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Integrating human immunodeficiency virus and tuberculosis drug treatment

by

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Submitted in fulfillment of the requirements for the degree Doctor of Philosophy (by publication) in the Discipline of Pharmaceutical Sciences.

DECLARATION BY SUPERVISOR

As the candidate's supervisor I, Prof. Julia Hillary Botha, agree to the submission of this thesis.

Signed: Julia Botha

Date: 14/11/2014

Declaration

I, **TANUJA NARAYANSAMY GENGIAH,** declare that,

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Dedication

For my mother Sally, father Narayan and sister Santhana whose unwavering support sustains me and without whom nothing in my life would be possible.…

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List of abbreviations

ABC	Abacavir
AE	Adverse event
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
ARV	Antiretroviral
ART	Antiretroviral therapy
ASP	Adherence Support Program
AST	Aspartate aminotransferase
AUC	Area under the curve
BOV	Between occasion variability
BHIVA	British HIV Association
CAMELIA	Cambodian Early versus Late Introduction of Antiretrovirals (ANRS 12154)
CI	Confidence interval
CDC	Centre for Disease Control
CL	Clearance
CL/F	Apparent clearance
Cmax	Maximum concentration
Cmin	Minimum concentration (trough)
CNS	Central nervous system
CRF	Case report form
CV	Coefficient of variance
CYP450	Cytochrome P450 monooxygenase enzyme
d4T	Stavudine
DAIDS	Division of AIDS
ddI	Didanosine
ddI-EC	Didanosine enteric coated
DILI	Drug induced liver injury
DME	Drug metabolizing enzymes
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
DLV	Delaviridine
DOT	Directly observed therapy
EC	Enteric coated
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
EFV CL/F	Efavirenz apparent clearance
ENCORE 1	Safety and efficacy of reduced dose efavirenz (EFV) with standard dose EFV plus two nucleotide reverse transcriptase inhibitors (N(t)RTI) in antiretroviral-naïve HIV-infected individuals
F	Bioavailability
FDA	Food and Drug Administration
GCP	Good Clinical Practice

GEE	Generalized estimating equations
GI	Gastrointestinal
HAART	Highly active antiretroviral therapy
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IOV	Inter-occasion variability
IRIS	Immune reconstitution inflammatory syndrome
LCMS/MS	Liquid chromatography –tandem mass spectrometry
MAF	Minor allele frequency
MDR	Multi-drug resistant
NAT 2	N-acetyltransferase 2
NONMEM	Non-linear mixed effects modelling
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NtRTI	Nucleotide reverse transcriptase inhibitors
NVP	Nevirapine
OBJ	Objective function
OATP	Organic anion-transporting polypeptide
PI	Protease inhibitor
PK	Pharmacokinetic
PR	Paradoxical reaction
RFB	Rifabutin
RFP	Rifapentine
RIF	Rifampicin/rifampin
RSE	Residual standard error
RTV	Ritonavir
SAPIT	Starting Antiretroviral Therapy at Three Points in Tuberculosis Trial (CAPRISA 003)
SLCO1B1	Solute carrier organic anion transporter family, member 1B1
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
START	Starting Tuberculosis and Antiretroviral Therapy Trial (CAPRISA 001)
STRIDE	A Strategy of Immediate Versus Deferred Initiation of Antiretroviral Therapy for AIDS Disease-free Survival (A5221)
3TC	Lamivudine
TB	Tuberculosis
TDM	Therapeutic drug monitoring
UGT	Uridine 5'-diphospho-glucuronosyltransferase
Vd	Volume of distribution
VL	Viral load
VPC	Visual predictive check

WHO	World Health Organization
ZDV	Zidovudine

Symbols and scientific units

cm	Centimeter
g	Gram
h	Hours/s
Ka	Absorption rate constant
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
µg	Microgram
β	Beta
Δ	Delta
θ	Theta
σ	Sigma
ω	Omega

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List of papers published

❖ First author publications (Chapter 2)

1. **Gengiah TN**, Gray AL, Naidoo K, Karim QA. Initiating antiretrovirals during tuberculosis treatment: a drug safety review. *Expert Opinion on Drug Safety*. 2011; 10 (4):559-74. *page 32*

2. **Gengiah TN**, Holford NH, Botha JH, Gray AL, Naidoo K, Abdool Karim SS. The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis. *European Journal of Clinical Pharmacology*. 2012; 68 (5):689-95. *page 52*

3. **Gengiah TN,** Botha JH, Yende-Zuma N, Naidoo K, Abdool Karim SS. Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent TB treatment. *Antiviral therapy*. Oct 15, 2014 :DOI: 10.3851/IMP2877 (Advanced publication). *page 63*

4. **Gengiah TN**, Abdool Karim SS. Should efavirenz be does higher when co-administered with rifampin? Journal Watch HIV/AIDS Clinical Care Feb 6, 2012. http://www.jwatch.org/ *page 84*

5. **Gengiah TN**, Botha JH, Soowamber D, Naidoo K, Abdool Karim SS. Low rifampicin concentrations in tuberculosis patients with HIV infection. *Journal of infection in developing countries*. 2014; 8(8):987-93. *page 88*

❖ Co-authored publications relevant to PHD Topic (Appendix A)

6. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray A, **Gengiah T**, Nair G, Bamber S, Singh A, Khan M, Pienaar J, El-Sadr,W, Friedland G, Abdool Karim Q. Timing of initiation of antiretroviral drugs during tuberculosis therapy. *New England Journal of Medicine*. 2010; 362(8):697-706. *page 128*

7. Gray A, Abdool Karim SS, **Gengiah TN**. Ritonavir/saquinavir safety concerns curtail antiretroviral therapy options for tuberculosis-HIV-co-infected patients in resource-constrained settings. *AIDS*. 2006; 20(2):302-3. *page 140*

8. Abdool Karim Q, Abdool Karim SS, Baxter C, Friedland G, **Gengiah T**, Gray A, Grobler, A. Naidoo, K. Padayatchi, N, El-Sadr, W. The SAPIT trial provides essential evidence on risks and benefits of integrated and sequential treatment of HIV and tuberculosis. *South African Medical Journal.* 2010; 100 (12): 808-9. *page 144*

9. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray AL, **Gengiah, T**, Gengiah, S, Naidoo, A, Jithoo, N, Nair, G, El-Sadr, W. M, Friedland, G, Abdool Karim, Q. Integration of antiretroviral therapy with tuberculosis treatment. *New England Journal of Medicine*. 2011; 365(16):1492-501. *page 148*

10. Naidoo A, Naidoo K, Yende-Zuma N, **Gengiah TN**, Padayatchi N, Gray AL, Bamber, S, Nair, G, Abdool Karim, S. S.et al. Changes to antiretroviral drug regimens during integrated TB-HIV treatment: results of the SAPiT trial. *Antiviral Therapy*. 2014; 19(2):161-9. *page 160*

Summary

The human immunodeficiency virus (HIV) and tuberculosis (TB) epidemics are major global public health challenges. Worldwide, approximately 42% of TB patients are also co-infected with HIV, and sub-Saharan Africa (SSA) is home to the majority of the world's infections of both HIV and TB. Dual infection has been shown to be associated with a higher risk of death. Integrating drug treatment for both diseases is therefore essential to improve survival. However, drug interactions between antiretroviral therapy (ART) and anti-TB medication remain a challenge to effective treatment integration. Although several drug interactions have been identified, only some are clinically relevant. The impact of significant interactions on public health outcomes is expected to be greatest when large numbers of patients are prescribed interacting drugs.

Efavirenz (EFV) is the most commonly prescribed nucleoside reverse transcriptase inhibitor (NNRTI) component of first line ART in sub-Saharan Africa, particularly when rifampicin (RIF) based TB treatment is co-administered. RIF is known to up-regulate cytochrome P450 (CYP450) drug metabolizing enzymes resulting in decreased exposure to concomitantly administered drugs that utilize similar metabolic pathways. Therefore, the concomitant use of EFV with RIF would be expected to increase EFV clearance while absorption of TB drugs may also be compromised by advanced HIV disease. The efficacy of both TB and HIV treatment may thus be compromised by pharmacokinetic interactions, while more recent evidence also implicates genetic variation in drug metabolism as a predictor of drug exposure.

To understand the significance of the EFV-RIF interaction better in a South African population, the pharmacokinetics of EFV during and after RIF-based TB treatment were investigated as an ancillary study of the 'Starting Tuberculosis and Antiretroviral Therapy' (START) trial (CAPRISA 001: NCT00091936). Participants were randomized to receive both ART and TB treatment simultaneously (integrated arm) or to initiate ART only on

completion of TB treatment (sequential arm). In both arms, the ART regimen included once daily enteric-coated didanosine (400 mg for participants >60 kg; 250 mg for participants <60 kg), lamivudine 300mg and efavirenz. Based on the expected drug interactions, when EFV was administered in the presence of TB treatment, participants weighing less than 50kg received 600mg and those weighing 50kg or more received 800mg daily. After TB treatment was successfully completed, all patients received EFV 600mg.

Blood samples for trough EFV plasma concentrations were obtained at the end of months 1, 2 and 3 during TB treatment and at the same time points after TB treatment was successfully completed. Additionally, approximated peak RIF concentrations were measured 2.5 hours post-dose at the end of months 1, 2 and 3 of TB treatment. The influence of single nucleotide polymorphisms, in CYP2B6, CYP2A6, and UGT2B7 on EFV concentrations, and in drug transporter genes (SLCO1B1) on RIF concentrations, was assessed post-trial from stored peripheral blood mononuclear cell (PBMC) samples.

EFV concentration-time data were analyzed using a population pharmacokinetic non-linear mixed effects model (NONMEM) to quantify the impact of RIF-based TB treatment on EFV clearance. Unexpectedly, there was an overall 29.5% reduction in EFV clearance during TB treatment. A bimodal distribution of EFV apparent clearance (CL/F) was evident and indicated that slow EFV metabolisers accounted for 21.9% of the population. EFV clearance after oral administration in fast metabolisers was 11.5 L/h/70kg off TB treatment and 7.6 L/h/70kg when on TB treatment. In slow metabolisers, however, the clearance estimates were 2.9 and 4.3 L/h/70kg in the presence and absence of TB treatment respectively.

Building on the findings of the NONMEM analysis and in response to the US FDA prescribing change in 2012, that approved an EFV dose increase from 600mg to 800mg in patients weighing 50kg and more when on concomitant RIF, the presence and

influence of pharmacogenetic polymorphisms of the CYP450 enzyme system on NNRTI plasma exposure during and after TB co-treatment and the effect of increasing the EFV dose was investigated. During TB treatment, median (IQR) EFV Cmin was 3.2 (2.6-6.3) µg/mL and 3.3 (2.4-9.5) µg/mL in the EFV 800mg and 600mg groups respectively, while off TB treatment Cmin was 2.0 (1.4 - 3.5) µg/mL. The frequency of the CYP2B6 *1, *6 and *18 haplotypes was 18.5%, 38.9% and 25.9% respectively. Polymorphisms in all three CYP2B6 genes studied (516T-785G-983C) were present in 11.1% of patients. Median (IQR) EFV concentrations in patients with the three mutations were 19.2 (9.5-20) µg/mL and 4.7 (3.5-5.6) µg/mL when on and off TB treatment. TB treatment, composite genotypes CYP2B6 516 GT/TT, CYP2B6 983 TC/CC or being a CYP2A6*9B carrier predicted median EFV Cmin > 4 µg/mL. Therefore, increasing the EFV dose to 800mg during TB treatment is unnecessary in African patients with these polymorphisms.

As a critical component of first line TB treatment concerns about sub-optimal TB drug bioavailability were examined for RIF. The influence of drug transporter gene polymorphisms on RIF concentrations was also assessed. Median RIF (IQR) $C_{2.5hr}$ was found to be 3.6 (2.8-5.0) µg/mL while polymorphism frequency of the SLCO1B1 (rs4149032) drug transporter gene was high (0.76) and was associated with low RIF concentrations as was male gender and having a low haemoglobin. Increased RIF dosage warrants urgent consideration in African TB-HIV co-infected patients.

