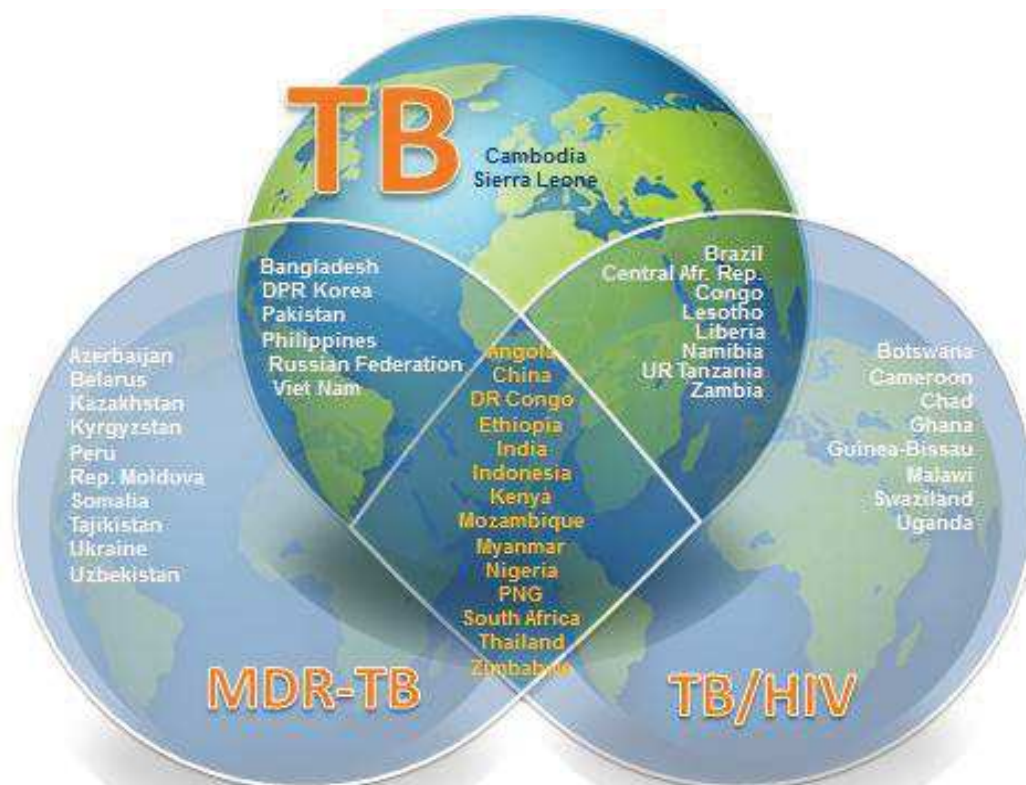


Figure 5. The three high burden countries' (HBC) lists (TB, MDR-TB and TB/HIV) of 30 countries each that will be used by WHO 2016–2020, from WHO, 2015.

http://www.who.int/tb/publications/global_report/high_tb_burden/countrylists2016-2020.pdf?ua=1 [3]

1.2.2 Burden of HIV-TB co-infection

HIV infection is still a global public health concern, especially in Africa and Asia. Since the beginning of the epidemic, more than 70 million people have been infected with the HIV virus and about 35 million people have died [18]. Globally, 36.7 million [34.0–39.8 million] people were living with HIV at the end of 2015. The burden of the epidemic continues to vary considerably between countries and regions with the Sub-Saharan Africa being the most severely affected, with nearly 1 in every 25 adults (4.4%) living with HIV and accounting for nearly 70% of the people living with HIV worldwide [18].

The estimated risk of developing active TB in people living with HIV ranges between 26 and 31 times greater than in those without HIV infection [19]. Overall, people living with HIV account for 1.2 million (11%) of all new TB cases. The proportion of TB cases living with HIV is highest in the WHO African Region (31%), and exceeds 50% in parts of southern Africa [16] (See Figure 6).

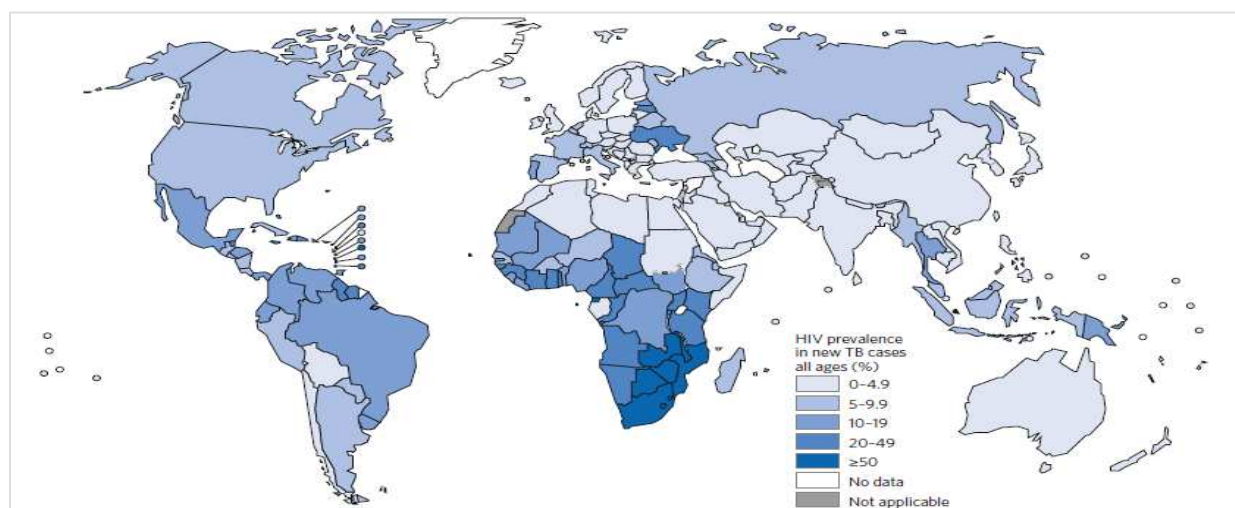


Figure 6. Estimated HIV prevalence in new and relapse TB cases, 2015, from WHO TB report 2016. http://www.who.int/tb/publications/global_report/en/ [16]

The overall TB mortality rates among HIV patients is still highest in the same regions with African region experiencing about 6 times higher mortality (30 per 100,000 population) as compared to the global average (5.3 per 100,000 population), with approximately 75% of all deaths occurring in Sub-Saharan Africa. In countries with a high prevalence of HIV infection, TB is a major cause of mortality and morbidity [20]. In addition to diagnosis and treatment of TB, early antiretroviral treatment (ART) of HIV infection has long been noted as the best protection against the risk of death from TB [21, 22]. Co-infection with HIV greatly increases mortality and risk of treatment failure [23]. These important co-morbidities clearly demonstrate that TB management needs an integrated approach and that every TB patient should be investigated for HIV, and every patient with HIV should be screened for active TB disease [24].

1.3 Tuberculosis diagnosis

Diagnosing active TB is the first step to being able to treat it and prevent transmission. In as much as diagnosis of TB should be “available free of charge to all persons with TB and populations at risk” [25], yet an estimated over four million people with TB went undiagnosed or unreported to national treatment programs in 2015, with India, Indonesia, and Nigeria accounting for almost half of this gap [16]. Even among those who do receive a TB diagnosis, often do so only after multiple health care visits and lengthy delays. Average delays of 28 to 30 days from when patients first contact a health care provider to diagnosis, even when patients present with overt TB symptoms have been reported [26]. Drug-susceptibility testing (DST) is not widely available and is used far too infrequently, even among those diagnosed with TB and living in countries with a high burden of drug-resistant TB (DR-TB). The end result is that 40% of people with TB do not receive a diagnosis or are not reported to national program, and DR-TB is detected in only 23% of people thought to have it [26].

Microscopy has been the commonly used method that detects the AFB although not specific as in to distinguish between live and dead bacilli, but also atypical mycobacteria from MTB. It is also simple, fast, and inexpensive. However, microscopy has low and variable sensitivity (20 to 60%) [27], especially among patients co-infected with HIV [28]. This low sensitivity is due to low level of detection. Nevertheless, it is widely implemented in limited resource countries.

On the other-hand, culture methods are the definitive diagnostic tests of TB, given their ability to distinguish between alive from dead bacilli. Its use is hindered by the long turn-around time. On average 3 weeks may be needed for mycobacterium growth to occur. This hinders its use as a point-of-care test. More so, the lack of appropriate laboratory infrastructure with good infection control mechanisms in many resource limited settings limit its use and left only in reference laboratories. Two types of culture media exist: 1) solid media e.g. Lowenstein-Jensen (LJ) and mycobacteria growth indicator tube (MGIT). Specifically, LJ tend to exhibit a slower growth and lower sensitivity as compared to MGIT [29, 30].

Molecular diagnostic methods can provide the data needed more rapidly, and in many cases it is more cost-effective than traditional culture methods [31, 32]. Nucleic acid amplification assays including polymerase chain reaction (PCR) have revolutionized the detection of MTB. It is a useful and sensitive tool for the early diagnosis of MTB in variety of clinical samples[33]. Xpert MTB/RIF is an automated PCR test (that is, a molecular test) utilizing the GeneXpert platform (Cepheid, Sunnyvale, CA, United States). Xpert MTB/RIF is a single test that can detect both MTB complex and rifampicin resistance within 2 hours after starting the assay, with minimal hands-on technical time. Given that all steps in the assay are automated and contained within its cartridge, that fact that it requires limited infrastructure and expertise to do, increases its access even in low and middle-income countries (LMICs). The fact that the assay's sample reagent, used to liquefy sputum, is tuberculocidal, it largely eliminates concerns about biosafety during the test procedure and allow the technology to be decentralized for use nearer to patients[34].

In an effort to enhance early detection of TB and prompt initiation of appropriate treatment, there has been a switch in the recommendations to Xpert MTB/RIF use rather than conventional microscopy, culture and DST as the initial diagnostic test in adults and children suspected of having TB or MDR-TB [35]. This also applies in case of non-respiratory specimens like cerebrospinal fluid, lymph nodes and other tissues from patients suspected of having TB. However, microscopy still remain key for treatment monitoring given that it is readily available in remote settings and despite not being able to differentiate alive from dead bacilli, it can show reduction in bacillary load with treatment [36].

Chest X-ray (CXR) is used to diagnose intra-thoracic lesions due to TB. CXR has high sensitivity for PTB and thus is a valuable tool to diagnose PTB and allow also to identify differential diagnosis of TB. However, CXR has poor specificity; although some CXR abnormalities are rather specific for PTB (for example, cavities), many CXR abnormalities that are consistent with pulmonary TB are also common in several other lung pathologies. Moreover, there is significant intra- and inter-observer variation in the reading of CXRs. Relying only on CXR for

TB diagnosis leads to over-diagnosis, as well as under-diagnosis [37, 38]. Rigorous efforts should always be made to base a TB diagnosis on bacteriological confirmation. WHO classifies TB diagnosis into bacteriologically confirmed TB, if it is based on bacteriological confirmation, or clinically diagnosed TB, if it is based on clinical assessment including CXR, only, the latter receiving an empirical TB treatment [37]. However, it should be noted that the low access to X-ray in limited resource countries, issues of poor quality and the fact that its costs are often not covered by National TB programs (NTP) limits its use among patients that would need it. In 2016, the WHO issued a summary of its existing recommendations on CXR as a screening tool for TB disease, indicating its sensitivity, its importance for diagnosing childhood TB, its additive value with Xpert MTB/RIF, its use in diagnosing TB in people with HIV, and its role in ruling out active TB before treating LTBI [37].

It is noteworthy that the lack of antibody or antigen sputum-based rapid test or non-sputum based point of care test is a limitation in the effort to diagnose TB, especially in patients with advanced HIV infection and children. This leaves many patients being started on empirical TB treatment.

However, recently, the WHO endorsed use of urine lipoarabinomannan (LAM) test for active TB screening and diagnosis among HIV/AIDS patients with advanced disease or with severe immune-suppression. LAM antigen is a lipopolysaccharide present in mycobacterial cell walls, which is released from metabolically active or degenerating bacterial cells and appears to be present only in people with active TB disease. Urine-based testing would have advantages over sputum-based testing because urine is easy to collect and store, and lacks the infection control risks associated with sputum collection. However, it suffers poor sensitivity [39].

1.4 Treatment of Tuberculosis

The aims of TB treatment are: to cure the patient and restore quality of life and productivity; to prevent death from active TB or its late effects; to prevent relapse of TB; to reduce transmission of TB to others; and to prevent the development and transmission of drug resistance [40]. Anti-TB treatment (ATT) is a combination of antibiotics, to avoid the risk of selection of resistant strains of MTB. It is of long duration so as to increase the chances of sterilization of quiescent bacilli and reduce the risk of TB recurrence. Given the long waiting times for the results of DST and their low access in countries with a high TB prevalence, TB treatment has for long been an empirical standardized treatment [41].

1.4.1 History of tuberculosis drug development

The first antibiotic to be discovered with proven activity against MTB was streptomycin. It is an antibiotic purified from *Streptomyces griseus*, discovered almost 70 years ago, thus providing the first hope of a TB-specific therapy [42, 43]. Nevertheless, uncertainties remained with regard to its ability to consistently cure patients, and this was closely followed by the realization that drug resistance develops rapidly when a single agent is used for the treatment of

TB. Following the launch of the Medical Research Council (MRC) TB unit in the United Kingdom in 1946, the unit conducted the first recorded randomized, controlled clinical trial designed to compare streptomycin plus bed rest versus bed rest alone [44]. The study showed that streptomycin plus bed rest achieved greater clinical improvement but only modest pathological improvement (as assessed by CXR) in comparison to bed rest alone. Importantly, improvement in TB was greatest in the first 3 months of therapy, after which many patients began to deteriorate due in part to the emergence of streptomycin resistance. In the 1950s, several other TB drugs with different mechanisms of action were discovered and developed (see Table 1), including *para*-amino salicylic acid, isoniazid (H), pyrazinamide (Z), cycloserine and kanamycin. This paved the way for combination therapy that, at that time, had a treatment duration of 18 months or more. In collaboration with the United States Public Health Service (USPHS) the MRC TB unit spent the next four decades developing the current short-course therapeutic regimen comprising H, rifampicin (R), Z and ethambutol (E). The introduction of R into clinical practice in the 1960s was a major breakthrough that allowed treatment duration to be shortened to 9 months, and then 6 months especially when used in a regimen that also contained Z. Through these developments, the USPHS (now known as the Center for Disease Control TB Trials Consortium) and the MRC TB unit firmly established the concept of the randomized, controlled clinical trial as the gold-standard methodology for the assessment of new drugs [45].

Table 1. Main tuberculosis drugs in Clinical use, their year of discovery and targets, from Zumla A., et al. 2013 [46].

Drug (year of discovery)	Target	Effect
First-line drugs		
Isoniazid (1952)	Enoyl-[acyl-carrier-protein] reductase	Inhibits mycolic acid synthesis
Rifampicin (1963)	RNA polymerase, beta subunit	Inhibits transcription
Pyrazinamide (1954)	S1 component of 30S ribosomal subunit	Inhibits translation and trans-translation, acidifies cytoplasm
Ethambutol (1961)	Arabinosyl transferases	Inhibits arabinogalactan biosynthesis
Second-line drugs		
<i>Para</i> -amino salicylic acid (1948)	Dihydropteroate synthase	Inhibits folate biosynthesis
Streptomycin (1944)	S12 and 16S rRNA components of 30S ribosomal subunit	Inhibits protein synthesis
Ethionamide (1961)	Enoyl-[acyl-carrier-protein] reductase	Inhibits mycolic acid biosynthesis
Ofloxacin (1980)	DNA gyrase and DNA topoisomerase	Inhibits DNA supercoiling
Capreomycin (1963)	Interbridge B2a between 30S and 50S ribosomal subunits	Inhibits protein synthesis
Kanamycin (1957)	30S ribosomal subunit	Inhibits protein synthesis
Amikacin (1972)	30S ribosomal subunit	Inhibits protein synthesis
Cycloserine (1955)	D-alanine racemase and ligase	Inhibits peptidoglycan synthesis

1.4.2 Treatment of drug-susceptible TB

Management of a patient diagnosed with active TB depends on whether or not the patient has already received anti-TB drugs in the past. Thus, the newly diagnosed drug-susceptible TB patient receives a six-month regimen that breaks down into an intensive two-month phase involving H, R, Z and E followed by four months of continuation phase with R and H with doses based on body weight [40]. (See Table2).

Table 2. Recommended doses of first-line anti-tuberculosis drugs for adults, from WHO report, 2017 [40]

Drug	Daily recommended dose	
	Dose and range (mg/kg body weight)	Maximum (mg)
H	5 (4-6)	300
R	10 (8-12)	600
Z	25 (20-30)	--
E	15 (15-20)	--

In patients who require retreatment due to treatment interruption or recurrence of disease, the current recommendation is that DST should be conducted first so as to inform the choice of treatment regimen [47]. Both R and H are the back-bone drugs within the standard 6-month WHO recommended first-line TB treatment regimen [47, 48]. It is important to note that the potency of this regimen is attributed mainly to rifampicin's strong bactericidal activity [49, 50] that is also based on its ability to inhibit transcription by binding with high affinity to bacterial DNA-dependent RNA polymerase [51-53]. The 6-month regimen (2HRZE/4RH) has a good efficacy with a TB recurrence rate of 5% [50, 54]. This regimen is recommended for use as a fixed dose combination (FDC), and administered once daily for both TB only and TB-HIV co-infected patients [47].

1.4.2.1 General Safety description of first line anti-tuberculosis drugs

A number of adverse effects have been reported in respect to each of the first line anti-TB drugs. These are summarized in Table 3. Pyridoxine is administered throughout the duration of TB treatment to prevent peripheral neuropathy due to H. In programmatic conditions, it is not necessary to systematically monitor liver function test or other laboratory tests for safety purposes.

Table 3. Adverse effects of first line anti-tuberculosis drugs, from Arbex MA, et al. 2010 [55].

Drug	Adverse effects
Isoniazid	Minor: Nausea, vomiting, and epigastric pain. Transitory and asymptomatic increase in hepatic enzyme levels, Arthralgia, Headache, insomnia, euphoria, agitation, anxiety, Somnolence, Acne, Cutaneous pruritus or fever Major: Psychosis, convulsive seizures, mental, confusion, coma, attempted suicides, hematological alterations or vasculitis, peripheral neuropathy, Clinical hepatitis and Lupus-like syndrome
Rifampicin	Minor: Gastrointestinal reactions: Nausea, anorexia, and abdominal pain. Orange-colored tears, sweat, and urine. Skin reaction: Pruritus, with or without Erythema. Flu-like syndrome, fatigue, dizziness, headache, dyspnea, and ataxia Major: Exanthema, hepatotoxicity, immunological reactions like Thrombocytopenia, leukopenia, eosinophilia, hemolytic anemia, agranulocytosis, vasculitis, acute interstitial nephritis, and septic shock.
Pyrazinamide	Minor: Gastrointestinal symptoms: Nausea, Vomiting, and anorexia. Hyperuricemia and arthralgia in non-gouty individuals. Exanthema, pruritus and dermatitis. Major: Severe exanthema, pruritus, Rhabdomyolysis with myoglobinuria and kidney failure. Acute arthritis in gouty individuals and hepatotoxicity
Ethambutol	Retro-bulbar neuritis with symptoms of blurred vision, decrease in visual acuity, presence of scotomas, and loss of the ability to discern the color green and, in some cases, the color red. Peripheral fiber impairment which manifests as a reduction in the visual field. Peripheral neuritis. Gastrointestinal symptoms (nausea, vomiting, abdominal pain, and hepatotoxicity), hematological symptoms (eosinophilia, neutropenia, and thrombocytopenia), cardiovascular symptoms (myocarditis and pericarditis), neurological symptoms (headache, dizziness, and mental confusion), hyperuricemia/gout (due to a reduction in the excretion of uric acid by the kidney), hypersensitivity (skin rash, arthralgia, and fever), and (occasionally) pulmonary Infiltrates.

1.4.2.2 Anti-tuberculosis drug induced liver injury

Drug-induced liver injury (DILI) is an important consideration during administration of ATT. DILI is ultimately a clinical diagnosis of exclusion given that histologic specimens of the liver are often not obtained. Its diagnosis is made plausible after the exclusion of other causes of liver injury, such as acute viral hepatitis[56]. Detection of DILI is based on measurement of hepatic enzymes [56]. More specifically, an increase in ALT, is more specific for hepatocellular injury than an increase in aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase [SGOT]), which can also signify abnormalities in muscle, heart, or kidney [57, 58]. Increases in alkaline phosphatase and/or bilirubin with little or no increase in ALT indicate cholestasis. Jaundice is usually detectable on the physical examination when serum bilirubin exceeds 3.0 mg/dl [56]. Overall, the strongest confirmation of the DILI is based on: a re-challenge with the suspected offending agent in cases with more than two-fold serum alanine aminotransferase (ALT) elevation, and discontinuation leading to a fall in ALT [59].

DILI may result from direct toxicity of the primary compound, a metabolite, or from an immunologically mediated response, affecting hepatocytes, biliary epithelial cells, and/or liver vasculature[56]. There are two types of DILI that have been reported, that is: 1) Predictable DILI: characterized by certain dose-related injury in experimental animal models, has a higher attack rate, and tends to occur rapidly. Injurious free radicals cause hepatocyte necrosis in zones farthest from the hepatic arterioles, where metabolism is greatest and antioxidant detoxifying capacity is

the least [60, 61]. 2) Unpredictable or idiosyncratic reactions: comprise most types of DILI and these hypersensitivity or metabolic reactions occur largely independent of dose and relatively rarely for each drug, and may result in hepatocellular injury and/or cholestasis. Hepatocyte necrosis is often distributed throughout hepatic lobules rather than being zonal, as is often seen with predictable DILI [62]. Metabolic idiosyncratic reactions may result from genetic or acquired variations in drug biotransformation pathways, with synthesis or abnormally slow detoxification of a hepatotoxic metabolite. Metabolic idiosyncratic reactions may have a widely variable latent period, but recur within days to weeks after re-exposure [63].

DILI may occur with all currently recommended regimens for the treatment of TB infection or disease [64]. This can occur with R, Z and H [65-67]. Metabolic idiosyncratic reactions appear to be responsible for most DILI from the ATT [56]. The onset of DILI bears some differences depending on the type of TB drug responsible.

Rifampicin-related DILI

R has been reported to occasionally cause dose-dependent interference with bilirubin uptake, resulting in subclinical, unconjugated hyperbilirubinemia or jaundice without hepatocellular damage. This may be transient and occur early in treatment or in some individuals with preexisting liver disease [68-71]. R occasionally can cause hepatocellular injury and potentiate hepatotoxicities of other anti-TB medications [72, 73]. The conjugated hyperbilirubinemia occasionally reported during treatment of TB using R-based combinations, is probably caused by R inhibiting the major bile salt exporter pump [74]. Asymptomatic elevated bilirubin may also result from dose-dependent competition with bilirubin for clearance at the sinusoidal membrane or from impeded secretion at the canalicular level [63, 68, 69]. Rare hepatocellular injury appears to be a hypersensitivity reaction, and it may be more common with large, intermittent doses [68]. Hypersensitivity reactions have been reported in combination with renal dysfunction, hemolytic anemia, or “flulike syndrome” [75, 76]. R-induced hepatotoxicity is characterized by cholestasis which may be insidious. Idiosyncratic hypersensitivity reaction to R, manifest as anorexia, nausea, vomiting, malaise, fever, mildly elevated ALT, and elevated bilirubin, usually occurs in the first month of treatment initiation [68, 76-78].

Isoniazid-related DILI

Two potential explanations for onset of isoniazid DILI have been raised. The first one is based on acetylation rate, a claim that is still controversial. . In contrast to early studies [79-81], slow acetylators may actually have greater cumulative mono-acetyl hydrazine (MAH) exposure, which could be further metabolized to other toxic intermediaries especially reactive metabolites[45]. Slow acetylators also had higher peak ALT than did fast acetylators and, when rechallenged with H, more frequently developed transaminase elevation of at least three times the upper limit of normal (ULN) [79]. Secondly, metabolic idiosyncratic mechanisms have been implicated in H-induced hepatotoxicity. The H metabolite acetyl-hydrazine covalently binds to liver macromolecules, a process mediated by microsomal enzymes [80]. Patients with homozygous cytochrome P450 2E1 c1/c1 host gene polymorphism, who have enhanced cytochrome P450 2E1 activity, in one study had a higher risk of

hepatotoxicity, particularly in slow acetylators [82]. R appears to enhance a metabolic hepatocellular idiosyncratic reaction in patients receiving H, perhaps by promoting the formation of toxic H metabolites [83, 84].

At presentation, some individuals may be asymptomatic, whereas others may experience symptomatic hepatotoxicity at varying serum transaminase concentrations. Constitutional symptoms may be seen early in severe hepatotoxicity, and may last from days to weeks. Nausea, vomiting, and abdominal pain are seen in 50 to 75% of patients with severe illness, whereas fever is noted in 10% and rash in 5% of patients. Overt jaundice, dark urine, and clay-colored stools are late signs of clinical worsening. Coagulopathy, hypoalbuminemia, and hypoglycemia signify life-threatening hepatic dysfunction. The regression of H hepatotoxicity usually takes weeks. Recovery is complete in most after discontinuation of H [85].

H induced hepatotoxicity occurs generally within weeks to months rather than the days to weeks of onset seen with hypersensitivity reactions [85, 86]. Unlike a classical hypersensitivity reaction, H re-challenge does not always elicit rapid recurrence of hepatotoxicity [85]. Approximately 60% of the hepatotoxicity incidence is reported to occur in the first 3 months of treatment, and 80% of the incidence in the first 6 months [87-89]. A retrospective case fatality review found that the median interval from treatment initiation to symptom onset was 16 weeks [90]. Most H-associated hepatotoxicity is age associated, with symptomatic transaminase elevation: ranging from 0% in <14 years to 0.28% in those >65 years in the Seattle study [91]; 0.44% in <35 years to 2.08% in >49 years having AST elevation more than five times the ULN in the Tennessee study, a difference that was statistically significant [89]. However, the differences in the findings among these studies have been attributed to differing definitions of hepatotoxicity, patient selection, and inability to exclude confounding causes of hepatotoxicity [56]. The severity of H-related hepatitis has been reported to also increase with age, with higher mortality in those older than 50 years [85, 90, 92].

Combined Rifampicin and Isoniazid related DILI

The rate of symptomatic hepatitis with the combination of R and H has been estimated at 2.55% in a meta-analysis that included patients with TB disease, a higher incidence than in regimens containing one or the other drug [93]. It is thought that the observed elevation in transaminases may partly or fully be due to H, a phenomenon that is supported by studies reporting transaminase elevations above the upper-limit of normal ranges being seen more commonly in people with LTBI on H alone as compared to those on R alone [73], or even the absence of significant transaminase elevations even with intake of R alone for 4-months [72]. Secondly, that perhaps the observed ALT elevations may actually be equivalent to what we would normally expect even without any treatment administration. This is supported by results of a study that showed a non-significant difference in geometric mean of serum ALT between the placebo and R groups [94].

Pyrazinamide-related DILI

Transaminase elevation more than four times the ULN were reported with administration of Z and either E (58%) or levofloxacin (18%) or ofloxacin (41%) for treatment of LTBI after exposure to MDR TB [65, 66]. The half-

life ($t_{1/2}$) of Z is notably longer than that of either H or R, approximately 10 hours [82]. In patients with preexisting hepatic disease, $t_{1/2}$ is increased to 15 hours [101]. Z, a nicotinic acid derivative, is de-amidated to pyrazinoic acid in the liver and subsequently metabolized to 5-hydroxy-pyrazinoic acid by xanthine oxidase [95], aldehyde oxidase [96], and xanthine dehydrogenase [97, 98]. In addition, 5-hydroxy-pyrazinamide may be generated during metabolism [99]. The kidneys clear metabolites of Z, hence requiring intermittent dosing in patients with renal insufficiency [100]. Z may exhibit both dose-dependent and idiosyncratic hepatotoxicity. Historically, daily use of Z at 40 to 50 mg/kg commonly resulted in hepatotoxicity, and a relationship to dose was noted [101]. It is thought that there might be shared mechanisms of injury for H and Z, because there is some similarity in molecular structure. This is implied in the evidence that patients who previously had hepatotoxic reactions with H have had more severe reactions with R and Z given for LTBI [102]. Z may induce hypersensitivity reactions with eosinophilia and liver injury [102] or granulomatous hepatitis [103].

There has been few reports of ethambutol-related liver cholestatic jaundice, with unclear circumstances [104]. Otherwise, E has not been linked to liver injury.

Factors associated with onset of DILI during TB treatment

An increased risk of hepatotoxicity has been associated with: being woman [105-108] though contradicted in other studies [87, 109]; alcohol use [87, 110, 111], contrary to other two studies [106, 109]; having abnormal baseline transaminases [107]; NAT2 slow acetylators as determined by phenotypic assays [83, 110, 112] or by genotypic assays [79, 113]; malnutrition or hypoalbuminemia [110, 114-116]; presence of HLA-DQB1*0201 [116]; gene polymorphisms at loci of genes coding for cytochrome P450 2E1 and for glutathione S-transferase [79, 117]; higher EFV concentrations [113, 118]; CYP2B6*6 genotype [113, 118]; extensive TB disease [105, 110]; and patients under-going liver transplant [119]. In a meta-analysis, the presence of R in a multidrug treatment regimen increased the incidence of significant hepatotoxicity for adults from 1.6 to 2.55% and in children from 1.0 to 6.9% [93]. Z is considered as a contributor to increased incidence or severity of hepatotoxicity [107, 109, 120, 121], although some studies could not demonstrate this link [122-125]. The role of hepatitis B infection on incidence of TB DILI has been demonstrated in some studies [126, 127] but not on others [110]. Hepatitis B virus carriage has been linked to incidence of more severe hepatic disease from treatment-associated liver injury [126, 128]. Additional studies are needed, but the limited data leave sufficient concern that hepatitis B may be a risk factor for more frequent or severe hepatotoxicity during treatment of TB disease [45]. The role of Hepatitis C Virus infection on DILI during treatment for TB disease was shown in a study conducted in Florida, in which patients received at least 5 days of H, R or rifabutin, or Z, and had not received alcohol or drugs of abuse for at least 10 days before starting anti-TB therapy. Hepatotoxicity was reported in 30% versus 11% of patients with and without hepatitis C-infection respectively. Hepatitis C was independently associated with fivefold risk of transaminase elevation of at least 120 U/L, or of serum bilirubin of at least 1.5 mg/dl. Co-infection with both hepatitis C and HIV elevated the risk of hepatotoxicity more than 14-fold [129].

1.4.2.3 Treatment outcomes of drug susceptible tuberculosis

Assessment of therapeutic response is based on systematic microscopic examination of sputum at the end of the intensive phase (two months), at five months and at the end of treatment (6 months)[130]. A patient is considered cured if he has received full treatment and is smear-negative twice between the end of the intensive phase and the end of treatment. One is considered to have treatment failure if smear-positive at five months or later [41]. Needless to say, microscopy is poorly adapted to this evaluation of therapeutic response given its inability to differentiate live from a dead bacillus. Indeed, smear-positive persistence is not uncommon after five months of treatment in patients with large pulmonary cavities [131]. Although accessibility to Xpert MTB/RIF is increasing, it is also not adequate in differentiating dead from live bacilli and therefore not recommended for treatment monitoring. The only test to assess response is culture but it is poorly available and with long delays that make difficult treatment decision.

There is no effective early surrogate markers of TB treatment efficacy yet. Culture conversion at week 8 though used commonly in TB chemotherapeutic trials, has been reported to be a poor surrogate marker for treatment efficacy [132-134] and therefore create a challenge in precise estimation of new anti-tuberculosis drug or regimen efficacy. The delayed culture conversion beyond week 8 in East African populations [135, 136], has challenged the use of week-8 culture conversion rate, with researchers suggesting the use of months-3 instead of month-2 culture conversion endpoint as a surrogate of treatment efficacy within the phase II East African trials [133]. Because of the weakness of month-2 culture conversion, phase 3 confirmatory trials still rely on relapse free cure as the primary endpoint for efficacy which implies very long post treatment follow-up for a minimum of 12 months, which is time consuming and costly, something that could explain why there are so few randomized clinical trials (RCT) in TB.

1.4.2.4 Adherence to TB treatment

The effectiveness of the 6-month TB treatment regimen is based on attaining good patient adherence. This is also key in prevention of onset of rifampicin resistance [137, 138]. Directly Observed Treatment (DOT) and the use of FDC, is strongly promoted by WHO to enhance adherence [41]. Treatment administration approaches currently being recommended by the WHO are: a) Community- or home-based DOT is recommended over health facility-based DOT or unsupervised treatment; b) DOT administered by trained lay providers or health-care workers is recommended over DOT administered by family members or unsupervised treatment; c) Video observed treatment (VOT) can replace DOT when the video communication technology is available and can be appropriately organized and operated by health-care providers and patients [47].

In addition to DOT, patients are entitled to effective care and support interventions, which are: a) Health education and counselling on the disease and treatment adherence; b) A package of treatment adherence intervention such as social support (e.g. food, financial incentive, and transport fee); psychological support; home

visit or digital health communication (e.g. SMS, telephone call); medication monitor. The interventions should be selected on the basis of the assessment of individual patient's needs, provider's resources and conditions for implementation; c) Support in selection of a suitable treatment administration option, which include DOT, VOT, non-daily DOT (e.g. not every dose supervised treatment, weekly or a few times per week supervision), or unsupervised treatment. All of this is good in theory but represents a significant cost for the TB programs, which explains why it is often poorly implemented [47].

1.4.3 Drug-resistant tuberculosis

1.4.3.1 Burden of drug-resistant tuberculosis

Development of resistance against any of the anti-tuberculosis drugs arises by bacterial chromosomal mutations[29]. While these mutations are rare events, a mutation early in multiplication produces a clone of resistant bacilli that are found more frequently. The mutation rates were thus found to be about 2.6×10^{-8} for H and 2.2×10^{-10} for R, while the more useful estimates of the highest proportion of mutants that can be expected in an unselected bacterial population were found to be 3.5×10^{-6} for H and 3.3×10^{-8} for R[139]. A recent calculation has made these proportions somewhat greater [140]. During the initial phase of treatment, when bacterial populations are large, treatment is almost always with Z, known to prevent resistance emerging [141], and sometimes E as well as R and H. The origins of drug resistance appear to be due to 1) irregularity in drug taking by mechanisms described elsewhere [142]; while attempts to simulate the emergence of resistance by irregular drug taking have failed [143], resistance does emerge, although rarely, in relapse cultures [144, 145]; 2) inadequate dosage, particularly of R [146], leading to a slow response and eventual resistance; and 3) the prescription of single-drug treatment for financial reasons by private practitioners, a common but regrettable practice in some countries[147]. Resistant strains therefore have their own epidemiology, and are capable of creating disastrous epidemics, with treatment that is expensive and of low efficacy.

1.4.3.2 Multi and extensive-drug resistant tuberculosis

Multi-drug resistant TB (MDR-TB) is defined as resistance to rifampicin and isoniazid, the two most effective anti-TB drugs. In 2015, WHO estimated 480 000 new cases of MDR-TB and an additional 100 000 people with R-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment. India, China and the Russian Federation accounted for 45% of the combined total of 580 000 cases. There were about 250 000 (range, 160 000–340 000) deaths from RR-TB in 2015[16]. In May 2016, WHO issued guidance [148] that people with RR-TB should be treated with second line drugs.

There is evidence on the growing resistance against second line anti-TB drugs and Z. By the end of 2015, extensively drug-resistant TB (XDR-TB) had been reported by 117 WHO Member States. XDR-TB is defined as

MDR-TB plus resistance to at least one fluoroquinolone and a second-line injectable agent. The average proportion of MDR-TB cases with XDR-TB was 9.5% (95% CI: 7.0–12.1%), similar to estimates for previous years (9.7% in 2014 and 9.0% in 2013) [16]. The proportion of MDR-TB cases with resistance to any fluoroquinolone for which testing was done – including ofloxacin, levofloxacin and moxifloxacin – was 21.0% (95% CI: 8.8–33.3%). A total of 51% (30–70%) of patients with MDR-TB have resistance to a fluoroquinolone or a second-line injectable agent, or both [16]. In a recent multi-country project assessing the level of resistance to both Z and fluoroquinolone in five countries – Azerbaijan, Bangladesh, Belarus, Pakistan and South Africa and coordinated by WHO, substantial variations in levels of resistance were noted among the different settings (3.1–42.1%) [149]. Resistance to Z was reported to be significantly associated with R resistance (0.5–4.2% among R-susceptible cases and 36.7–81.3% among R-resistant cases). Resistance ranged from 1.0% to 16.6% for ofloxacin, from 0.5% to 12.4% for levofloxacin and from 0.9% to 14.6% for moxifloxacin when tested at 0.5 µg/ml. The study recommended that the presence of R resistance, which currently is easily identified because of the wide availability of new rapid molecular technology, should prompt attention to the possibility of the simultaneous presence of resistance to Z and, in some settings, the earlier generation fluoroquinolones. Resistance to the latest generation fluoroquinolones at the clinical breakpoint is still uncommon, a finding that supports current WHO recommendations to use moxifloxacin or gatifloxacin in the treatment of MDR-TB [149].

1.4.3.3 Treatment of drug-resistant tuberculosis

The currently recommended medicines for RR-TB and MDR-TB treatment are categorized into groups A-D, as summarized below. Group A. Fluoroquinolones (Levofloxacin, Moxifloxacin, Gatifloxacin); Group B. Second-line injectable agents (Amikacin, Capreomycin, Kanamycin, Streptomycin); Group C. Other core second-line agents (Ethionamide / prothionamide, Cycloserine / terizidone, Linezolid, Clofazimine); Group D. Add-on agents and not part of the core MDR-TB regimen (D1: Z, E, high-dose H; D2: Bedaquiline, Delamanid; D3: *p*-aminosalicylic acid, Imipenem–cilastatin, Meropenem, Amoxicillin-clavulanate, (Thioacetazone))[150]. WHO recommends both shorter and longer MDR-TB regimens based on different circumstances[150]. Most of these drugs are repurposed or old TB drugs abandoned before due to toxicity..

The longer MDR-TB regimens of 18-24 months apply to both adults and children and covers all forms of RR-TB, that is those who are H susceptible, MDR-TB, resistant to other medicines from firstline group (poly-resistant). In patients with RR-TB or MDR-TB, a regimen with at least five effective TB medicines during the intensive phase is recommended, including Z and four core secondline TB medicines – one chosen from Group A, one from Group B, and at least two from Group C6 (conditional recommendation, very low certainty in the evidence). If the minimum number of effective TB medicines cannot be composed as given above, an agent from Group D2 and other agents from Group D3 may be added to bring the total to five. In patients with RR-TB or MDR-TB, it is recommended that the regimen be further strengthened with high-dose H and/or E (conditional recommendation, very low certainty in the evidence). It is recommended that any patient – child or adult – with

RR-TB in whom H resistance is absent or unknown be treated with a recommended MDR-TB regimen. It could either be a shorter MDR-TB regimen, or a longer MDR-TB regimen to which isoniazid is added [150].

A shorter MDR-TB regimen of 9–12 months may be used instead of the longer regimens in adults and children with RR-TB or MDR-TB who were not previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents was excluded or is considered highly unlikely. The shorter MDR-TB treatment regimens are standardized in content and duration and split into two distinct parts. The first is an intensive phase of four months (extended up to a maximum of six months in case of lack of sputum smear conversion) and included the following drugs: gatifloxacin (or moxifloxacin), kanamycin, prothionamide, clofazimine, high-dose H, Z, and E. This is followed by a continuation phase of five months with the following medicines: gatifloxacin (or moxifloxacin), clofazimine, pyrazinamide and ethambutol (with or without prothionamide kept in the continuation phase in earlier studies).