In conclusion, concomitant RIF-containing TB treatment unexpectedly reduced EFV CL/F with a corresponding increase in EFV exposure. Polymorphisms of EFV metabolizing enzymes were frequent in this population and contribute to this outcome. While in South Africa where TB-HIV co-treatment is associated with elevated EFV concentrations, peak RIF concentrations were alarmingly low and well below the recommended target range of 8 to 24 µg/mL. Increased RIF dosage may be warranted in African TB-HIV co-infected patients whilst the need for EFV dose increase is not supported by these data.

Recommendations for public health benefit, in this generalized epidemic in South Africa, include the consideration of an EFV dose reduction as a cost saving to improve life-long treatment sustainability, and a RIF dose increase to curb TB treatment failure and future development of multiple-drug resistant (MDR) TB.

Structure of the PhD thesis

The PhD thesis is structured in accordance with guidance provided by the College of Health Sciences, University of KwaZulu-Natal for the **thesis by publication** format. There is a single reference list in the Vancouver format for references cited in chapters 1 and 3. The chapters are divided as follows:

Chapter 1: Introduction

This chapter provides an overview and appraisal of the TB and HIV epidemics, the need for co-treatment integration and barriers associated with co-treatment. It also provides a review of the literature assessing the EFV-RIF drug-drug interaction and the influence of pharmacogenetics on EFV pharmacokinetics. Chapter 1 ends with the outline of the rationale for the work undertaken and the study objectives.

Chapter 2: Publications

This chapter contains the four, first authored publications arising from the findings of the PhD work and includes one commentary to Journal Watch. The PhD candidate's contribution to each publication is summarised and a brief discussion of each publication is presented. Each article stands alone and details its own methodology, statistical considerations and references. There may be unavoidable duplication of aspects of the discussion in Chapters 2 and 3.

Chapter 3: Conclusion

This chapter provides an overarching discussion of the major findings from the PhD and compares and contrasts these with current knowledge, detailing the novel contribution of the work. Future recommendations for research and practice along with study limitations are also described in this chapter.

Appendices:

The appendices contain summaries of the five co-authored publications that have contributed to advancing TB and HIV treatment integration, some of which, have had a direct impact on policy and practice. A summary of the START trial, regulatory approvals for the START trial and the PhD study analysis, informed consent forms, assay information and NONMEM code can also be verified in this section.

CHAPTER 1: INTRODUCTION

1.1 Background and literature review

1.1.1 The HIV and TB epidemics in Southern Africa

Between 2001 and 2013, there was a 38% reduction in annual incident HIV infections [1]. Despite this remarkable success, in 2013, the HIV epidemic remains a major public health burden with 35 million people living with HIV, 2.1 million new infections and 1.5 million AIDS related deaths globally [2]. Notably, the majority of all infections are found in sub-Saharan Africa, which is reported to be home to 24.7 million HIV infected individuals [2].

Developing countries, in particular, face a higher burden of HIV disease and increased susceptibility to other infectious opportunistic diseases like TB [3]. The proportion of TB cases co-infected with HIV is highest in African countries (Figure 1) [4] . In South Africa, 73% of patients diagnosed with active TB are estimated to also be HIV-positive [3], whereas this proportion is estimated to be at around 42% globally. Further, HIV-positive patients show a 20-37 fold increased risk of developing active TB than those who are HIV-negative [5]. Mortality rates are also high among TB-HIV co-infected individuals. In 2013, 360 000 people died of HIV-associated TB, making TB the leading cause of death in HIV-infected individuals, with African women bearing a disproportionate burden of the mortality risk when co-infected [4].

Since 2004, there have been tremendous gains in treatment coverage for HIV and it is estimated that by 2015, approximately 15 million people will be on ART. Despite this achievement, many TB-HIV co-infected individuals are not yet receiving ART. In sub Saharan-Africa, which houses 75% of the world's TB infections, only two countries, Kenya and Malawi, are delivering ART to more than 50% of those TB co-infected [1]. One of the main factors impacting on the suboptimal treatment coverage for dually infected patients is the complexity associated with integrating the treatment for both diseases.