Bedaquilline and delamanid were approved by FDA after phase 2 trials, and have received a conditional recommendation by WHO for use in the treatment of adults with drug-resistant TB in 2013 and 2014 respectively [151, 152].

Among patients with H mono-resistance or populations with known or suspected high levels of H mono-resistance, administration of 7RZE or 9RE as therapy in the continuation phase is an alternative to HR to prevent relapses [47].

Surgery has also been reported to have a role especially among patients with lung cavities and tuberculomas. Its need is demonstrated in the study from Tbilisi Georgia, that described pulmonary surgery in 137 patients with half having MDR/XDR TB. The study recommended the revisiting of the need for surgery especially in patients with extensive pulmonary lesions even those with bacterial cure or patients where bacterial cure is not possible [153].

1.5 Treatment of TB-HIV co-infection

1.5.1 Anti-retroviral treatment

Since 1987, more than 25 antiretroviral (ARV) drugs in 6 mechanistic classes for treatment of HIV infection have been approved by Food and Drug Administration (FDA) [154]. These 6 classes include the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), a fusion inhibitor (FI), a CCR5 antagonist, and integrase strand transfer inhibitors (INSTIs). In addition, two drugs, ritonavir (r) and cobicistat (COBI or c), potent CYP3A inhibitors, are used solely as pharmacokinetic (PK) enhancers (i.e. boosters) to improve the PK profiles of some ARV drugs highly metabolized by CYP3A (e.g. PIs and the INSTI elvitegravir [EVG])[155]. The two commonly used NNRTIs as part of the treatment of HIV patients in low-resource settings, that is efavirenz and nevirapine, received FDA approval in

1998 and 1996 respectively [154] and are available as FDC combined with 2 NRTIs, either zidovudine(ZDV) + lamivudine(3TC) or tenofovir disoproxil fumarate(TDF) + 3TC.

With focus on LMICs, in their June 2016 report, WHO fronted a “treat-all” recommendation, with removal of all limitations on eligibility for ART among people living with HIV; all populations and age groups are now eligible for treatment irrespective of CD4 count, including pregnant women and children. The same once-per-day combination pill is now recommended for all adults living with HIV, including those with TB, hepatitis, and other co-infections [36]. The recommended first-line ART for adults and adolescents consist of two NRTIs plus a NNRTI like EFV or INSTI like dolutegravir(DTG)[36]. However the DTG is not available today in many LMICs (See Table 4).

Table 4. WHO recommended first-line ART regimens for adults, pregnant or breastfeeding women, adolescents and children, from WHO report, 2016 [36]

First-line ART	Preferred first-line regimens	Alternative first-line regimens ^{a,b}
Adults	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV (or NVP) TDF + 3TC (or FTC) + DTG ^c TDF + 3TC (or FTC) + EFV ₄₀₀ ^{c,d} TDF + 3TC (or FTC) + NVP
Pregnant or breastfeeding women	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV (or NVP) TDF + 3TC (or FTC) + NVP
Adolescents	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV (or NVP) TDF (or ABC) + 3TC (or FTC) + DTG ^{c,d} TDF (or ABC) + 3TC (or FTC) + EFV ₄₀₀ ^{c,d} TDF (or ABC) + 3TC (or FTC) + NVP
Children 3 years to less than 10 years	ABC + 3TC + EFV	ABC + 3TC + NVP AZT + 3TC + EFV (or NVP) TDF + 3TC (or FTC) + EFV (or NVP)
Children less than 3 years	ABC (or AZT) + 3TC + LPV/r	ABC (or AZT) + 3TC + NVP

^a For adults and adolescents, d4T should be discontinued as an option in first-line treatment.

^b ABC or boosted protease inhibitors (ATV/r, DRV/r, LPV/r) can be used in special circumstances.

^c Safety and efficacy data on the use of DTG and EFV₄₀₀ in pregnant women, people with HIV/TB coinfection and adolescents younger than 12 years of age are not yet available.

^d Conditional recommendation, moderate-quality evidence.

^e EFV at lower dose (400 mg/day).

3TC lamivudine, ABC abacavir, AZT zidovudine, DRV darunavir, DTG dolutegravir, EFV efavirenz, FTC emtricitabine, LPV lopinavir, NVP nevirapine, r ritonavir, TDF tenofovir.

EFV has been widely used as part of triple-combination first-line therapy for over 18 years [156] and well incorporated in LMICs' national guidelines [157, 158]. It has a high virological efficacy, [159], available as a FDC at the dose of 600mg once-daily, and is compatible with TB treatment [160]. Recent data were reassuring regarding potential teratogenicity [161] and its safety profile is better compared to the widely used NNRTI alternative, nevirapine [162].

EFV, the commonly used NNRTI in TB-HIV high burden countries is mostly metabolized by hydroxylation to inactive metabolites 8-hydroxy EFV and 7-hydroxy efavirenz involving mainly CYP2B6 [163, 164]. The main metabolite, 8- hydroxyl EFV is further hydroxylated primarily by CYP2B6 to form 8, 14-hydroxy EFV. The

oxidative metabolites undergo conjugation by UDP-glucuronosyltransferase (UGT) pathway and are excreted in the urine as glucuronides[165]. There is a wide inter-individual variability in EFV concentrations[166] that is partially explained by genetic factors as shown by the strong association between *CYP2B6* 516G>T single nucleotide polymorphism (SNP) and EFV exposure [167]. The *CYP2B6* 516G>T is a common polymorphism that has been consistently associated with reduced enzyme activity, higher EFV exposure and increased toxicity [163, 167, 168].

1.5.2 Drug interaction between anti-tuberculosis and antiretroviral therapy.

Co-administration of both ART and ATT bear complex interactions especially with R which is a very potent inducer of many drug-metabolizing enzymes [169]. Specifically, EFV use among patients who are concurrently on R-based ATT bears three concerns; 1) drug interactions resulting in reduced plasma concentrations of EFV; 2) Overlapping toxicities of ATT and ART e.g DILI and central nervous system (CNS) events, necessitating discontinuation of therapy and increasing the risk of non-adherence; 3) Immunopathological reactions, termed "the immune reconstitution inflammatory syndrome," that can occur when ART is initiated in patients with TB [170].

1.5.2.1 Mechanism of interaction between anti-tuberculosis and antiretroviral drugs

Interactions between R and PIs [171-174], NNRTIs [173], INSTIs [174], and CCR5 receptor antagonists [171] are well documented at least for the newer drugs from clinical pharmacokinetic studies conducted in healthy volunteers. Despite these interactions and their potential clinical consequences, R-sparing regimens were ineffective in HIV-co-infected patients [171].

Rifamycins induce liver enzymes, and of the three rifamycins (rifampicin, rifabutin and rifapentine) R is the most potent inducer of the hepatic cytochrome P450 3A (CYP3A) system and reduces serum concentrations of protease inhibitors such as indinavir, nelfinavir, saquinavir, ritonavir, amprenavir, atazanavir and fosamprenavir. Rifabutin is the least potent inducer of CYP3A and rifapentine falls in between R and rifabutin in its capacity to induce CYP3A [46].

R activates hepatocyte pregnane X receptors, leading to induction of cytochromes. R also induces uridine diphosphate-glucuronosyl-transferases and P-glycoprotein transport, which are involved in the metabolism and transport of other drugs [175-177]. R interacts with numerous drugs metabolized by these and other hepatic enzymes, including warfarin, prednisone, digitoxin, quinidine, ketoconazole, itraconazole, propranolol, clofibrate, sulfonylureas, phenytoin, HIV protease inhibitors, and HIV NNRTIs [178]. Through induction of isoenzyme CYP2B6, R slightly enhances EFV drug metabolism and may lead to sub-therapeutic EFV plasma concentration [179].

The other cornerstone anti-TB drug, H, is metabolized mainly through N-acetyltransferase type 2 (NAT2) and was demonstrated *in vitro* to have an inhibitory effect on several CYP450 enzymes (CYP2C19, CYP1A2, CYP2A6, CYP2C19, and CYP3A4) [180]. H is cleared mostly by the liver, primarily by acetylation by NAT-2. Acetyl-isoniazid is metabolized mainly to mono-acetyl hydrazine (MAH) and to the nontoxic diacetyl hydrazine, as well as other minor metabolites [79]. Inter-individual variation in plasma elimination half-life ($t_{1/2}$), independent of drug dose and concentration, is considerable, with individuals with prolonged $t_{1/2}$ having extended exposure to the drug. Genetic polymorphisms of NAT-2 correlate with fast, slow, and intermediate acetylation phenotypes [79, 82, 181]. Microsomal enzymes (e.g., CYP 2E1) further metabolize H intermediates through phase 1 pathways [82]. From recent studies, there is some growing evidence that the inducing effect of combined R+H is less potent than R alone [182, 183]. Such effect could be different according to patient *CYP2B6 516 G>T* genetic polymorphism.

Co-administration of EFV with R revealed EFV Area Under the Concentration vs time curve (AUC), maximum concentration (C_{max}) and minimum concentration (C_{min}) reduced by 26, 20 and 32% among healthy volunteers, a finding that led to the FDA recommendation of an increase in efavirenz dosing to 800 mg once a day when combined with TB drugs [179, 184]. Reduction in mid-dose EFV plasma concentrations to below 1000 ng/mL during co-administration with R, have been associated with increased risk of virological failure in HIV-infected patients, while concentrations above 4000 ng/mL have been associated with risk of central nervous system (CNS) side effects [166, 185]. It is important to note that as already mentioned, genetic polymorphism of *CYP2B6* influence greatly the EFV plasma concentrations even without co-administration with ATT. Other factors have also been reported to influence EFV concentrations although to a lesser extent: gender, body weight, having hepatitis B surface antigen, and baseline white blood cell counts [169, 182, 186-188].

R is also known to significantly lower plasma concentrations of DTG, mainly metabolized by UGT, hence may necessitate an increase in DTG dose to 50mg twice-daily. To date, there are very few studies and limited clinical experience with this combination, particularly in TB co-infected patients [189, 190]. More evidence on DTG interaction with R is expected from the ongoing studies [191, 192]. In contrast, R does not affect concentrations of TDF or 3TC, mainly eliminated unchanged through the kidney.

1.5.3 TB-HIV coinfection treatment recommendation

Concomitant use of ART and ATT improves survival rates in HIV-infected individuals [171]. Delaying initiation of ART until ATT is completed significantly increases mortality across the spectrum of immunodeficiency. Clinical trials have shown that early initiation of ART in TB-HIV co- patients reduces mortality in patients with very advanced HIV infection [193, 194]. The WHO currently recommend the use of the same EFV-based ART, with TDF, and either lamivudine (3TC) or emtricitabine (FTC) for treatment of TB-HIV coinfection (TDF + 3TC (or FTC) + EFV). Further recommendations are: 1) ART should be started in all TB patients living with HIV regardless of their CD4 cell count; 2) TB treatment should be initiated first, followed by ART as soon as possible

within the first 8 weeks of treatment. HIV-positive patients with profound immunosuppression (e.g. CD4 counts less than 50 cells/mm³) should receive ART within the first 2 weeks of initiating ATT. On the other-hand, ART should be started in any child with TB disease as soon as possible and within 8 weeks following the initiation of ATT, regardless of the CD4 cell count and clinical stage [47]. Rifabutin is used as a substitute for R in the treatment of active TB in patients receiving HIV-protease inhibitor-based ART [46], as R is contra-indicated, although its cost still limits its usage in many LMICs [195]. If rifabutin is not available, ritonavir boosted lopinavir (LPV/r) can be used for the duration of TB treatment by doubling the standard dose of LPV/r or increasing the boosting dose of ritonavir (r). For children, using a triple NRTI regimen (such as AZT + 3TC + ABC) should also be considered [36].

1.5.4 Key Safety considerations for ART and ATT co-administration

A number of cross-cutting adverse events (AEs) have been reported with co-administration of ATT and ART [196]. See Table 5.

Table 5. Adverse events attributed to anti-tuberculosis and antiretroviral drugs, from Zumla A. et al. 2015 [196]

	Antiretroviral drugs	Adverse event
Isoniazid; linezolid	Didanosine; stavudine	Peripheral neuropathy
Bedaquiline; isoniazid; para-aminosalicylic acid; pyrazinamide; rifampicin	Efavirenz; etravirine; maraviroc; nevirapine; ritonavir and protease inhibitors	Liver dysfunction
Amikacin; amoxicillin and clavulanate; fluoroquinolones; isoniazid; kanamycin; rifampicin; streptomycin; thiacetazone	Abacavir; efavirenz; etravirine; nevirapine	Skin rash
Bedaquiline; pyrazinamide; thiacetazone	--	Arthromyalgia
Amikacin; capreomycin; kanamycin; streptomycin	Indinavir; Tenofovir	Renal dysfunction
Amikacin; capreomycin; kanamycin; streptomycin	--	Vestibular and auditory dysfunction
Amoxicillin and clavulanate; bedaquiline; clarithromycin; dofazimine; ethionamide; fluoroquinolones; linezolid; prothionamide; para-aminosalicylic acid; terizidone; thiacetazone	Didanosine; protease inhibitors; stavudine; zidovudine	Gastrointestinal disorders, nausea, vomiting, diarrhoea, and abdominal pain
Cycloserine; fluoroquinolones; terizidone	Efavirenz	Psychosis

1.5.4.1 CNS toxicity

The main precaution with use of EFV is onset of CNS AEs, which typically resolve after a few weeks, although in some cases, they can persist for months or not resolve at all [197]. Marzolini et al., and Haas et al., reported that EFV CNS adverse events were more common in those patients with higher EFV concentrations [166, 198]. A recent systematic review comparing the risk of discontinuation due to AEs associated with EFV compared to

other ARV drugs in ART found that EFV was well tolerated, with over 90% of patients remaining on an EFV-based ART after an average follow-up time of 78 weeks. While the relative risk of discontinuation was higher for EFV compared to most other first-line options, absolute differences were less than 5%, and there was no difference in the risk of severe clinical AEs. The rate of suicidal ideation was low (0.6%), and no suicides were reported [199]. In another study in South eastern United States, significant differences with regard to EFV discontinuation due to CNS AEs across polymorphisms in CYP2B6 and CYP2A6 defined EFV metabolizer categories (Hazard ratio= 4.9; 95% CI:1.9–12.4; P=0.001 for slow versus extensive metabolizers) were reported. Significant racial differences were noted (Hazard ratio in Whites was 6.5; 95% CI:2.3–18.8, P=0.001 and in Blacks 2.6; 95% CI: 0.5–14.1,P=0.27), with the positive predictive value of slow metabolizer genotypes for EFV discontinuation of 27% in Whites and 11% in Blacks [200]. In children, CNS AEs demand closer monitoring, as characterizing symptoms in younger children may be more difficult.

A randomized trial comparing standard-dose EFV at 600 mg/day with the reduced dose of 400 mg/day in non-pregnant adults found that fewer EFV-related AEs were reported with the lower dose, including fewer CNS AEs, a finding that informs the new recommendation to use the lower dose as part of first-line ART [201]. Despite concerns about the potential risk of teratogenicity associated with the use of EFV during pregnancy, an update of a systematic review in 2014 found no overall increase in the incidence of birth defects with first-trimester EFV exposure compared with other ARV drugs [202].

1.5.4.2 Drug-induced liver injury during ART and ATT co-administration

Recent studies have noted a higher incidence of DILI among TB-HIV co-infected patients treated with both ART and ATT (30%) as compared with HIV patients with ART only (15.7%)[169]. The independent role of HIV infection alone on onset of TB DILI is difficult to assess, but appears to be slight [129, 203].

1.5.4.3 TB associated Immune reconstitution inflammatory syndrome (TB-IRIS)

Case definitions

IRIS, is defined as a transient worsening or exacerbation of symptoms and lesions during therapy documented by clinical examination and radiological investigations[204]. Tuberculosis-associated IRIS can present as one of 2 syndromes: (1) Paradoxical TB-associated IRIS), or 2) Unmasking TB-associated IRIS (see Figure 7).

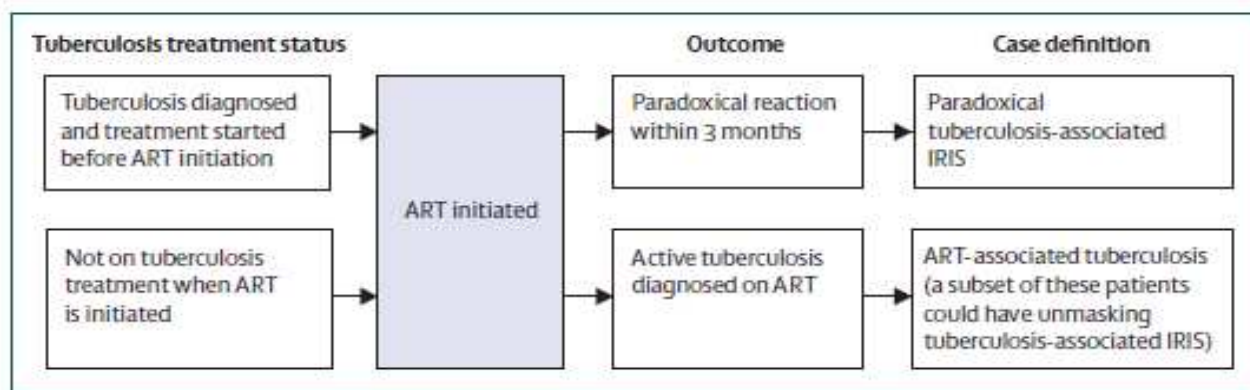


Figure 7. Schematic representation showing the different forms of tuberculosis-associated IRIS and ART-associated tuberculosis, from Meintjes G., et al.2008 [205]

Paradoxical worsening of TB after initiation of ART has been reported in about 2–23% [206, 207] of treated TB patients without HIV infection; it occurs more frequently in patients with extra-pulmonary and disseminated TB. In HIV-seronegative patients with paradoxical reactions, there was a lower baseline absolute lymphocyte count, and a greater increase in lymphocytes during a paradoxical response [207]. The incidence of TB IRIS among HIV-positive patients with active TB treated with ART varies between 11% and 45% [204]. In patients taking ART, IRIS against TB antigens probably account for one third of all HIV-related IRIS events [208], particularly in developing countries where HIV and TB co-infection is very common. The data for Africa was limited by the lack of consensus on the case definition for TB-IRIS that could be used in LMICs [204]. In this regard, a group of scientists met and reviewed the definition of different forms of TB-IRIS with an intent to ensure consistence and standardization in how researchers report [205]. The following definitions were agreed upon for use in LMICs and perhaps internationally.

The proposed case definition for paradoxical TB-IRIS has 3 components[205]:

(A) Antecedent requirements: Both of the two following requirements must be met; 1) Diagnosis of TB should have been made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive PTB, smear-negative PTB, or extra-pulmonary TB; 2) the patient's condition should have stabilized or improved on appropriate ART before ART initiation eg, cessation of night sweats, fevers, cough, weight loss.

(B) Clinical criteria: The onset of TB-IRIS manifestations should be within 3 months of ART initiation, re-initiation, or regimen change because of treatment failure. Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria: 1) New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement e.g., tuberculous arthritis; 2) New or worsening radiological features of TB; 3) New or worsening CNS TB; 4) New or worsening serositis.

Minor criteria: 1) New or worsening constitutional symptoms such as fever, night sweats, or weight loss; 2) New or worsening respiratory symptoms such as cough, dyspnoea, or stridor; 3) New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible, that is: 1) Failure of tuberculosis treatment because of tuberculosis drug resistance; 2) Poor adherence to tuberculosis treatment; 3) Another opportunistic infection or neoplasm; 4) Drug toxicity or reaction.

The following provisional case definitions for unmasking tuberculosis-associated IRIS has been suggested [205]: Patient is not receiving ATT when ART is initiated and then presents with active TB within 3 months of starting ART AND one of the following criteria must be met: 1) Heightened intensity of clinical manifestations, particularly if there is evidence of a marked inflammatory component to the presentation. Examples include TB lymphadenitis or TB abscesses with prominent acute inflammatory features, presentation with PTB that is complicated by respiratory failure due to adult respiratory distress syndrome, and those who present with a marked systemic inflammatory syndrome related to TB; 2) Once established on ATT, a clinical course that is complicated by a paradoxical reaction.

Pathogenesis of TB IRIS

The pathogenesis of TB-IRIS is generally poorly understood. It is generally thought to be the restoration of the immune responses to antigens (viable or not) producing exuberant inflammatory reactions. Given the fact that shortly after the initiation of highly active ART (HAART) there is a rapid recirculation of memory cells [209], these memory cells may play a role in the development of IRIS. Such reactions do not require the presence of viable organisms because they can occur after successful TB treatment. AFB identified in IRIS events are often not found to be viable. In certain TB-IRIS patients, rapid restoration of the cutaneous tuberculin reaction has been observed [210]. The immune-pathogenic mechanisms causing IRIS seem to be determined by the pathogen. For example, mycobacterial IRIS is associated with delayed-type hypersensitivity responses to mycobacterial antigens, whereas there is evidence of a CD8 T-cell response in herpes virus IRIS. Furthermore, the association of different cytokine gene polymorphisms with mycobacterial or herpes virus IRIS provides evidence for differences in pathogenic mechanisms as well as in genetic susceptibility to IRIS [211].

Risk factors for TB-IRIS include: starting ART within 6 weeks of starting TB treatment; extra-pulmonary or disseminated disease; a low CD4 lymphocyte count and a high viral load at the start of ART; a good immunological and virological response during HAART; black ethnic origin, and purified protein derivative conversion [204, 205].

1.6 Strategies for TB control

Currently, there are two key global strategies that constitute clear TB control strategies: END-TB Strategy; and Sustainable Development Goals (SDGs). The End-TB strategy was launched in 2015 to replace the STOP-TB strategy, with a goal of ensuring a world free of TB, Zero deaths, disease and suffering due to TB and a complete end to the global TB epidemic [212]. This strategy is synonymous with the third goal of the United Nations

Sustainable Development Goals (SDG) adopted by countries as on 25th September 2015 and a replacement for the Millennium Development Goals, that include reduction in TB incidence rate, number of deaths and TB-affected families facing catastrophic costs due to TB by 90%, 80% and 0% by 2030 respectively [213]. The End-TB strategy has milestones of reduction in TB incidence rate, number of deaths and TB-affected families facing catastrophic costs due to TB by 95%, 90% and 0% by 2035 respectively [213]. In order to attain to the 2035 targets, the End TB strategy stipulates the need to ensure availability of new tools from the research pipeline, in particular: 1) Better diagnostics, including new point-of-care tests; 2) Safer, easier and shorter treatment regimens; 3) Safer and more effective treatment for LTBI; 4) Effective pre- and post-exposure vaccines.

With reference to development of new TB diagnostic tests, the 2017 pipeline report points out important progress made, which include: the launch of a more sensitive Xpert MTB/RIF Ultra assay for diagnosing TB and detecting R resistance; further evidence of the effect of urine LAM testing for people with HIV; a sputum LAM assay that could revolutionize treatment monitoring; and several rapid tests inching towards launch that could bring TB and R resistance testing closer to patients (GeneXpert Omni, TrueNat) or expand susceptibility testing to more drugs (Xpert XDR, RealTime MTB RIF/INH, and FluoroType MDR) [214]. More so, research towards discovering safe, effective and shorter treatments should be intensified. The projected impact of these interventions on global TB incidence rates so as to attain the 2035 target are demonstrated in the figure 8.

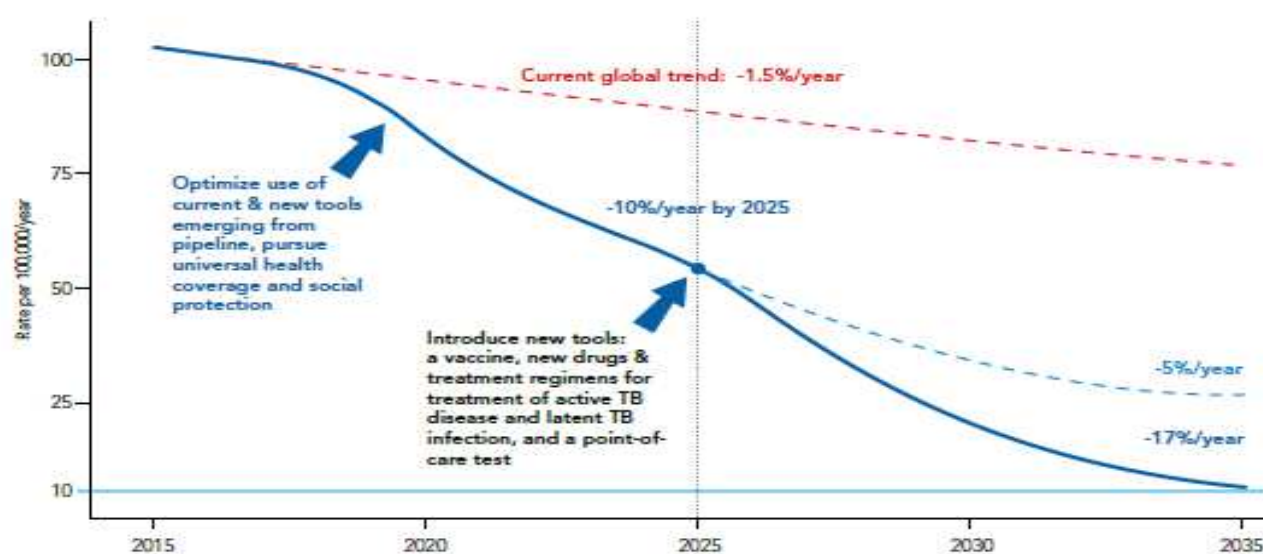


Figure 8. Desired decline in global TB incidence rates to reach the 2035 targets, from WHO: The End TB strategy. URL: http://www.who.int/tb/End_TB_brochure.pdf [212]

The following global priority indicators and targets for monitoring the implementation of the End TB strategy were recommended to guide and indicate progress at a country level: 1) Treatment coverage ($\geq 90\%$); 2) TB treatment success rate ($\geq 90\%$); 3) Preventive treatment coverage ($\geq 90\%$); 4) TB affected households facing catastrophic costs; 5) Uptake of new diagnostics and new drugs ($\geq 90\%$) [212]. All these strategies cannot stand in isolation without the already enforced strategies like the prevention and treatment of TB in people living with HIV using the “Three I’s”, that is, Isoniazid preventive treatment, Intensified case finding for active TB, and TB

Infection control, which are key public health strategies to decrease the impact of TB on people living with HIV [215]. Also, to be successful in achieving TB elimination, there should be a strong funding commitment by funders and governments for further investment in TB prevention, diagnosis, and treatments that are safe and shorter [216].

In a nutshell, it is worth noting that the current TB elimination strategies, are hinged on the historical approaches that are based on understanding the key drivers of the TB epidemiology so as to achieve TB control. The 4 main TB epidemiological and control scenarios were originally described by Karel Styblo (1921–1998) with his famous model [217, 218] but recently simplified by Rosella Centis [219]. These scenarios are discussed below: 1) In the absence of interventions, an infectious (e.g., a sputum smear-positive) patient infects 10 persons a year for 2 years before healing spontaneously or dying. So, given a 10% lifetime probability of acquiring disease given the infection and the 50% probability of becoming sputum smear-positive, a case generates another case, contributing to keeping the epidemic level stable; This forms the basis why TB services (that is, diagnostic, preventive and curative services) should be accessible to all people irrespective of their socio-economic status, something that has been described as an ethical and human right approach that should guide the pursuit of END-TB targets[25] 2) With rapid/early diagnosis and effective treatment, only five individuals are infected and, as a result, only 0.25 cases are generated by the original infectious case. This would mean that we need four sputum smear-positive cases to generate a new infectious one. This shows that an adequate implementation of the directly observed treatment short course strategy, could have a substantial impact on the epidemic in the absence of factors working in an opposite direction, like HIV or any other factor increasing the probability of acquiring TB disease given infection (e.g., diabetes, stress as observed in war areas or in migrants/refugees, etc.). This scenario, also strengthens the case for more research towards developing more sensitive POC diagnostics, shorter and effective TB treatments but also the need to not only emphasize coverage but also quality of TB services with implementation of a comprehensive care package to TB patients as recently recommended by WHO [47]; 3) If irregularities or interruptions occur during treatment, it is sufficient to prevent death and prolong the infectious period, so that one case can produce up to 1.5 new infectious cases, which in turn will gradually result in a more severe drug resistance pattern. This scenario could risk a global drug-susceptible TB epidemic replacement with drug-resistant TB, a phenomenon that has been recently reported [220]. Ensuring reliable TB drug supplies by governments is a key action needed for TB control; 4) Under the hypothetical scenario of a population with TB co-infected by HIV not undergoing ART, one infectious case generates another two cases, again contributing to reducing the speed of decline in TB incidence. This scenario emphasizes that in addition to pursuing the End-TB targets, there should be a concurrent pursuit of the 90-90-90 targets defined by the Joint United Nations Programme on HIV/AIDS (UNAIDS) which include: 90% of persons living with HIV must be diagnosed, 90% of those diagnosed should be on sustained therapy and 90% of those on therapy should have an undetectable viral load [221]. These HIV control targets continue to be monitored by employing the cascade of care analysis to identify and address major gaps in the continuum and quality of HIV care, from diagnosis to successful virological suppression after antiretroviral therapy [26, 222]. Actually, a recent global HIV care cascade showed that a considerable number of HIV positive patients are still not diagnosed (54%), not treated though diagnosed (41%), and with no viral load

(VL) suppression despite being on ART (32%) between 2014/2015 [26]. Meaning that this could still affect TB control. Inadequate TB control has already been highlighted as a barrier to achievement of the 90–90–90 targets in children and so calls for specific attention to TB care in HIV-infected children [223].

CHAPTER 2

JUSTIFICATION, OBJECTIVES AND METHODS

2.1 Justification

2.1.1 Justification for a shorter treatment regimen

Shorter, effective and safe treatments for DS-TB are needed to facilitate the fight against TB especially if the End-TB 2035 target of $\geq 90\%$ treatment success rate at country level and overall reduction in TB deaths by 95% compared to 2015 is to be attained [212]. An effective TB treatment should : cure the patient and restore quality of life and productivity; prevent death from active TB or its late effects; prevent relapse of TB; reduce transmission of TB to others; and prevent the development and transmission of drug resistance [40]. However, even with use of an effective treatment, treatment success can only be assured once there is good adherence. Today, good TB treatment adherence is still being limited by the long duration of TB treatment, with the current treatment of tuberculosis involving taking drugs daily for 6 months[47]. There is general agreement that shortening treatment from 6 to 4 months or less would be of great benefit [224]. A shorter treatment would have several advantages, including shorter exposure to toxic drugs and better adherence, thereby increasing cure rates.

A long duration of therapy is also fraught with the risk of non-adherence, which in turn increases the likelihood of acquiring drug resistance and continuing transmission of disease within the community [225, 226]. Cost-effectiveness studies in South Africa and Brazil have also projected that introducing a four-month regimen could result in significant cost savings for both the health service and patients [227, 228]. While there is hope that shortening treatment might be achieved with the development of new anti-tuberculosis drugs, the prospects for the new drugs currently in clinical development (which are at the beginning of phase II) suggest that it is likely to be many years before any treatment shortening to less than 6 months can be obtained because of the limited number of drugs in the developmental pipeline [229] and the time required to conduct the necessary phase II and phase III trials (see Figure 9).

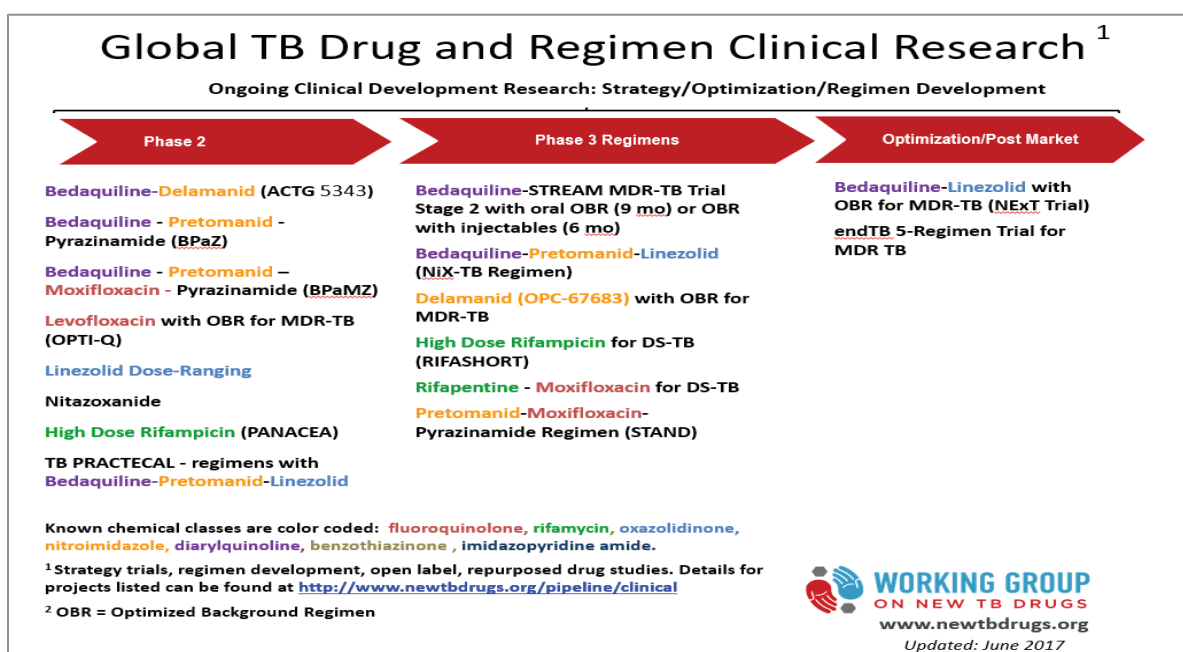


Figure 9. List of on-going clinical trials, from Working group on new TB drugs [229]

A major set-back in TB treatment shortening followed the fact that the most promising and advanced drug in the pipeline, moxifloxacin, despite having demonstrated an accelerated elimination of tubercle bacilli from sputum in Phase II trials [230, 231] and in the mouse model [232], together with other quinolones have failed in their objective to shorten treatment as per the three recently published Phase III clinical trials [233-235]. It is therefore essential to see whether improved results, and in particular treatment shortening, can be obtained with any of the existing drugs.

2.1.2 Justification for high-dose rifampicin

Shorter regimens with use of the same standard doses of all drugs of the current 6-month regimen (2HRZE/4HR) [50, 236], had unacceptably high relapse rates [237] even when used specifically among patients with non-cavitary TB and culture conversion after 2 months [238].

A compelling case for an increase in dose size of R has been made by Peloquin on a basis of the observed dramatic acceleration of bacterial killing and no colonies observed by 3 and 4 months in infected Mice, when the R dose was increased 2-fold to 25 mg/kg [239], with a similar trend also observed in experiments with the guinea pig [240]. Although both R and Z are capable of killing persisting bacilli [237, 241], increase in the dose size of Z is almost certain to result in unacceptable levels of liver toxicity [242] while an increase in R dose offers the possibility of treatment shortening. The choice of the current dose size of R of about 10 mg/kg was empirical, based on the dose size just sufficient to be effective [243].

Additional evidence on potential of using high R doses derives from studies of the early bactericidal activity (EBA) of drugs. As already described by Phillip[244], clinical trial phase 2 development for TB drugs typically follows a sequential two-step or three-step pathway, with the first step being a 14-day monotherapy trial (a new drug can only be given for a maximum of 14 days as monotherapy owing to the risk of acquiring drug resistance), which is

used to demonstrate the microbiological activity of the agent alone at different doses. This is often followed by a 7-day to 14-day study of drugs in combination. These trials are followed by longer-duration studies of drugs in combination (traditionally 8 weeks). The 14-day studies are considered phase IIA (early bactericidal activity trial) and 8-week studies as phase IIB, and in each case all patients are given standard treatment after completing the experimental therapy so that the total duration of treatment is not less than the standard 6 months. The primary endpoints



of such studies are microbiological intermediate endpoints, that is, measuring the speed at which bacilli are killed using serially collected sputum during the first days and weeks of treatment, and include the decline in the number of colony-forming units growing on solid culture media, increase in time to positivity in liquid culture media, time

to stable negative culture conversion, or proportion of participants with negative sputum cultures at a specific time during treatment. In this regard, EBA studies have shown the accelerated bactericidal activity of R when used at higher doses by the fall in colony forming counts in the sputum caused by the drug either singly or in combination. A comparison of the therapeutic margins for H and R shows that the usual 300 mg dose of H is about 20 times the minimal effective dose size (when the EBA = 0) whereas, the corresponding therapeutic margin for R is only four [245]. In view of the variability in the absorption of R by individual patients, it is not surprising that a proportion of these will have inadequate dosage. In an earlier EBA study [245] and in a recent study at Stellenbosch University, S. Africa [246], an increase in R dose size to 20 mg/kg (1200 mg) resulted in a substantial linear increase in EBA (see Figure 10). While this is probably a measure of bactericidal activity against multiplying bacilli in cavities, it is likely to result in increased sterilizing activity against more dormant bacilli.

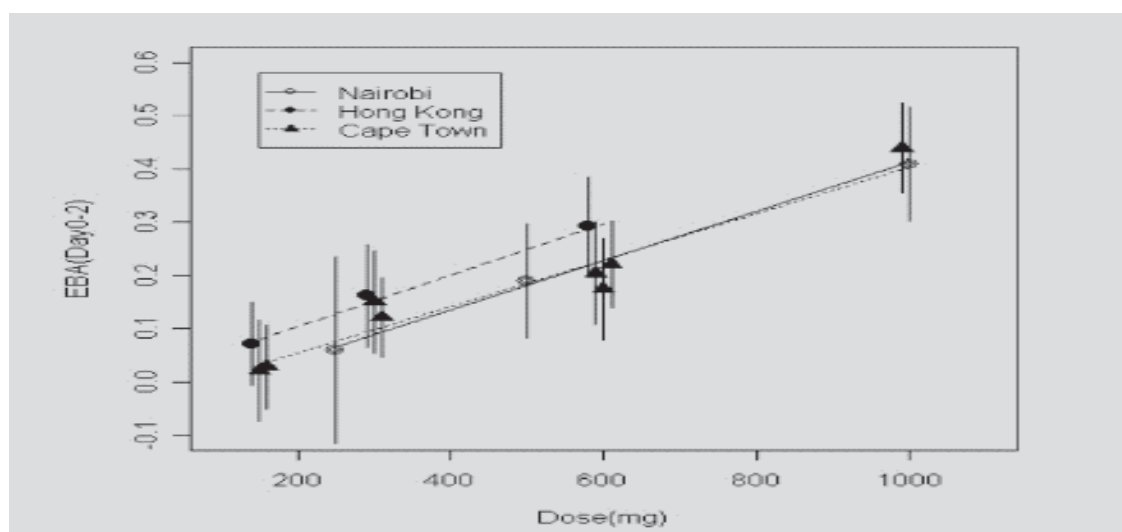


Figure 10. EBA over 2 days related to rifampicin dose size, from Diacon A. H, et al.2007 [246]

Two trials, investigating the pharmacokinetics of R have been carried out in Indonesia. One was an open label phase II randomized clinical trial (RCT) [247] and the second a double blind RCT [248] in which R was given at doses of 450mg and 600 mg. In both trials the authors report a more than dose-proportional increase in the mean AUC-24 h and C_{max} of R without affecting the incidence of serious adverse events (SAE). Little is known about the PK of dose sizes above 600 mg. It has been reported that the half-life following a single dose increased from 2.6 hr for a dose of 300 mg to 5.1 hr for a dose of 900 mg, but this difference was smaller after 6 or more doses had been given [249]. Also, using population PK, Goutelle et al. modeled concentrations of R in plasma and airway epithelial lining fluid and used the model to assess the ability of R to achieve predefined targets for bacterial killing. The authors found that 1200 mg R was associated with better results at achieving target values than 600 mg R [250]. However earlier studies have shown a non-linear increase in PK parameters with increasing R doses [246, 251].