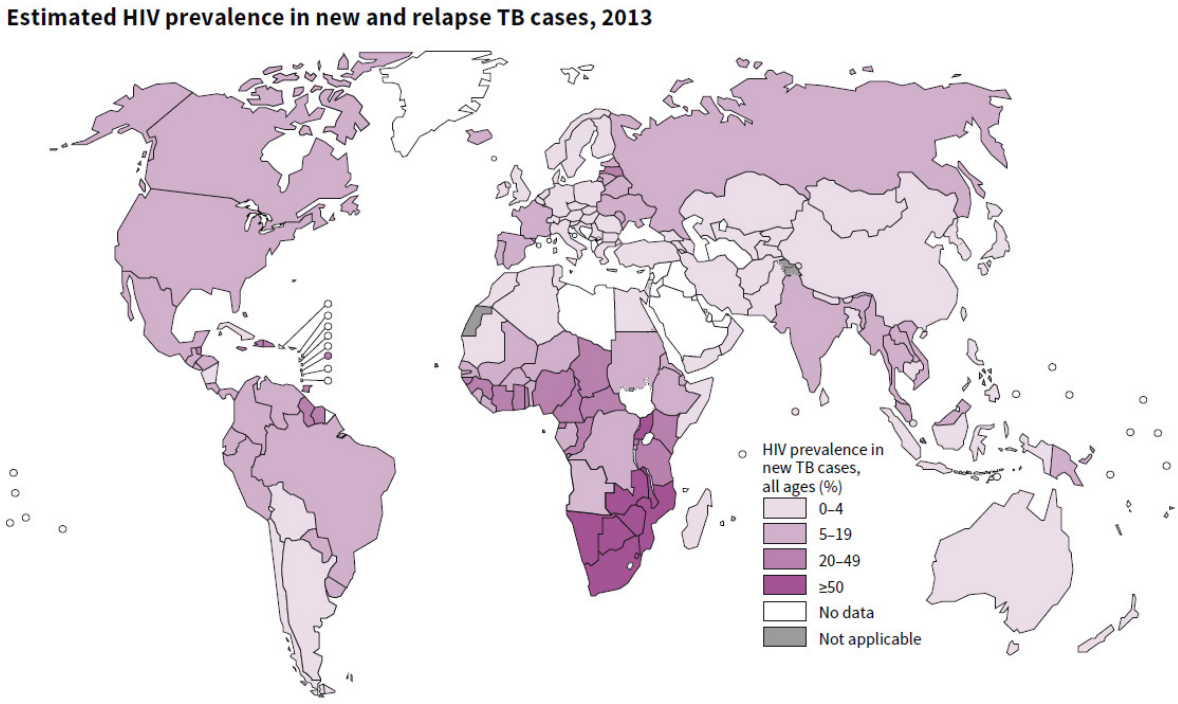

Figure 1: Estimated HIV prevalence in new and relapse TB cases from the 2014 Global TB report [4].

1.1.2 The traditional barriers to HIV and TB treatment integration

Strategies, policies and treatment guidelines to effectively integrate the management of both diseases are critical, but have been impeded by the lack of rigorous and consistent evidence for treatment integration early on in the HIV epidemic [6].

Barriers to integrated HIV and TB treatment have traditionally included a lack of rigorous empiric evidence on how to safely manage co-infected patients [7], including the lack of clear clinical guidance on immune reconstitution inflammatory syndrome (IRIS) detection and management; fear of potential drug-drug interactions; fear of additive drug toxicities and tolerability issues and the assumed adherence challenges associated with high pill burdens [8, 9]. Facilities to treat HIV and TB have also traditionally been separate, thus further complicating the integration of HIV and TB treatment.

Balancing the need for treatment (Figure 2) meant outweighing the risks associated with co-treatment versus the risks associated with delaying HIV treatment to after TB cure is achieved.

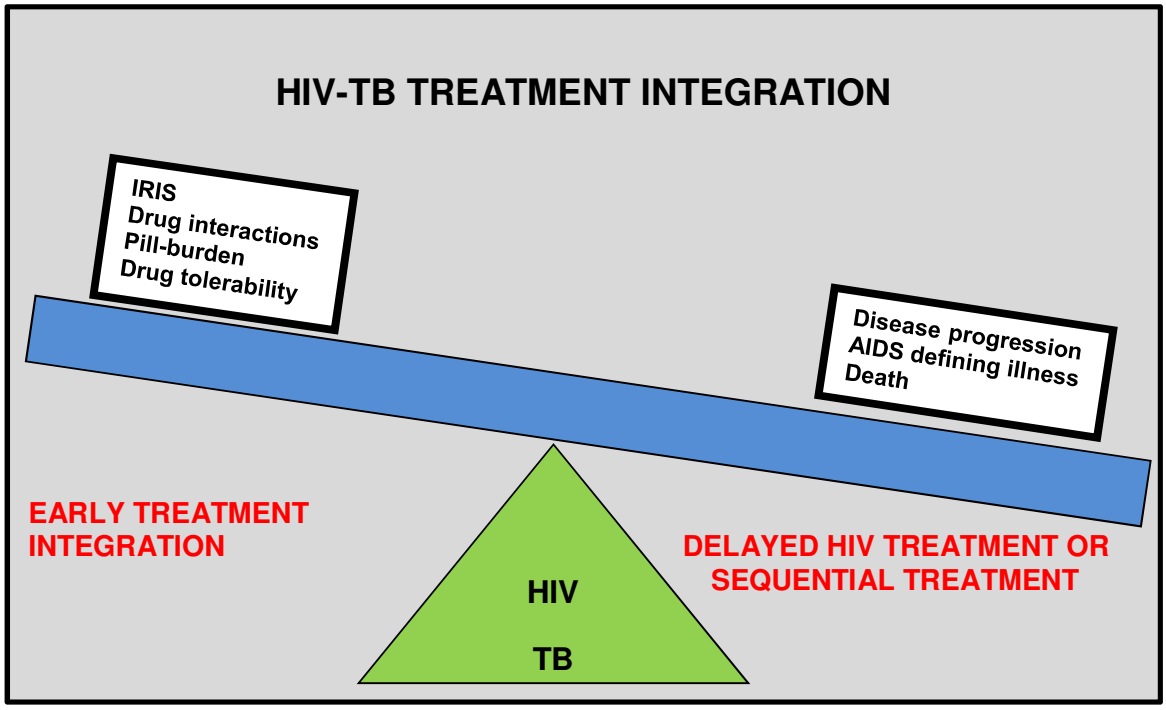

Figure 2: Balancing the scales in early vs delayed HIV-TB treatment integration

1.1.3 The need for HIV and TB treatment integration

Prior to 2010, due to lack of rigorous clinical trial evidence, it was not known whether TB and HIV treatment could be concurrent, how co-administration should be timed and whether it was safe to administer the treatments together. The indecision on how to treat TB and HIV co-infected individuals at a provider level was influenced by the barriers described above as well as the lack of firm guidance from global policies.

In 2010, results from the CAPRISA 003, 'Starting antiretrovirals at three time points in tuberculosis treatment' (SAPiT) trial provided the first clinical trial evidence on how best to treat TB-HIV co-infected individuals [10]. This study of 642 South African patients showed a 56% relative reduction in risk of death in patients who received integrated treatment compared to those who initiated their HIV treatment after completing TB treatment. The trial was halted early so that all patients could benefit from integrated treatment. These survival benefits rapidly contributed to informing TB-HIV co-treatment policy and practice [11].

Further data published from the SAPiT study in 2011 indicated that when deciding on the timing of integrated TB and HIV treatment, early initiation of ART (four weeks after the start of TB therapy) in patients with CD4 counts less than 50 mm^3 was associated with increased AIDS-free survival. While deferral of ART to the first four weeks of the continuation phase of TB treatment, in patients with higher CD4 counts, reduced the risk for IRIS and other adverse events without increasing the risk for HIV disease progression or death [12].