Although the evidence of the efficacy of higher doses of R is very persuasive, very little is known about the potential toxicity of higher doses of R. No AEs were encountered in the treatment of 48 leishmaniasis patients given 1200 mg daily for 4 weeks [252, 253]. A daily R dose of 900 mg daily for 3 weeks has also been used without AEs in 239 brucellosis patients [254] and in staphylococcal infections [255]. In a trial of the treatment of PTB, a

R dose of 1200 mg was given daily or intermittently to 91 patients and no SAEs were reported [256]. A R dose as high as 1800 mg was also given, in a PK study, but intermittently [257] with no SAE reported. Another study on the potential toxicity of increased size doses of another rifamycin, rifapentine, has been carried out within a CDC study [258] with 150 patients. Treatment was discontinued in 6%, 4% and 6% in the 600, 900 and 1200 mg treatment arms respectively. Only one discontinuation, in the 1200mg arm, was due to an adverse event (AE) possibly associated with the study therapy.

In summary SAEs due to R appear to be sporadic and not dose related. Nevertheless the data are limited and the possibility of dose related AEs cannot be excluded at this time and merits further systematic investigation. More so, there is no data on the effect of high dose R on EFV during co-administration among TB-HIV infected patients.

As already depicted, in the failure of phase III fluoroquinolone trials [233-235], month-2 culture conversion is not a good surrogate marker and its use within high R dose trials need to be evaluated.

We hypothesize that optimization of the current treatment regimen especially with use of high-dose R, might result in a shorter TB treatment duration (3-4 months). An exploration of safety of R use at higher dose sizes and its impact on PK profile of EFV, and resultant impact of ART efficacy among either HIV negative or HIV/TB co-infected patients within the global TB and HIV high-burden countries, offers the prospect of a substantial improvement in TB control efforts by shortening of the treatment period. This study also reports results from an exploration for potential surrogate markers for TB chemotherapy efficacy.

2.2 Objectives

2.2.1 General objective

To establish the safety and efficacy of high-dose R, and also its interaction and impact on ART efficacy when co-administered with EFV among TB patients with or without HIV in TB/HIV high-burden settings.

2.2.2 Specific objectives

- 1) To assess the safety of using high-dose R in HIV negative pulmonary tuberculosis (PTB) patients.
- 2) To describe the pharmacokinetics of EFV during co-administration with ATT in high HIV and TB burden countries.
- 3) To assess the safety of high-dose R and EFV during co-administration among HIV-positive TB patients
- 4) To determine the effect of high-dose R on the EFV plasma concentration and effectiveness in HIV/TB co-infected patients.
- 5) To establish the predictors of week-8 culture non-conversion and the effect of culture media on early treatment efficacy estimation in TB chemotherapeutic trials.

2.3 Materials and Methods

2.3.1 Site and population

2.3.1.1 Mbarara

The studies were implemented in Mbarara (Uganda), at the Epicentre Mbarara Research Centre, with Mbarara Regional Referral Hospital (MRRH) and surrounding health facilities as the main sources of TB patients.

Uganda had an estimated population of 39 million people by end of 2015. Uganda is still among the 30 TB-HIV high-burden countries globally [3]. However, the country has recorded a twist in the above statistics following the recent country-wide TB survey by ministry of health, whose results were availed in August 2017[259] and with findings that may influence its position in the global rankings of high-burden countries. The survey reported an overall TB prevalence of 253 per 100,000 people (159 per 100,000 in 2015) and TB incidence of 234 per 100,000 people (161 per 100,000 in 2015). The HIV prevalence among TB patients was 27%. From the same survey, prevalence of TB among men is 4 times higher than in women (734 versus 178 per 100,000), and the burden of TB highest among people aged 35-44, whereas age group 15-24 has the highest missed cases. The prevalence of TB was highest in urban areas than rural (504 versus 370 per 100,000) [259]. Based on 2015 statistics, the country had an overall incidence of MDR/RR-TB of 4.9 (2.6-7.2) per 100 000 population. MDR/RR-TB was detected in 1.6% (0.78-2.4) versus 12% (5.9-18) of new cases and previously treated cases respectively. No XDR-TB case was notified by 2015. Treatment success rate for all forms of TB is about 75%. The country implemented the 6-month TB treatment in 2014. The National TB budget is mainly funded by international donors (78%), with a 19% funding gap [16].

Mbarara town, in Mbarara district is the largest urban center and main business capital of Western Uganda, a region commonly referred to as *"the land of milk and honey"*, given its historical and present legacy as a cattle-keeping area, although its biggest population also depend on subsistence farming. In the 2014 Census, Mbarara district had a population of 472,629, of which 195,158 stay in urban setting[260]. On 7th August 2015 it was granted city status. Built on an elevated basin that forms part of the African Rift Valley and nestled amongst hills and shallow valleys, Mbarara was almost destroyed in the internal Ugandan conflicts of 1972, 1979 and again in 1985 before emerging into the vibrant and bustling town. Its position as a gateway to the Great Lakes Region. Mbarara is 180 miles south-west of Kampala.



2.3.1.2 Epicentre

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and vaccination studies, to mention but a few. The primary role of Epicentre in Mbarara is to conduct high standard clinical research, with important Good Clinical Practice (GCP) requirements, on critical public health burdens in Uganda and Africa. To reach this objective, Epicentre collaborates with the MUST, MRRH.



Picture: EMRC building

A full-time research team is now based at the Epicentre Mbarara Research centre (EMRC). In 2009, Epicentre started a research program on TB in collaboration with the MUST and MRRH. The Epicentre laboratory offer microscopy, XpertMTB/RIF, GenoType MTBDRplus 2.0 (Hain Lifescience, Nehren), solid and liquid culture based methods for TB.

The Laboratory receives technical support from Institute of Tropical Medicine (ITM) Antwerp, and participates in external quality control with NHLS in South Africa.

The research targeted majorly adults with DS-TB with or without HIV co-infection. Detailed eligibility criteria are specified under each study sections.

2.3.2 Study designs

We conducted 2 open-label phase II randomized clinical trials (1 single site and 1 International multi-centre trial), 1 nested prospective cohort of an open-label phase II, International multicentric RCT, and 1 systematic review (see Table 6).

Table 6. Summary of the different studies conducted per study objective

Objective	Chapter number	Thematic area addressed	Study/sites	Study design	Sponsor
1	3.1	Safety of high- R dose in HIV negative pulmonary tuberculosis (PTB) patients	RIFATOX TRIAL ISRCTN55670677, Mbarara in Uganda, Nepal and Kathumandhu	Open-label, phase II, International Randomized Controlled Clinical trial	St. Georges University of London
2	3.2	Pharmacokinetics of efavirenz during co-administration with anti-tuberculosis treatment in high HIV and tuberculosis burden countries	SYSTEMATIC REVIEW, Global TB- HIV high-burden countries	Systematic review	NA
3 and 4	3.3	Safety and pharmacokinetics of high-dose rifampicin and efavirenz during co-administration among HIV/TB co-infected patients	RIFAVIRENZ- ANRS12292 Trial (NCT01986543), Uganda.	Phase-2, randomized, open-label therapeutic trial	ANRS
5	3.4	Challenges related to early surrogate markers of TB chemotherapy efficacy within phase 2 trials	RIFATOX TRIAL (ISRCTN55670677), Uganda	Nested prospective cohort of RIFATOX Trial	NA

CHAPTER 3

STUDIES

Chapter 3.1

Safety of high-dose rifampicin among HIV-negative TB patients

Published article related to this chapter

Amina Jindani, Gabor Borgulya, Ilona Westermann de Patiño, Tomás Gonzales, Rosario Alvarez de Fernandes, Bhabana Shrestha, **Daniel Atwine**, Maryline Bonnet, Marcos Burgos, Faisal Dubash, Nanita Patel, Anna M. Checkley, Thomas S. Harrison and Denny Mitchison. (2016): A randomised Phase II trial to evaluate the toxicity of high-dose rifampicin to treat pulmonary tuberculosis. *The International Journal of Tuberc Lung Dis*, 2016. 20 (6): 832-838

(Full Published Manuscript attached at the end of this chapter)

Presentations related to this chapter

Atwine D. et al. Is it safe to double the dose of rifampicin to shorten tuberculosis treatment duration? In. Epicentre Scientific Conference, Kampala, 5th July 2017. URL: <http://epicentre.msf.org/en/uganda-scientific-day-2017> (Oral presentation).

Atwine D. et al. An International Multicentre Controlled Clinical Trial to Evaluate the Toxicity of High Dose Rifampicin in the Treatment of Pulmonary Tuberculosis (RIFATOX). In. Annual Grande doctors' Conference, 11-13th August 2016, Kampala, Uganda (Oral presentation).

Atwine D. et al. An International Multicentre Controlled Clinical Trial to Evaluate the Toxicity of High Dose Rifampicin in the Treatment of Pulmonary Tuberculosis (RIFATOX). In. MSF at 30 years in Uganda Exhibition, 2015, Kampala, Uganda (Poster presentation).

3.1.1 Justification and objectives

The reluctance to investigate higher doses of R is due to the fear of serious hepatotoxicity. Little is known about the potential toxicity of a higher dose of R, especially in combination with other potentially liver toxic drugs like H and Z. Available data is derived from non-comparative cohort studies [253-255, 257, 261, 262].

Thus, the objective was to assess the safety of higher doses of R at 15 or 20 mg/kg/daily, for the first 16 weeks, versus the standard 10 mg/kg dose, when given as part of the otherwise standard 26 week treatment, to HIV-negative patients with newly diagnosed, smear-positive PTB. A secondary objective was to determine if the increased dose resulted in more rapid lung sterilization assessed by culture conversion rate after 8 weeks' treatment.

3.1.2 Methods, Results and Conclusion

This was a phase II, multicentre open-label randomized controlled trial.

Newly diagnosed, microscopy positive and HIV negative adults in Santa Cruz, Bolivia; Kathmandu, Nepal; and Mbarara in Uganda, were randomized to either **Control Regimen (R10)**: Eight weeks of daily HEZ and R at the usual dose of 10 mg/kg, followed by 18 weeks of HR; **Study Regimen 1 (R15)**: same regimen using R at 15mg/kg for the first 16 weeks ; and **Study Regimen 2 (R20)** using the same regimen with R at 20mg/kg .

The primary end-point of the trial was the occurrence of any grade 3 or 4 adverse event (AE) during the first 16 weeks of chemotherapy, with a focus on the risk of drug related hepatotoxicity.

Patients took their medication under drug observation. Clinical and laboratory monitoring including liver function test were carried out at 2, 4, 8, 12 and 16 weeks after starting treatment. Sputum microscopy and culture for *MTB* was done at baseline and 8 weeks, using LJ medium.

A total of 300 patients were enrolled (100 in Mbarara) with similar baseline characteristics in the 3 groups. Overall, AEs classified as serious, occurred in 3 patients, with one of the 3 patients having drug related hepatotoxicity (R15 group). ALT levels increased as the dose of rifampicin increased but there was no difference in grade 3 ALT increase between the 3 study regimens: 1.0% (1/100), 2.0% (2/100) and 4.0% (4/100), in the R10, R15 and R20 groups, respectively (trend test $p=0.15$). No grade 4 increases in ALT were reported in this trial.

Culture conversion rates were 75% (69/92), 82.5% (66/80) and 83.1% (76/91) in the R10, R15 and R20 groups, respectively (trend test $p=0.16$).

In conclusion, this study demonstrates the safety of an increase in R dosage from the standard 10 mg/kg to 15 mg/kg and 20 mg/kg in HIV-negative PTB patients. These results suggest that a daily dose of 20 mg/kg R for 4 months is safe enough to be taken forward to larger Phase III trials. The results are consistent with those reported by the PanACEA MAMS-TB Trial [263] in groups of 62 patients each, treated for 12 weeks with rifampicin at up

to 35 mg/kg/day. Grade 3, or higher, AE were reported in 12% R10, 12% R20, and 14% of the R35 (35 mg/kg/day) group.

Increasing R doses also showed a non-significant increasing trend in culture conversion at 8 weeks. However, it is important to note that the trial was not powered to determine an effect on culture conversion.

3.1.3 Involvement in this work

I was both the principal investigator and the clinical investigator for the Mbarara site. As principal investigator participated in the review of the trial documents and development of site specific documents for the Mbarara site; implementation of the trial in the Mbarara site; coordination of the trial activities; and overseeing the communication with the Ugandan regulatory authorities. As clinical investigator, I was involved in patient recruitment, eligibility checking, randomization, patients' clinical assessment and management, patient follow-up, AE detection and reporting. Since all trial sites data management processes were handled at Mbarara site, I also ensured that proper data management procedures are followed. Overall, I ensured that the conduct of the trial was in accordance to good clinical practice (GCP) guidelines. Furthermore, I participated in the, review of the manuscript and dissemination of the results of the trial in national conferences, that is: Epicentre Scientific Conference, Kampala, 5th July 2017 (URL: <http://epicentre.msf.org/en/uganda-scientific-day-2017>), the Annual Grande doctors' Conference, 11-13th August 2016, Kampala, Uganda and at MSF at 30 years in Uganda exhibition, 2015, Kampala, Uganda. (Further details included in PHD Portfolio at the end of the book)

A randomised Phase II trial to evaluate the toxicity of high-dose rifampicin to treat pulmonary tuberculosis

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SUMMARY

SETTING: Randomised Phase IIB clinical trial.

OBJECTIVES: To assess whether increasing the dose of rifampicin (RMP) from 10 mg/kg to 15 or 20 mg/kg results in an increase in grade 3 or 4 hepatic adverse events and/or serious adverse events (SAE).

METHODS: Three hundred human immunodeficiency virus negative patients with newly diagnosed microscopy-positive pulmonary tuberculosis (TB) were randomly assigned to one of three regimens: 1) the control regimen (R10), comprising daily ethambutol (EMB), isoniazid (INH), RMP and pyrazinamide for 8 weeks, followed by INH and RMP daily for 18 weeks; 2) Study Regimen 1 (R15), as above, with the RMP dose increased to 15 mg/kg body weight daily for the first 16 weeks; and 3) Study Regimen 2 (R20), as above, with RMP increased to 20

mg/kg. Serum alanine transferase (ALT) levels were measured at regular intervals.

RESULTS: There were seven grade 3 increases in ALT levels, 1/100 (1%) among R10 arm patients, 2/100 (2%) in the R15 arm and 4/100 (4%) in the R20 arm (trend test $P = 0.15$). One (R15) patient developed jaundice, requiring treatment modification. There were no grade 4 ALT increases. There was a non-significant increase in culture negativity at 8 weeks with increasing RMP dosage: 75% (69/92) in R10, 82.5% (66/80) in R15 and 83.1% (76/91) R20 patients ($P = 0.16$).

CONCLUSIONS: No significant increase in adverse events occurred when the RMP dose was increased from 10 mg/kg to 15 mg/kg or 20 mg/kg.

KEY WORDS: tuberculosis; treatment; rifampicin; toxicity.

THE INTRODUCTION OF NEW DRUGS for routine treatment of drug-susceptible tuberculosis (TB) will take many years. Meanwhile, there may be an opportunity to improve treatment by the better use of available drugs, helped in part by the use of systems for grading adverse events.^{1,2} Rifampicin (RMP, R) is responsible for killing the majority of the tubercle bacilli in tuberculous lesions.³ When introduced in the 1970s, it was given at the lowest dose with proven efficacy, and this dosage has not been changed since.

The standard 6-month regimen recommended by the World Health Organization (WHO) is highly effective and safe.⁴ However, its efficacy depends on the administration of RMP throughout the 6-month period.^{4,5} In 1981, Wallace Fox wrote, 'It is clear that 6-month regimens are too long. Higher dosage schedules, particularly of RMP, might be given.'⁶ In 2003, Peloquin suggested that the current dose of 10

mg/kg was suboptimal, and recommended that higher doses be investigated.⁷ Higher RMP dosing is supported by animal models and early bactericidal activity data.^{8,9}

The reluctance to investigate higher doses of RMP is due to the fear of serious hepatotoxic adverse effects. Little is known about the potential toxicity of a higher dose of RMP, as most data are derived from non-comparative cohort studies.^{10–15} A systematic review of 14 randomised trials using higher doses of RMP showed that hepatotoxicity was rarely observed.¹⁶ However, the authors state that 'additional data on safety will be needed'.

The objective of the present study was to assess the safety of higher doses of RMP, 15 or 20 mg/kg/daily, vs. the standard 10 mg/kg dose when prescribed for the first 16 weeks of the standard 26-week treatment regimen to human immunodeficiency virus (HIV) non-infected patients with newly diagnosed micros-

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copy-positive pulmonary TB. A secondary objective was to determine if the increased dose resulted in more rapid lung sterilisation, assessed by the culture conversion rate after 8 weeks of treatment. We report the results in 300 patients included in an open-label, randomised clinical trial, the RIFATOX Trial.

STUDY POPULATION AND METHODS

The study protocol was approved by the Oxford Tropical Research Ethics Committee (OxTREC, University of Oxford, Oxford, UK) and the ethics and regulatory review bodies of each participating centre.

Population

Newly diagnosed microscopy-positive adults in Santa Cruz (Bolivia), Kathmandu (Nepal) and Mbarara (Uganda) were invited to participate provided they met the eligibility criteria and provided informed consent. All enrolled patients were tested for hepatitis C antibody and hepatitis B surface antigen.

Eligibility criteria

Eligible patients were aged 18–65 years, had two sputum samples positive for tubercle bacilli on microscopy, had received less than a month of previous anti-tuberculosis chemotherapy and had a reliable and accessible home address.

Patients were excluded if they were critically ill, had extra-pulmonary TB, were alcoholic, were pregnant or had psychiatric illness, blood disorders, diabetes, epilepsy, peripheral neuritis, haemoglobin <7 g/dl, serum alanine transferase (ALT) levels >5 times the upper limit of normal (ULN), creatinine clearance <30 ml/min, and isoniazid (INH, H) or RMP resistance (GenoType® MTBDR_{plus} test [Hain Lifescience, Nehren, Germany] or drug susceptibility testing on culture). HIV-positive patients were excluded because of possible interactions between antiretrovirals (ARVs) and high-dose RMP, leading to the reduction in blood levels of some ARVs.

Randomisation

A randomisation schedule was created by an independent person. Patients were randomised in a 1:1:1 ratio in blocks of nine patients to the three treatment groups, each comprising 100 subjects. Participating centres were supplied with a batch of sealed, serially numbered opaque envelopes, each containing a slip of paper showing the allocated regimen. No attempt was made to conceal the treatment regimen after randomisation from patients, researchers or health care staff. However, the laboratory staff and an independent clinician who would make a clinical assessment of any serious adverse event (SAE) were blinded to the regimen.

Stopping rule

The following stopping rule was applied to the trial: if, at review by the Data and Safety Monitoring Committee, ≥ 5 grade 4 events had occurred in one arm of the study, that arm would be discontinued.

Treatment

Three hundred eligible patients were randomised to the following regimens: 1) the control regimen (R10), comprising 8 weeks of daily ethambutol (EMB, E), INH, RMP and pyrazinamide (PZA, Z), followed by 18 weeks of daily INH and RMP (2EHRZ/4HR); 2) Study Regimen 1 (R15), comprising 8 weeks of daily EHRZ, followed by 18 weeks of daily HR, supplemented by 300 mg RMP for the first 16 weeks (2EHR₁₅Z/2HR₁₅/2HR); and 3) Study Regimen 2 (R20), comprising 8 weeks of daily EHRZ, followed by 18 weeks of daily HR, supplemented by 450 mg or 600 mg of RMP for the first 16 weeks (2EHR₂₀Z/2HR₂₀/2HR).^{*} The drugs given were those used in routine practice in Kathmandu and Santa Cruz. In Kathmandu, the drug formulations are EHRZ (275/75/150/400 mg) and HR (150/300 mg), and are supplied by the Global Drug Facility. RMP 150/300 mg was manufactured by Ranbaxy, Mumbai, India. In Santa Cruz, the formulations are E 400 mg (MacLeods, Mumbai, India), HR 150/300 mg, Z 400 mg and R 300 mg (Lupin, Mumbai, India). In Mbarara, drugs were imported for the purposes of the trial (EHRZ 275/75/150/400 mg, HR 150/300 mg and R 150 mg/300 mg), and were manufactured by Lupin, India.

Doses were calculated at enrolment according to WHO weight bands: 35–39, 40–54, 55–69 and ≥ 70 kg,⁴ with targets of 5 mg/kg for INH, 25 mg/kg for PZA and 15 mg/kg for EMB. The target dose for RMP and the range for each weight band are shown in Table 1.

Follow-up

For the first 8 weeks, the patients attended the treatment centre daily for direct observation of doses ingested. Thereafter, the patients either attended 6 days a week (Kathmandu) or were given a supply of the drugs to be taken under the supervision of a designated family or community member, known as the Domiciliary Treatment Monitor (DTM) (Santa Cruz and Mbarara). The type of supervision was recorded on the treatment card for the first 16 weeks: C for treatment centre, D for DTM, T for unsupervised and N for not taken.

The patients were interviewed regarding symptoms and signs, and serum ALT levels were measured at 2, 4, 8, 12 and 16 weeks. Patients could also attend at any time if they experienced side effects. A doctor

^{*}Numbers before the letters indicate the duration in months of the phase of treatment; numbers in subscript indicate the trial regimen.

Table 1 RIFATOX Trial: target range of RMP drug dosages per kg by weight band

Weight kg	Control regimen		Study regimen 1		Study regimen 2	
	10 mg/kg (FDC)		15 mg/kg (add 2 RMP capsules)		20 mg/kg (add 3 or 4 RMP capsules)	
35–39	7.7	8.6	15.4	17.1	19.2	21.4*
40–54	8.3	11.3	14.0	18.8	16.7	22.5*
55–69	8.7	10.9	13.0	16.4	17.4	21.8
≥70		10.7		15.0		20.0

*Received 3 capsules.

RMP = rifampicin; FDC = fixed-dose combination.

who was independent of the trial and blinded to the regimen was appointed to interview patients who reported symptoms or adverse reactions to assess their causality.

Sputum microscopy and *Mycobacterium tuberculosis* culture were performed pre-treatment and at 8 weeks using Löwenstein-Jensen medium. Drug susceptibility testing (DST) against INH and RMP were performed using either standard phenotypic tests (Kathmandu and Santa Cruz) or the Hain MTBDR_{plus} test (Mbarara).¹⁷

Data management

Data were collected on case report forms. From Kathmandu and Santa Cruz, the forms were sent to St George's, University of London, London, UK, where they were checked for missing and atypical results. Once these issues had been resolved, the forms were sent to Epicentre, Mbarara, where double data entry and data validation was carried out using Voozanoo software version 3.4.2 (Epiconcept, Paris, France).

Site monitoring

The sites were regularly monitored by the Trial Manager as well as local monitors.

Outcome measures

Primary outcome

The primary outcome was the occurrence of any grade 3 or 4 adverse event (AE) of the 'Table for grading severity of adult adverse experiences' (US Department of Health and Human Services, Division of AIDS [DAIDS])¹ and/or SAE² during the first 16 weeks of chemotherapy.

Secondary outcomes

Secondary outcomes were as follows: 1) culture conversion at the end of 8 weeks of chemotherapy, 2) treatment modification as a result of an SAE and/or a grade 3 or more AE, 3) any increase in ALT level at any time during treatment, and 4) the number of observed doses of chemotherapy ingested.

Statistical methods

Sample size

The decision to enrol a total of 300 patients was a pragmatic one based on the consideration that this

would be sufficient to assess whether moving forward to a Phase III trial with higher doses of RMP could be justified on safety criteria. A study with 100 controls and 200 test cases has 82% power to detect the difference between a major AE rate of 5% in controls and 16% in test cases at two-sided 5% significance level.

Analysis

Safety analysis was performed among patients who received at least one dose of treatment. Hepatotoxicity was assessed using serial ALT measurements and graded using the DAIDS¹ system according to the range of normal levels for each laboratory. Non-hepatic AEs were similarly graded. Efficacy was assessed by the rate of sputum culture negativity after 8 weeks of treatment.

Adherence

Adherence to prescribed doses was ensured by observing drug ingestion by the medical staff and DTM.

RESULTS

Patient enrolment

Enrolment began in February 2011 and ended in May 2013. Three hundred patients were enrolled (100 per arm); 150 in Bolivia, 50 in Nepal, and 100 in Uganda.

Baseline characteristics, and treatment supervision

The Figure shows the populations available for analysis. Baseline characteristics were similar in the three groups (Table 2). Two thirds were males. The median age was approximately 30 years, and median weight approximately 50 kg. Less than 3% of patients were positive for either hepatitis B surface antigen or hepatitis C antibody. The median RMP dose was respectively 9.6, 15 and 18.8 mg in the three RMP groups.

Safety

Primary outcome analysis

A total of seven grade 3 AEs were recorded in respectively 1.0% (1/100), 2.0% (2/100) and 4.0% (4/100) of the R10, R15 and R20 patients (trend test $P = 0.15$). Of the 1800 scheduled ALT tests, results were available on 1719 occasions. Table 3 shows the

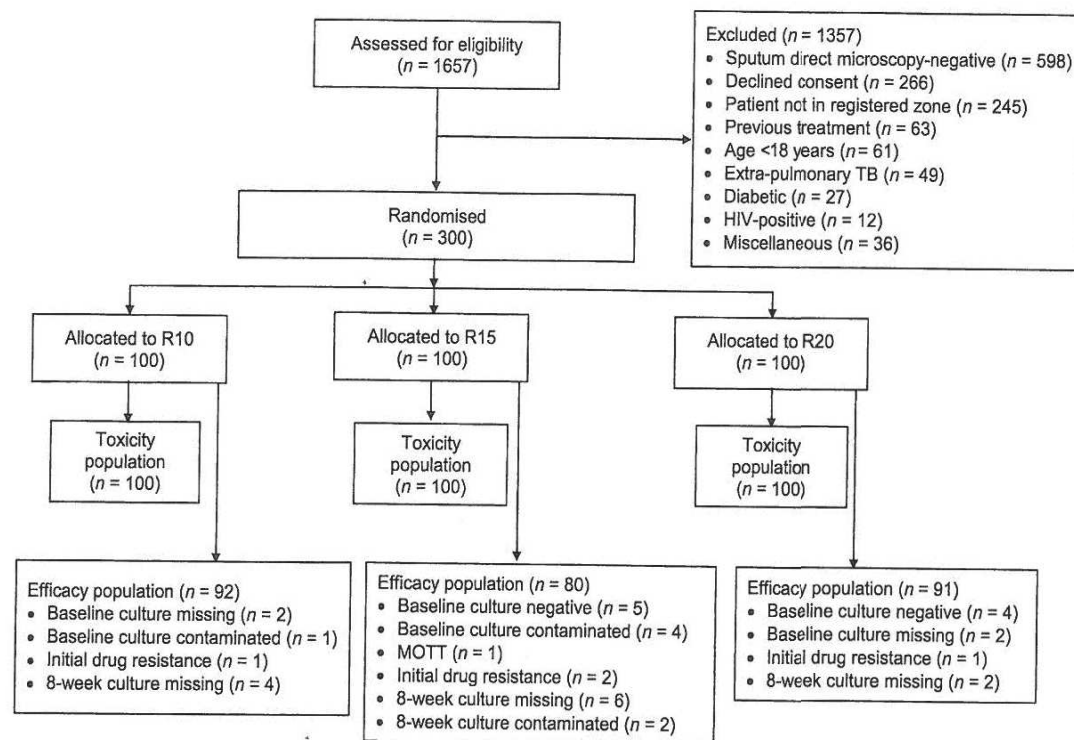


Figure Consort diagram. TB = tuberculosis; HIV = human immunodeficiency virus; MOTT = mycobacteria other than tuberculosis.

results in each grade. The majority remained within the laboratory's normal range and are graded as 0. ALT levels increased as the dose of RMP increased, most to grades 1 and 2.

One patient (R15) who received 750 mg daily RMP developed jaundice after 34 doses with a grade 3 ALT (192 international units [IU]) increase and a cutaneous hypersensitivity reaction, necessitating interruption of treatment. When all symptoms and signs had subsided and ALT levels had returned to normal, he was restarted on the standard regimen and completed 6 months of treatment. In the remaining six cases, ALT levels returned to normal at the next visit without treatment interruption. There were no ALT

increases to grade 4. No increases above grade 2 were recorded in patients positive for hepatitis B surface antigen or hepatitis C antibody.

Table 4 shows the frequency of non-hepatic AEs reported in all three regimens. SAEs occurred in two patients. One (R15) patient, diagnosed with carcinoma of the oesophagus after 12 weeks of treatment, was withdrawn from the trial. His culture result at 8 weeks was positive. A second (R20) patient died of septicaemia at 12 weeks after starting treatment. His culture result at 8 weeks was negative. For all other events, none was greater than grade 2, and all were treated symptomatically, without treatment interruption.

Table 2 Baseline characteristics

	Regimen*		
	R10 (n = 100)	R15 (n = 100)	R20 (n = 100)
Male sex, n	66	69	70
Age, years, median (range)	28.5 (18–67)	27.5 (16–67)	30 (19–66)
Body weight, kg, median (range)	50.7 (35–87)	53 (39–79)	51.55 (38–81)
Baseline ALT, IU/l, median (range)	17.1 (4–160)	19.1 (5–133)	17 (5–110)
Haemoglobin, g/l, median (range)	11.65 (6–15.0)	12.1 (8–18.1)	11.8 (8.3–15.9)
Creatinine clearance, median (range)†	79.16 (40.75–149.23)	78.64 (35.24–233.80)	81.1 (40.1–250.5)
Hepatitis B-positive, n	1	1	2
Hepatitis C-positive, n	2	2	3
Hepatitis B and C-positive, n	1	2	0
Daily RMP dose, median (range)	9.6 (7.7–11.8)	15.0 (12.5–18.3)	18.8 (14.4–25.5)

* R10 = 2EHRZ/4HR; R15 = 2EHR₁₅Z/2HR₁₅/2HR; R20 = 2EHR₂₀Z/2HR₂₀/2HR, where numbers before the letters indicate the duration in months of the phase of treatment; numbers in subscript indicate the study regimen.

† Calculated as: $\frac{(140 - \text{age}) \times \text{weight} \times 1.23 (\times 0.85 \text{ if female})}{\text{serum creatinine } [\mu\text{mol/l}]}$.

ALT = alanine transferase; IU = international units; RMP, R = rifampicin; E = ethambutol; H = isoniazid; Z = pyrazinamide.

Table 3 Grading of ALT values recorded at any time during the trial by study regimen

Grade [†]	ALT units			Total (n = 1800) n (%)
	R10* (n = 600) n (%)	R15* (n = 600) n (%)	R20* (n = 600) n (%)	
0	553 (92)	501 (84)	518 (86)	1572 (87)
1	29 (5)	51 (9)	42 (7)	122 (7)
2	2 (0.3)	8 (1.3)	8 (1.3)	18 (1)
3	1 (0)	2 (0.3)	4 (0.6)	7 (0.4)
4	0	0	0	0
Results not available	15 (2.5)	38 (6.3)	28 (5)	81 (5)

* R10 = 2EHRZ/4HR; R15 = 2EHR₁₅Z/2HR₁₅/2HR; R20 = 2EHR₂₀Z/2HR₂₀/2HR, where numbers before the letters indicate the duration in months of the phase of treatment; numbers in subscript indicate the study regimen.

[†] Based on DAIDS grading as follows: grade 1 = laboratory range 1.25–3.0 × ULN; grade 2 = >3.0–5.0 × ULN; grade 3 = >5.0–10.0 × ULN; grade 4 = >10.0 × ULN.

ALT = alanine transferase; E = ethambutol; H = isoniazid; R = rifampicin; Z = pyrazinamide; DAIDS = Division of AIDS (National Institutes of Health, Bethesda, MD, USA); ULN = upper limit of normal.

Efficacy

Baseline cultures were missing in all three arms (Figure) and, together with those with initial INH resistance, these participants were excluded from the analysis as late screening failures. The 8-week culture result was unavailable for 16 patients, either because they were contaminated or they were not performed because patients could not provide sputum, leaving 92 R10, 80 R15 and 91 R20 patients for analysis.

Culture negativity rates were respectively 75% (69/92), 82.5% (66/80) and 83.1% (76/91) (trend test $P = 0.16$). A logistic regression of culture conversion was performed using dose as a continuous predictor and treatment centre as nominal predictor. The analysis showed a 0.71-fold (29%) reduction in the odds of having a positive result given a 5 mg/kg increase in dose (confidence interval [CI] 0.47–1.06, $P = 0.09$).

Adherence

In Kathmandu, 76% of all doses ingested were observed by the medical staff, whereas 80% of the supervision in Mbarara was carried out by the DTM. In Santa Cruz, 66% of dose ingestion was observed by the medical staff and 30% by the DTM.

DISCUSSION

In this trial, RMP at daily doses of 15 mg/kg and 20 mg/kg for the first 16 weeks in microscopy-positive HIV-negative patients with pulmonary TB showed a rising, but statistically non-significant, trend in ALT levels that returned to normal without treatment interruption. Increasing RMP doses also showed a non-significant increasing trend in culture conversion at 8 weeks.

The occurrence of jaundice and increase of ALT to 192 IU (grade 3) necessitated treatment interruption in only one patient who was receiving RMP at 15 mg/kg. It is possible that some of the hepatic AEs observed in this trial are attributable to the use of INH or PZA rather than RMP. In a trial comparing

two 8-month regimens with the standard 6-month regimen, where RMP was given at the standard dose,⁵ 10/1355 (0.7%) trial patients experienced hepatic side effects, leading to an interruption of treatment of >7 days. Only 4/10 patients were on RMP, whereas all were on INH, suggesting that some of these hepatic events were more likely to be related to INH. Similar conclusions were made in two other reports.^{17,18}

Patients infected with hepatitis B and hepatitis C ($n = 14$) had no recorded increases in ALT value. However, the numbers are too small to allow any comment on safety. All but one of the increases in ALT were transient, returning to normal at the next recorded test. Almost identical results are reported in an abstract.¹⁹ Similar results are reported by the PanACEA MAMS-TB Trial²⁰ in groups of 62 patients, each treated with 12 weeks of RMP at up to 35 mg/kg/day. Grade 3 or higher AEs were reported in 12% of the R10, 12% of the R20, and 14% of the R35 (35 mg/kg/d) group.

Culture negativity rates at 8 weeks showed an increase (albeit non-significant) with increasing dose. However, although the trial was not powered to determine an effect on culture conversion, the CI of the logistic regression does not exclude the possibility of a substantial improvement with increasing dose. One limitation of our study was that it was an open-label trial without a placebo, and patients and staff were aware of treatment

Table 4 RIFATOX Trial: number of adverse events, other than hepatic, occurring throughout the trial

Toxicity	R10 (n = 100)	R15 (n = 100)	R20 (n = 100)
Haematological	0	0	1
Gastrointestinal	6	3	2
Cutaneous	2	6	3
Arthralgia	8	5	4
Other	4	8	5
Total	20	22	13

allocations. However, as we used microbiological and laboratory (ALT) outcomes, and the clinician responsible for decisions relating to treatment modification was blinded to study allocation, we feel that bias had been minimised. Second, the drugs used were those in use for routine treatment in each country. This allowed us to carry out the trial as a pragmatic, operational study at a minimum cost and within a short time-frame. Third, the trial was conducted in HIV-negative patients whose tolerability level may have been different from that of HIV-positive patients. Fourth, the absence of pharmacokinetic assessments failed to give an indication of blood levels attained with higher doses of RMP and whether there were any interracial differences. However, as the patients were from different races, the similar outcomes in all three arms supports the external validity of the trial. Finally, methods used for treatment administration and directly observed treatment differed between centres, although, importantly, this did not result in a difference in losses to follow-up or in the number of doses taken.

CONCLUSION

A daily dose of 20 mg/kg RMP for 4 months appears to be safe and well tolerated. Although the treatment groups are relatively small, these encouraging results suggest that a daily dose of 20 mg/kg RMP for 4 months is safe enough to be taken forward to larger Phase III trials. However, caution should be exercised in patients whose liver function tests are compromised for any reason, in those with viral hepatitis infection and in pregnant women.

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Trial Steering Committee: H Hodgson, D Moore, M Bonnet, M Burgos.

Data and Safety Monitoring Committee: G Davies, S White, J Porter.

Conflicts of interest: none declared.

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A RANDOMISED CONTROLLED PHASE IIB TRIAL TO EVALUATE TOXICITY OF HIGH DOSE RIFAMPICIN IN THE TREATMENT OF PULMONARY TUBERCULOSIS (RIFATOX TRIAL)



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St George's
University of London

St George's, University of London and MSF, Funded the Trial.



BACKGROUND

- The 6-month TB regimen recommended by WHO is effective and safe, **But still long**.
- Much of its efficacy depend on Rifampicin (R) being given throughout the 6 months.
- Following introduction of R (in 1970s), it was given at the lowest dose at which efficacy had been proven.
- Use of higher doses of R in animal studies and early human studies have shown faster sterilization of the lungs and increase bactericidal effect
- There is a potential to shorten the duration of treatment from 6 to 4 months by increasing the dose of R
- The safety of higher doses of R and its potential efficacy in humans had not been explored.

OBJECTIVES

Primary objective: To assess if an increase of rifampicin from 10 mg/kg to 15 and 20 mg/kg, results in an increase in grade 3 or 4 hepatic adverse events and/or serious adverse events.

Secondary: To determine if the increased dose resulted in more rapid lung sterilization assessed by culture conversion rate after 8 weeks' treatment.

METHODS

- Study Period:** Between February 2011 and May 2013,
- Design:** An International Multi-centre randomised controlled Phase IIB trial.
- Sample size:** 300 patients
- Sites:** Nepal, Bolivia, Uganda (Epicentre).
- Eligibility:** Newly diagnosed drug susceptible TB, adult, HIV negative and non-pregnant patient.
- 3 allocation arms:**
 - Control Regimen : (2EHR10Z/4HR10). Usual dose of rifampicin 10mg/Kg.
 - Study Regimen 1: (2EHR15Z/2HR15/2HR) For the first 4 months, the dose of rifampicin was 15mg/kg.
 - Study Regimen 2: (2EHR20Z/2HR20/2HR) For the first 4 months, the dose of rifampicin was 20mg/kg.

Outcome measures:

Occurrence of grade 3 or 4 adverse events during the first 4 months of chemotherapy.
8 weeks sputum culture conversion using Lowenstein-Jensen culture

Follow-up duration:

- First 4 months of chemotherapy.
- Weekly in first 2 months and monthly for 2 months.
- ALT done at 0, 2, 4, 8, 12 and 16 weeks.

RESULTS

Figure 1: study profile

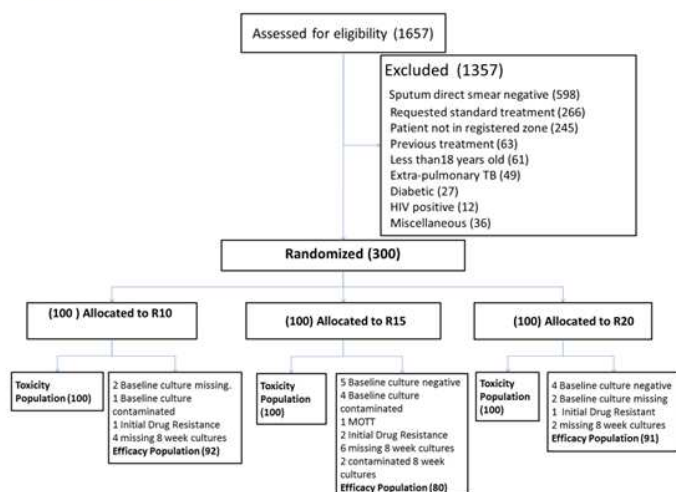


Table 1. Baseline Participants' Characteristics.