Other studies have also confirmed these findings. In the Cambodian early versus late introduction of antiretrovirals (CAMELIA) trial published in 2011, 661 Cambodian patients were randomized to either early treatment (2 weeks after beginning TB treatment) or later treatment (eight weeks after TB treatment start) with concomitant ART [13]. Mortality was significantly reduced in the earlier treatment group compared to the later treatment group but the risk of IRIS was reported to be higher. The authors concluded: "… initiating ART 2 weeks after the start of TB treatment significantly improved survival among HIV-infected adults with CD4+ T-cell counts of 200 mm^3 or lower" [13].

The multi-center, 'A strategy of immediate versus deferred initiation of antiretroviral therapy for AIDS disease-free survival (STRIDE) trial', conducted in 809 patients from various countries whose CD4 counts were less than 250 cells/mm^3, compared earlier ART (within

two weeks after TB treatment start) with later ART (between eight and 12 weeks after the initiation of TB treatment [14]. This study found that overall earlier ART did not decrease the risk of new AIDS defining illness, but in patients with very low CD4 counts of less that 50cells/mm^3 earlier ART was associated with lower rates of new AIDS defining illness and death [14].

With regards to non-IRIS related reports of grade 3 or 4 adverse events, two of these three major clinical trials showed similar rates between early and late treatment arms and the majority of patients tolerated co-treatment fairly well [14, 15]. In the CAMELIA trial, although serious adverse events were similar between treatment arms, hepatotoxicity accounted for 43% of all serious drug related toxic events and drug toxicity was found to be the second most common cause of death in this trial, after TB itself [13].

The latest UNAIDS report [2] shows that after 2010 (Figure 3), there was a concerted effort by the top 10 countries most affected by dual epidemics, to prioritize TB-HIV treatment integration with South Africa leading the way. Undoubtedly, the results of the three major clinical trials discussed have played a central role in informing policy and practice.

In summary, from these large clinical trials, we have learnt that the goals for combined treatment of HIV and TB are as follows:

1) To reduce morbidity and mortality associated with both diseases

2) Ensure safe timing of ART initiation after TB treatment is commenced. This is best guided by the immune status of the patient, using the CD4 cell count as a guide in order to reduce the risk of IRIS.

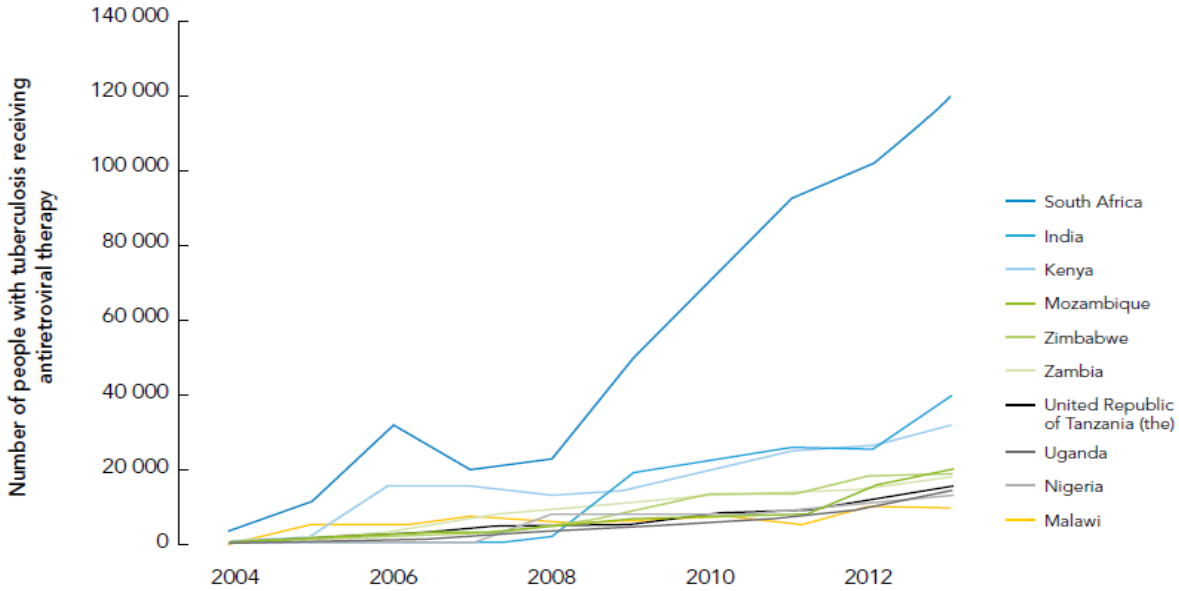

Figure 3:TB patients receiving antiretrovirals in the 10 countries representing more than 80% of TB-HIV co-infected patients [2]

1.1.4 Drug interactions between TB and HIV drugs

In South Africa, standard first line TB drugs used for treatment of uncomplicated pulmonary TB are dosed daily and are dependent on body weight [16]. Prior to 2008, TB treatment was dosed for five days of the week with drug breaks allowed on weekends [17]. The weekday only dosing strategy was standard of care when the data for the PhD study was collected.

The typical first-line TB treatment course comprises of a minimum of a two month intensive treatment phase containing a four drug regimen (RIF, isoniazid, ethambutol and pyrazinamide) followed by a minimum four month continuation treatment phase consisting of two drugs only, RIF and isoniazid [16].

RIF is a critical and potent component of first-line, multi-drug TB therapy because of its early sterilizing activity against *Mycobacterium TB* in the intensive phase of treatment and because of its sustained activity against persistent bacilli throughout the continuation phase of TB treatment [18, 19].

First line ART consists of a combination treatment of at least three drugs consisting usually of an appropriate non-nucleoside reverse transcriptase inhibitor (NNRTI) with two nucleoside reverse transcriptase inhibitors (NRTI). Second line treatment consists of a protease inhibitor backbone with appropriate NRTIs (Table 1), again comprising a minimum three drug combination [20].

Table 1: Approved antiretroviral agents registered for use in South Africa (2014)

Class	Drug
Nucleoside reverse transcriptase inhibitors (NRTI)	Lamivudine, stavudine, didanosine, emtricitabine, zidovudine, abacavir,
Nucleotide reverse transcriptase inhibitors (NtRTI)	Tenofovir
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Efavirenz, nevirapine, etravirine
Protease inhibitors (PI)	Lopinavir/ritonavir, ritonavir, indinavir, nelfinavir, saquinavir, fosamprenavir, atazanavir, darunavir
Integrase inhibitors	Raltegravir

Adapted from: South African Medicines Formulary, 2014 [21]

Typically, a drug interaction occurs when a substance (usually another drug) affects the activity of one drug when both drugs are administered together. The effect of co-administration could be synergistic (enhanced) or antagonistic (diminished), or a new effect could be produced that neither drug exhibits on its own. Drug interactions can also exist between drugs and certain foods or between drugs and plants or 'herbal medication' [22]. Following oral administration (Figure 4), drug interactions may be mediated at four stages of drug disposition. The most significant factors affecting the co-administration of antiretroviral (ARV) and TB drugs include: malabsorption due to disease, drug-drug interactions including

competition between two drugs for protein binding site and changes in hepatic elimination that are drug-induced [23, 24].

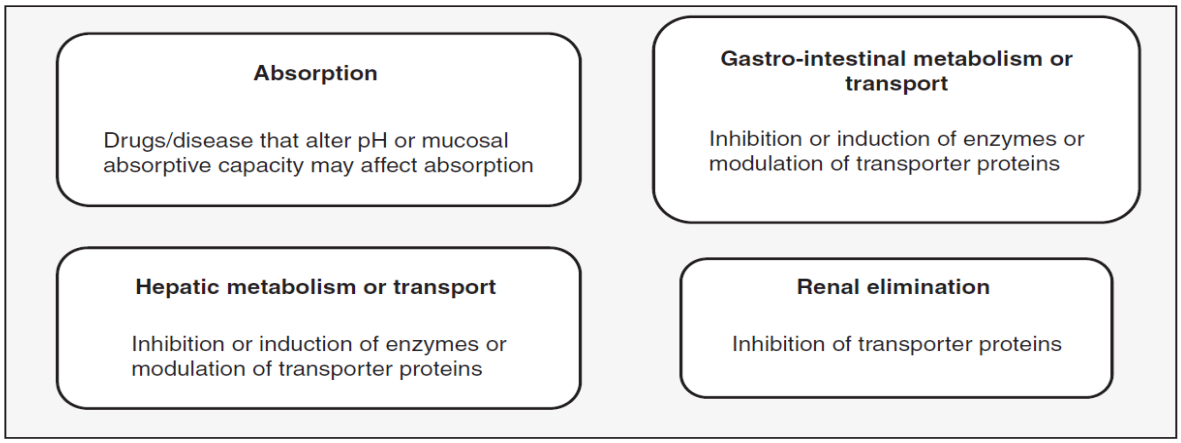

Figure 4: Drug interactions following oral administration may be mediated at four stages of drug disposition (from Gengiah et al. [24] Paper I)

Malabsorption of TB drugs has been shown to occur in patients with advanced HIV disease when the gut integrity is compromised [25-28]. This may have a potentially detrimental impact on TB treatment outcomes due to lower drug exposure and is an important factor to consider when co-treating patients. However, drug-drug interactions that influence gastrointestinal and hepatic metabolism of TB and HIV drugs are most likely to result in clinically significant interactions [29-31].

The rifamycin class of TB antimicrobials consist of rifampicin (RIF), rifabutin (RFB) and rifapentine (RFP).The main drug-drug interactions expected between TB treatment and ARVs are most frequently related to alterations in hepatic elimination involving the rifamycin class of anti-TB drugs with the NNRTI and the PI classes of ARV drugs [30, 32].

TB-HIV drug interactions occur due to substrate activity and either inhibition or induction of the hepatic cytochrome (CYP450) monooxygenase enzyme system, by either class of drug, resulting in changes in metabolism of one or both interacting drugs [30]. The CYP450 isoforms

that appear most frequently associated with TB-HIV drug interactions are CYP-3A4, 2B6, 2C19, 1A2 and 2D6 [23, 30, 33], (Figure 5). Polymorphisms of these enzymes affect the functional ability of these enzymes to metabolize drugs optimally [31]. Additionally, modulation of the P-glycoprotein cellular transport system in the intestinal mucosa can also increase the efflux of drugs from cells and reduce optimal absorption and plasma exposure of affected drugs [30].

The resultant effect following hepatic- or transporter-mediated pharmacokinetic interactions may impact the treatment outcome in two ways, depending on the potency of the effect: sub therapeutic concentrations may result in treatment failure and higher concentrations may be associated with treatment-limiting toxicity [30, 34].

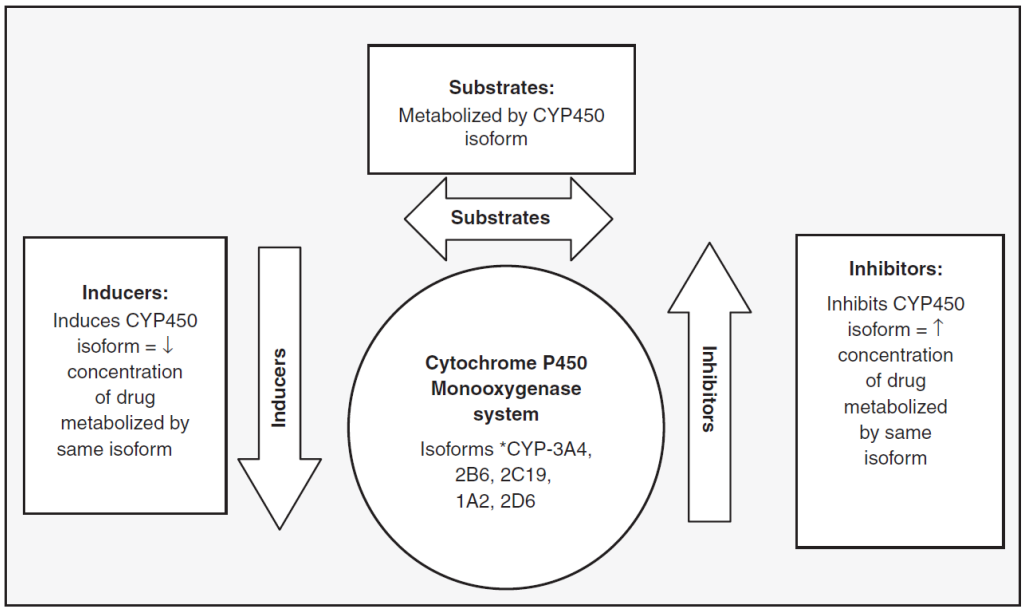

Figure 5: Possible metabolic drug interaction and the CYP450 system (from Gengiah et al [24] Paper I)