		Regimen		
		R10 (n = 100)	R15 (n = 100)	R20 (n = 100)
Gender	Male	66	69	70
Age (Years)	Median	28.5	27.5	30
Body Weight (Kg)	Median	50.7	53	51.55
	40 - <55	64	59	67
	55 - <70	30	37	30
	≥70	6	4	3
Baseline ALT (IU/L)	Median	17.1	19.1	17
	Range	4 - 160	5 - 133	5 - 110
Haemoglobin (g/L)	Median	11.65	12.1	11.8
*Creatinine Clearance	Median	79.16	78.64	81.1
Hepatitis B	Positive	1	1	2
Hepatitis C	Positive	2	2	3
Hepatitis B & C	Positive	1	2	0

SAFETY RESULTS

Figure 2. ALT distribution by study regimen overtime

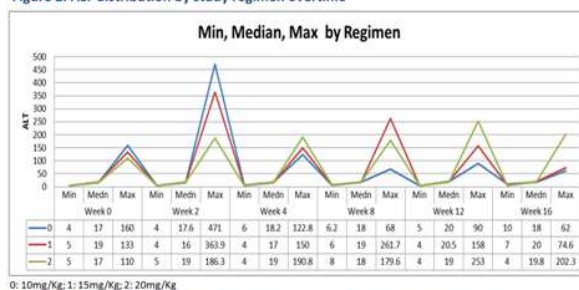


Table 2. Number of adverse events, other than hepatic, occurring throughout the trial.

TOXICITY	R10	R15	R20
Haematological	0	0	1
Gastrointestinal	6	3	2
Cutaneous	2	6	3
Arthralgia	8	5	4
Other	4	8	5
TOTAL	20	22	13

- There were 7 (grade 3/severe) increases in serum ALT levels,
 - 1.0% (1/100) R10, 2.0% (2/100) R15 and 4.0% (4/100) R20, (trend test p=0.15).
 - One case, with jaundice (R15), required treatment modification.
 - No Life threatening events occurred.

CULTURE CONVERSION AT 8 WEEKS

- Non-significant increase in culture conversion at 8 weeks:
 - 75% (69/92) R10 vs 82.5% (66/80) R15 vs 83.1% (76/91) R20, (p=0.16).
- Baseline higher bacteria loads ($\geq 3+$ nb colonies) and lower culture conversion among African patients

Table 4. Grade 3 culture at baseline and culture negativity at 8 weeks

	R10		R15		R20		Total	
	Baseline $\geq 3+$	8w cult conv %	Baseline $\geq 3+$	8w cult conv %	Baseline $\geq 3+$	8w cult conv %	Baseline $\geq 3+$	8w cult conv %
Mbarara	90	38	70	67	73	67	78	80
Kathmandu	44	94	36	100	53	94	44	96
Santa Cruz	11	95	15	87	9	93	12	90

CONCLUSION

- No significant increase in adverse events occurred when rifampicin dose was increased from 10mg/kg to 15mg/kg or 20mg/kg.
- A daily dose of 20mg/kg rifampicin for 4 months appears to be safe and well tolerated.
- There was a trend of higher rates of culture conversion at 2 months in patients on 20mg/Kg
- A larger, phase III trial to start in 2016
- The factors responsible for the slow culture conversion in the African patients require further exploration.

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Chapter 3.2

Pharmacokinetics and safety of efavirenz during co-administration with anti-tuberculosis treatment in high HIV and tuberculosis burden countries: a systematic review

Atwine D., Bonnet M. and Taburet AM. Pharmacokinetics of efavirenz during co-administration with antituberculosis treatment in high HIV and tuberculosis burden countries: A systematic review

3.2.1 Justification and objectives

Co-administration of both EFV-based ART and ATT for TB-HIV co-infection continues to be the practice among high TB and HIV burden countries despite the reported drug-drug interactions especially between EFV and R. This is majorly because of EFV's good efficacy [162], tolerability, cost, formulation as a FDC administered at 600mg dose once-daily, but also being well incorporated among LMICs and high HIV burden countries' national guidelines [157-159]. Dolutegravir despite its improved tolerance compared to EFV, its limited experience in HIV-TB co-infected patients will make EFV-based ART regimen the cornerstone of HIV treatment in those patients for many years [36, 264]. Meaning that clinicians and pharmacologists may have to continue facing the challenges of drug-drug interaction between ART and ATT regimens and this will continue to be an important consideration in developing and implementation of new drugs or regimens for either HIV or TB.

Despite attempts made previously in describing the pharmacokinetics and pharmacogenetics of EFV with RH-based ATT co-administration [165, 265-267], an extensive focus on the world's highest TB/HIV burden countries that may be affected most by drug-drug interactions is lacking. We therefore conducted a systematic review to gather existing information on the PK of EFV when combined with ATT among TB and HIV high-burden countries. We assessed the effect of body weight, gender, EFV dosing and the CYP2B6 homozygous slow metabolizer genetic polymorphism, *CYP2B6 516 G>T*, on the EFV concentrations during R and H co-administration, and the effect of the EFV pharmacokinetic parameters on the virological response, CNS and hepatic toxicity.

3.2.2 Methods, results and conclusion

The detailed methodology, results and conclusions of this systematic review are as presented in the attached complete manuscript **(for submission soon to Clinical Pharmacokinetics journal)**.

3.2.3 Involvement in this work

In this systematic review, I wrote the protocol based on PRISMA guidelines [268], developed the search strategy, developed all data collection tools, did the systematic search of Cochrane Library, EMBASE.COM and MEDLINE (via OvidSP), screened articles, extracted data from included articles onto a standardized data extraction form and validated by a second reviewer. I designed a database designed using Epi Info™ software (V7.2, 1600 Clifton Road Atlanta, GA 30329-4027 USA), entered data, performed the analysis with Stata software (v. 13, College Station, Texas, USA) and wrote the manuscript, all with support of my PhD Directors.

Pharmacokinetics of efavirenz during co-administration with antituberculosis treatment in high HIV and tuberculosis burden countries: A systematic review

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Abstract

Background: Efavirenz (EFV) and Rifampicin-Isoniazid (RH) are cornerstone drugs in tuberculosis (TB)-HIV co-infection treatment but their co-administration bares complex interactions with efficacy and safety challenges. We reviewed recent data on EFV and RH interaction in TB/HIV high-burden countries.

Methods: We conducted a systematic review of studies conducted in the 41 high TB/HIV-burden countries between 1990 and 2016, on EFV pharmacokinetics (PK) during RH co-administration in co-infected patients. Article screening and data collection were done by 2 reviewers.

Results: Of 119 records retrieved, 22 were included (14 Africa, 7 Asia, 1 inter-continental), all observational studies reporting either EFV mid-dose (C_{12}) or pre-dose (C_{min}) concentrations. Of 20 studies in adults, 19 had average concentrations between 1000-4000ng/mL on RH. Increased concentrations on versus off RH were reported in 6/10 African studies (3.7 – 33.3 %) and 2/4 Asian studies (9.5 – 26.3%). Sub-therapeutic concentrations frequency $\geq 20\%$ reported in 5/13 studies during RH. Five of 8 studies reporting virological response $> 80\%$, had $< 20\%$ of the population with sub-therapeutic concentrations. High frequency of supratherapeutic concentrations ($\geq 20\%$) were reported in all 7 studies reporting these data. The *CYP2B6* 516G>T polymorphism was consistently associated with higher plasma EFV concentrations, whatever the ethnic background of the patients. Effect of EFV and RH co-administration on hepatic and central nervous system toxicity was noted in 2/8 and 3/9 studies, respectively.

Conclusions:

These findings support the use of 600mg EFV in co-infected patients in high-burden countries. *CYP2B6* loss of function genes frequency may explain the variability of EFV concentrations in African and Asian populations and should be considered prior to EFV-400mg implementation in TB-HIV co-infected patients.

BACKGROUND

HIV infection is still a global public health concern, especially in Africa and Asia. Sub-Saharan Africa remains most severely affected, with nearly 1 in every 25 adults (4.4%) living with HIV and accounting for nearly 70% of the people living with HIV worldwide [1]. The estimated risk of developing tuberculosis (TB) in people living with HIV ranges between 26 and 31 times greater than in those without HIV infection [2]. The highest TB incidence rates among HIV patients are reported in Africa and Asia. The overall TB mortality rate among HIV patients is about 6 times higher in Africa (30 per 100,000 population) than the global average (5.3 per 100,000 population), with approximately 75% of all deaths occurring in Sub-Saharan Africa [3].

The recommended first-line anti-retroviral treatment (ART) for adults and adolescents consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or an integrase inhibitor (INSTI)[4]. Recommended WHO NRTI backbone was zidovudine and lamivudine and recently tenofovir +lamivudine, those two drugs being eliminated unchanged through the kidney, which makes drug-drug interactions unlikely [4]. The recommended NNRTI is efavirenz (EFV) given its proven high virological efficacy, its availability as a fixed-dose combination (FDC) administered at 600mg dose once-daily, under generic formulation preferably in the night so as to minimize central nervous system (CNS) adverse events, and to ensure good adherence. EFV is well incorporated among low income and high HIV burden countries' national guidelines [5-7]. There is reassuring data regarding its safety in pregnancy [8] and improved efficacy compared to the former widely used NNRTI, nevirapine [9]. Although dolutegravir an HIV-integrase inhibitor may be available in resource limited countries shortly, despite improved tolerance compared to EFV, limited experience in HIV-TB co-infected patients will make EFV-based ART regimen the corner stone of HIV treatment in those patients for many years [4, 10].

EFV is mostly metabolized by hydroxylation to inactive metabolites 8-hydroxy EFV and 7-hydroxy efavirenz involving mainly CYP2B6 [11, 12]. The main metabolite, 8- hydroxy efavirenz is further hydroxylated primarily by CYP2B6 to form 8, 14-hydroxy efavirenz. The oxidative metabolites undergo conjugation by UDP-glucuronyltransferase (UGT) pathway and are excreted in the urine as glucuronides [13]. Mid-dose (12h) EFV plasma concentrations below 1000 ng/mL have been associated with increased risk of virological failure in HIV-infected patients, while concentrations above 4000 ng/mL have been associated with risk of CNS side effects [14, 15]. There is a wide inter-individual variability in EFV concentrations [15] that is partially explained by genetic factors as shown by the strong association between CYP2B6 516G>T single nucleotide polymorphism (SNP) and EFV exposure [16]. The CYP2B6 516G>T is a common polymorphism that has been consistently associated with reduced enzyme activity, higher EFV exposure and increased toxicity [11, 16, 17]. On the other hand, there is no clear evidence supporting the association with gender and body weight [13, 18].

With regard to drug susceptible TB, a six-month regimen broken down into an intensive two-month phase involving isoniazid (H, 5 mg/kg), rifampicin (R, 10 mg/kg), pyrazinamide (Z, 25 mg/kg) and Ethambutol (E, 15 mg/kg) followed by

four months of continuation phase with R and H [19]. Both R and H are the cornerstone drugs within this regimen [20, 21] that has a very good efficacy [22]. This regimen is used with FDC, and administered once daily [21].

Rifampicin has a strong bactericidal activity [22, 23] given its ability to inhibit transcription by binding with high affinity to bacterial DNA-dependent RNA polymerase [24-26] and the best sterilizing drug to prevent relapses of TB. R is also a potent inducer of several liver or gut drug metabolizing enzymes, especially isoenzymes of cytochrome P450 (CYP), mainly isoenzyme CYP3A4 and CYP2B6. This results in enhanced NNRTI drug metabolism and may lead to sub-therapeutic NNRTI plasma concentration during co-administration [27]. In healthy volunteers, EFV Area Under the Curve (AUC), maximum concentration (C_{max}) and minimum concentration (C_{min}) are reduced by 26, 20 and 32% when co-administered with R as compared to EFV alone, respectively, which led to the Food and Drug Authority (FDA) recommendation of an increase in EFV dosing to 800 mg once a day when combined with TB drugs [27, 28]. However, due to the potential of increased risk of CNS toxicity with the increase of EFV dose and reassuring virological response in co-infected patients receiving EFV at 600mg once daily in high HIV burden countries, it is recommended to maintain EFV at usual dose (600mg/day once daily) [14, 29-33]. The other cornerstone anti-TB drug, isoniazid, is metabolized mainly through N-acetyltransferase type 2 (NAT2) and was demonstrated in vitro to have an inhibitory effect on several cytochrome P450 enzymes (CYP2C19, CYP1A2, CYP2A6, CYP2C19, and CYP3A4) [34]. From recent studies, there is some growing evidence that the inducing effect of combined R and H (RH) is less potent than R alone [35, 36]. Such effect could be different according to patient CYP2B6 516 G>T genetic polymorphism [37].

However, despite attempts made previously in describing the pharmacokinetics (PK) and pharmacogenetics of EFV with RH co-administration [13, 38-40], an extensive focus on the world's highest TB/HIV burden countries that may be affected most by drug-drug interactions is lacking.

We conducted a systematic review to gather existing information on the pharmacokinetics of EFV when combined with anti-TB treatment among TB and HIV high-burden countries. We assessed the effect of body weight, gender, EFV dosing and the CYP2B6 homozygous slow metabolizer genetic polymorphism, CYP2B6 516 G>T, on the EFV concentrations during RH co-administration, and the effect of the EFV PK results on the virological response, CNS and hepatic toxicity.

METHODS

Study eligibility criteria

A study was considered eligible for inclusion if it was a randomized controlled trial, cohort, case-control or cross-sectional study, that report PK of the EFV (minimum or mid-dose concentrations at least) following co-administration with RH in TB/HIV co-infected patients for at least 4 weeks (that is to ensure a minimum steady state) and conducted among the 41 TB/HIV high burden countries according to the WHO Post-2015 list [41]. Studies that enrolled patients with comorbidities

that require co-administration of other drugs with known interaction with EFV, and studies enrolling patients on anti-TB prophylaxis, or using other rifamycins like rifapentine and rifabutin were excluded. Only studies published in English were included.

Search strategy

We conducted this review according to PRISMA guidelines [42]. We identified relevant articles through a systematic search of Cochrane Library, EMBASE.COM and MEDLINE (via OvidSP) published from 1st January 1990 to 31st August 2016 in the English language. The choice of 1990 as start point was based on the consideration that in most of the TB/HIV high-burden countries, access to antiretroviral treatment took place after 1990, with EFV receiving FDA approval in 1998 [43]. We also searched the Web-of-science and carried out manual searches (hand searching) to retrieve other reports of studies that are reported in journals, conference proceedings, bibliographies of review articles and retrieved articles, monographs, and sources other than those mentioned above. We used the following abbreviated search strategy: ("Efavirenz" or "Stocrin" or "Sustiva") and ("Rifampicin" or "RIFAFOUR" or "RIFAMPIN" or "RIFAMYCIN") and ("pharmacokinetics" or "drug assay" or "plasma drug concentration" or "Ctough" or "pharmacology" or "drug interaction" or "drug-drug interaction" or "Efavirenz concentration" or "Rifampicin concentration" or "non-nucleoside reverse transcriptase inhibitors concentration").

Bibliography search and screening of titles and abstracts were done by one reviewer (DA), duplicate records were eliminated and full texts of potentially relevant articles retrieved. The selection was validated by a second reviewer (MB), blinded to the initial assessment. Full texts retained through this process were independently screened by two reviewers (DA and AMT). Disagreements were examined by the third reviewer (MB).

Records with inaccessible full text but with author contact details were retrieved after contacting authors by email. Abstract only records were excluded from further data collection processes.

Data collection and analysis

Data collected from each study were recorded by one reviewer (DA) in a standardized data extraction form (see Appendix 1) and validated by the second reviewer (AMT). Authors were contacted for clarification whenever needed. All discrepancies were discussed and resolved by consensus between the three reviewers (DA, AMT, and MB).

Data extraction forms were entered into a database using Epi Info™ software (V7.2, 1600 Clifton Road Atlanta, GA 30329-4027 USA) and analysis used the Stata software (v. 13, College Station, Texas, USA). We performed descriptive presentation of the studies' and patients' characteristics. EFV mid-dose concentrations measured 12h post dose or pre-dose concentration (Cmin) measured before next dose intake were the PK parameters chosen as a surrogate of EFV exposure

based on their availability in all selected articles. They were presented with or without RH co-administration globally, by gender, body weight, and EFV dose. Proportions of patients with sub-therapeutic (<1000ng/mL) or supra-therapeutic EFV concentrations (>4000ng/mL)[15] and the attributable rate percent (AR%) defined by the difference in proportions of patients with sub-therapeutic or supratherapeutic concentrations during EFV co-administration with anti-TB drugs and EFV alone, were calculated and presented per study.

EFV concentrations and frequency of CYP2B6 homozygous slow and extensive metabolizer genetic polymorphism, CYP2B6 516 G>T were presented by study.

RESULTS

Of the total 119 records retrieved, 22 records were included in the analysis (Fig I). Of these, 20 studies had data on at least EFV C12 or Cmin during RH co-administration [14, 32, 35, 44-60], while 1 study only reported body weight-specific EFV C12 data during RH co-administration [61] and one reported only proportions of patients with sub-therapeutic EFV concentrations [62].

General characteristics of studies

Table I shows characteristics of the 22 included studies. All were published between 2006 and 2016. They were all observational studies and 64% were conducted exclusively in Africa. The majority included adult patients (91%). The 2 children studies were conducted in South Africa. EFV was systematically administered at bed-time to improve the tolerability and reduce adverse events [63], except for 2 adult studies when it was taken in the morning [46, 64] and a 6-month anti-TB treatment was used and administered daily at recommended dosing in all studies except one [48]. Slightly more than half of the studies (59%) reported EFV concentrations on and off RH within the same patients. All studies had EFV concentrations during RH co-administration, with sampling done during intensive phase or continuation phase. Individual study-specific characteristics are shown in Appendix 2.

Effect of RH co-administration on the PK of EFV in HIV/TB co-infected patients

Overall, 19/20 studies reported that the average EFV concentrations were in the alleged therapeutic window (1000-4000 ng/mL) during RH co-administration irrespective of the geographical region (Table II). One study in Thai patients [45] reported an average EFV concentration >4000 ng/mL although without specifying the proportion of patients with supratherapeutic concentrations. Of the 14 studies conducted among adult patients that reported EFV concentrations on and

off RH, 10 were in Africa and 4 in Asia. Among studies conducted in Africa, with RH co-administration, an increased EFV concentration was noted compared to the off RH period in 6 studies ranging from 3.7% up to 33.3% across studies and countries.

Among studies in Asia, increased EFV concentration with RH co-administration was noted compared to the off RH period in 2 studies ranging from 9.5% up to 26.3% while reduced concentrations were seen in 2 studies ranging from 3.6% up to 22.2% across, studies and countries.

In the two studies among the South African children, the earlier study by Ren (2009) indicated a slight increase in median C12 with RH co-administration (0.8%), while a recent study by McIlleron (2013) indicated a decrease of 16.3%.

Sub-therapeutic EFV concentration and virological response

Fig II shows the proportion of patients with sub-therapeutic EFV levels on or off RH as reported in different studies. Of the 13 studies reporting information on the proportion of patients with sub-therapeutic EFV concentrations, 10 studies had comparative concentration with and without RH. On RH, 5/13 studies reported concentrations <1000ng/ml in more than 20% of patients but only one study in South-Africa reported a proportion of patients with sub-therapeutic concentrations higher during RH co-administration by > 10% as compared to without RH. Two studies (one in adult and one in children) reported the very high proportion of patients (>50%) with sub-therapeutic concentrations either with or without RH.

Table III shows results of virological response with EFV and RH co-administration. Out of 10 studies (8 in adults, 2 in children) reporting both EFV exposure and virological response, 8 had a virological response > 80% between 6 and 12 months follow-up (6 in adults, 2 in children). Of them, 5 studies had less than 20% of patients with sub-therapeutic concentrations (4 in adults, 1 in children). In contrast, both studies (all in adults) with virological suppression lower than 80% had more than 20% of patients with sub-therapeutic levels.

Supra-therapeutic EFV concentration and safety

Fig III shows that all the 7 studies, reported a proportion of approximately 20% or above among the patients on RH with supra-therapeutic EFV concentrations (>4000ng/mL). Only 5 studies had comparative results in same patients on and off RH and reported all higher proportions on versus off RH, although the difference was highly variable ranging from ~1% up to 23%. Interestingly, the 3 studies conducted in Africa reported higher EFV concentration on versus off RH [56, 60, 64].

Table IV shows the hepatic adverse events among HIV-TB co-infected patients. Of the 8 studies that assessed and reported data on hepatic events, the 4 African adult studies reported incidence of any grade alanine aminotransferase (ALT) rises

ranging between 2.8-30% among TB-HIV co-infected patients [32, 46, 55, 56], though a relationship between ALT rise and EFV/RH co-administration was demonstrated in only one study [55]. Among the 3 Asian studies [53, 58, 62], incidence of any grade ALT rises ranged between 0-16.7%, with a significant relationship with EFV/RH co-administration reported only in one study [58]. The only children study conducted in South Africa reported all grade ALT rises in 2.5% of children [35].

Table IV shows the central nervous system adverse events among HIV-TB co-infected patients. Nine studies that is, 6 African, 2 Asian, 1 international study assessed and reported data on incidence of any grade CNS symptoms. Among African studies, 2/6 showed a significant relationship between CNS adverse events and supratherapeutic EFV plasma concentrations [50, 57]. Of the 2 Asian studies 1 reported a significant relationship between developing incidence of CNS side-effects of any grade with having at least one-time supratherapeutic EFV concentrations [58].

EFV concentration by body weight, EFV dose, and Gender

Three studies reported plasma EFV concentrations stratified by body weight when given at a 600 mg dose with anti-TB drugs co-administration [54, 55, 61]. Two studies reported lower EFV concentrations in patients with weight > 50Kg than in patients with weight < 50Kg, with median C12 (interquartile range, IQR) of 2060 ng/mL (IQR: 1425, 3575) vs 2859 (IQR: 1787, 4749) in Cambodian patients [61] and mean Cmin of 1860ng/mL vs 2080ng/mL in a study that included African, Latin American and Asian sites [54]. On the other hand, in the third study in Ethiopia the median (IQR) C12 was slightly higher in patients with weight > 50Kg compared to those with weight < 50Kg: 1515ng/mL (962, 3019) vs 1345ng/mL (765, 3058) [55]. However, in the same study, without co-administration, there was a trend towards lower concentrations in patients with higher body weight: 1233ng/mL (848, 1670) vs 1410ng/mL (1067, 2155) [55].

Only one study in South Africa included patients on high EFV dose (800mg) [32]. A higher median (IQR) C12 of EFV during RH co-administration was noted in the patients on 800mg EFV (2900 ng/mL, IQR: 1800, 5600) as compared to the group on 600mg EFV (2400ng/mL, IQR: 1200, 5100).

Only one study presented plasma EFV concentrations stratified by gender during co-administration with anti-TB drugs. The mean Cmin was lower for males than for females (1870 ng/mL vs 2370 ng/mL) [54].

The frequency of CYP2B6 slow metabolizers and effect on EFV concentration during anti-TB treatment co-administration

A total of 9 studies (6 from Africa and 3 from Asia) reported EFV concentrations according to the CYP2B6 G516T genetic polymorphism encoding for a defective CYP2B6 enzyme, and 8/9 studies (5 from Africa and 3 from Asia) reported the frequency of CYP2B6 homozygous slow metabolizer genetic polymorphism within the studies' populations. Most of the

studies reported only frequencies for the most frequent polymorphism CYP2B6 516 G>T (CYP2B6*6 allele). In all studies, except for one conducted in Tanzania [49], patients who carried this loss of function variant allele had higher EFV concentrations both off and on RH as compared to those carrying the wild-type gene as shown in figure IV, panels A and B respectively. The frequency of slow metabolizers ranged between 10% in one study in Rwanda [59] and 28% in another study in Ethiopia [55].

DISCUSSION

In this review, we note that, 19 out of the 20 selected studies reported average EFV concentrations within the alleged therapeutic range (1000-4000 ng/mL) during RH-based standard TB drug co-administration, and many of which also showed an increase in EFV concentrations during RH co-administration. This observation was demonstrated to be dependent on CYP2B6 and NAT2 genetic polymorphism. Indeed, patients who are slow metabolizers for CYP2B6, had higher concentrations of EFV (>4000 ng/ml). In those patients, R has little effect on minor drug metabolizing enzymes involved in EFV biotransformation, although H which is metabolized by the polymorphic NAT2 was demonstrated to inhibit these enzymes [37, 65]. Except in Ethiopia, we observe a reduction of EFV concentration during co-administration with anti-TB drugs in the Eastern African region and an increase in the Southern African region. This variability may partially be explained by the difference in the frequency of CYP2B6 polymorphisms [30, 66]. In the study done in Rwanda, the frequency of the CYP2B6 983 T>C polymorphism associated with extensive metabolizing activity was particularly high (83%) in the study population [59] that experienced a major decrease in concentrations during RH co-administration compared to EFV alone (33.3%); and the frequency of slow metabolizing genotypes was higher (28%) in the Ethiopian study population [55] where an increase of EFV concentrations during co-administration (17.4%) was also observed.

Studies from only three countries (Cambodia, Thailand and India) were available for South East Asia with discordant results on effect of RH co-administration on EFV PK even within studies conducted in the same country. This is demonstrated in studies conducted in Thailand, in which one reported an increase [45] while another reported a decrease [52] in EFV concentrations during RH co-administration. Similar intra-country variability was observed in adult studies conducted in Tanzania [46, 49], and in the two children studies in South Africa [35, 47], and could partially be explained by the difference in sample size and methodology between the studies. The influence of age on CYP2B6 expression has not been well established although some studies hypothesized that it may also depend on sex, as significant increase in liver CYP2B6 is more linked to only males at higher age [67].

In this review, the lower EFV concentrations among males reported in one study [54] are in agreement with what has been reported in another study in Zimbabwe without anti-TB drugs co-administration that showed a mean EFV C12 lower in males than in females [68] although this study was excluded in this review given a non-specified timing of PK sampling. This might also be dependent on the genetic polymorphism (CYP2B6*6, *18 and CYP2A6*9) and age [67].

Although the proportion of patients with sub-therapeutic EFV levels was very high in some studies, there were no major differences in EFV concentrations between on and off anti-TB treatment. As expected, there was a trend of lower virological response in studies with very high proportion of patients with sub-therapeutic concentrations. However, some discordances between the EFV concentrations and the virological response in some studies [47, 55, 64], illustrate the difficulty to correlate the drug plasma concentration measured at one point of time with the virological response. This is in agreement with the PK/Pharmaco-dynamic results of the ENCORE1 study where patients were randomized to receive EFV once daily either at 400 mg or 600 mg. It was shown that despite predicted $C_{12} < 1000$ ng/mL in 5% and 2% for EFV 400 and EFV600 respectively, 1 patient in the EFV400 group and 3 in the EFV600 group had detectable plasma viral load at 48 weeks of therapy [69]. In addition, it also highlights the limitation of the commonly used sub-therapeutic threshold of 1000ng/mL for mid-dose EFV concentration, which is based on very low level of evidence [15]. Furthermore, the use of the same threshold for studies reporting C_{12} (mid dose concentration) or C_{min} (trough concentration) concentrations overestimated the proportion of patients with sub-therapeutic concentrations [47].

The occurrence of supratherapeutic EFV levels was very common both in African and Asian studies. However, higher increases in proportion of patients with supratherapeutic EFV levels with RH co-administration than without, were noted in African studies (range: 10-23%) as compared to Asia (3.0%) and in one inter-continental study (0.8%). Despite the low frequency of slow-metabolizer CYP2B6 genes among study patients, their presence was almost always associated with supratherapeutic EFV concentrations, something that could partly explain the observed supratherapeutic EFV variability.

Higher EFV concentrations during co-administration with anti-TB treatment could increase the occurrence of adverse events. Marzolini et al., and Haas et al., reported that EFV CNS adverse effects were more common in those patients with higher EFV concentrations [15, 70]. However, due to the very low number of studies reporting both safety and PK data during co-administration with anti-TB treatment, it is very difficult to draw strong conclusions based on this current review. In addition, the lack of information or standardization in reporting safety information between studies, especially for CNS makes the interpretation even more difficult. Nevertheless, we note that no clear correlation could be made between EFV supratherapeutic levels and occurrence of CNS adverse events during RH co-administration within the exclusively African studies [14, 50, 60]. This lack of association might also be biased by the other common causes of neuropsychiatric disorders besides EFV treatment in HIV infected patients [71, 72]. Some studies have however attempted to explain this disparity between plasma EFV concentrations and onset of CNS adverse events on grounds of the high lipophilic nature of EFV which allows it to penetrate the blood brain barrier easily and so give disproportionate EFV concentrations between plasma and brain [62]. Interestingly it was recently suggested that among 563 patients who had initiated EFV-containing regimens at an HIV primary care clinic in the South-eastern United States, slow metabolizer genotypes were associated significantly with EFV discontinuation for reported CNS symptoms although this association was considerably stronger in Whites than in Blacks [73].

Regarding, hepatotoxicity, the reported overall incidence of ALT rises to any grade among TB-HIV co-infected patients was higher among the African adult studies (2.8-30%) as compared to Asian studies (0-16.7%). Nevertheless, only one adult study revealed a significant relationship between any grade ALT rise with EFV and RH co-administration [58]. In this review, it was not possible to distinguish the individual drug contribution of R and H on ALT rises of any grade given that in all studies RH was administered within FDC, but also since the timing of onset was not well clarified in all studies. In one children study conducted in South Africa, all grade ALT rises were noted in 2.5% of children, with only 1 child suffering a grade 3 elevation in ALT in the month after completion of anti-TB treatment, which turned to normal without treatment adjustment [35].

With the few studies available, it was not possible to satisfactorily assess the effect of body weight and EFV dose during co-administration with anti-TB drugs. Two of three studies showed a decrease of EFV concentration among patients with high body weight (>50Kg) as previously reported in patients on EFV alone [37, 52, 68] and children [35].

This systematic review has some limitations, i) the great heterogeneity between studies with regard to study designs, PK parameters explored, and reporting, hindered any potential meta-analysis; ii) The small sample sizes for TB-HIV co-infected populations in many studies, may have contributed to the observed variability in EFV exposure due to RH co-administration even within same country; iv) The effect of body weight, gender, and EFV dose could not be properly assessed due to the very small number of studies and their low sample sizes; v) Most papers never correlated sub-therapeutic and supra-therapeutic rates of EFV during RH co-administration with CYP2B6 metabolizer genes, and so hindered clear quantification of changes attributable to various CYP2B6 genetic polymorphisms. vi) The few children studies could not allow a thorough evaluation and conclusions on EFV exposure with RH co-administration. vii) Analysis of safety information was limited by the very few number of studies correlating both safety and PK data and by the variability in the assessment of CNS adverse events, with only one study using a standard scale [50]. Viii) No study utilized R doses higher than 10 mg/kg, leaving the PK, safety and efficacy implications related with co-administration of ART and anti-TB regimens utilizing higher R doses to remain unknown.

In conclusion, this systematic review supports the current recommendation for co- administration of ART regimen with EFV 600mg daily and anti-TB treatment in TB-HIV high burden countries. This systematic review is important as ritonavir/cobicistat boosted PI cannot be used with R, and yet sufficient data is not yet available on potential use of raltegravir or dolutegravir [74, 75]. The interpretation and management of elevations in ALT and CNS adverse events should done not only in the context of EFV and RH interaction but also looking at other independent predictors like advanced disease, liver diseases, adherence and patients' demographic characteristics. Despite the reported good PK, efficacy and safety profile of the 400mg dose of EFV as compared to 600mg in HIV patients [69, 76], its use among TB/HIV co-infected patients who are having concurrent TB treatment will necessitate further PK and pharmacogenetic exploration in both African and Asian populations. Finally, there is need for research that explores the safety and effect of high R doses on EFV PK as a strategy towards developing treatment shortening regimens.

DECLARATIONS

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Conflict of Interest

Author AD, Author MB and Author AMT declare that they have no conflict of interest.

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Table I. Characteristics of the selected studies and of the HIV-TB co-infected patients included

Characteristic	Number of studies	
Study types, n (%)	22	
Prospective non-randomized comparative study		6 (27.3)
Cohort from randomized clinical trial		8 (36.4)
Prospective cohort		6 (27.3)
Case-Control		1 (4.6)
Cross-sectional		1 (4.6)
Geographical setting of studies, n (%)	22	
Africa		14 (63.6)
Asia		7 (31.8)
Africa/Latin America		1 (4.6)
Study population, n (%)	22	
Adult		20 (90.9)
Children		2 (9.1)
Age in years, median [range]		
Adult studies	17	35 [31.0-40.5]
Children studies	2	7.4 [6.3-8.5]
Gender, male		
Adults, n (%)	20	
<50%		5 (25.0)
50-70%		11 (55.0)
>70%		4 (20.0)
Children, n (%)	2	
<50%		1 (50)
50-70%		1 (50)
Body weight in kilograms		
Adult studies, median [range]	15	53.3 [50.0-57.4]
Children studies, median [range]	2	20.7 [18-23.4]
Sample size for selected studies, median [IQR]	22	131 [45-270]
Adult studies, n (%)	20	
<30		3 (15.0)
30-100		5 (25.0)
>100		12 (60.0)
Children, n (%)	2	
<30		1 (50.0)
30-100		1 (50.0)
Patient groups, n (%)	22	
HIV-TB co-infected and HIV-mono infected (2 parallel groups)		7 (31.8)
HIV-TB co-infected on and off anti-TB drugs		13 (59.1)
HIV-TB co-infected on anti-TB drugs (no off anti-TB drugs period)		2 (9.1)
Anti-TB treatment administration frequency for 6 months, n (%)	22	
Daily		21 (95.5)
Thrice weekly		1 (4.5)
EFV intake, n (%)	22	
Evening		20 (90.9)
Morning		2 (9.1)
EFV Pharmacokinetic parameter reported*, n (%)	22	
C12 (mid-dose concentration)		16 (72.7)
Cmin (Ctrough, pre-dose concentration)		6 (27.3)
CNS toxicity assessed, n (%)	22	8 (36.4)
Hepatotoxicity assessed, n (%)	22	7 (31.8)
Virological response assessed following standard anti-TB drugs co-administration, n (%)	22	12 (54.5)

HIV: Human Immuno-virus; TB: Tuberculosis; EFV: Efavirenz; C12: Mid-dose concentration; Cmin: Ctrough, pre-dose concentration; CNS: Central Nervous System; IQR: inter-quartile range

Table II. Concentrations of EFV co administered with RH and without RH or after the discontinuation of TB treatment, measured at steady state (≥ 4 weeks) in 20 selected studies. Concentrations (ng/mL) are reported as mean (SD), median (p25, p75) or median [range]

Region/study description				On RH			Off RH			% Diff. in EFV conc. between on and off-RH**
	Author/Country	Ref. no	Comparison group	n	Cmin	C12	n	Cmin	C12	
AFRICA										
5 Adult studies, with on/off RH data in same patient	Semvua, 2013; (Tanzania)	[46]	S	21	2600 (1600, 4200)		21	2400 (1600, 3500)		8.3%
	Bhatt, 2015 ; (Mozambique)	[60]	S	235		2700 (1701, 6965)	199		2604 (1742, 4412)	3.7%
	Bienvenu, 2014;(Rwanda)	[59]	S	21		1800 (1400, 2300)	21		2700 (1500, 3100)	-33.3%
	Friedland, 2006; (South Africa)*	[64]	S	19	1730 [350, 27180]		19	1380 [570, 3980]		25.4%
	Orrell, 2011;(South Africa)	[32]	S	34		2400 (1200, 5100)	34		2200 (1400, 3700)	9.1%
5 Adult studies, with on-RH data in TB-HIV co-infected group and off-RH data in HIV only patient group	Ngaimisi, 2011 ; (Tanzania)	[49]	P	54		1148 (895, 2270)	128		1614 (1140, 2692)	-28.9%
	Habtewold, 2015; (Ethiopia)	[55]	P	60		1515 (856, 3039)	187		1290 (934, 1869)	17.4%
	Cohen, 2009; (South Africa)	[57]	P	40		2400 (1300, 3100)	102		1800 (1400, 4400)	33.3%
	Mukonzo, 2014; (Uganda)	[51]	P	130		1916 (1467, 3098)	78		2312 (1638, 3063)	-17.1%
	Mukonzo, 2013; (Uganda)	[50]	P	118		1820 (1420, 3210)	50		2410 (1640, 3060)	-24.5%
2 Children studies with on/off RH data in same patient	Ren, 2009; (South Africa)	[47]	S	15	830 (590, 6570)	1240 (910, 7380)	15	860 (610, 3560)	1230 (850, 4180)	0.8
	McIlleron, 2013 ; (South Africa)	[35]	S	32		1640 (1210, 4400)	32		1960 (1320, 2930)	-16.3
2 African studies in adults, without off RH data	Yimer, 2011 ; (Ethiopia)	[44]	S	67		1318 (977, 1995)				
	Gengiah, 2015 ; (South Africa)	[56]	S	29	3100 (2600, 4800)					
ASIA										
3 Adult studies, with on/off-RH data in same patient	Ramachandran, 2013 ^c ; (India)	[48]	S	51	2300 (2500)		49	2100 (1900)		9.5%
	Uttayamakul, 2010 ; (Thailand)	[45]	S	65		4420 (5970)	65		3500 (2670)	26.3%
	Borand, 2014; (Cambodia)	[58]	S	401		2667 (1753,4494)	401		2766 (1941, 3976)	-3.6%
1 Adult study, with on-RH data in TB-HIV co-infected group and off-RH data in HIV only patient group	Manosuthi, 2013; (Thailand)	[52]	P	101		2100 (1300, 3500)	38		2700 (1800, 5400)	-22.2%

1 adult study without off-RH data	Manosuthi, 2009 ; (Thailand)	[53]	None	71		3540 (3780)				
AFRICAN/ LATIN AMERICA:										
1 study without off-RH data	Luetkemeyer, 2013; (International)	[54]	S	505	1960 (1240, 3790)					

* Geometric mean (90% confidence intervals) or geometric mean [range]); SD: standard deviation; IQR: interquartile range; RH: rifampicin and Isoniazid; C12: mid-dose concentration;

Cmin: Ctrough, pre-dose concentration; Ref.no: reference number corresponding to the cited study.

S= PK comparisons done in same patients; P= PK comparisons done in different patients;

**This was calculated as the difference in mean or median C12 or Cmin during on RH and off RH, as a fraction of the mean or median C12 or C_{min} during on RH, and expressed as a percentage. This estimates the change in EFV plasma concentration attributable to RH co-administration.