RIF is an inducer of both CYP 450 enzymes and P-glycoprotein [30, 35-37], and is known to potently induce CYP3A while it is a strong inducer of CYP2B6 [38]. The relative extent of CYP3A induction is: RIF > rifapentine >rifabutin [38] but RIF's concentration is not influenced by CYP3A induction. RIF is also the most commonly available rifamycin and is used

throughout the course of first-line TB treatment. Therefore, the 'RIF effect' on concomitant ARV drugs needs to be anticipated and managed for the entire six to nine months of the TB treatment course.

Both efavirenz (EFV) and nevirapine (NVP) are metabolized by the CYP450 enzyme system. The CYP2B6 isoform is primarily responsible for EFV metabolism and the CY3A4 isoform is primarily responsible for NVP metabolism and to a less significant extent for EFV metabolism [33]. Both EFV and NVP also have the ability to induce the enzymes that are responsible for their own metabolism and may increase the clearance of co-administered drugs that share these metabolic pathways [33].

The other first–line TB drugs, ethambutol and pyrazinamide, although metabolized in the liver, do not significantly influence the CYP450 monooxygenase enzyme system in humans [39], although conversely, isoniazid is known to competitively inhibit CYP2C19 and CYP3A [40].

Protease inhibitors (PIs) are also associated with many clinically relevant drug interactions [30]. PIs are mostly substrates of CYP3A4 and P-glycoprotein, with the exception of nelfinavir which is metabolized by CYP2C19 [29]. Ritonavir (RTV) has the ability to potently inhibit CP3A4 and P-glycoprotein efflux pumps and this property has been used to therapeutic advantage in combination with other PIs. These combinations of low-dose RTV and PIs, commonly referred to as boosted PIs, show enhanced activity by virtue of increased plasma concentrations and increased likelihood of viral suppression [41].

Co-administering un-boosted PIs with RIF have been shown to result in a greater than 90% reduction in PI trough concentrations [42, 43]. Boosting with low-dose ritonavir (RTV) may not be sufficient to overcome the RIF effect [44-46] and higher doses of RTV (super-boosted PIs) may need to be considered [45].

In the South African TB-HIV context, the majority of patients requiring co-treatment would receive a RIF-based first line TB treatment regimen and an NNRTI containing first line ART regimen, with EFV being the preferred NNRTI to use during TB co-treatment [47-49]. Drug interactions between these drugs would therefore have the highest public health impact and are thus the focus of further study.

1.1.5 Focusing on the RIF-EFV drug-drug interaction

EFV was first registered by the FDA in 1998 for use in the treatment of HIV [50] and there has subsequently been extensive clinical experience with this popular NNRTI. Study of population PK based on modelling 16 phase I studies in 2002 predicted a lower clearance in Asian and Black patients relative to Caucasians and identified interactions with several drugs including fluconazole, ritonavir, azithromycin, indinavir and RIF [51].

EFV has been shown to have high inter-patient and low intra-patient variability in PK parameters, with a suspected inverse correlation of concentrations with viral load and a direct correlation with concentration and toxicity, making it a drug with required characteristics for therapeutic drug monitoring (TDM) [52].

In subsequent clinical studies, hepatic clearance of EFV appears to be 28% higher in white non-Hispanics than in African Americans. Being Hispanic and having higher adherence was associated with increased EFV exposure, however, if previously NNRTI exposed, then increases in EFV clearance were associated with virological failure [53]. Another trial has shown that Asian patients exhibit lower EFV clearance compared to South African, South American and other Western country patients [54].

As reported previously, RIF up-regulates cytochrome P450 (CYP450) drug metabolizing enzymes resulting in decreased exposure to concomitantly administered drugs that utilize

similar metabolic pathways [23, 37, 55]. The often cited study by Marzolini et al [56] demonstrated that steady state/trough EFV concentrations below 1.0 µg/ml are associated with virological failure and those above 4.0 µg/ml are associated with central nervous system (CNS) toxicity. The goal therefore during EFV co-treatment with RIF is to maintain concentrations within therapeutic range of 1.0-4.0 µg/ml to reduce the risk of virological failure with possible emergence of drug resistance and to monitor for treatment limiting toxicity when the therapeutic range is exceeded.

The first available data demonstrating a deleterious effect of RIF on EFV was published in 2002 by Lopez-Cortez et al. in a small group of Spanish patients (Table 2) [57]. Pharmacokinetic (PK) interactions and adverse event reports for studies from a variety of populations, that have monitored combined EFV and RIF treatment since 2002, are summarised in Table 2. Body weight and racial differences are demonstrated as important determinants of EFV disposition [57-59]. In addition, there is high inter-patient variability in EFV PK parameters, especially in the presence of TB treatment [49, 58, 60]. The variability in patients' EFV exposure is also unusual. Several studies have demonstrated that patients of similar weight and ethnicity to their cohort present with extremely high EFV concentrations, which could be up to five times the upper limit of the therapeutic range [60-63].

Only one other South African study assessed the EFV 800mg dose in Black, African patients [64]. This study found that EFV concentrations were significantly increased in the higher dose and no significant deleterious effect of RIF on EFV 600mg concentrations were observed when regimens were co-administered.