Table III. Presentation of virological response with EFV concentrations during RH co-administration

Studies	Reference number	VL threshold (copies/mL)	Follow-up (months)	% patients with Sub-therapeutic levels*	% patients with VL suppression
Adult studies, N=8					
Mariana, 2016 (Indonesia)	[62]	<40	3 to 6	72.2	27.8
Manosuthi, 2009 (Thailand)	[53]	<50	12	3.1	83.9
Habtewold Abiy, 2015 (Ethiopia)	[55]	<50	12	38.6	84.1
Bhatt, 2015 (Mozambique)	[60]	<50	6	8.9	85.5
Friedland, 2006 (South Africa)	[64]	<100	6	31.6	80
Borand, 2014 (Cambodia)	[58]	<250	6	5.3	91
Luetkemeyer, 2013 (Botswana, Brazil, Haiti, Kenya, Malawi, South Africa, Thailand, Uganda, Zimbabwe, Peru, USA)	[54]	<400	12	27.3*	71.4
Orrell, 2011 (South Africa)	[32]	<50	12	12	92
Children studies, N= 2					
Ren , 2009 (South Africa)	[47]	<50	6	60*	84.6
McIlleron, 2013 (South Africa)	[35]	<400	6	17.4	87.0

All results based on C12 unless otherwise indicated (*) in case of Cmin based results; VL: viral load;

RH: Rifampicin and Isoniazid

Table IV. Hepatic and Central nervous system adverse events among HIV-TB co-infected patients

Study	Ref no	Number of HIV-TB coinfected patients included in the analysis, N	% of patients with all grade ALT increase	% of patients with all grade CNS events	Relationship between ALT rise with EFV/RH co-administration	Relationship between CNS events with EFV/RH co-administration
Mariana, 2016; (Indonesia): P	[62]	18	16.7	55.6	No relationship despite the higher incidence of all-grade ALT rise among HIV-TB patients (16.7%) versus HIV alone (3.7%). No patient developed supratherapeutic EFV concentrations.	No relationship noted. Since no patient had supratherapeutic EFV plasma concentrations, Authors attributed the high onset of CNS events to EFV's high lipophilic nature allowing it to easily penetrate the blood brain barrier.
Habtewold, 2015; (Ethiopia): P	[55]	208	30.0	No data	Relationship with EFV/RH co-administration noted. Incidence of grade ≥ 3 ALT rise higher in HIV-TB patients on EFV/R co-administration (30%) than in HIV patients on EFV alone (15.7%). The role of high EFV plasma concentration and CYP2B6*6 genotype noted in both patient groups. NAT2 slow-acetylator genotype, as determined by sequencing of NAT-2 coding region predicted liver toxicity in TB-HIV co-infected on isoniazid. Overlapping drug toxicity (ART and anti-TB drugs) and disease effect (TB-HIV coinfection) could not be ruled-out.	Not applicable
Borand, 2014; (Cambodia): S	[58]	540	8.7	0.9	No relationship noted with grade ≥ 3 transaminase elevation ($p=0.30$), instead a significant relationship was noted between the risk of any grade hepatotoxicity with having consistent supratherapeutic EFV concentrations ($p<0.001$, OR =1.52 [1.33-1.74] but not with intermittent EFV levels $>4\,000$ ng/mL, as compared with those in normal ranges.	No relationship noted with CNS events grade ≥ 3 , $p=0.30$, but a significant relationship was noted between developing a CNS side-effect irrespective of grade with having at least one time supratherapeutic EFV levels as compared to those with normal ranges, $p<0.001$, OR= 2.72 [2.05-3.62]
Mukonzo, 2013; (Uganda): P	[50]	138	No data	74.0	Not applicable	Relationship with EFV/RH co-administration noted. All grade CNS symptoms during ART were significantly predicted by EFV plasma concentrations consistently. No significant differences in incidence of CNS symptoms between patients on EFV with RH (74%) and those without R (72%) co-treatment ($p=0.73$) was noted. No treatment discontinuation occurred due to severe CNS events.
Luetkemeyer, 2013; (International): S	[54]	780	No data	5.9		No relationship with EFV/RH co-administration noted. EFV Cmin $>4\,000$ ng/ml was not significantly associated with occurrence of grade 3 or higher CNS events.
Friedland, 2006; (South Africa): S	[64]	19	No data	36.8		No clear association was observed between onset of all grade CNS symptoms and plasma EFV levels.

McIlleron, 2013; (South Africa): S	[35]	40	2.5	0	No relationship with EFV/RH co-administration noted. Only 1 child suffered a grade 3 elevation in ALT in the month after completion of anti-TB treatment, which turned to normal without treatment adjustment. This child had an average mid-dose interval concentration of 17.7 mg/l during anti-TB treatment, which dropped to 4.14 mg/l a month after stopping RH and isoniazid. There was a low incidence of liver toxicity with use of R 10 mg/kg.	No relationship with EFV/RH co-administration noted. Though assessed, no grade 3 or 4 CNS events recorded. Subtle effects were not recorded in the study. Lack of CNS events was linked to good tolerability given the night administration of EFV..
Bhatt, 2015 ; (Mozambique): S	[60]	302	No data	2.0	Not applicable	No relationship with EFV/RH co-administration noted. No significant association between occurrence of grade 2 or higher CNS adverse events reported within the first 12 weeks of ART and EFV concentrations >4000 ng/ml at week 12 , p=0.293
Orrell, 2011; (South Africa): S	[32]	72	2.8	No data	No relationship with EFV/RH co-administration noted. Both grade 2 and 3 events of raise in ALT occurred during EFV co-administration. The absence of ALT elevation events before ART initiation signified that these events were EFV-related. No direct link made to EFV interaction.	Not applicable
Cohen, 2009; (South Africa): P	[57]	137	No data	35.8	Not applicable	A relationship with EFV/RH co-administration noted. About 31% of those with CNS symptoms (all grade) had high EFV concentrations. No significant associations between EFV concentrations and other neuropsychiatric symptoms.
Semvua, 2013; (Tanzania): S	[46]	25	4.0	No data	No relationship with EFV/RH co-administration noted. Only ALT rises of grade 1 noted, with no link with EFV interaction established.	Not applicable
Gengiah, 2015; (South Africa): S	[56]	20	9.0	5.0	No relationship with EFV/RH co-administration noted. Approximately 67% of all events of transaminase rise (grade 3 or 4) occurred during ATT alone, and only 26.7% during ATT/ART. No link to interaction and events during ART resolved without drug cessation.	No relationship with EFV/RH co-administration noted. All the reported 3 CNS toxicity events (all grade), were from 1 patient on EFV 800mg and had a Cmin=2100ng/ml at the time of onset of CNS events. Symptoms ceased with a switch from morning to night administration of EFV.
Manosuthi, 2009; (Thailand): S	[53]	71	0	No data	No relationship with EFV/RH co-administration noted. No NNRTI-associated hepatitis with EFV co-administered with RH.	Not applicable

S: same patient comparisons (only HIV-TB co-infected). P: Parallel patients' comparisons (both HIV only and HIV-TB co-infected), ALT: Alkaline aminotransferase, CNS: Central nervous system, EFV: Efavirenz, RH: Rifampicin and Isoniazid, TB: tuberculosis, ART: Antiretroviral therapy, Cmin: minimum EFV concentration, Ref no: reference number

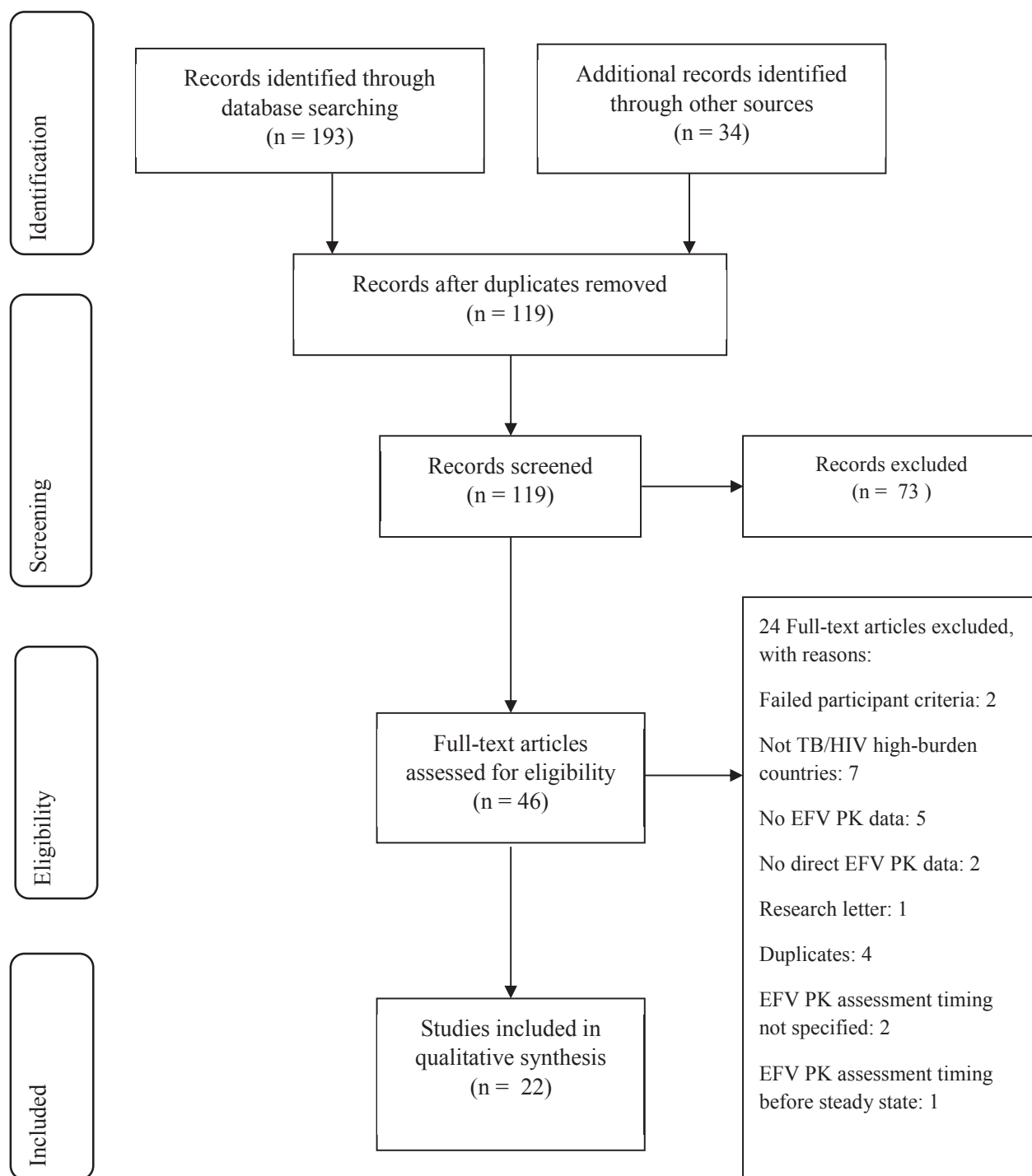


Fig I. Flow chart showing the selection of relevant papers.

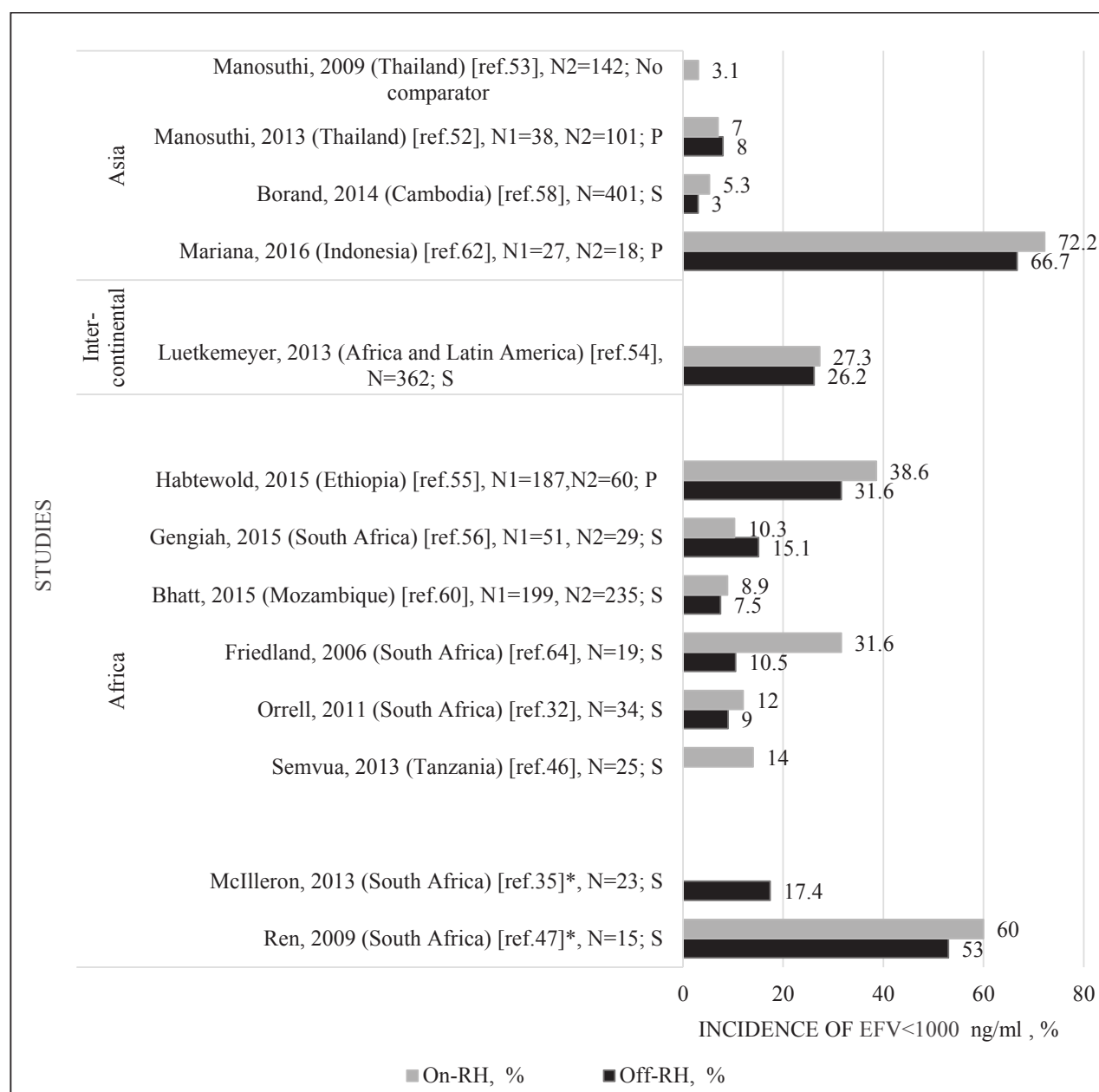


Fig II. Proportion of patients with sub-therapeutic efavirenz Plasma concentration

*Children studies; S= on and off RH EFV sub-therapeutic concentrations assessed in the same patient; P= on and off RH EFV sub-therapeutic concentrations assessed in different patient groups (HIV-TB co-infected Versus HIV only). N= Sample size on which the EFV sub-therapeutic rates both with or without RH co-administration is based; N1= Sample size on which the EFV sub-therapeutic rates during ART without RH co-administration is based; N2= Sample size on which the EFV sub-therapeutic rates during ART with RH co-administration is based.

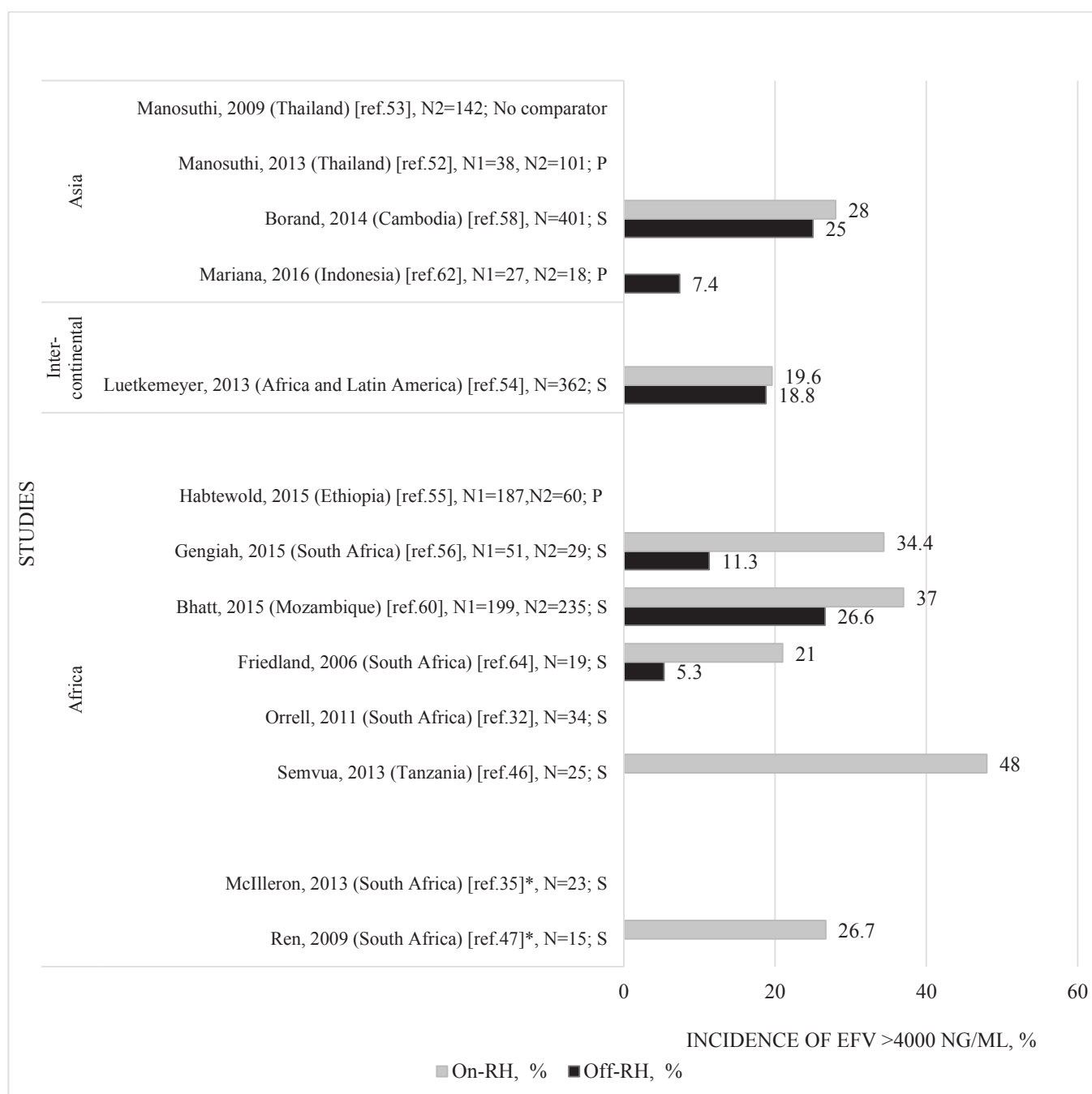
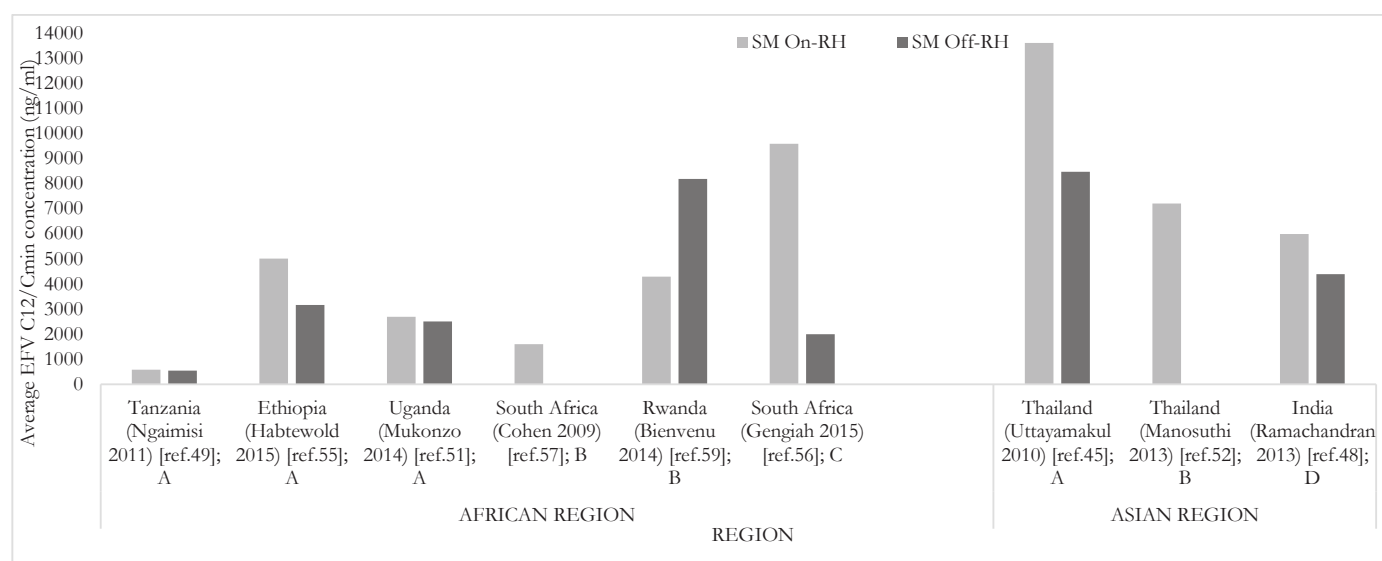


Fig III. Proportion of patients with supra-therapeutic efavirenz plasma concentration

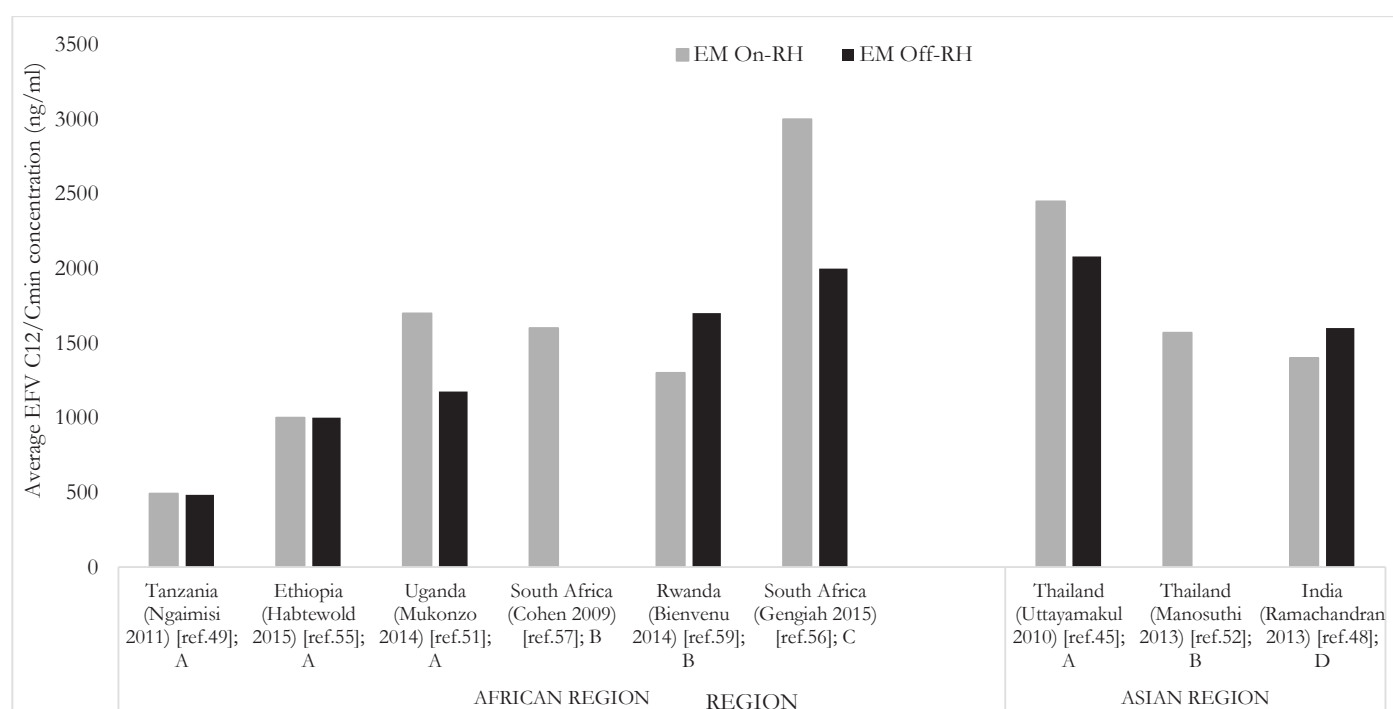
*Children studies; S= on and off RH sub-therapeutic EFV concentrations assessed in the same patient; P = on and off RH sub-therapeutic EFV concentrations assessed in different patient groups (HIV-TB co-infected Versus HIV only). N= Sample size on which the EFV sub-therapeutic rates both with or without RH co-administration is based; N1= Sample size on which the EFV sub-therapeutic rates during ART without RH co-administration is based; N2= Sample size on which the EFV sub-therapeutic rates during ART with RH co-administration is based.

IVa



SM freq%	16.7	28.1		10	13	11.1		13.9	11.8	18.9
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IVb



EM freq%	37.5			45	49	50		38.5	45	33.7
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Fig IV. Efavirenz plasma concentrations on and off rifampicin co-administration among patients with CYP2B6 homozygous slow metabolizer genetic polymorphism (IVa) and extensive metabolizer genetic polymorphism, CYP2B6 G516T (IVa).

RH: Rifampicin and Isoniazid. Off-R: off rifampicin. SM: Slow metabolizer. EM: Extensive metabolizer; EFV: Efavirenz; Ref: reference number; C12: efavirenz mid-dose concentrations. Cmin: minimum efavirenz concentration; A: C12, mean, B: C12, median, C: Cmin, median, D: Cmin, Mean

Appendix I. Study specific characteristics, N=22

Study ID	Study design	Ref. no	N	PK	Patients' groups	Hepatic toxicity assessed	CNS toxicity assessed	Virological f-up month	Male (%)	Age, median/mean (years)	Body weight, median/mean (Kg)
Adult Studies, N=20											
Mariana, 2016 (Indonesia)	Prospective randomized non comparative study	[62]	45	C12	A	Yes	Yes	6	81.5	32	54
Habtewold, 2015 (Ethiopia)	Prospective randomized non comparative study	[55]	493	C12	A	Yes	No	6	52.6	35	50
Mukonzo, 2014 (Uganda)	Prospective randomized non comparative study	[51]	263	C12	A	No	No		50	37	50
Borand, 2014 (Cambodia)	Cohort from a randomized clinical trial	[58]	540	C12	B	No	Yes	6, 12	65	35	45
Bienvenu, 2014 (Rwanda)	Prospective cohort	[59]	21	C12	B	No	No	1.5	51.3	38	
Mukonzo, 2013 (Uganda)	Prospective randomized non comparative study	[50]	197	C12	A	No	Yes		44.7	33.8	53.6
Luetkemeyer, 2013; (Botswana, Brazil, Haiti, Kenya, Malawi, South Africa, Thailand, Uganda, Zimbabwe, Peru, USA)	Cohort from a randomized clinical trial	[54]	543	Cmin	B	No	Yes	12	63	34	52.8
Manosuthi, 2013 (Thailand)	Prospective randomized non comparative study	[52]	139	C12	A	No	No		78	37	54
Borand, 2013 (Cambodia)	Cohort from a randomized clinical trial	[61]	482	C12	C	No	No		93		
Semvua, 2013 (Tanzania) ^d	Prospective cohort	[46]	25	Cmin	B	Yes ^a	No		56	32	48.4
Orrell, 2011*** (South Africa)	Prospective cohort	[32]	72	C12	B	Yes ^a	Yes	12	35		60
Cohen, 2009 (South Africa)	Cross-sectional study	[57]	142	C12	A	No	Yes	6	72.5	40.3	65.2
Friedland, 2006 (South Africa) ^d	Prospective cohort	[64]	20	Cmin	B	Yes	Yes	6	25	31	59.4
Ramachandran, 2013 (India) ^c	Cohort from a randomized control trial	[48]	55	Cmin	B	No	No	6	46	34.8	
Uttayamakul, 2010 (Thailand)	Cohort from a randomized control trial	[45]	124	C12	B	No	No	6-12	64.6	35.9	53.3
Manosuthi, 2009 (Thailand)	Cohort from a randomized control trial	[53]	71	C12	C	No	No	12	64.8	35.7	52.9
Bhatt, 2015 (Mozambique)	Cohort from a randomized control trial	[60]	270	C12	B	Yes	Yes	3-12	59.3	33	52.3
Yimer, 2011 (Ethiopia)	Case-control: liver toxicity vs no liver toxicity	[44]	353	C12	B	No	No		58.6		
Ngaimisi, 2011 (Tanzania)	Prospective randomized non comparative study	[49]	182	C12	A	No	No		36.6	40.5	
Gengiah, 2015 (South Africa)	Cohort from a randomized control trial	[56]	58	Cmin	B	Yes ^b	No		53.6	32	57.4
Children studies , N= 2											
Ren, 2009 (South Africa)	Prospective cohort	[47]	15	Cmin	B	No	No	6	60	6.3	18
McIlleron, 2013 (South Africa)	Prospective cohort	[35]	40	C12	B	Yes	No	6	38	8.5	23.4

^a On and off RH values not reported. ^bNumber of patients with adverse events not clear. ^cATT given thrice a week throughout the 6 months of treatment. ^dEfavirenz intake in the morning.

Note: Same R-dose of 10mg/kg used, *Also used EFV= 800mg. ***Calculated as an average of the 3 medians provided for the different SNPs (41, 54 and 67 for GG, GT and TT respectively). **Only for On-RH group. For off-RH it is 234 cells/ml.

A: 2 different groups: HIV-TB co-infected and HIV infected without TB

B: HIV-TB co-infected patients on and off antituberculosis drugs C: One group of HIV-TB co-infected patients on antituberculosis drugs

D: Two groups of HIV-TB co-infected patients on antituberculosis drugs with two different dose of efavirenz

Chapter 3.3

Safety and pharmacokinetics of high-dose rifampicin and efavirenz during co-administration among HIV-positive TB patients

Presentations related to this chapter:

Atwine D., Baudin E., Muyindike E., Kananura K., Nyehangane D., Orikiriza P., Nanjebe D., Logoose S, Rinah A., Mwanga-Amumpaire J., Taburet AM., Bonnet M. Eight weeks safety results of high-dose Rifampicin in HIV-Tuberculosis co-infected patients in Uganda: RIFAVIRENZ-ANRS 12292 Trial. [Abstr MOPEB0340] July 2017. in International AIDS Conference. July 2017. Paris, France. (Poster Presentation and obtained an ANRS scholarship for the presentation)

Atwine DW., Baudin E., Gelé T., Muyindike W., Kenneth M., Kyohairwe R., Kananura K., Nyehangane D., Orikiriza P., Furlan V., Taburet AM., Barrail-Tran A., Bonnet M., and the ANRS12292 Rifavirenz study group. Efavirenz pharmacokinetics with high-dose rifampicin in tuberculosis-HIV infected patients. [Abstract number 329] submitted to the CROI conference March 2018, Boston, United States.

Article under writing

3.3.1 Justification and objectives

Despite the already demonstrated safety of doses of R as high as 15, 20 and 35mg/kg [262, 263, 269] among HIV-negative patients with PTB, there is no corresponding data on the safety of higher doses of R when co-administered with EFV-based ART in HIV-TB co-infected patients[65, 66]. In addition, the uncertainty of the drug-drug interactions that could arise with co-administration of EFV and high R doses and their impact on both ART and ATT efficacy [270].

We report the results of the ANRS12292 RIFAVIRENZ trial that compared the PK parameters of EFV in HIV-TB co-infected patients, with and without co-administration of R, when using two different dosing regimen of R (10 and 20mg/Kg/day) and EFV (600 and 800mg daily), respectively. Secondly, we report results on: the safety of concurrent administration of ATT with high dose R and EFV-based ART in HIV-TB co-infected patients by week-8 after ATT initiation and throughout the 28 weeks of study follow-up; HIV virological response after starting ART; eight weeks MTB culture conversion after starting ATT; outcomes of ATT; and patients' adherence to the ART and ATT. Also, the PK of R and H and the pharmacogenetic analysis planned in this trial are still ongoing and are not presented in this report. The manuscript could not be submitted before the completion of the PHD due to these pending results. So we detail in the PhD book the results available as per the Trial objectives.

3.3.2 Methods

The RIFAVIRENZ Trial was approved by the Research Ethics Committee of Mbarara University of Science and Technology, Uganda National Council for Science and Technology (UNCST) and National Drug Authority (NDA) for Uganda.

The study design was a single site phase-2, open-label RCT among Ugandan patients conducted between 2014 and 2017. The trial enrolled patients from Mbarara Regional Referral Hospital and other surrounding health facilities within Mbarara district in Uganda.

3.3.2.1 Study population

Patients were eligible if they were; ≥ 18 years of age, newly diagnosed with pulmonary tuberculosis confirmed by XpertMTB/RIF test, HIV positive, ART naïve, for women of childbearing age: having a negative urine test for pregnancy on the day of enrolment, and accept to take a barrier contraception during the trial. Participants had to be stable enough to receive ambulatory treatment, weight > 35 Kg, having a readily accessible home address and providing informed consent to participate in the trial. A patient was excluded if the patient had RR-TB based on

XpertMTB/RIF result confirmed by Genotype MTBDR plus assay, had concomitant opportunistic infection requiring additional medication, had Karnofsky score <80%, alanine aminotransferases (ALT) or bilirubin > 5.0 x ULN (hepatitis grade 3 or 4), grade 4 clinical sign or biological result according to the DAIDS grading scale, not able to give his/her informed consent or unable to cooperate with sampling procedures, patients receiving or requiring medications that may interfere with study drugs, having a formal contraindication to any drug used in the trial, and pregnant or lactating women.

Written informed consent was obtained from all patients prior to their participation in the study.

3.3.2.2 Randomization

A randomization schedule was created by the trial statistician and patients were randomized in a 1:1:1 ratio to each of the three treatment groups. A fixed randomization method was used thus ensuring that the probability of being assigned in one of the three arms remained constant throughout the study. A block-randomization ensured that a comparable set of patients is included in each group at all times. The study site was supplied with a batch of sealed and serially numbered opaque envelopes each containing a slip of paper showing the allocated regimen. There was no attempt to conceal the treatment after randomization from patients, researchers, or healthcare staff.

3.3.2.3 Treatment allocation

PTB and ART-naïve patients were randomized to one of the 3 treatment arms below:

- Arm 1: 8 weeks R20mg/Kg + H + Z + E and EFV600mg/day +tenofovir-lamivudine fixed dose combination (TDF/3TC);
- Arm 2: 8 weeks R20mg/Kg + H+Z+ E and EFV800mg/day + TDF/3TC;
- Standard arm: 8 weeks R10mg/Kg + H+Z+ E and EFV600mg/day + TDF/3TC.

All ATT doses were calculated according to WHO weight-band recommendation[195]. ART treatment was started after first 2 weeks or 4 weeks of ATT for patients with baseline CD4<50 and ≥ 50 cells/mm³, respectively. At 8 weeks, all patients were switched to standard R (10mg/kg) and EFV (600mg) doses (see Figure 12).

Patients took ART in the evening during the first two weeks in order to facilitate the tolerance to EFV and then switched to morning intake for organisational reason, that is, in order to perform the EFV PK assessment during the day. For patients who could not tolerate the EFV after this switch, were returned to the evening intake and the PK assessment done during the night. Pyridoxine and cotrimoxazole were administered to all patients. Only WHO pre-qualified drugs were used in this study.

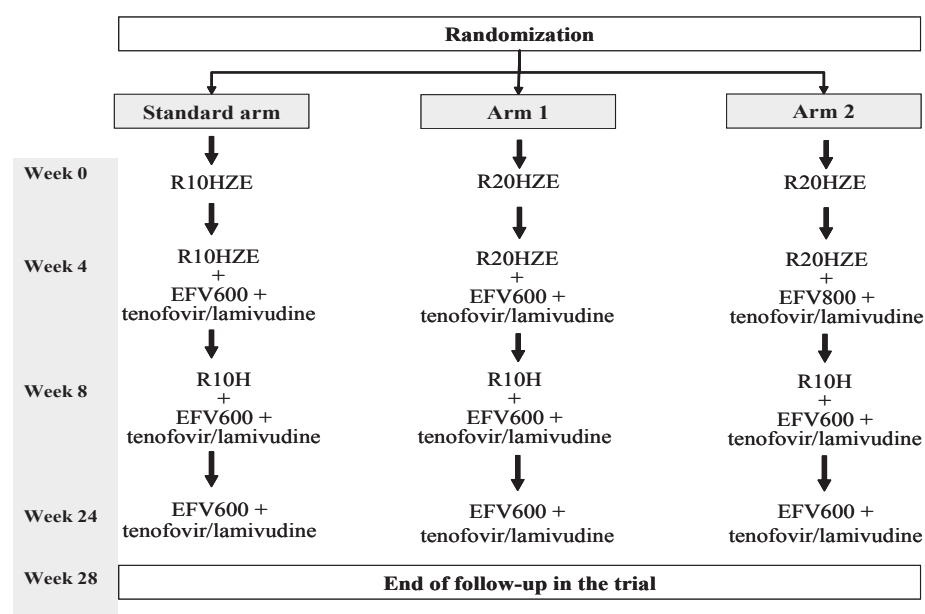


Figure 11. Study scheme

3.3.2.4 Follow-up

Patients were interviewed regarding symptoms and signs, and liver function test (AST, ALT, and bilirubin), complete blood count evaluations were carried out at baseline, week 2, 4, and 8 after starting treatment. Patients could also attend at any time if they experienced side effects. AEs were assessed using the DAIDS severity grading scale.

Patients were followed up to week 28, (that is, 4 weeks after stopping TB treatment) with weekly visits during the first 8 weeks and then 4 weekly visits. Treatment was observed at the trial clinic by the nurse, and at home by a DTM. The treatment adherence rate for both ATT and ART for each inter-visit time intervals was calculated as a percentage of the total dose intake under supervision out of the total dose received.

Sputum smear-microscopy was performed using fluorescence microscopy using LED-based microscope and auramine staining on both early morning and spot samples at weeks 8, 16 and 24 as recommended by the International Union Against Tuberculosis and Lung Disease guidelines at the laboratory of EMRC [271]. The GenXpert® MTB/RIF system (Cepheid, Sunnyvale, USA) was used in accordance to the manufacturer guidelines (www.cephheid.com) during screening of patients and in cases of smear-positivity at week 8 and beyond. The GenoType MTBDR_{plus} (HAIN lifesciences GmbH, Nehren, Germany) was used to confirm resistance to R in

sputum specimens collected at screening and collection visits according to the manufacturer guidelines (www.hain-lifescience.de).

Sputum specimens were tested with two MTB culture methods, that is, Lowenstein-Jensen (LJ) and mycobacteria growth indicator tube (MGIT) at enrolment and after eight weeks. Culture was also performed at week 24 though only in culture-positive patients at week 8. Sample decontamination was done using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method. The decontaminated pellet was then suspended into 2.5 mL of sodium chloride. For LJ, two drops of decontaminated sample were inoculated into one LJ medium and the LJ tube incubated for 56 days before reporting a negative result. Whenever growth would be detected, then smear microscopy would be performed to confirm the presence of acid fast bacilli. The number of colonies on the whole tube would then be estimated and reported. For MGIT, 500 µL from decontaminated sample were inoculated in MGIT tube. MGIT tubes were also incubated for 56 days before reporting a negative result. Whenever a MGIT tube was found positive, inoculation on blood agar was processed to check contamination and smear microscopy done for AFB detection. Distinction between MTB and non-tuberculosis mycobacteria (NTM) was confirmed using the P-nitrobenzoic acid and Bioline Ag MPT64 Rapid (Standard Diagnostics Inc, Kyonggi-do, Korea). In case of positive culture results at week 8, the MTB isolate were sent to the national TB laboratory in Kampala for DST.

The patients' CD4 counts were measured at the screening visit and at week 28. CD4 cell count and percentage results were generated using the BD FACSCount™ system (BD Biosciences, San Jose, USA) according to the manufacturer guidelines (http://www.bdbiosciences.com/documents/BD_FACSCount_Brochure.pdf).

For HIV-1 RNA, a blood sample was centrifuged locally and serum stored at -80°C at Epicentre research laboratory until shipment to the laboratory of the Joint Clinical Research Centre in Kampala for analysis using the automate PCR amplification and detection COBAS® AMPLICOR Analyzer (Roche Molecular Diagnostics, Indianapolis, USA) In addition, resistance mutations to NNRTI and NRTI were systematically searched in patients with more than 500copies/mL 24 weeks after starting ART and in specific cases according to protocol specific decisional algorithms at the MRC reference laboratory in Entebbe (Uganda).

3.3.2.5 PK procedures

All PK sampling was performed at therapy steady state: at least 2 weeks for RH and 4 weeks for EFV, respectively. Patients were admitted at the Epicentre TB research clinic for the evening prior and during the PK sampling.

PK sampling was performed at 8 time points over 24 hours, that is: -0.5 h (30 minutes prior to treatment administration), then 1, 2, 3, 4, 8, 12, 24 hours after treatment administration. For each PK sample collection for R, H (at week 2) and R, H, EFV (at week 8), 6 ml of blood were drawn, resulting in a total 48 ml of blood /patient/day.

For PK of EFV alone (at week 28), 4 mL of blood were collected, resulting in a total of 32 mL. After collection, fresh blood samples were sent to the Epicentre laboratory. As R and H are not stable in plasma at room temperature and at -20°C , all samples were immediately deep frozen at -70°C . After centrifugation within 40 min after blood collection, 500 μL of plasma exactly measured was half diluted with the ascorbic acid solution for rifampin assay and remaining plasma stored frozen at -70°C until shipment to the pharmacy department of the Bicêtre hospital in Paris (France) for analysis. Assays of EFV in plasma samples were performed using validated High Performance Liquid Chromatography (HPLC). Assays of R and H are still ongoing. The analytical laboratory was accredited for the purpose of quantifying drug concentrations in biological samples including assay development.

3.3.2.6 *Trial Endpoints*

The primary endpoint was the PK parameters of EFV with (4 weeks after starting EFV) and without R (4 weeks after completion of R): trough concentrations before drug intake (C_{\min}), maximal concentration (C_{\max}), time to achieve the C_{\max} (T_{\max}) and area under the curve of concentrations vs time at steady state during a 24-hour dosing interval (AUC_{0-24}).

The secondary endpoints include: 8 weeks MTB culture conversion; ATT outcome based on standard WHO case definitions; HIV-1 RNA after 4, 12 and 24 weeks on ART (in ART naïve patients, a reduction of 2 logs copies/mL is expected after 4 weeks of ART. After 12 and 24 weeks, the HIV-1 RNA is expected to be below 400 and 50 copies/mL, respectively); resistance mutations to NNRTI and NRTI in patients with HIV-1 RNA > 500 copies/mL 24 weeks after ART; proportion of patients with more than 95% adherence rate for ART and ATT, respectively; serious adverse event (SAE); major AE of interest: biological hepatitis, rash, neurological and psychiatric disorders.

3.3.2.7 *Statistical analysis*

We used a non-inferiority approach to show that the decrease in AUC of EFV when given with R20mg/Kg compared to EFV alone (EFV+R - EFV) is not greater than 30%. In each study arm, the EFV pharmacokinetic parameters with R were to be compared with the parameters without R in same patients. Therefore, with a number of 28 patients, at the 5% significance level (one sided), we will have 80% power to reject the null hypothesis that the decrease in AUC will be greater than 30%, assuming a standard deviation of the differences of the log AUC of 0.29 (reflecting the 15% intra-patient variability of EFV clearance based on the data from the PECAN study), and an expected difference of AUC of 20%, value in between the PECAN results (decrease of 13%) and the 26% decrease in AUC reported in the manufacturer's insert package [272]. The same sample size was estimated for each arm, including the arm using R10mg/Kg. There was a 20% increase in the sample size to take into account potential withdrawals between the PK assessments, resulting in 34 patients per study arm and a total of 105 patients.

Data were double data entered using Voozanoo (Epiconcept, Paris, France) software version 3.4.2 and analyzed using Stata version 3.0.

From individual plots of drug plasma concentrations versus time the following pharmacokinetic parameters were estimated using the software Win Nonlin v6.1 (Pharsight Corporation). Geometric mean ratios (GMR) of log transformed C_{min} and AUC of EFV with TB drugs over the C_{min} and AUC of EFV without TB drugs was calculated and presented with 90% confidence intervals (CI) in each study arm according to current FDA and EMA guidelines for drug-drug interaction studies. The limits of the confidence interval in each arm were compared to the 0.70-1.43 interval owing to the EFV therapeutic range.

Proportions were calculated to describe patients with week 8 *MTB* culture conversion, 24 week ATT outcomes, virological response at weeks 4 (HIV-1 RNA reduction of at least 2 logs), 12 (< 400copies/mL) and 24 (< 100copies/mL) after starting ART, and anti-TB and ART adherence above 95% and 80%. The safety analysis was performed on all patients who received at least one dose of treatment. The frequency of AEs and proportion of patients affected within the first 8 and 28 weeks post initiation of ATT were calculated by treatment regimen. The diagnosis of hepatotoxicity was based on rises in transaminases (ALT and AST) while that of CNS toxicity was based on symptom and clinical assessments.

3.3.3 Results

3.3.3.1 Characteristics of the patients

A total of 98 patients were randomized between April 2014 and August 2016.

Four populations were considered for the analysis (see Figure 13):

- Safety population: all patients exposed to the trial intervention (randomized patients).
- Modified intention to treat population: randomized patient after exclusion of one patient who was not HIV infected after PCR.
- EFV+R population: all patients who were exposed to EFV and anti-tuberculosis drug and had PK at week 8
- PK population: all patients who had samples drawn for PK at week 8 and 28 for comparison of EFV PK parameters on and off TB drugs. The GMR (primary endpoint) was analyzed in this population.

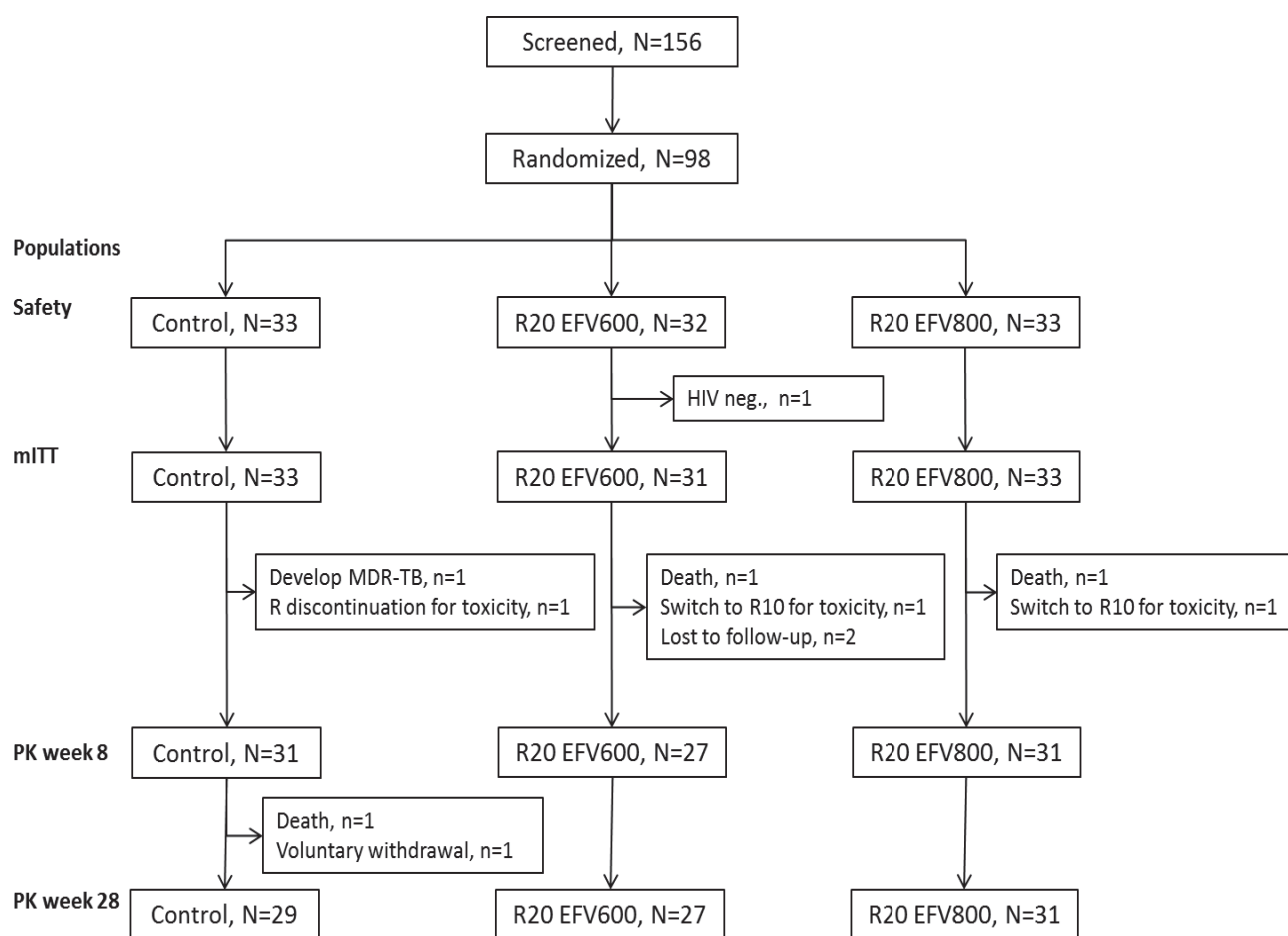


Figure 12. Study profile

Participants' baseline characteristics were similar in the 3 groups (Table 9). Three-quarters were men, with a median age of approximately 32 years and a median weight of approximately 52kg. Six (5%) of patients were positive for either hepatitis B surface antigen or hepatitis C antibody.

All patients had a good adherence >95% for both anti-TB treatment and ART for each inter-visit time intervals.

Table 7. Baseline participant's characteristics (mITT population)

	R10HZE EFV600 N=33	R20HZE EFV600 N=31	R20HZE EFV800 N=33
Males, n (%)	29 (87.9)	22 (71.0)	20 (60.6)
Age in years, Median [p25; p75]	34.1 [29.6; 38.1]	33.4 [28.0; 36.6]	32.3 [27.8;43.1]
Weight in kg, Median [p25; p75]	51.9 [49.2;56]	53.8 [48.2;59.1]	54.1 [50.6;58.0]
BMI in kg/m ² , Median [p25; p75]	18.6 [17.9;20.6]	20.5 [17.5;21.0]	19.6 [18.3;21.4]
Smear positive, n/N (%)	31/33 (93.9)	27/31 (87.1)	26/32 (81.3)
MTB culture positive (LJ or MGIT), n (%)	31 (93.9)	30 (96.8)	31 (93.9)
Hemoglobin, median [p25;p75]	11.1 [9;12.6]	12.4 [10.7;13.8]	10.7 [9.5;12.4]
CD4 cell count, median [p25;p75]	120 [66;252]	211 [69;334]	144 [86;367]
CD4 cell count <50, n/N (%)	7/33 (21.2)	4/27 (12.9)	4/33(12.1)
HIV1RNA (log copies/ml), median [p25;p75]	5.5 [4.6 ;5.8]	5.2 [4.5;5.7]	5.1 [4.8 ;5.9]
Radiological			
Presence of cavities, n (%)	14 (42.4)	16 (51.6)	12 (36.4)
Extend of lung disease			
Normal, n (%)	2 (6.1)	1 (3.2)	4 (12.1)
Minimal disease, n (%)	9 (27.3)	15 (48.4)	12 (36.4)
Moderately advanced, n (%)	16 (48.5)	14 (45.2)	14 (42.4)
Far advanced, n (%)	6 (18.2)	1 (3.2)	3 (9.1)

*ALT normal range: Male= 0-45 IU/L; Female= 0-34 IU/L

*AST normal range: Male= 0-35 IU/L; Female= 0-31 IU/L

IQR: interquartile range, HBV: Hepatitis B virus, HCV: Hepatitis C virus

3.3.3.2 Pharmacokinetics of efavirenz

Table 8 presents the main EFV PK parameters of interest (C_{\min} (H24), C_{12} , AUC_{0-24} at week 8 and week 28) and Table 9 the GMR week8/week28 of the C_{24} , C_{12} , and AUC.

The median C_{12} of EFV was consistently within the normal therapeutic range (1000-4000 ng/ml) during EFV with R (week 8) and without R (week28) co-administration, irrespective of treatment arm. The proportion of patients with sub-therapeutic EFV concentrations (<1000 ng/ml) were consistently higher during R co-administration (CR: 29%, SR1:14.3%, SR2: 29%) as compared to during EFV alone (CR: 21.4%, SR1:7.4%, SR2: 20%). No major differences were noted across treatment arms. The lower margins of the 90% confidence interval of the geometric mean ratios of either the AUC or C_{\min} week 8/week 28 irrespective of treatment arms were within the predefined range [0.70 to 1.43].

Table 8. Pharmacokinetic parameters of EFV

PK parameters	R10 EFV600 (CR)	R20 EFV600 (SR1)	R20 EFV800 (SR2)
Week 8 (On R), N	31	28	31
C ₁₂ median [p25;p75]	1605 [982.6; 3593]	1822.2 [1190.8; 2671.4]	1896.4 [938.8; 3270.1]
< 1000ng/mL, n (%)	9 (29.0)	4 (14.3)	9 (29.0)
C _{min} median [Range]	1077 [233; 9407]	1188 [498; 12212]	1032 [214; 11555]
C _{max} -ng/mL, median [p25;p75]	2325 [939; 19819]	2953 [786; 14 027]	2877 [952; 14872]
AUC-ng.h/mL, median [Range]	40 198 [13 406; 314 509]	47 505 [16 180; 308 410]	44 466 [12 943; 326 311]
Week 28 (off R)			
C ₁₂ , n median [p25;p75]	28 1629 [1050; 2686]	27 1989 [1336; 2845]	30 1420 [1042; 2920]
< 1000ng/mL, n (%)	6/28 (21.4)	2/27 (7.4)	6/30 (20.0)
C _{min} , n median [Range]	29 1137 [324; 8049]	27 1496 [457; 17967]	31 1028 [408; 11299]
C _{max} -ng/mL, median [p25;p75]	2692 [945; 11935]	3105 [841; 22128]	2300 [966; 13203]
AUC-ng.h/mL, median [Range]	38918 [14346; 214 301]	49 574 [13365; 486 759]	35 169 [14 236; 265 682]

C_{min}: minimum concentration/Pre-dosing concentration; C₁₂: Mid-dose concentration; C_{max}: maximum concentration; AUC: area under the concentration versus time curve; CR: control regimen SR1: study regimen 1; SR2: study regimen 2; On R: on rifampicin co-administration; Off R: off rifampicin co-administration

Table 9. Geometric mean ratio (GMR) week8/week28 of the C_{min} and AUC.

Pk parameters	R10 EFV600 (CR)	R20 EFV600 (SR1)	R20 EFV800 (SR2)
Geometric mean (GM) ratio (GMR), N	29	27	31
GM AUC at week 8, ng.h/mL	43374	48169	47627
GM AUC at week 28, ng.h/mL	45231	55475	42601
GMR AUC W8/W28	0.96 [0.84; 1.10]	0.87 [0.75; 1.00]	1.12 [0.96; 1.30]
GM C _{min} at week 8, ng/mL	1254	1377	1433
GM C _{min} at week 28, ng/mL	1360	1651	1651
GMR C _{min} W8/W28	0.92 [0.79; 1.08]	0.83 [0.72; 0.96]	1.16 [0.97; 1.39]

GM: geometric mean; GMR: geometric mean ratio; AUC: area under the curve; C_{min}: minimum concentration/Pre-dosing concentration; CR: control regimen SR1: study regimen 1; SR2: study regimen 2.

Figure 13 shows individual EFV AUCs on and off R in the 3 arms. There was huge inter-patient variability in the EFV plasma concentrations as shown by the individual EFV C_{min} at week 8 and week 28 by treatment arm (Figures 14A, 14B and 14C). Interestingly in some patients, EFV concentrations are higher on R than off R.

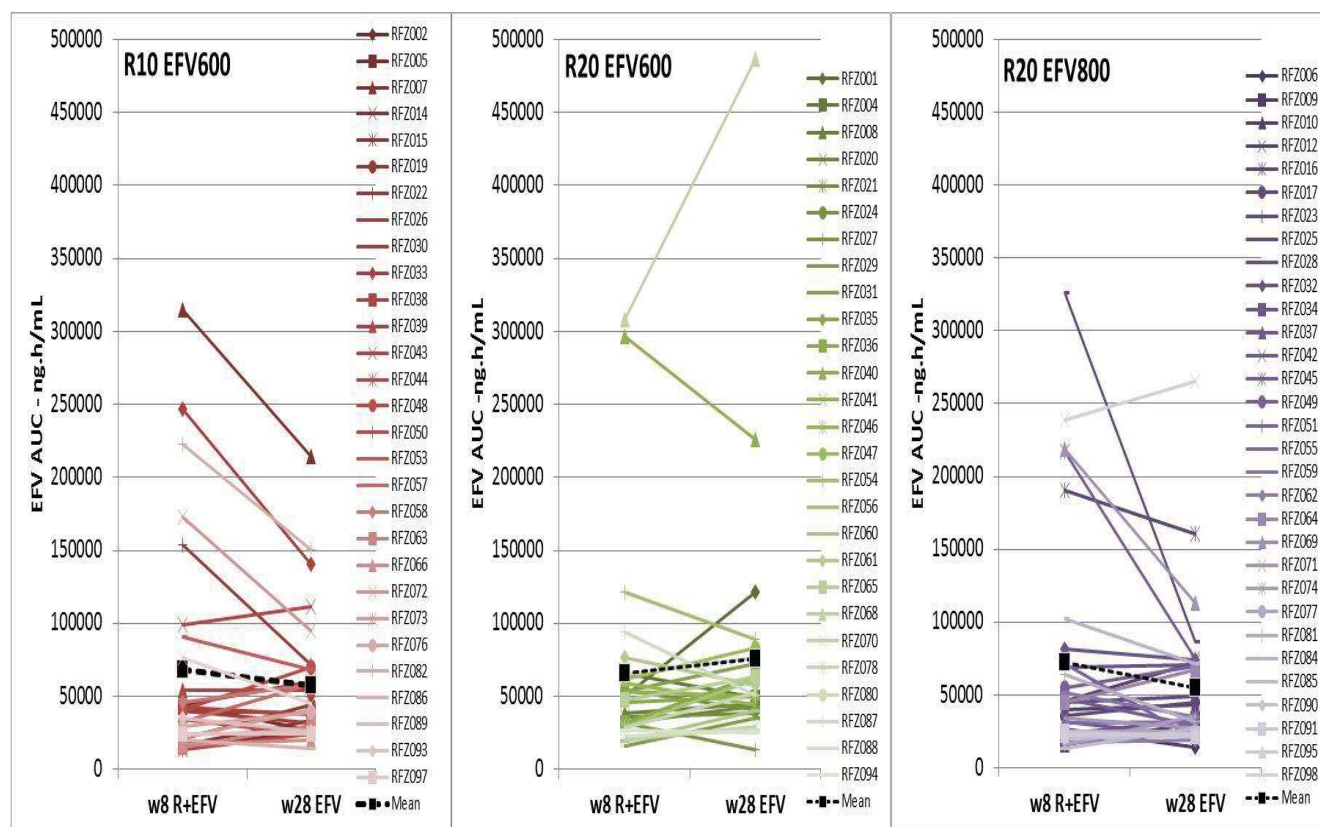


Figure 13. Efavirenz AUC per treatment arm



Figure 14. Shows individual Cmin on and off R in the three treatment arms.

Figure 14A. Individual efavirenz Cmin at week 8 and week 28 among patients in the R10EFV600 arm;

Figure 14B. Individual efavirenz minimum concentrations at week 8 and week 28 among patients in the R20EFV600 arm; Figure 14C. Individual efavirenz Cmin at week 8 and week 28 among patients in the R20EFV800 arm

3.3.3.3 Safety

Of the 98 patients included in the safety analysis, 18 patients (6 per treatment arm) developed at least one SAE during the 28 weeks of trial follow-up, with 3 deaths (1 per treatment arm). Of the 18 patients with at least 1 SAE, 15 had SAEs within the first 8 weeks of anti-TB treatment (4-6 weeks of ATT and ART co-administration), that is, 5 from each treatment arm had grade 3 or 4 adverse event, with 2 of the 15 patients resulting in death unrelated to treatment (see Table 10).

Serious adverse events and grade 3 or 4 hepatic or CNS adverse events occurring during the trial intervention are shown in Table 10. Elevated transaminases (ALT or AST) of grade 3 or 4 were observed in 6 patients (2 patients from each arm) within the first 8 weeks.

Table 10. Patients with at least one serious adverse event

	R10 EFV600 N=33	R20 EFV600 N=32	R20 EFV800 N=33
Overall SAEs (weeks 0 to 28), n (%)	6 (18.2)	6 (18.8)	6 (18.2)
Overall SAEs (weeks 0 to 8), n (%)	5 (15.2)	5 (15.6)	5 (15.2)
Leading to death, n (%)	1 (3.0)	1 (3.1)	1 (3.0)
0 to 28 week, n	1	1	1
0 to 8 week, n	1		1
Grade 3 or 4 increase ALT or AST, n	2 (6.1)	2 (6.2)	2 (6.1)
ALT grade ≥ 3 , n	2	2	2
AST grade ≥ 3 , n	2	2	2
Hyperbilirubinemia grade ≥ 3 , n (%)	3 (9.1)	2 (6.3)	0 (0.0)
CNS AE grade 2, n (%)	1 (3.0)	1 (3.1)	2 (6.2)

ALT: alanine aminotransferase AST: aspartate aminotransferase CNS: central nervous system AE: adverse event

SAE: serious adverse event (grade 3 and 4)

Figure 15 shows the timing of onset of elevated transaminases grade 3 or 4 with respect to time of initiation of antiretroviral treatment within the first 8 weeks. In the control arm all rises in ALT/AST of grade 3 or 4 were noted during anti-tuberculosis treatment alone (before ART initiation), with equal distribution noted pre-and post-ART initiation within the R high-dose treatment arms.

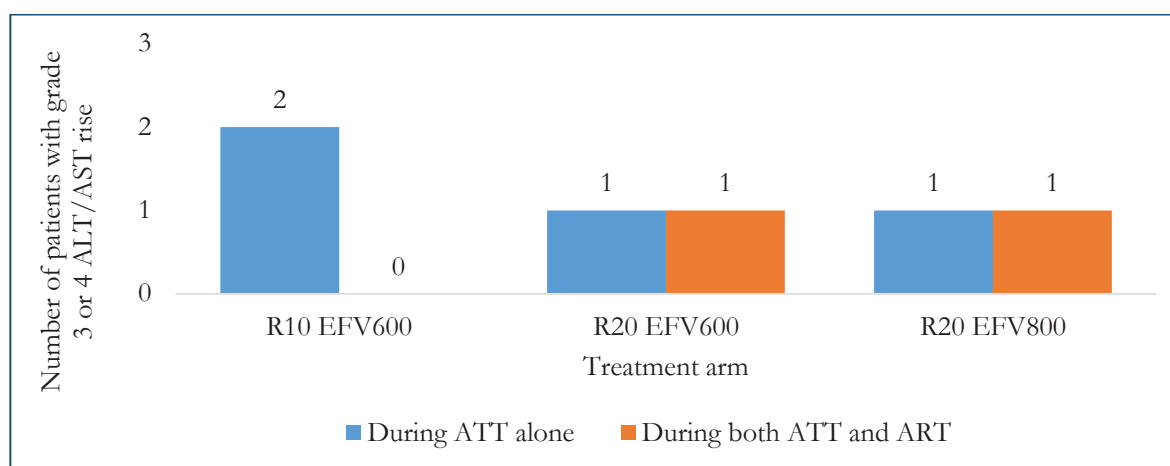


Figure 15. Onset of grade 3 or 4 elevated transaminases during first 8 weeks before and after ART initiation per treatment arm. ALT: Alanine aminotransferase enzyme; AST: Aspartate aminotransferase enzyme.

Of the 6 patients with grade 3 or 4 elevated transaminases, 2 had their treatment interrupted with subsequent change of treatment arm from R 20mg/kg to R 10mg/kg dose. No grade 3 or 4 CNS adverse events were reported. Only 4 patients (4.1%) had grade 2 CNS AEs within the first 8 weeks of treatment with a non-significant distribution across treatment arms, $p=0.780$ (see Table 2). An increasing trend in median ALT was shown irrespective of treatment arm during the first 8 weeks after initiation of ATT (see Figure 16).

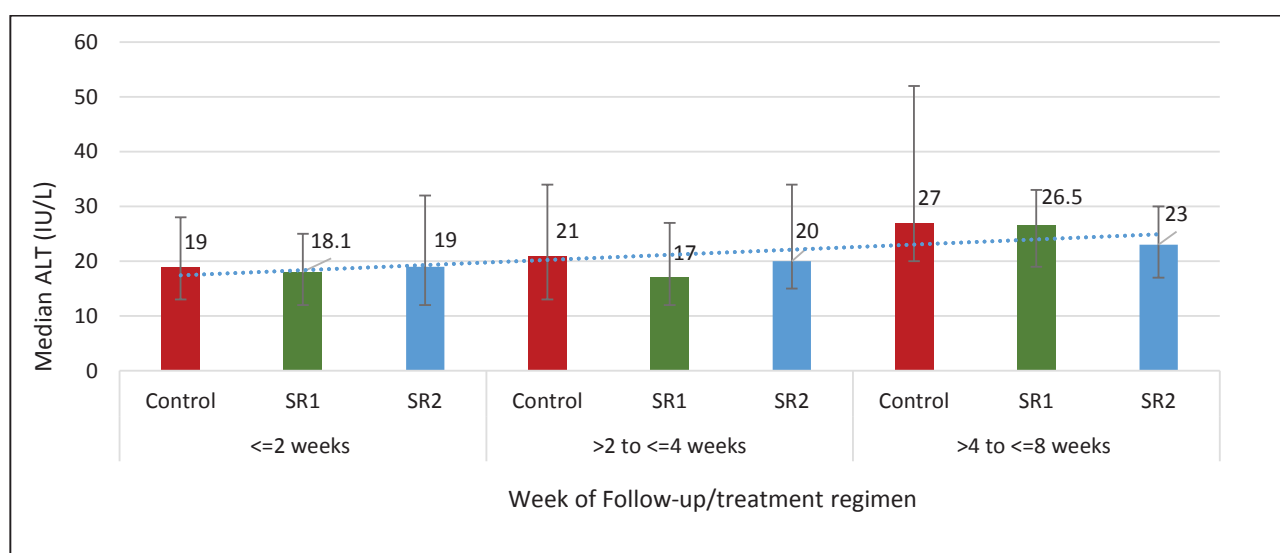


Figure 16. Evolution in median ALT per treatment regimen during study intervention (first 8 weeks) ALT: Alanine aminotransferase enzyme; EFV: Efavirenz; R: Rifampicin. The numbers on the bars are the medians, while the grey error lines on the bars represent the interquartile range (25th to 75th percentile). The dotted blue line is the trendline.

Other adverse events rather than hepatic and CNS

Overall, 12 patients developed non-hepatic grade 3 or 4 adverse events, although with no increasing trend across treatment arms. Specifically, equal number of patients developed thrombocytopenia (grade ≥ 3) in both R 10mg/kg arm (4) and in the two arms with R 20mg/kg (4). Two patients died, 1 following onset of hyponatraemia and 1 following Sepsis (see Table 11).

Table 11. Non-hepatic grade 3 and 4 adverse events

	R10 EFV600 N=33	R20 EFV600 N=32	R20 EFV800 N=33
Hyponatraemia	1*	0	0
Leukopenia	0	1	0
Respiratory distress	0	1	0
Sepsis	0	0	1*
Thrombocytopenia	4	1	3
Total	5	3	4

*Leading to death

3.3.3.4 Efficacy of antiretroviral treatment

The Virological response 24 weeks after ART initiation was reported based on two cut-offs, that is 400 and 100 copies/ml. We used a viral load threshold of 100 copies/mL because this was the lower limit of quantification of the laboratory. In a modified Intention to treat population (mITT), basing on a 400 copies/ml threshold, virological suppression was achieved in more than 80% of patients across treatment arms (CR: 96.6%; SR1:81.5%; SR2: 83.9%). For the 100 copies/ml threshold, virological suppression was <80% for the 2 high-dose rifampicin arms at 24 weeks post ART initiation (CR: 93.1%; SR1: 74.1%; SR2: 67.7%) (see Table 12).

Table 12. Level of reduction in HIV1 RNA at 4, 12 and 24 weeks after ART initiation, across treatment arms– mITT population after exclusion of 3 patients with baseline resistance to NNRTI

	R10HZE EFV600	R20HZE EFV600	R20HZE EFV800
Reduction HIV1 RNA ≥ 1 log 4 weeks after ART*	27/31 (87.1)	26/28 (92.9)	27/27 (100)
HIV1RNA<400 copies/ml			
12 weeks after ART	26/28 (92.9)	26/28 (92.9)	25/29 (86.2)
24 weeks after ART	28/28 (100)	22/27 (81.5)	26/29 (89.7)
HIV1RNA<100 copies/ml 24 weeks after ART	27/28 (96.4)	20/27 (74.1)	21/29 (72.4)

With exclusion of 3 patients with baseline NNRTI resistance, we note good early virological suppression at week 4 and week 12 after ART initiation. Of the patients with virological non-response by 24 weeks post ART initiation no relationship with EFV concentration could be demonstrated.

3.3.3.5 Efficacy of tuberculosis treatment

Week 8 MGIT culture conversion was >85% in the high dose R arms unlike for the control arm (80%). Overall, treatment success was noted in 88% vs 90% vs 94% in CR, SR1 and SR2 respectively. Three patients died during treatment (1 per treatment arm) (see Table 13).

Table 13. Number of patients with positive MTB culture at week 8 and End of TB treatment outcomes – mITT population

	R10 EFV600	R20 EFV600	R20 EFV800
Week 8 culture conversion*			
Lowenstein-Jensen, n/N (%)	28/31 (90.3)	23/26 (88.5)	24/27 (88.9)
MGIT, n/N (%)	24/30 (80.0)	24/28 (85.7)	26/30 (86.7)
TB Treatment outcome, N	33	31	33
Cured	22 (66.7)	19 (61.3)	24 (72.7)
Treatment completed	7 (21.2)	9 (29.0)	7 (21.2)
Treatment failure	1 (3.0)	0	0
Death	1 (3.0)	1 (3.2)	1 (3.0)
Default	1 (3.0)	1 (3.2)	0
Transferred-out**	1 (3.0)	1 (3.2)	1 (3.0)
Treatment success (cured + completed)	29 (87.9)	28 (90.3)	31 (93.9)

MGIT: Mycobacteria growth indicator tube

*After exclusion of contaminated, not done and non-mycobacteria culture results

** The 3 patients transferred out were patients withdrawn by the study investigator due to safety reasons.

3.3.4 Discussion and Conclusion

3.3.4.1 Pharmacokinetics

Overall the EFV concentrations including the 75th percentile of the interquartile range were in the expected range when compared to other studies conducted in sub-Saharan Africa [273, 274] and most patients having mid-dose concentrations in the alleged 1000-4000 ng/mL therapeutic range. The proportion of patients with sub-therapeutic concentrations is low at week 8 and decreased by week 28. The observed sub-therapeutic concentrations of EFV during R co-administration has been linked to the induction of isoenzyme CYP2B6 by R with resultant enhancement

in NNRTI drug metabolism [179]. However, in our study, similar proportions of patients with EFV sub-therapeutic concentrations (29%) with regard to C12 were noted for both patients in R10 mg/kg with EFV 600mg/day and those in R 20mg/kg and EFV 800mg/day treatment arm, hence suggesting a potential non-increased induction of isoenzyme CYP2B6 with higher R doses. However, this is pending clarification when pharmacogenetic analysis is completed. Whatever the treatment arm, a number of patients had higher EFV concentrations on vs off R as previously described in African population [56, 60, 64].

3.3.4.2 Safety of high-dose R in HIV-TB co-infected patients

We note that co-administration of a double-dose of R with EFV (600 or 800mg) among HIV/TB co-infected patients was well tolerated with very few severe transaminitis occurring during the first 8 weeks of TB treatment (4-6 weeks of co-administration of ATT with ART) and no severe CNS disorders throughout the study.

The incidence of elevated ALT seen in this study among HIV positive TB patients, that is, 6/98 (6.1%) is comparable to that previously reported among HIV negative TB patients (7%) in Rifatox trial [275] and lower than rates reported in other African [169, 276, 277] and Asian countries [278]. The distribution of the ALT rises of grade 3 or 4 shows no increasing trend with co-administration with ART as noted by 4 of the 6 events occurring prior to ART initiation. The study also revealed onset of hyperbilirubinemia though no clear R-dose dependence can be concluded given that in the arm with R 20mg/kg and EFV 800 mg, grade 3 or 4 rises in bilirubin were not noted. This is consistent with the documented occasional dose dependent interference effect of R on bilirubin uptake occurring early in treatment with resultant transient subclinical, unconjugated hyperbilirubinemia or jaundice without hepatocellular damage [68-71]. The overall mild increase in mean ALT reported across treatment arms during the first 8 weeks of TB treatment (Figure 3) is typical of the expected idiosyncratic hypersensitivity reaction to R, and usually occurring in the first months of treatment initiation [68, 76-78]. Nonetheless, the role of H in inducing liver injury could not be excluded [169] given that no NAT2 genotypic sequencing was done.

The overall absence of onset of CNS adverse events of grade ≥ 3 in this study, further confirms the safety of using EFV at 600mg dose even with dose of R as high as 20mg/kg. This is consistent with the already established safety of EFV among HIV-TB co-infected patients [199]. Few all grade CNS adverse-effects were observed in our study as compared to the incidence reported in Ugandan patients within another study [279]. No specific adverse events questionnaire was administered to patients and events were collected on the basis of spontaneous information given by the patient or following clinician's observation during follow-up visits. It is likely that AEs were under-reported by these patients.

3.3.4.3 Efficacy of antiretroviral therapy

Despite the known virological and immunological efficacy of EFV at 600 mg [36], the effect of R co-administration on EFV dose is not expected to greatly affect its efficacy. Nevertheless, in as much as our study showed good virological efficacy at 4 and 12 weeks post ART initiation, decline was noted at 24 weeks especially in the two high dose R arms despite all patients (irrespective of treatment arm) being on standard treatment between week 8 and 28. These surprising data which could not be fully explained by adherence given that the adherence rates for ART was good (>95%) throughout the 28 weeks of follow-up in our study and irrespective of treatment arm, have warranted an ongoing further investigation on the effect of high R doses on the late virological failure.

3.3.4.4 Efficacy of anti-tuberculosis therapy

On the other-hand we noted a slight increase in the week 8 conversion rate, and treatment success rates in the two treatment arms with R 20 mg/kg, a finding that supports the rifampicin dose-dependent bactericidal effect already demonstrated in an earlier EBA study [245] and in a recent study at Stellenbosch University, S. Africa [246], in which an increase in rifampicin dose size to 20 mg/kg (1200 mg) resulted in a substantial linear increase in EBA. Also, treatment success rates were good (>85%) for all arms.

3.3.4.5 Limitations

This study had some limitations: 1) It was not powered for safety and efficacy determination; 2) Due to laboratory inability to provide HIV viral load results below the 100 copies/ml, limited the use of a lower threshold at week 24 post ART initiation; 3) The trial only evaluated R doses up to 20 mg/kg limiting the generalizability of the results in cases of higher R doses like 35 mg/kg which is already evaluated in HIV negative TB patients; 4) We only evaluated EFV at 600 and 800mg and not lower doses (specifically EFV400) or any other ART drug including the new dolutegravir.

3.3.4.6 Conclusion

A double-dose of R in co-administration with EFV (600 or 800mg) among HIV/TB co-infected patients is safe, with no significant effects on EFV blood concentrations, and with resultant good ATT and early ART efficacy. Additional consideration is required especially to investigate the observed decreased virological suppression at 24 weeks post ART initiation among the high R dose with EFV 600 mg or 800mg. There is still need to establish the safety of R

doses as high as 35 mg/kg and their effect on the PK of EFV during co-administration before HIV-TB co-infected patients could be included in the on-going phase III treatment shortening efficacy trials like Rifashort trial. This study was limited in that it was not powered to evaluate treatment efficacy. These results will need to be further interpreted in line with the viral genotyping results and R and H PK results, and pharmacogenetic findings.

3.3.5 Involvement in this work

In this study, I was the South principal investigator. I participated in the protocol writing and grant submission. I also participated in the development of all Trial documents, implementation, patient recruitment, randomization, patients' clinical assessments, AE detection and reporting, sample shipments, coordination of collaborating laboratories and recruitment sites, scientific advisory board and Data safety management board meetings, data analysis, interpretation of results and dissemination at international conference (IAS, 2017 in Paris). I also rotated in the pharmacology laboratory in Bicetre Hospital under supervision of my PhD Co-director to be acquainted with some of the processes involved in PK analysis.

Eight weeks safety results of high-dose Rifampicin in HIV-Tuberculosis co-infected patients in Uganda:

RIFAVIRENZ-ANRS 12292 Trial.

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BACKGROUND

- A 6-month WHO recommended TB treatment regimen consist of 4 drugs: Rifampicin (10mg/Kg) + Isoniazid(H) + pyrazinamide(Z) + ethambutol(E).
- Rifampicin(R) is a highly bactericidal and cornerstone drug.
- Optimization of R doses may shorten Tuberculosis (TB) treatment to lower than the current 6 months
 - Linear increase of the bactericidal effect with dose
 - Non-linear increase of the pharmacokinetic parameters with dose
 - Safety of R doses of up to 35mg/kg among HIV negative TB patients
- No data exists in HIV-TB co-infected patients on anti-retroviral therapy (ART).

OBJECTIVES

The RIFAVIRENZ trial compared the pharmacokinetic parameters of efavirenz (EFV) in same HIV-TB co-infected patients with and without anti-TB treatment using rifampicin (R) at 10 or 20mg/Kg/day and EFV at 600 or 800mg/day during the first 8 weeks of treatment in HIV-TB co-infected patients.

We present here the safety of high dose R in co-administration with EFV-based ART in HIV-TB co-infected patients by week-8 of TB treatment.

METHODS

- Phase-2, randomized, open-label therapeutic trial (NCT01986543)
- Sample size of 28 patients per arm calculated for the primary pharmacokinetic endpoint

Eligibility criteria

- Inclusion**
 - ≥ 18years
 - New TB case XpertMTB+
 - HIV+ ART naïve
 - Negative pregnancy test and physical contraception
 - >35Kg
 - Written informed consent
- Exclusion**
 - R resistance
 - Treatment of opportunistic infection
 - Karnofsky score < 80%
 - Transaminase > 5xULN
 - Grade 4 laboratory/clinical sign
 - Treatment interfering with R or EFV
 - Contraindication to R or EFV
 - Patient unable to give consent

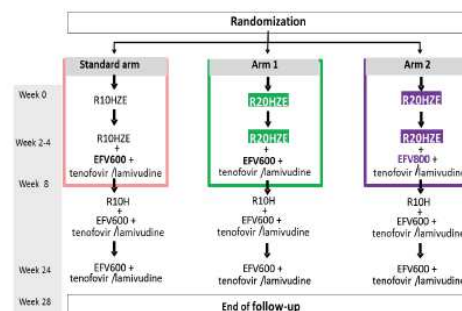


Figure 1. Trial Design

- ART was initiated at weeks 2 and 4 for patients with CD4<50 or ≥50 cells/ml respectively.
- At week 8: All patients were switched to R-10mg/kg and EFV 600mg.

Safety Monitoring

- Hepatitis B (HBV) surface Ag and hepatitis C antibodies (HCV) at baseline
- Weekly clinical assessments
- Full blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin monitoring after 2, 4 and 8 weeks
- Adverse event (AE) assessed using the DAIDS severity grading scale
- Adverse event of specific interest
 - Grade 3 (≥ 5xULN) and 4 (≥ 10xULN) increase of ALT or AST
 - Grade 2 or more neuropsychiatric AE : greater than minimal interference with usual social & functional activities

RESULTS

Table1. Baseline characteristics

	R10 EFV600 N=33	R20 EFV600 N=32	R20 EFV800 N=33
Males, n (%)	29 (87.9)	23 (71.9)	20 (60.6)
Age in years, median [IQR]	34.1 [29.6-38.1]	33.7 [28.3-38.1]	32.3 [27.8-43.1]
Weight in kg, median [IQR]	51.9 [49.2-56.0]	53.8 [48.4-59.1]	54.1 [50.9-58.1]
Chest cavities, n (%)	14 (42.4)	17 (53.1)	12 (36.4)
CD4 in cell/mm ³ , median [IQR]	120 [66-252]	219 [70-357]	144 [86-367]
HIV-1 RNA in log copies/mL, median [IQR]	5.5 [4.6-5.8]	5.1 [4.5-5.7]	5.1 [4.8-5.9]
ALT*, median [IQR]	22 [15-29]	19 [13-37]	19 [11-35]
AST*, median [IQR]	37 [28-58]	27 [20-65]	37 [26-52]
HBV surface Antigen, n (%)	2 (6.1)	1 (3.1)	1 (3.0)
Anti-HCV antibody, n (%)	1 (3.0)	0	0

*ALT normal range: Male= 0-45; Female= 0-34
*AST normal range: Male= 0-35; Female= 0-31
IQR: interquartile range

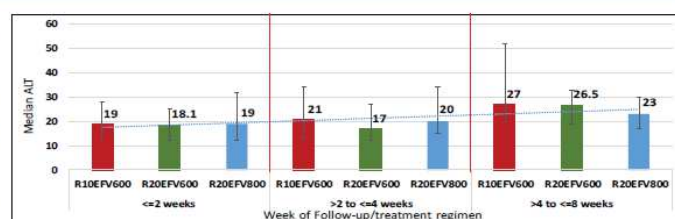


Figure 3. Evolution in median ALT per treatment regimen during first 8 weeks

Table 3. Non-hepatic grade 3 & 4 adverse events

	R10 EFV600 N=33	R20 EFV600 N=32	R20 EFV800 N=33
Hyponatraemia	1*	0	0
Leukopenia	0	1	0
Respiratory distress	0	1	0
Sepsis	0	0	1*
Thrombocytopenia	4	1	3
Total	5	3	4

* Leading to death

CONCLUSIONS

- First study with 20 mg/kg R and EFV in HIV-TB co-infected patients.
- Co-administration of a double-dose of rifampicin with efavirenz (600 or 800mg) among HIV/TB co-infected patients was well tolerated with very few severe transaminitis and no severe neuropsychiatric disorders.

ACKNOWLEDGEMENTS:

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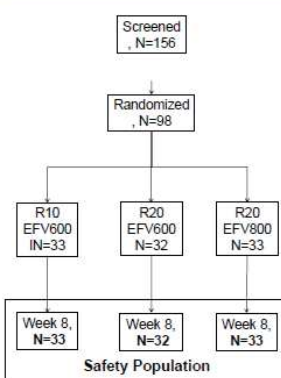


Figure 2: study profile

SAFETY RESULTS

15 patients, 5 from each arm had grade 3 or 4 AE: 2 leading to death unrelated to treatment

Table 2. Hepatic and neuropsychiatric adverse events

	R10 EFV600 N=33	R20 EFV600 N=32	R20 EFV800 N=33
Grade 3 or 4 increase ALT or AST, n(%)	2 (6.1)	2 (6.2)	2 (6.1)
ALT grade ≥3, n	2	2	2
AST grade ≥3, n	2	2	2
Hyperbilirubinaemia grade ≥3, n(%)	2 (6.1)	3 (9.4)	0 (0)
Neuropsychiatric, grade 2, n(%)	1 (3.0)	1 (3.1)	2 (6.2)*

* p=0.780

- 2 patients from each arm had grade 3 or 4 increases in ALT or AST within the first 8 weeks:
 - 1 before ART initiation and 1 during co-administration for both high dose R arms
 - 2 before ART initiation for the control arm
- 2 patients had their treatment arm changed from 20mg/kg-10mg/kg of R dose
- No grade 3 or 4 neuropsychiatric AEs

EFVIRENZ PHARMACOKINETICS WITH RIFAMPIN DOUBLE DOSE IN TB-HIV INFECTED PATIENTS

Abstract Number:

329

Abstract Type:

General Abstract

Authors:

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Background:

There is increasing interest towards a potential reduction of tuberculosis (TB) treatment duration with use of high-dose rifampicin (R) among HIV-negative patients. Little is known among HIV-positive patients on antiretroviral therapy (ART). The ANRS 12292 Rifavirenz phase 2 trial evaluated efavirenz (EFV) pharmacokinetics (PK) in Ugandan HIV/TB co-infected patients receiving high-dose R (20 mg/kg) as part of their standard TB treatment for the first 2 months.

Methods:

Newly diagnosed, confirmed pulmonary TB, ART-naïve, adults were randomized to 3- regimens administered QD. All patients were started on TB treatment and initiated on ART 2-4 weeks after. They received isoniazid(H)/pyrazinamide(Z)/ethambutol(E) and tenofovir/lamivudine at standard dosing during the first 8 weeks with R20mg/kg and EFV600mg (group G1); R20mg/kg and EFV 800mg (G2); R10mg/kg and EFV600mg (Control C). At 8 weeks of follow-up, all patients were switched to standard R and EFV doses. Drug intake was observed. Blood samples were drawn 4 weeks after EFV initiation and 4 weeks after R discontinuation. EFV plasma concentrations were assayed by validated High Performance Liquid Chromatography assay. PK parameters were estimated by a model-independent method. The 90% confidence interval (CI) of the geometric mean ratios (GMR) of PK parameters with and without TB treatment was compared to the predefined 0.70-1.43 range for concentrations to remain within the therapeutic window. HIV-viral load (VL) was monitored 4, 12 and 24-26 weeks after ART initiation and mycobacterial sputum culture (Mycobacteria Growth Indicator Tube) 8

weeks after starting TB treatment.

Results:

Of 97 included patients (G1 31; G2 33; C 33), 87 were evaluable for PK. Median age, weight and CD4 count were 33 years, 53.6 kg and 141 cells/ μ L, respectively and 77% were males. EFV PK parameters are summarized in the table below. TB culture conversion was 85.7% (G1), 86.7% (G2) and 80.0% (C). At 12 weeks post-ART initiation, 92.6%, 86.2% and 92.6% of patients had VL < 400 copies/mL, respectively. No relationship could be evidenced between VL decline and EFV concentrations. During the first 8 weeks, 6 (2 per arm) and 4 (G1=1; G2=2; C=1) patients had alanine aminotransferase increase > grade 3 and neuropsychiatric events > grade 2, respectively.

Conclusion:

Despite a trend to lower EFV concentrations when R dosing was doubled, concentration remained in the therapeutic window and there was no sign of decreased tolerance.

Clinical:

(F) Clinical Pharmacology

Keywords:

Drug interactions

Efavirenz

Pharmacokinetics

Rifampicin

Median (range) unless otherwise indicated	EFV PK parameters		
	EFV+R (week 8)	EFV alone (week 28)	GMR [90% CI] EFV+R/EFV
Control group (C): R10mg/kg and EFV 600mg, n=29 patients			
C24-ng/mL	1077 (233; 9407)	1137 (324; 8049)	0.92 [0.79; 1.08]
C24<1000ng/mL, n (%)	14 (48)	11 (38)	
AUC- μ g.h/mL	40.2 (13.4; 314.5)	38.9 (14.3; 214.3)	0.96 [0.84; 1.09]
Group 1: R20mg/kg and EFV 600mg, n=27 patients			
C24-ng/mL	1188 (498; 12212)	1496 (457; 17967)	0.83 [0.72; 0.96]
C24<1000ng/mL, n (%)	12 (44)	5 (18)	
AUC- μ g.h/mL	47.5 (16.2; 308.4)	49.6 (13.4; 486.8)	0.87 [0.75; 1.00]
Group 2: R20mg/kg and EFV 800mg, n=31 patients			
C24-ng/mL	1032 (214; 11555)	1028 (408; 11299)	1.16 [0.97; 1.39]
C24<1000ng/mL, n (%)	13 (42)	15 (48)	
AUC- μ g.h/mL	44.5 (12.9; 326.3)	35.2 (14.2; 265.7)	1.12 [0.96; 1.30]

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Chapter 3.4

Challenges related to early surrogate markers of TB chemotherapy efficacy within phase 2 trials.

Published article related to this chapter

Atwine Daniel, Orikiriza Patrick, Taremwa Ivan, Arnold Ayebare, Suzan Logoose, Juliet Mwanga-Amumpaire, Amina Jindani, Maryline Bonnet. Predictors of delayed culture conversion among Ugandan patients. BMC Infectious Diseases (2017) 17:299. URL. <https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-017-2335-7>

(Full Published Manuscript attached at the end of this chapter)

Presentations related to this chapter

Atwine D. Delayed tuberculosis culture conversion: Predictors and mycobacterial culture effect among Ugandan patients. In. Annual Grande doctors' Conference, 11-13th August 2016, Kampala, Uganda (Oral presentation)

Atwine D. Delayed tuberculosis culture conversion: Predictors and mycobacterial culture effect among Ugandan patients. In. 47th Union World Conference on Lung Health, 26 to 29 October 2016 - Liverpool, UK [Abstr ID: SOA-505-27] (Oral presentation)

3.4.1 Justification and objectives

Culture conversion at month-2, is globally used as proxy indicator of TB chemotherapy efficacy in phase 2 clinical trials [29, 135, 280].

In addition to the treatment effect, several factors can affect the culture conversion at month-2, such as genetic polymorphism [281], age above 45 years, high pre-treatment sputum bacterial load, drug resistance extent of the radiographic involvement or presence of lung cavities, baseline time-to-detection (TTD) of TB, infection with W-Beijing genotype of *M. Tuberculosis*, smoking, and alcohol abuse [282-287]. Treatment interruption, irregularity in drug intake, and inadequate dosage, particularly of rifampicin may also lead to delayed culture conversion [142, 146, 288]. This may explain variability of culture conversion at month-2 across sites in multicenter clinical trials or amongst trials.

Although not much explored, the variations in culture conversion may also be influenced by type of culture media [280, 289]. Specifically, the solid culture medium (Löwenstein-Jensen [LJ]) tend to exhibit a slower growth and lower sensitivity as compared to liquid culture in a mycobacterium growth indicator tube (MGIT) [29, 30]. There is no clear guidance whether both solid and liquid based culture media should be used in tuberculosis clinical trials for outcome assessment. This is particularly important for some sites from high TB burden and LMICs which still do have limited access to liquid culture medium. Therefore, the impact of parallel use of different culture media within the same geographical patient population on the outcome evaluation of tuberculosis treatment regimen needs further assessment.

We present results from a sub-study of the Rifatox trial [270]. This sub-study took advantage that within the Rifatox trial, the Ugandan site used both LJ and MGIT cultures for all patients during the 6 months treatment as per site guidelines unlike other international sites that used LJ alone and only up to month 2, so as to compare TB detection rates on different culture media, within and across R-based treatment regimens over a 6-month treatment follow-up period, and to establish predictors of month-2 culture non-conversion.

3.4.2 Methods, results and conclusion

The study is a nested cohort of the Rifatox trial using data of the patients enrolled from the Mbarara site.

We compared the culture conversion after 2, 4 and 6 months between different culture methods and by study arms and we identified the baseline patients' characteristics associated with month 2 culture conversion;

Overall, using both LJ and MGIT, 45% (45/100) had converted on culture by month-2. Culture conversion was 50% (17/34), 40.6% (13/32) and 44.1% (15/34) for patients in the 10, 15 and 20mg/Kg rifampicin arms, respectively, $p=0.721$. It increased to 91.2% (31/34), 83.3% (25/30) and 86.7% (26/30) at month-4 ($p=0.685$). All patients were culture negative at month-6.

The overall estimates of culture conversion by LJ were higher than with MGIT at months 2 (56.0% vs 48.4%, $p=0.0707$) and month-4 (98.9% vs 88.4%, $p=0.0391$). Although none showed statistical significance the differences in conversion rates between LJ and MGIT at month-2 seem more higher within patients in high-dose rifampicin arms (10% for 15mg/Kg and 14.1% for 20mg/Kg) than patients in the control arm (-1.5%).

Patients with baseline time to detection below 14 days had a 2-fold increased risk of month-2 non-conversion as compared to those with time to detection of 14 days and above, (aRR=2.1, [95% CI: 1.18-3.93], $p=0.013$). Patients working in “social service jobs”, which in this study accounts mostly for motor-bike passenger riders, had a 3-fold increased risk of month-2 culture non-conversion (RR=3.0, [95%CI:1.11-8.19], $p=0.031$) as compared to other patients.

In conclusion, we observed that the low culture conversion at Month 2 was due to delayed culture conversion. It confirms the slow bacteriological response to treatment or the potential presence of persister bacilli populations reported among African patients something that has complicated the estimation of early TB chemotherapy efficacy in a number of multi-centric clinical trials [29, 135, 136, 275]. No significant difference was observed in the conversion rates across 10, 15 and 20mg/kg rifampicin-based regimens using LJ, MGIT or combination of LJ and MGIT. As expected, LJ which is known to be a less sensitive culture method than MGIT increased by an average of 10% the Month 2 culture conversion compared to MGIT. Surprisingly, this effect of culture media used, seem higher in the high rifampicin dose groups, a phenomenon that may perhaps be explained by a faster reduction in LJ sensitivity following a more rapid decline in bacilli population due to high doses of rifampicin [146]. The month-2 culture conversion used in phase 2 clinical trials as surrogate marker of treatment efficacy is influenced by the culture method. Multi-centric TB therapeutic trials using early efficacy endpoint should use the same culture method across sites.

3.4.3 Involvement in this work

I developed the research synopsis for this sub-analysis, developed data collection tools, extracted data from the trial database, analyzed data and wrote the manuscript. I also disseminated the results through local and international conferences, that is Grande doctors' Conference in Kampala, and Union World Conference on Lung Health in Liverpool, UK.

RESEARCH ARTICLE

Open Access

Predictors of delayed culture conversion among Ugandan patients



Daniel Atwine^{1,2,5*}, Patrick Orikiriza^{1,2}, Ivan Taremwa¹, Arnold Ayebare¹, Suzan Logoose¹, Juliet Mwanga-Amumpaire^{1,2}, Amina Jindani⁴ and Maryline Bonnet^{1,3}

Abstract

Background: Estimates of month-2 culture conversion, a proxy indicator of tuberculosis (TB) treatment efficacy in phase-2 trials can vary by culture-type and geographically with lower rates reported among African sites. The sub-study aimed at comparing TB detection rates of different culture media, within and across rifampicin-based regimens (R10, 15 and 20 mg/Kg) over a 6-month treatment follow-up period, and to establish predictors of month-2 culture non-conversion among HIV-negative TB patients enrolled at RIFATOX trial site in Uganda.

Methods: Unlike in other Rifatox Trial sites, it is only in Uganda where Lowenstein-Jensen (LJ) and Mycobacteria growth indicator tube (MGIT) were used throughout 6-months for treatment monitoring. Conversion rates were compared at month-2, 4 and 6 across cultures and treatment-type. Binomial regression analysis performed for predictors of month-2 non-conversion.

Results: Of the 100 enrolled patients, 45% had converted based on combined LJ and MGIT by month-2, with no significant differences across treatment arms, $p = 0.721$. LJ exhibited higher conversion rates than MGIT at month-2 (58.4% vs 56.0%, $p = 0.0707$) and month-4 (98.9% vs 88.4%, $p = 0.0391$) respectively, more so within the high-dose rifampicin arms. All patients had converted by month-6. Time-to-TB detection (TTD) on MGIT and social service jobs independently predict month-2 non-conversion.

Conclusion: The month-2 culture conversion used in phase 2 clinical trials as surrogate marker of treatment efficacy is influenced by the culture method used for monitoring mycobacterial response to TB treatment. Therefore, multi-centric TB therapeutic trials using early efficacy endpoint should use the same culture method across sites. The Time-to-detection of MTB on MGIT prior to treatment and working in Social service jobs bear an increased risk of culture non-conversion at month-2.

Trial registration: ISRCTN ISRCTN55670677. Registered 09th November 2010. Retrospectively registered.

Keywords: Delayed culture conversion, Efficacy, HIV-negative TB patients, Time-to-detection, Treatment failure

Background

Culture conversion at month-2, is globally used as proxy indicator of tuberculosis (TB) chemotherapy efficacy in phase 2 clinical trials [1–3].

In addition to the treatment effect, several factors can affect the culture conversion at month-2, such as genetic polymorphism [4], age above 45 years, high pre-treatment sputum bacterial load, drug resistance extent of the radiographic involvement or presence of lung cavities, baseline

time-to-detection (TTD) of TB, infection with W-Beijing genotype of *M. Tuberculosis*, smoking, and alcohol abuse [5–10]. Treatment interruption, irregularity in drug intake, and inadequate dosage, particularly of rifampicin may also lead to delayed culture conversion [11–13]. This may explain variability of culture conversion at month-2 across sites in multicenter clinical trials or amongst trials.

Although not much explored, the variations in culture conversion may also be influenced by type of culture media [3, 14]. Specifically, the solid culture medium (Löwenstein-Jensen [LJ]) tend to exhibit a slower growth and lower sensitivity as compared to liquid culture in a mycobacterium growth indicator tube (MGIT) [1, 15]. There is no clear

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guidance whether both solid and liquid based culture media should be used in tuberculosis clinical trials for outcome assessment. This is particularly important for some sites from high TB burden and limited resource countries which still do have limited access to liquid culture medium. Therefore, the impact of parallel use of different culture media within the same geographical patient population on the outcome evaluation of tuberculosis treatment regimen needs further assessment.

We present results from a sub-study of the Rifatox trial that evaluated the safety of high-dose rifampicin (R) (15 and 20 mg/Kg) administered during the first 4 months of HIV negative TB patients' treatment in Uganda, Nepal and Bolivia [16].

This sub-study took advantage that within the Rifatox trial, the Ugandan site used both LJ and MGIT cultures for all patients during the 6 months treatment as per site guidelines unlike other international sites that used LJ alone and only up to month 2, so as to compare TB detection rates on different culture media, within and across rifampicin-based treatment regimens over a 6-month treatment follow-up period, and to establish predictors of month-2 culture non-conversion.

Methods

Study design

We conducted a secondary analysis of data of all 100 patients enrolled from the African site (Mbarara, Uganda) within the Rifatox Trial [16].

Participants

The Mbarara site of the Rifatox trial was at Epicentre Mbarara Research Centre and was the only African site. The setting is endemic of TB and HIV. The trial consecutively recruited participants from the outpatient department (OPD) at Mbarara Regional Referral Hospital (MRRH).

Patients were eligible for enrolment in the trial if HIV negative, aged 18 years and above, with two sputum samples positive for tubercle bacilli on microscopy; having received less than a month of previous anti-tuberculosis chemotherapy; with a firm and accessible home address and if they consented to participation. Patients were excluded if they were critically ill, had extra-pulmonary TB, alcoholism, psychiatric illness, blood disorders, diabetes, epilepsy, HIV positivity, peripheral neuritis, pregnancy, a hemoglobin <7 g/dl, serum ALT levels >5 times the upper limit of normal (ULN), a creatinine clearance <30 ml/min, and rifampicin resistance.

Study procedures

Socio-demographic characteristics specifically age, sex, district of residence, marital status and occupation, were collected using a standardized questionnaire which was administered in the local language. All patients

underwent a physical examination by a medical doctor to record clinical data.

Patients were randomized to one of the 3 treatment regimens, that is: 1) Control Regimen (CR): 2 months of daily ethambutol (E) isoniazid (H) pyrazinamide (Z) Z and R at the usual dose of 10 mg/kg followed by 4 months of RH; 2) Study Regimen 1 (SR1): The regimen as above but with an increase in the dose of R to 15 mg/kg body weight daily for the first 4 months and a standard dose R (10 mg/kg) was given for the last 2 months; 3) Study Regimen 2 (SR2): The regimen as in SR1 but with an increase in the dose of R to 20 mg/kg body weight daily for the 4 months. Treatment was given under Direct Observation (DOT) by Domiciliary Treatment Monitor (DTM) or study nurse.

Patients provided spot sputum specimen at baseline, months 2, 4 and 6 for smear microscopy, LJ and manual MGIT (Becton, Dickinson, Franklin Lakes, NJ) cultures. Resistance testing was performed on decontaminated sample at baseline using GenoType MTBDRplus 2.0 (Hain Lifescience, Nehren).

In the laboratory, the specimens were decontaminated using the N-acetyl-L-cysteine and sodium hydroxide (1.5% final concentration). The decontaminated sputum was inoculated into two homemade LJ medium tubes and one MGIT tube. Negative culture results were reported after 56 days of incubation. Growth in the LJ and MGIT cultures, were checked for Acid fast bacilli and contamination using Ziehl-Neelsen (ZN) microscopy and blood agar culture respectively. ZN positive cultures were differentiated between *Mycobacterium tuberculosis* (MTB) and non-tuberculosis mycobacterium (NTM) using the SD TB Ag MPT64 Rapid system (SD Bioline, Kyonggi-do, South Korea). Generally, the Time-to-positivity/detection was recorded for all TB patients using MGIT cultures.

Patients had weekly clinical assessment during first 2 months and then monthly with regular monitoring of liver function tests [16].

Statistical analysis

Data were double-entered in a Voozanoo database (Epicentre, Paris, France) and all statistical analysis was performed using Stata® software (v. 12, College Station, Texas, USA).

Relevant summary statistics were used to describe participants' baseline characteristics. The proportion of patients with culture conversion out of those with prior baseline culture positivity was calculated at months-2, 4 and 6 by treatment regimen and by culture method (LJ, MGIT and combination of LJ + MGIT) after exclusion of patients with culture contaminated result from the denominator. Patients with contaminated samples were excluded in the subsequent analysis. Comparison of culture conversion between LJ and MGIT culture methods at each time point was performed using Mc' Nemar

exact test for matched data, while Pearson Chi2 was used to compare the culture conversion rates between different treatment regimens used. Our dependent variable was culture non-conversion at month-2, defined as: having a MTB growth on either LJ or MGIT culture at month-2. Independent variables used included: 1) baseline patients' socio-demographics (age, sex, district of residence, marital status and occupation using definitions adapted from those used in the Uganda Demographic Health Survey reports [17]; 2) Baseline Clinical data, that is, body mass index (BMI) defined as low if $<18.5 \text{ kg/m}^2$ [18], sputum bacterial load using the World Health Organization (WHO) grading for LJ culture [19], time-to-detection of MTB on MGIT culture at baseline categorized into a binary variable using a cut-off of 14 days [20], hemoglobin level defined as low if $<11 \text{ g/dL}$ for males and $\leq 10.4 \text{ g/dL}$ for females [21].

We fitted univariable and multivariable binomial regression models to establish the predictors of culture non-conversion at month-2. Variables associated with $P < 0.4$ in univariable analysis were included in the initial multivariable model after establishing the absence of multi-collinearity. The final model was systematically adjusted on treatment regimen, age, gender and baseline colony density. A 5% significance level was used. Tests for interaction and goodness-of-fit were performed.

Results

One hundred enrolled patients in Uganda had their sputum evaluated at different time points (Figure 1). The participants were predominantly males (80%) with a mean age of 36.2 years. They had a mean body weight of 51 kg and 44% were underweight ($\text{BMI} < 18.5 \text{ Kg/m}^2$). Predominantly, patients had high bacillary loads of 2+ and above (87%) and high colony density of 3+ and above in LJ (80%). Overall, mean TTD of MTB on MGIT was 13.4 ± 10.6 days (Range: 3–51 days), with 65 out of 95 (68.4%) patients showing MTB growth within 14 days from MGIT inoculation at baseline. Majority of patients with baseline colony density of $>2+$ or $\leq 2+$, had a TTD on MGIT of less than 14 days (79%) and above 14 days (72%), respectively, $p < 0.0001$. Three patients (3%) had isoniazid mono-resistance (Table 1).

Overall, using both LJ and MGIT, 45% had converted on culture by month-2. Culture conversion was 50%, 40.6% and 44.1% for patients in the 10, 15 and 20 mg/Kg rifampicin arms, respectively, $p = 0.721$ (Table 2). It increased to 91.2%, 83.3% and 86.7% at month-4 ($p = 0.685$). All patients were culture negative at month-6.

Contamination was reported in 5/99 (5.1%), 2/93 (2.2%) and 0/90 (0%) of the patients with LJ done at month-2, 4 and 6 respectively. On the other-hand, contamination was reported in 3/85 (3.5%) patients with MGIT performed at

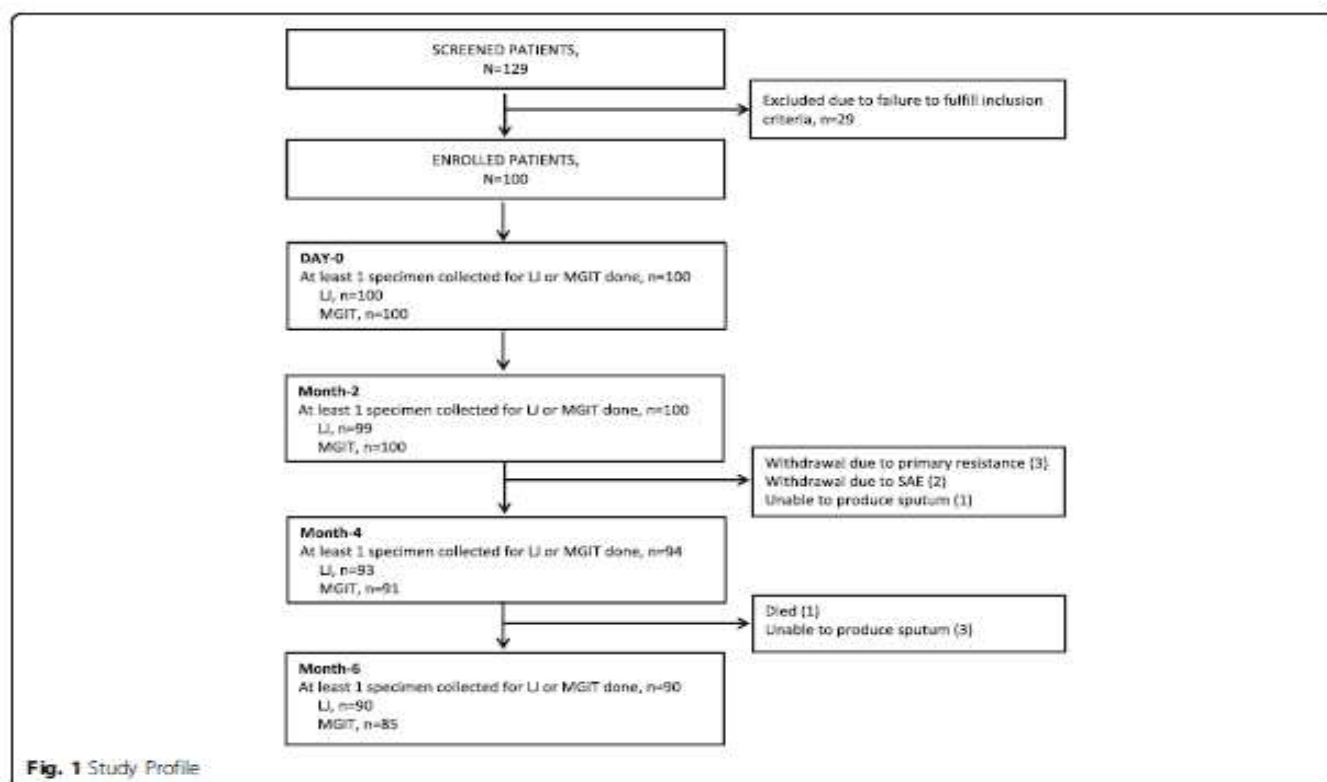


Table 1 Participants' baseline characteristics

Characteristic	Number	
Mean age in years (SD)	100	36.2 (11.6)
Age categories, n (%)		
18–24		20 (20.0)
25–49		64 (64.0)
> =50		16 (16.0)
Gender, male, n (%)	100	80 (80.0)
Marital status, n (%)	92	
Married		54 (58.7)
Separated/Divorced		8 (8.7)
Single/widowed		30 (32.6)
Occupation ^a , n (%)	92	
Peasant		16 (17.4)
Professional		9 (9.8)
Service		18 (19.6)
Unskilled manual		37 (40.2)
Student		6 (6.5)
Business		6 (6.5)
Transport mode to clinic, n (%)	91	
Motorbike		34 (37.4)
Car		16 (17.6)
Taxi		41 (45.1)
District of residence, n (%)	100	
Bushenyi		9 (9.0)
Isingiro		21 (21.0)
Mbarara		70 (70.0)
Relationship with Domiciliary treatment monitor, n (%)	97	
Parental family		42 (43.3)
Own family		22 (22.7)
Friends/in-laws		33 (34.0)
Current smoker, n (%)	89	35 (39.3)
TB treatment regimen, n (%)	100	
Standard regimen (R10mg/kg)		33 (33.0)
Regimen 1 (R15mg/kg)		33 (33.0)
Regimen 2 (R20mg/kg)		34 (34.0)
Clinical Parameters		
Mean body weight in kg (SD)	100	51.2 (7.8)
Mean BMI in Kg/m ² (SD)	99	19.0 (2.4)
BMI <18.5, n (%)		44 (44.4)
Biochemical parameters		
Mean hemoglobin in g/dl (SD)	100	11.7 (1.8)
Hemoglobin grade ^b		
Mild anemia, n (%)		13 (13.0)
Moderate anemia, n (%)		11 (11.0)

Table 1 Participants' baseline characteristics (Continued)

Severe anemia, n (%)		6 (6.0)
Biological Parameters		
Baseline smear positivity, n (%)	100	100 (100.0)
Scanty		4 (4.0)
1+		9 (9.0)
2+		25 (25.0)
3+		62 (62.0)
Baseline LJ positivity, n (%)	100	98 (98.0)
Colony density, n (%)		
Negative		2 (2.0)
1+		4 (4.0)
2+		14 (14.0)
3+		56 (56.0)
4+		24 (24.0)
Baseline MGIT positivity, n (%)	100	95 (95.0)
Baseline combined LJ/MGIT positivity, n (%)	100	100 (100.0)
Mean Time-to-detection (TDD) of MTB on MGIT in days (±SD)	95	13.4 ± 10.6
TDD of MTB on MGIT in days, <14 days, n (%)	95	65 (68.4)
Primary Isoniazid resistance, n (%)	100	3 (3.0)

SD standard deviation, BMI body mass index, LJ Löwenstein-Jensen, MGIT, mycobacterium growth indicator tube, MTB mycobacterium tuberculosis complex

^aOccupation: 1) Peasant represents people earning from agriculture; 2) professional represents those working in certified, managerial or technical jobs; 3) Social service representing mainly transport services like motor-bike riders and those in unskilled manual (like bar/restaurant attendants, house maids, truck attendants); 4) Students representing those still in school and not working; 5) Business representing those involved in their own businesses.

^bHemoglobin: Mild (male:10–10.9 g/dl, Female: 9.5–10.4), Moderate (Male: 9.0–< 10.0, Female: 8.5–< 9.5), Severe (Male:7.0–< 9.0, Female: 6.5–< 8.5) based on Division of AIDS (DAIDS) guideline

month-6. No MGIT contamination was noted before month 6 follow-up. None of the patients had contamination on both LJ and MGIT at the same visit.

The overall estimates of culture conversion by LJ were higher than with MGIT at months 2 (56.0% vs 48.4%, $p = 0.0707$) and month-4 (98.9% vs 88.4%, $p = 0.0391$) (Table 2). Although none showed statistical significance the differences in conversion rates between LJ and MGIT at month-2 seem more higher within patients in high-dose rifampicin arms (10% for 15 mg/Kg and 14.1% for 20 mg/Kg) than patients in the control arm (–1.5%).

TDD of MTB on baseline MGIT culture and occupation were significantly associated with the culture non-conversion at month-2 after adjusting for age, gender, treatment regimen, and baseline colony density. Patients with baseline TDD below 14 days had a 2-fold increased risk of month-2 non-conversion as compared to those with TDD of 14 days and above, (aRR = 2.1, [95% CI: 1.18–3.93], $p = 0.013$). On the other-hand, within occupations, patients working in “social service jobs”, which in this study accounts mostly for motor-bike passenger

Table 2 Culture conversion by culture method and treatment arm at month-2, 4 and 6

Month	Culture	Overall	Treatment Arm			p-value ^b
			CR	SR1	SR2	
		Converted, n/N, %	Converted, n/N, %	Converted, n/N, %	Converted, n/N, %	
Month-2	LJ	51/91 (56.0)	17/32 (53.1)	14/27 (51.9)	20/32 (62.5)	0.697
	MGIT	46/95 (48.4)	18/33 (54.6)	13/31 (41.9)	15/31 (48.4)	0.601
	% difference	7.6	-1.5	10	14.1	
	p-value ^a	0.0707	1.0000	0.1250	0.2891	
	Both LJ and MGIT	45/100 (45.0)	17/34 (50.0)	13/32 (40.6)	15/34 (44.1)	0.721
Month-4	LJ	88/89 (98.9)	31/31 (100.0)	28/29 (96.6)	29/29 (100.0)	0.652
	MGIT	76/86 (88.4)	29/32 (90.6)	24/27 (88.9)	23/27 (85.2)	0.913
	% difference	10.5	9.4	7.7	14.8	
	p-value ^a	0.0391	0.5000	0.6250	0.2500	
	Both LJ and MGIT	82/94 (87.2)	31/34 (91.2)	25/30 (83.3)	26/30 (86.7)	0.685
Month-6	LJ	87/87 (100.0)	33/33 (100.0)	26/26 (100.0)	28/28 (100.0)	NA
	MGIT	78/78 (100.0)	29/29 (100.0)	26/26 (100.0)	23/23 (100.0)	NA
	% difference	0.0	0.0	0.0	0.0	
	p-value ^a	1.0000	1.0000	1.0000	1.0000	
	Both LJ and MGIT	88/88 (100.0)	34/34 (100.0)	26/26 (100.0)	28/28 (100.0)	NA

^aExact McNemar p-value^bFishers' exact p-value

Converted culture: converted, CR control regimen (10 mg/kg rifampicin), SR1 study regimen1 (15 mg/kg rifampicin), SR2 study regimen2 (20 mg/kg rifampicin), LJ Löwenstein-Jensen, MGIT mycobacterium growth indicator tube

riders, were significantly associated with a 3-fold increased risk of month-2 culture non-conversion (RR = 3.0, [95% CI:1.11–8.19], $p = 0.031$) as compared to those in formal professional jobs (Table 3).

Discussion

This sub-analysis of the data from Ugandan site within the Rifatox trial, shows that the low culture conversion at month-2 was due to delayed culture conversion and not treatment failure. Indeed, 87.2% of patients converted by month-4 and 100% by month-6 without initiation of second-line treatment. Culture non-conversion at month-2 suffers a lower specificity in predicting TB relapse or treatment failure [22] a phenomenon partially observed in our study given that all month-2 non-converters finally converted by month 6. No significant differences were observed in the conversion rates across 10, 15 and 20 mg/kg rifampicin-based regimens using LJ, MGIT or combination of LJ and MGIT. Despite the low sample size on which this sub-analysis was performed, these results are consistent with what was reported in the Rifatox trial using LJ culture only [16]. However, given the good safety results of high-dose rifampicin shown in two recent trials [16, 23] and the difficulty to rely on month-2 culture conversion as surrogate endpoint of treatment efficacy [24–26], a phase3 efficacy trial of 4 month regimens based on high-dose rifampicin is under implementation. This Rifashort trial

(NCT02581527) will evaluate 1200 mg and 1800 mg rifampicin daily in the reduction of treatment duration for pulmonary tuberculosis from 6 months to 4 months.

The observed combined LJ/MGIT month-2 culture conversion rate (45%), was lower than that reported (61%) in another trial that involved patients in Central Uganda [14]. The slow conversion rates may partly be explained by the fact that majority of the patients (>80%) had high baseline bacillary load and colony density. The absence of association between baseline sputum bacterial load and the absence of culture conversion at Month 2 is possibly hidden by the selection of smear-positive patients and by a very high proportion of patients with high bacterial load overall. This is a reflection of late medical consultation or delayed TB diagnosis among TB patients. Delayed culture conversion may potentially elongate patients' period of infectiousness, a challenge in TB control efforts. Unfortunately, since patients were not followed up after completion of treatment in the Rifatox phase2 trial, it was not possible to report on whether delayed sputum converters also had a higher risk of relapse than early converters. On a positive side, this will also be assessed within the Rifashort trial.

Our study noted a 2-fold increase in risk of culture non-conversion at month-2 in patients with baseline TTD of MTB on MGIT below 14 days as compared to those with TTD of 14 days or above [10]. This difference may partially be due to the high bacillary colony density prior to

Table 3 Results of univariable and multivariable binomial regression analysis of predictors of month-two culture non-conversion

Characteristic	Converted, n (%)	Non-converted, n (%)	Unadjusted RR [95% CI]	Adjusted RR [95% CI]
Age in years, n = 100				
18–45	36 (43.4)	47 (56.6)	1.2 [0.70–2.06]	0.6 [0.32–1.32]
> 45	9 (52.9)	8 (47.1)	1.0	1.0
Gender, n = 100				
Female	10 (50.0)	10 (50.0)	1.0	1.0
Male	35 (43.8)	45 (56.2)	1.1 [0.70–1.82]	1.1 [0.60–2.06]
Marital status, n = 92				
Married	26 (48.2)	28 (51.8)	1.0 [0.67–1.61]	
Separated/Divorced	2 (25.0)	6 (75.0)	1.5 [0.88–2.57]	
Single/widowed	15 (50.0)	15 (50.0)	1.0	
Occupation, n = 92				
Peasant	11 (68.8)	5 (31.2)	0.9 [0.29–3.04]	0.8 [0.26–2.65]
Professional	6 (66.7)	3 (33.3)	1.0	1.0
Social service	3 (16.7)	15 (83.3)	2.5 [0.97–6.44]	3.0 [1.10–8.19]
Unskilled manual	17 (46.0)	20 (54.0)	1.6 [0.61–4.28]	*1.5 [0.56–3.78]
Student	3 (50.0)	3 (50.0)	1.5 [0.44–5.09]	2.1 [0.61–7.11]
Business	3 (50.0)	3 (50.0)	1.5 [0.44–5.09]	1.3 [0.40–4.36]
District of residence, N = 100				
Bushenyi	5 (55.6)	4 (44.4)	1.0	
Isingiro	8 (38.1)	13 (61.9)	1.4 [0.62–3.11]	
Mbarara	32 (45.7)	38 (54.3)	1.2 [0.57–2.62]	
Current Smoker, n = 89	15 (42.9)	20 (57.1)	1.1 [0.74–1.62]	
TB treatment regimen, n = 100				
Standard regimen	17 (50.0)	17 (50.0)	1.0	1.0
Regimen 1	13 (40.6)	19 (59.4)	1.5 [0.55–3.87]	0.8 [0.45–1.34]
Regimen 2	15 (44.1)	19 (55.9)	1.3 [0.49–3.29]	1.1 [0.69–1.84]
Body mass index in kg/m ² , n = 99				
18.5 and above	25 (45.5)	30 (54.5)	1.0	
< 18.5	20 (45.5)	24 (54.5)	1.0 [0.70–1.44]	
Colony density at baseline, n = 98				
1+	2 (50.0)	2 (50.0)	1.0	1.0
2+	8 (57.1)	6 (42.9)	0.9 [0.27–2.71]	0.5 [0.12–2.01]
3+	23 (41.1)	33 (58.9)	1.2 [0.43–3.22]	0.5 [0.11–1.82]
4+	11 (45.8)	13 (54.2)	1.1 [0.38–3.09]	0.4 [0.09–1.44]
Time-to-detection (TDD) of MTB on MGIT at baseline				
Less than 14 days	24 (36.9)	41 (63.1)	1.6 [0.98–2.54]	2.1 [1.18–3.93] ^b
14 days and above	18 (60.0)	12 (40.0)	1.0	1.0
Hemoglobin levels, n = 100				
Normal	28 (40.0)	42 (60.0)	1.0	
Low	17 (56.7)	13 (43.3)	0.7 [0.46–1.13]	
Missed at least a dose in first 2 months, n = 100	3 (23.1)	10 (76.9)	1.5 [1.04–2.13]	

Multivariable model characteristics: N = 87, deviance = 100.63, Binomial model-based goodness of fit, $p = 0.0177$, Logit model-based goodness of fit, $p = 0.5291$. RR crude risk ratio, aRR adjusted risk ratio, 95% CI 95% Confidence Interval, LJ Löwenstein-Jensen, MGIT mycobacterium growth indicator tube, NA not applicable, MTB mycobacterium tuberculosis * $p = 0.031$; ^b $p = 0.013$

treatment (80% > 2+). However, it is interesting to note that the significant association remained after adjustment on baseline colony density. On the other-hand, baseline colony density showed no significant association with culture non-conversion at month-2, a finding that could be explained by an overwhelmingly higher proportion of patients with high colony density (>80%) in this study. The 3-fold increase in risk of culture non-conversion at month-2 noted in our study among patients employed in social service jobs (mainly commercial motor-bike passenger riders) as compared to those in formal professional employment, shows the role of social factors and the need for their consideration within TB treatment efficacy trials. The Motor-bike passenger riders have a great social sphere of interaction, and may play a key role in TB transmission within urban and semi-urban settings. Further exploration into the reasons underlying the increased vulnerability of this population group is needed.

This sub-study confirms the slow bacteriological response to treatment or the presence of persister bacilli populations reported among African patients, something that has complicated the estimation of early TB chemotherapy efficacy in a number of multi-centric clinical trials [1, 2, 16, 27]. Indeed, this delayed conversion among Ugandan patients, offers more evidence to the suggestion made in the retrospective study of several phase 3 TB trials conducted by the British Medical Council in Hong Kong and East Africa, recommending the use of month-3 instead of month-2 culture conversion endpoint as a surrogate of treatment efficacy within the phase II East African trials [26].

As expected, LJ which is known to be a less sensitive culture method than MGIT had a 10% higher month-2 culture conversion rate as compared to MGIT. The significantly high overall difference in conversion rates between LJ and MGIT especially at month4, only confirms the declining sensitivity of LJ in detecting TB with declining bacillary loads during treatment follow-up, a finding also consistent with other studies [1, 15, 28]. It highlights the necessity to consistently use the same culture methods for early treatment efficacy endpoints across sites within a TB clinical trial. This approach, will not affect the comparisons of the conversion rates between treatment arms. Likewise, culture conversion rates across clinical trials can easily be compared or pooled as long as they are based on the same culture method. Also, our results do not support the need to combine LJ and MGIT cultures in the same trial for the microbiological outcome assessment. However, this would require to be further assessed in a larger study. Surprisingly, this effect of culture media used, seem higher and faster in the high rifampicin dose groups, with bigger differences in TB conversion rates between LJ and MGIT manifesting as earlier as month-2 of treatment follow-up, a phenomenon that may perhaps be explained

by a faster reduction in LJ sensitivity following a more rapid decline in bacilli population due to higher doses of rifampicin [13].

The study has several limitations: i) Since it was a sub-study from the Ugandan site only, numbers are very small. The small sample size reduced the power to show a difference of culture conversion between arms or between culture methods and may have hindered significance of some predictor variables of non-conversion. This also may have contributed to the high deviance and inadequate goodness of fit seen with the multivariable binomial regression model ($p = 0.0177$), although a good fit was achieved on a re-run of the final model with a logit-based model ($p = 0.5291$). ii) Our study being limited to only adult HIV negative TB patient population limits its generalizability to the HIV positive population. Similarly, the study enrollment was limited to smear-positive patients and the results cannot be generalized to smear-negative culture positive TB patients. Also, being a sub-analysis within a single site, findings cannot be generalized to other sites from different regions. iii) Some variables like chest radiological findings were not collected within the Rifatox trial and could not be explored in the analysis of predictors of non-conversion. iv) Finally, because the Rifatox trial was a phase 2 safety trial, post treatment culture follow-up was not part of the trial procedures and so, we could not establish whether the delayed converters had a higher relapse rate than early converters.

Conclusions

The month-2 culture conversion used in phase 2 clinical trials as surrogate marker of treatment efficacy is influenced by the culture method used for monitoring mycobacterial response to TB treatment. Therefore, multi-centric TB therapeutic trials using early efficacy endpoint should use the same culture method across sites. The Time-to-detection of MTB on MGIT prior to treatment initiation and working in Social service jobs bear an increased risk of culture non-conversion at month-2.

Additional file

Additional file 1: Dataset. (CSV 14.4kb)

Abbreviations

ALT: Alanine aminotransferase; BMI: Body mass index; DOT: Directly observed treatment; DTM: Domiciliary treatment monitor; E: Ethambutol; HIV: Human immunodeficiency virus; H: Isoniazid; LJ: Lowenstein-Jensen; MGIT: Mycobacteria growth indicator tube; NTM: Non-tuberculosis mycobacterium; Z: Pyrazinamide; RH: Rifampicin and isoniazid; R: Rifampicin; Rlt: Risk ratio; SR1: Study regimen one; SR2: Study regimen two; TB: Tuberculosis; ULN: Upper limit of normal

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Availability of data and materials

The dataset from which the information presented in this manuscript originates has been submitted as Additional file 1.

Authors' contributions

DA MB AJ conceived the study, and participated in its design and coordination and drafted the manuscript. DA MB performed the statistical analysis and drafted the manuscript. JM, PO participated in the statistical analysis and drafted the manuscript. PO IT and AA carried out the laboratory procedures of the study and participated in manuscript writing. SI participated in data collection, study monitoring, and manuscript writing. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Ethics approval and consent to participate

The Rifatox Trial which is the source of the data used in this sub-analysis was approved by the Oxford Tropical Research Ethics Committee (OxTREC), Research Ethics Committee of Mbarara University of Science and Technology, Uganda National Council for Science and Technology (UNCST) and National Drug Authority (NDA) for Uganda. Written informed consent was obtained from all patients prior to their participation in the study.

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CHAPTER 4

DISCUSSION

In this thesis, we hypothesized that optimization of the current treatment regimen especially with use of high-dose rifampicin, might result in a shorter TB treatment duration (3-4 months).

4.1 Safety of high-dose rifampicin

The reluctance to investigate higher doses of R has been due to the fear of serious hepatotoxicity. Within an environment of limited knowledge on the potential toxicity of a higher R doses, and with most available data derived from non-comparative cohort studies [253-255, 257, 261, 262], it becomes hard to implement high R doses in phase 3 trials or even in clinical care despite reports of good efficacy. In this thesis, we first of all sought to establish safety of high dose R in the population of HIV negative TB patients before involving HIV-TB co-infected patients, based on the premise of no existing data on the potential effect of high R doses on efficacy of EFV-based ART, given the already documented drug-drug interactions [169] and overlapping toxicities as a result of anti-tuberculosis and ART co-administration [170]. The RIFATOX, randomized clinical trial demonstrated the safety of an increase of up to double the R dose (20 mg/kg) for the first 16 weeks in microscopy-positive HIV-negative patients with pulmonary TB. This was based on the observed rising, but statistically non-significant trend in ALT levels that returned to normal without treatment interruption. We noted that of the 7 patients with grade 3 rise in ALT in the whole study, only one (on R 15mg/kg) experienced jaundice resulting in R discontinuation, while others resolved by the next clinic visit.

Given the high-burden of HIV-TB coinfection in Africa and Asia, there is need to also establish the safety of high R doses in such patients on ART. Notably, EFV-based ART regimens will continue to be a commonly used by clinicians given their affordability and good efficacy [171] which benefit out-weighs the known risk of cross-cutting AEs during co-administration with ATT [196]. In the RIFAVIRENZ, randomized clinical trial, we also demonstrated the safety of R 20mg/kg, as supported by the absence of an incremental trend in incidence of both grade 3 or 4 hepatic and non-hepatic AEs. But also that, treatment reduction from R 20mg/kg to R 10mg/kg was only observed in 2 of the 4 patients that had ALT rise of grade ≥ 3 . The overall absence of onset of CNS grade ≥ 3 AEs in this study, also confirms the safety of using EFV at 600mg dose even with R at double dose. This is consistent with the already established safety of EFV 600mg among HIV-TB co-infected patients [199].

Although the treatment groups were relatively small particularly in the RIFAVIRENZ trial, these results suggest that a daily R dose of 20 mg/kg is safe enough to be taken forward to larger Phase III trials. However, caution should be exercised in patients whose liver function tests are compromised for any reason, in those with viral hepatitis infection and in pregnant women, since there is no evidence on safety of higher R doses in these populations. These results in HIV negative TB patients have been confirmed within another study using R as high as 35 mg/kg [290].

4.2 Drug-drug interaction between rifampicin and efavirenz and effect of doubling the dose of rifampicin

In order to better understand the potential effect of high R doses on the PK of EFV, it was important to explore the known evidence and try to appreciate the existing variations and their predictors, within the TB-HIV high-burden countries. This would also guide future implementation of new anti-TB and ART regimens with adjusted R and EFV doses respectively. In our systematic review of publications from studies conducted in the WHO high TB/HIV-burden countries on PK of EFV during RH co-administration, we noted that the average EFV PK parameters were within the therapeutic range. This review affirmed the safety and good ART efficacy when EFV 600mg was co-administered with the currently recommended RH-based 6-month anti-TB treatment utilizing R at 10mg/kg. Although higher EFV concentrations especially in patients with CYP2B6 loss of function gene were noted during co-administration with anti-TB treatment and that they could increase the occurrence of CNS adverse events [166, 198], in our systematic review, no such strong correlation could be concluded among the exclusively African studies [185, 274, 279]. This highlights the need for further research to generate conclusive evidence on the aetiology of CNS disorders beyond what could be attributed to supratherapeutic EFV concentrations especially in patients on concurrent treatment for TB and HIV [291, 292]. The CYP2B6 slow-metabolizer genes frequency was lower than that of extensive metabolizer genes among both African and Asian populations. The review suggests that CYP2B6 loss of function slow-metabolizer genes frequency could be the most likely explanation for the variability of EFV concentrations among African and Asian populations and should therefore be considered prior to EFV-400mg implementation in TB-HIV co-infected patients.

It is important to note that in the systematic review, no study was found to have utilized R doses beyond 10 mg/kg, hence leaving a gap of knowledge on the level of interaction expected with co-administration of R doses above 10 mg with EFV-based ART. The RIFAVIRENZ TRIAL, offered the first assessment of the effect of high-dose R (15 and 20 mg/kg) on the EFV metabolism and its effect on treatment outcomes in co-infected patients. Similar to the systematic review findings when R was used at 10 mg/kg, the RIFAVIRENZ trial demonstrated that the R doses up to 20 mg/kg does not seem to affect significantly the EFV concentrations, given that the lower margins of the 90% confidence interval of the geometric mean ratios of either the AUC or C_{min} at week 8/week 28 irrespective of treatment arms were within the predefined 0.70 to 1.43 range for concentrations, and also resulted in good efficacy outcomes for anti-tuberculosis treatment as shown by a month-2 culture conversion rate >90%, and with good virological suppression during the first 12 weeks post ART initiation. However, based on this study, we emphasize the need for further investigation given the observed low virological suppression at 24 weeks post ART initiation among patients in the R 20mg/kg dose arms with EFV 600 mg or 800mg despite being on standard R and EFV doses after 8 weeks post ART initiation. This would be important before higher R doses can be used in TB-HIV co-infected

patients. We also noted that sub-therapeutic EFV rates did not differ between the high R dose arm and the standard treatment arm during co-administration with ART. This finding further suggests that EFV exposure may be greatly influenced by CYP450 genetic polymorphisms rather than just the drug-drug interactions. In addition to this, as observed in the systematic review, the role of CYP2B6 loss of function genes frequency in the EFV exposure will be of great importance to further interpret the RIFAVIRENZ trial results in addition to adherence.

4.3 Efficacy of high dose Rifampicin

Despite the fact that both RIFATOX and RIFAVIRENZ trials were not powered for efficacy, we noted an increasing trend in week-8 culture conversion rates with increase of R to 20 mg/kg. The R dose-response relationship has also been demonstrated with use of R 35 mg/kg [290]. In this study by Boree, et al, R35 mg/kg exhibited a significantly faster month-2 culture conversion in liquid media, hence giving more strength to its potential use in shorter regimens for DS-TB. Time to stable culture conversion in liquid media was faster in the 35 mg/kg rifampicin group than in the control group (median 48 days *vs* 62 days, adjusted hazard ratio 1.78; 95% CI 1.22–2.58, $p=0.003$), but not in other experimental arms. There was no difference in any of the groups in time to culture conversion on solid media. In the same study, treatment arms containing SQ109 and moxifloxacin failed to show superiority to the standard of care [290].

4.4 Early efficacy markers for tuberculosis chemotherapeutic trials

Having a good early surrogate marker for TB treatment efficacy in phase II trials, ensures confident decisions for evaluating potential candidate drugs or regimen in large phase III trials and averts potential enormous losses on research investment, time and disappointing results in such large trials. The case in point is the 3 quinolone phase 3 trials which despite proven efficacy based on week-8 culture conversion in Phase II trials [230, 231] failed in their objective to shorten treatment [233-235]. Here, we will address three key questions with regard to use of month-2 culture conversion as a surrogate marker for TB treatment efficacy: 1) *Does the performance of month-2 culture conversion differ across HIV status?*; 2) *What influences month-2 culture conversion among both TB and HIV-TB co-infected populations?*; 3) *What is the impact of the culture media type used on early treatment response indicators, and in the context of shorter TB treatment regimens?*. With the same context within which the two phase II randomized clinical trials (RCTs), that is RIFATOX (in HIV negative TB patients) and RIFAVIRENZ (in HIV-TB co-infected patients) were implemented, that is among Ugandan patients, same Ugandan site, using up to double the normal R dose (that is 20 mg/kg), same patient catchment area, same laboratory facility and used MGIT-based month-2 culture conversion as an early surrogate

marker for treatment efficacy, avails an opportunity to explore the above questions surrounding the usage of month-2 culture conversion as an early efficacy marker within high-dose R-based trials and whether it will have a role in future shorter treatments.

4.4.1 Does the performance of month-2 culture conversion differ across HIV status?

Based on the reported month-2 conversion rates from both RCTs as summarized in Table 14 below, we note a discordance in MGIT-based month-2 culture conversion rates between RCTs, which seem to suggest a differential treatment response with a more delayed culture conversion among the HIV negative TB patients than in HIV-TB co-infected patients. It is not clear if patient enrolment based on smear positivity in RIFATOX versus Xpert positivity for the RIFAVIRENZ trial could have influenced this result. The potential influence of the extent of the disease based on CXR, cannot be demonstrated given that CXR was only done in RIFAVIRENZ trial but not in RIFATOX trial.

Though the reasons for this disparity in treatment response between HIV negative and positive TB patients is beyond the scope of this the current research, but what is surprising is that, common in both patient populations; month-2 non-converters never failed on treatment, and got cured by month 6, even when their treatment was not modified in any way.

Table 14. Month-2 culture conversion rates reported within 2 high R dose trials, that is, among HIV negative TB patients (RIFATOX trial) and HIV-TB co-infected patients (RIFAVIRENZ trial) in Uganda (Extracted from Chapters 3.1 and 3.3).

Study(population)	MGIT-based month-2 culture conversion among Ugandan patients, % (only Ugandan site)		
	R10	R15	R20
RIFATOX (HIV negative TB)*	54.6	41.9	48.4
RIFAVIRENZ (HIV-TB co-infected)	80.0		85.7** and 86.7***

*P=0.601; **For the R20 EFV600 arm; ***For R20 EFV 800

There was only 1 patient in RIFAVIRENZ trial who despite being sputum culture-negative at month-2, was diagnosed with RR-TB from a bone-marrow specimen during investigations into his recurrent anemia episodes. This further affirm the lower specificity of culture non-conversion at month-2 in predicting treatment failure [293] among Ugandan drug-susceptible TB patients irrespective of HIV status. Important to note is that, despite the controversies in literature with respect to the role of HIV status on month-two sputum conversion, with some in support [294] while others differing [282], the observed wide disparities in MGIT-based month-2 conversion rates in these two high-dose R trials among Ugandan patients seem to suggest a differential scenario that needs further investigation especially

through establishing factors associated with this high month-2 culture conversion in HIV-TB co-infection within the same setting as we have done for the HIV negative TB population [295], but also to establish its predictability of TB relapse and recurrence. Also, this disparity based on HIV status, questions the applicability of the suggestion made in the retrospective study of several phase 3 TB trials conducted by the British Medical Council in Hong Kong and East Africa, recommending the use of months-3 instead of month-2 culture conversion endpoint as a surrogate of treatment efficacy within the phase II East African trials [133]. Although this could apply to the HIV negative TB population who according to our study suffered delayed month-2 conversion rates, but this may not be the case for the HIV-TB co-infected population. However, given the search for shorter TB regimens, the focus should be on even much early (less than 2 months) biomarkers or surrogate markers.

4.4.2 What influences month-2 culture conversion among both TB and HIV-TB co-infected populations?

As reported in Chapter 3.4 of the thesis, from our analysis of the RIFATOX data for the Ugandan site, the observed low month-2 culture conversion rates could not be explained by treatment regimen. Contrary to other studies [282], baseline colony density revealed no influence on month 2 culture non-conversion potentially because the study population was almost homogeneous in the respect that over 80% of the participants had high baseline bacillary loads and colony density ($\geq 2+$). On the other-hand, pre-treatment time-to-detection (TTD) of MTB on MGIT of <14 days independently predicted month-2 culture non-conversion as compared to TTD of 14 days and above, something that confirms results in previous reports [283]. However, though TTD has also been associated with TB recurrence and relapse [280, 286], this could not be evaluated in this thesis since RIFATOX trial lacked an extended post-treatment follow-up. The influence of socio-economic indicators, precisely the type of occupation was noted as an important predictor of month-2 culture non-conversion. Beside the need to consider these factors during efficacy analysis in future phase II trials, the question necessitating further research is on whether the same or different factors do influence month-2 culture conversion among HIV-TB co-infected patients in the context of high R dose research.

4.4.3 What is the impact of the culture media type used on early treatment response indicators, and in context of shorter treatment regimens?

As reported in Chapter 3.4 of the thesis, we demonstrated that the reported month-2 culture conversion rates in phase II trials, vary across different culture media used, something that could affect interpretation and comparability of treatment efficacy between phase II trials in a bid to facilitate decisions for progression to phase II trials. In our study, we demonstrated that LJ (solid media) always gave culture conversion rates that are higher than those of MGIT (liquid

media) with differences becoming wider or more pronounced in all patients by month-4 of treatment follow-up of the 6 month TB treatment. Beyond the already known higher sensitivity of MGIT over LJ [29, 30], the fact that significantly wider difference in MTB detection between LJ and MGIT tend to occur earlier (by month 2) among patients on high R dose regimens (15 or 20 mg/kg) as compared to those on R10 mg/kg, may imply an early or faster reduction in LJ sensitivity following an early R dose-dependent faster clearance of tubercle bacilli population [146]. In this analysis, it was evident that in the environment of high R dose use, its usage as a treatment monitoring culture tool could be down-played because of this early loss of sensitivity and so could give over-estimated early indication of treatment efficacy. Though not easily accessible, with few but increasing number of laboratories with liquid culture infrastructure among LMICs, it may offer a more reliable early indication of treatment response given its ability to detect even persister populations of TB. Based on these results, we also recommend that the same culture media to be used across sites especially within the context of multi-centric TB chemotherapeutic trials so as to enable inter-site comparisons.

With the much anticipated and soon to come shorter TB treatments, early treatment efficacy indicators, that is earlier than 2 months will be needed so as to guide early treatment and diagnostic decisions. The question of whether month-2 culture conversion will still have a place as a surrogate marker for treatment efficacy within the context of future 4 months or less TB treatment lies in balance and cannot be answered by the results of our phase 2 high-dose R trials (RIFATOX and RIFAVIRENZ). However, our results suggests the evaluation of early markers like pre-treatment TTD and less than 2 months culture conversion, especially in studies evaluating the use of high-dose R or other drug compounds in shortening TB treatment that have a long post-TB treatment follow-ups. Also, given the observed disparities in month 2 culture conversion rates across HIV status, it is paramount that this should be considered in future evaluation of efficacy markers appropriate in shorter treatment durations.

4.5 Perspective of TB treatment shortening

RIFASHORT trial- NCT02581527 is one of the only two ongoing phase III trials globally, with a focus to shorten TB treatment duration to 4 months in HIV negative patients with drug-susceptible tuberculosis by using high doses of rifampicin or rifapentine with or without moxifloxacin [229]. Epicentre in Mbarara is participating as one of the Trial sites. It is an international multicentre open-label 3-arm controlled clinical trial comparing a standard 6-month control regimen with two 4-month treatment regimens utilizing either R 20 mg/kg or R 35 mg/kg, for the treatment of TB. The trial is supported by safety data from phase 2 trials that have explored safety, pharmacokinetics and bactericidal effects of high R doses, in patients with TB [263, 270, 296]. The second ongoing Phase III trial TBTC 31/ACTG A5349 (NCT02410772) utilizes another rifapentine given at 1200 mg as part of two four-month regimens

for the treatment of DS-TB. The first experimental regimen in this trial simply replaces R with rifapentine and reduces the continuation phase to two months. The second experimental regimen is the same as the first, but replaces ethambutol with moxifloxacin and continues moxifloxacin for the continuation phase[229].

There is currently no treatment shortening Phase III clinical trial enrolling TB-HIV co-infected patients. Both ongoing and some completed trials on high R doses have only included HIV negative TB patients [263, 270, 296]. The inclusion of TB-HIV co-infected patients in phase III trials will necessitate additional evidence on the magnitude of interaction between ART and high R doses and its impact on safety and efficacy of ART. Our results from the RIFAVIRENZ-ANRS 12292 Trial (ClinicalTrials.gov Identifier: NCT01986543) offer the initial hope for future involvement of HIV-TB co-infected patients into treatment shortening phase III trials. However, prior to their inclusion in phase III trials, more RCTs evaluating R-doses higher than 20mg/kg may need to be done. On the other hand, within the ongoing RIFASHORT trial, the potential of R 20 mg/kg and R 35 mg/kg in shortening TB treatment to 4 months among HIV negative TB populations will finally be concluded, with results expected by late 2019.

The research progress towards TB treatment shortening using high R doses may be affected by the parallel advances in HIV treatments, especially with the introduction of DTG and recommendation of EFV at 400mg as alternatives to EFV 600 in the first-line regimen [36]. Nevertheless, though the effect of R on EFV PK when administered at the standard dose of 600 mg/day is well documented and with still good ART efficacy [297], it is not clear whether the same consistent efficacy will be seen for the lower 400-mg dose of EFV when co-administered with normal or high-dose R. On the other-hand, the safety and efficacy of DTG among TB/HIV co-infected patients using R has not been established [298]. Therefore, given the expected increasing adoption of DTG among HIV and TB high-burden countries, it is pertinent that phase II RCTs be conducted to evaluate the safety, efficacy and pharmacokinetics of DTG with use of high R doses among HIV-TB co-infected patients, as part of the preliminary steps in fast-tracking TB treatment shortening among HIV-TB co-infected patients.

4.6 Conclusion

In conclusion, we have demonstrated that doubling R dose is safe and with good TB treatment outcomes in both TB and HIV-TB infected patients. Our study findings support the progression to phase III efficacy trials focused on treatment shortening to 4 months with use of at least double the normal R doses among HIV negative TB patients. We recommend further preliminary phase II trials to investigate the safety and effect of high R doses up to 35 mg/kg (as already utilized in RIFASHORT trial) on the EFV and dolutegravir-based ART regimen efficacy among HIV-TB

co-infected patients prior to their involvement in treatment shortening phase III trials. We also recommend further studies to investigate the effect of higher R doses on persister bacilli clearance. Studies evaluating new and existing biomarkers and early surrogate markers for treatment efficacy that would be appropriate for future shorter treatment durations are needed to ensure a more accurate prediction of late TB treatment efficacy (TB recurrence, relapse and treatment failure).and should be incorporated within the ongoing and future TB treatment shortening RCTs.

SUMMARY

The recommended 6-month treatment for drug-susceptible tuberculosis (DS-TB) is too long, hence threatening patient adherence, with resulting treatment failure and drug-resistance. Consequently, this complicates efforts towards achieving the TB control and elimination goal. Currently, no new drug is expected in the short term for reducing treatment duration. We hypothesized that optimization of the current treatment regimen with use of high-dose rifampicin (R), above the 10mg/kg current dose, might result in a shorter TB treatment duration (3-4 months). Clinical trials were conducted to investigate two research areas: first, Safety of high-dose R in HIV-negative TB and in HIV-TB co-infected patients; secondly, drug-drug interaction between high-dose R and antiretroviral drugs. A 3rd research area addressed the use of 2 months sputum culture conversion as surrogate marker for treatment efficacy in TB trials.

A Phase II, open-label randomized controlled trial (RIFATOX) was conducted with sites in Bolivia, Peru and Uganda. Three hundred HIV-negative smear-positive TB patients were randomised between three regimens differing only by R dose during the first 16 weeks of the standard 24 weeks treatment: R at 10 mg/kg and two high-R doses (15mg/kg or 20mg/kg). Liver function and bacteriological response were monitored. There was no significant increase in hepatotoxicity with high-dose R. Using data from the cohort of patients from the Ugandan site only, we showed that month-2 culture conversion, a commonly used surrogate marker in phase 2 trials was influenced by the culture method used with significantly higher conversion rates noted with solid versus liquid media within patients on high-R dose regimens. We therefore recommend use the same culture method across sites within multi-centric TB trials.

We conducted a systematic review to gather existing information on the pharmacokinetics, adverse effects and efficacy of efavirenz (EFV), the most commonly used antiretroviral drug, co-administered with R-based TB regimens among high TB/HIV-burden countries. Twenty-two articles published between 2006 and 2016 were analyzed. With the use of 600mg EFV daily, plasma concentrations on average were above the minimum therapeutic concentrations during R co-administration with good safety and efficacy. No clear relationship between supratherapeutic EFV concentrations and occurrence of neurological and hepatic adverse events was observed.

Then, we conducted a phase-2, randomized, open-label pharmacokinetic trial (ANRS12292 RIFAVIRENZ trial) among 97 DS-TB patients and antiretroviral therapy (ART)-naïve Ugandan patients. These were randomized between 3 drug regimens: 2 using R (20mg/Kg) with ART initiation 2-4 weeks later with EFV600mg/day or 800mg/day) and a control regimen using R10mg/kg and EFV600mg/day. At 8 weeks, all patients were switched to standard R and EFV doses. All patients had intensive pharmacokinetic sampling 4 weeks after EFV-R co-administration, and 4 weeks after R discontinuation. Despite a trend of lower EFV concentration when the R dose was doubled, concentrations remained within the therapeutic window. Treatment with high-dose R was well tolerated. Virological efficacy was high during the first 12 weeks on ART but reduced in the R arms after 24 weeks. We conclude that, use of high-dose R at 20mg/Kg is safe and could be evaluated in larger trials towards shortening of treatment for DS-TB patients. Due to late virological failures in patients on R 20 mg/kg and standard EFV dose, comprehensive efforts through additional research are needed to fast-track the inclusion of TB-HIV co-infected patients in phase 3 trials.

Short summary

The 6 months treatment for tuberculosis (TB) is too long, a challenge to patients and need shortening. Unfortunately, there are no promising new drugs to achieve this. Another option is using higher than usual doses of one of the existing TB drugs especially rifampicin. This needs evidence on whether it would kill TB germs faster, without increasing dangerous side-effects or interfering with efavirenz, the commonly used HIV drug in Africa and reducing their ability to adequately kill and reduce numbers of HIV viruses in blood.

In our investigations, we found that up to double the normal dose of rifampicin was safe either in TB mono-infected patients or in HIV-TB co-infected patients. We also demonstrated in HIV-TB co-infected patients that a double dose of rifampicin did not impair very much the amount of efavirenz in the body.

Additional work is needed to explain the decreased efficacy of efavirenz in patients who received the rifampicin double dose and standard efavirenz dose.

Résumé

Le traitement actuel de la tuberculose chimio-sensible est de 6 mois, ce qui est beaucoup trop long et entraîne des défauts d'adhésion au traitement, des échecs thérapeutiques par sélection de bactéries résistantes. Puisque dans le futur proche, aucun nouveau traitement ne permettra de raccourcir la durée de traitement, d'autres voies doivent être recherchées, entre autres l'augmentation de la posologie de rifampicine (R) au-delà des 10 mg/kg actuellement recommandés qui pourrait permettre un traitement de 3-4 mois.

Notre travail, réalisé dans le cadre d'essais cliniques, a consisté d'une part à étudier la tolérance d'une dose élevée de R au sein du traitement antituberculeux standard chez des patients tuberculeux séronégatifs ou séropositifs pour le VIH, et d'autre part l'interaction entre une double dose de R (20mg/kg) et le traitement antirétroviral, en particulier l'efavirenz (EFV). Enfin un autre axe de recherche a consisté à étudier si la négativation des cultures de crachat à 2 mois pouvait être un marqueur de l'efficacité du traitement antituberculeux dans les essais cliniques. Afin de répondre à ces objectifs, les études suivantes ont été réalisées.

Une étude ouverte, de Phase II, randomisée, contrôlée (RIFATOX) a été mise en place (Bolivie, Pérou and Ouganda). Trois cent patients tuberculeux, séronégatifs pour le VIH ont été randomisés entre 3 schémas thérapeutiques se différenciant par la posologie de la R durant les 16 premières semaines : R à 10, 15 ou 20 mg/kg. La fonction hépatique et la réponse bactériologiques ont été monitorées. La toxicité hépatique n'était pas plus importante à dose élevée de R. En utilisant les résultats des patients ougandais, nous avons montré que la négativation des cultures à deux mois était influencée par le type de milieu de culture utilisé, avec négativation plus importante en milieu solide qu'en milieu liquide pour les traitements avec dose élevée de R. Ces résultats nous amènent à recommander que le même milieu de culture soit utilisé dans tous les sites lors d'essais multicentriques visant à étudier l'efficacité de traitement antituberculeux.

Une étude bibliographique « systématique » a été réalisée afin de colliger les informations existantes sur l'efficacité, la tolérance et les interactions pharmacocinétiques de l'EFV associé au traitement antituberculeux à posologie standard dans les pays à forte endémie. Vingt-deux articles publiés entre 2006 et 2016 ont été sélectionnés. Aucune relation entre des concentrations élevées d'EFV et la survenue d'effets indésirables neurologiques ou hépatiques n'a pu être mise en évidence.

Au vu de ces informations, un essai pharmacocinétique de phase 2, ouvert (ANRS12292 RIFAVIRENZ trial) a randomisé 97 patients tuberculeux vivant avec le VIH et naïfs de traitement antirétroviral entre 3 bras de traitement : R20mg/kg+EFV 600mg, R20mg/kg +EFV 800 mg et R10mg/kg+EFV600mg (bras contrôle). R était associé au traitement antituberculeux à posologie standard et EFV associé à deux analogues nucléosidiques (tenofovir+lamivudine) a été initié 2-4 semaines après le traitement antituberculeux. Après 8 semaines de suivi, tous les patients ont reçu les traitements à posologie standard. Des prélèvements sanguins ont été réalisés pour étudier la pharmacocinétique de l'EFV associé à R ou administré seul (4 semaines après arrêt du traitement antituberculeux). Malgré une légère diminution des concentrations d'EFV dans le bras R20 mg/kg+EFV 600mg, les concentrations sont restées dans la zone des concentrations thérapeutiques. Quelque soit le bras de traitement, le traitement a été bien toléré. Cependant s'il n'y avait pas de différence sur l'efficacité virologique mesurée à 12 semaines, elle était diminuée à 24 semaines dans le bras R20 mg/kg+EFV 600mg. La pertinence clinique de ce résultat est en cours d'évaluation pour que des patients tuberculeux vivant avec le VIH puissent être inclus dans de futurs essais de phase 3.

Résumé court

La durée du traitement antituberculeux actuellement de 6 mois est trop longue et doit être raccourcie. Pour atteindre cet objectif, malheureusement, aucun nouveau médicament ne sera disponible prochainement. Une autre possibilité est d'augmenter la posologie des médicaments actuels en particulier de la rifampicine. Cependant il est nécessaire de vérifier que la tolérance reste acceptable et qu'il n'y a pas d'interaction avec les médicaments prescrits dans le VIH en Afrique, en particulier l'efavirenz. Dans notre travail réalisé en Ouganda, nous avons montré qu'une double dose de rifampicine au sein d'un traitement standard était bien tolérée aussi bien chez des patients mono-infectés par la tuberculose que ceux co-infectés par le VIH. Par ailleurs la dose augmentée de rifampicine modifie très peu la quantité d'efavirenz dans l'organisme lorsque ces traitements sont associés. Ce travail devra néanmoins être poursuivi pour comprendre la diminution d'efficacité virologique observée.

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PhD Training

Certified short trainings under-taken

2016	Field Management Training (FMT), Epicentre, Uganda
2016	Research Communication and Internationalization Training of Trainers, MUST/LUND University, Uganda
2015	Systematic Review and Meta-analysis Course, University of Bern, Switzerland,
2015	Mentorship Training and Students Supervision Course, JCRC/COHRE
2014	Pharmaco-epidemiology, Erasmus MC University Medical Center, Rotterdam,
2014	Primary and Secondary Prevention Research, Erasmus MC University Medical Center, Rotterdam
2014	Mbarara Training Program for Researchers, MSF- Epicentre, Uganda
2014	Leadership and Management Course, IMPLINT- UGANDA

Oral Presentations

- Is it safe to double the dose of rifampicin to shorten tuberculosis treatment duration? (Epicentre Scientific Conference, Kampala, 5th July 2017)
- Delayed tuberculosis culture conversion: Predictors and mycobacterial culture effect among Ugandan patients. (Annual Grande doctors' Conference, 11-13th August 2016, Kampala, Uganda)
- An International Multicentre Controlled Clinical Trial to Evaluate the Toxicity of High Dose Rifampicin in the Treatment of Pulmonary Tuberculosis (RIFATOX). (Annual Grande doctors' Conference, 11-13th August 2016, Kampala, Uganda.)
- D Atwine, et al. Delayed tuberculosis culture conversion: Predictors and mycobacterial culture effect among Ugandan patients. In 47th Union World Conference on Lung Health, 26 to 29 October 2016 - Liverpool, UK [Abstr ID: SOA-505-27]

Poster presentations

- Daniel A., et al. Eight weeks safety results of high-dose Rifampicin in HIV-Tuberculosis co-infected patients in Uganda: RIFAVIRENZ-ANRS 12292 Trial. (IAS conference for HIV science, 23rd-26th July 2017, Paris, France)

- Delayed conversion or Treatment failure: Diagnosis, Predictors, and Outcomes of month-two non-conversion among immuno-competent TB patients in Uganda. (National Tuberculosis Stakeholders' meeting, 2015)
- **Atwine D.** et al. An International Multicentre Controlled Clinical Trial to Evaluate the Toxicity of High Dose Rifampicin in the Treatment of Pulmonary Tuberculosis (RIFATOX). In. MSF at 30 years in Uganda Exhibition, 2015, Kampala, Uganda (Poster presentation).

Lecturing

Quantitative research methods	Master in Health Information Technology, Uganda, 2016 -2017
Clinical Research	Master in Public Health class, Mbarara University of Science and Technology, Uganda, 2016
Epidemiology	Master in Public Health class, Mbarara University of Science and Technology, Uganda, 2015
Systematic review and meta-analysis	Researchers at Epicentre Mbarara research Centre, Uganda, 2016

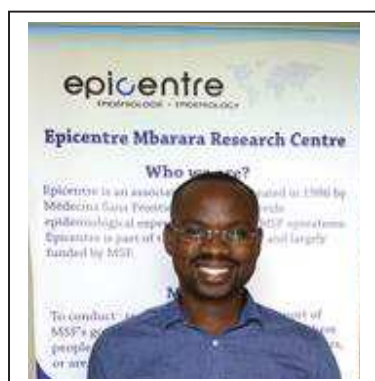
Students' supervisory activities

2014	Namugerwa Shadia, Bsc Pharmacy	Magnitude, self-reported reasons and perceived solutions for missed ART dose among pediatric patients treated from ISS-Clinic, MRRH.
2014	Aziiz Kapande, Bsc Pharmacy	Patient and provider perceptions and clinical outcomes following prescribing medicines that is out of stock at Mbarara Regional Referral Hospital.
2017	Benedict Agaba, MSc Health Information Technology	Improving of diabetic patients' self-management through a mobile phone intervention: a situation analysis
2017	Nyangoma Miria, MSc Health Information Technology	Effect of an innovative information delivery intervention on tuberculosis patients' early post-disclosure experiences at household level

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- Member of UNION.
- Member of IAS

About the Author



Atwine Daniel was born in Ssembabule district, Uganda. He is a second born in a family of 7 children. He completed his high school from Muntuyera High School in 2000. Having excelled, he joined Mbarara University of Science and Technology under a government sponsorship, and graduated with a Bachelors in Human Medicine and Surgery in 2006. He then joined MSF-Epicentre in November 2007 as a Clinical investigator in an International Multicentric EDCTP trial evaluating 4 Artemisinin-based combinations in treatment of uncomplicated malaria in Children. His interest in research grew, that he decided to build his career further by undertaking an Advanced Master in Public Health Methodology (MPHM) at the Université Libre de Bruxelles (ULB) in Belgium between 2009 and 2010, under CUD international scholarships. He

utilized this opportunity to also do a Post-graduate Diploma in advanced methodologies and also in communication. Having excelled with a Grande distinction master, he was further motivated to return to his parent organization (Epicentre) in Uganda in 2010, to further his new research interests and to apply his acquired skills. At this point, with the desire to share these skills, he engaged into occasional lecturing of post-graduate students at Mbarara University of Science and Technology. His turning point from Malaria into TB research started in 2010. He was first engaged in TB diagnostic studies which he coordinated and then to TB chemotherapeutic Trials from 2011. He has been the Ugandan principal investigator of RIFATOX, RIFAVIRENZ, and now RIFASHORT Trials. All these are focussed on TB treatment shortening with use of high rifampicin doses. In the Interim, he under-took an MPhil in Science and Technology at Stellenbosch University in South Africa with focus on research uptake, evaluation, impact assessment and science policy and communication, sponsored by DRUSSA. He started his PhD studies in 2013 as described in this thesis. Atwine's PhD was directed by Dr. Maryline Bonnet and Dr Anne-Marie Taburet. During his PhD, he attended a number of courses nationally and internationally, specifically at University of Erasmus, Netherlands in Pharmaco-epidemiology and University of Bern, Switzerland in systematic review and meta-analysis. Other non-scientific courses taken in Uganda included: Leadership and management, Research communication, students mentorship, among others. He has been involved in students' research supervision, and acted as an External examiner at University of CapeTown in the course of Health Innovation. Atwine is adventurous and is open to new knowledge that can support his career dreams. Atwine is a licensed medical doctor and public health specialist by the Uganda medical and dental practitioners' council. He is a member of UNION, IAS, and African biomedical engineering consortium (ABEC). Atwine has continued to actively mentor young researchers, both students and non-students, something that he loves to do and desires to be known for in the future. His passion is in inspiring the young generation of professionals into research, and promoting biomedical research outputs on the African continent. In the field of Tuberculosis, his passion is on identifying innovative treatments that could lead to a shortening of treatment. Atwine is a God fearing man, married to a fellow doctor with two children. He loves to see people happy and will try to just get that out of them despite what they are going through. He loves making new collaborations, to play a guitar, piano, singing Christian songs and praying. He enjoys being with his family, travelling and he plays volley ball.