Table 2: Pharmacokinetic interactions between EFV and RIF in TB-HIV co- infected adult patients

Study	Race (N)	EFV dose On TB Rx	Median EFV conc on RIF	Median EFV conc off RIF	Key Pharmacokinetic effect	Adverse event reports
Lopez-Cortes et al, 2002 [57] (Spain)	W (24)	600mg (n=8) 800mg (n=8) 800mg (n=8)	NA	NA	18%↓ Mean trough concs 24%↓ Mean peak concs 10%↓ AUC	CNS toxicity (n=6), rash (n=2), transient raised ALT/AST (n=3)
Pedral-Sampaio, 2004 et al [65] (Brazil)	W(49)	600mg (49)	NA	NA	NA	CNS toxicity (n=7), rash (n=1), transient raised ALT/AST (n=4), IRIS (n=7)
Brennan-Benson et al, 2005 [61] (England)	W (1) AF(8)	800mg (n=8) 600mg (n=1)	11.68 µg/ml	NA	↑ Trough median concs 11.68 µg/ml (IQR: 5.37 -19.6 µg/ml)	CNS toxicity (n=6), transient raised ALT/AST (n=1)
Manosuthi et al, 2005, 2006 [66, 67] (Thailand)	As (84)	600mg (n=42) 800mg (n=42)	3.02 µg/ml 3.39 µg/ml	NA	EFV>4 µg/ml: 40%: 600mg grp 45%: 800mgg grp	CNS toxicity (n=1), rash (n=1), transient raised ALT/AST (n=1)
Lopez-Cortes et al, 2006 [68] (Spain)	W (80)	800mg (n=80)	1.39 µg/ml	EFV 600mg: 1.28 µg/ml	EFV 800mg +RIF concs similar to EFV 600mg –RIF	CNS toxicity (n=16), rash(n=6), transient raised ALT/AST (n=5)
Friedland et al, 2006 [60] (South Africa)	AF(20)	600mg (n=20)	1.73 µg/ml	1.38 µg/ml	EFV concs inter-subject variability: On RIF: CV 157%, Off RIF: CV 58%	CNS toxicity (n=7), hepatotoxicity: (n=1)
Matteelli et al, 2007 [58] (Italy)	W (n=29)	800mg (n=16) Controls (n=13): EFV 600mg no RIF	1.5 µg/ml	1.6 µg/ml	Mean EFV CL/F/Kg: EFV 800mg: 0.269 L/h/kg Controls: 0.167 L/h/kg EFV conc inter-subject variability: On RIF: CV 93%, Off RIF: CV 62%	CNS toxicity (n=2)

Study	Race (N)	EFV dose On TB Rx	Median EFV conc on RIF	Median EFV conc off RIF	Key Pharmacokinetic effect	Adverse event reports
Sathia et al, 2008 [63] (India)	As (n=20)	600mg(n=3), if <50kg 800mg (n=7), if ≥50 kg	1.77 µg/ml	2.19 µg/ml	50%: had therapeutic EFV conc when on RIF 30%: were over therapeutic range on RIF	NA
Stohr et al, 2008 [59] (England)	W (n=225) AF (n=114)	On RIF (n=56) 800mg (n=48) 600mg (n=8) No TB RX (n=272)	NA	NA	On RIF: ↓ EFV conc by 35%	NA
Manosuthi et al 2009 [49] (Thailand)	As (n=121)	600mg (n=121)	3.54 µg/ml	NA	EFV conc inter-subject variability: On RIF: CV 107% 4.3%: had below -therapeutic EFV conc when on RIF	Rash: (n=3), hepatotoxicity: (n=1)
Orrell et al, 2009 [64] (South Africa)	AF (n=72)	600mg (n=34) 800mg (n=38)	2.4 µg/ml 2.9 µg/ml	2.2 µg/ml	EFV 800mg: 3% had below -therapeutic EFV concs when on RIF EFV 600mg: 12% had below -therapeutic EFV concs when on RIF	Hepatotoxicity: (n=2)
Leutkemeyer et al, 2013 [69] (Multi-country)	W (n=26) AF (n=403) His (n=108) As (n=5)	600mg (n=780)	1.96 µg/ml Blacks: 2.08 µg/ml	1.80 µg/ml Blacks: 1.75 µg/ml	Weights <60Kg associated with higher EFV Cmin: 2.02 µg/ml ≥60kg: 1.68 µg/ml	CNS toxicity (n=46)
Borand, et al 2014 [62] (Cambodia)	As (n=540)	600mg (n=540)	2.79 µg/ml	2.77 µg/ml	45% had EFV concs in therapeutic range 3.3% had below -therapeutic EFV concs when on RIF	CNS toxicity (n=23), hepatotoxicity: (n=47)

Key: As: Asian, AfM: African American, AF=Black African, His: Hispanic, W: White, NA: not available, Conc: concentration, CL: Clearance, CL/F: Apparent clearance, CV: coefficient of variation, AUC: Area under the concentration-time curve over the administration interval, grp: group

Black, African patients in particular appear to have higher EFV exposure when on TB treatment and patients with a higher weight are more likely to have lower EFV concentrations when on EFV 600mg [69]. In the analysis, by Stohr et al [59], Black patients, many receiving EFV 800mg with RIF, had 48% higher EFV concentrations. In this study, EFV concentrations were found to be 59% higher in Blacks than in Whites and 52% higher with EFV 800mg.The probability of having an EFV trough concentration less than 1 µg/ml whilst on RIF-based TB treatment was doubled in White patients compared with Black patients (50% vs 23% respectively). In White patients, increasing the dose to 800mg resulted in an improved probability, from 48% to 64%, of attaining therapeutic concentrations, but in Blacks this only increased the probability of being in the toxic range [59].

The most notable side effects associated with EFV administration are CNS symptoms, rash and transient elevations of liver transaminases. CNS symptoms included: dizziness, impaired concentration, somnolence, abnormal dreams, and insomnia [70].

Symptoms usually begin during the first or second day of therapy and generally resolve after the first 2 to 4 weeks of therapy [70]. Potential for additive symptoms may occur if used concomitantly with alcohol or psychoactive drugs. In clinical trials, 2.1% of EFV-treated patients discontinued therapy because of nervous system symptoms [71]. In summary, even if high concentrations are achieved during dose modification, EFV is well tolerated by the majority of patients.

From the data presented in Table 2, it remains uncertain whether the EFV dose should be increased to 800mg when RIF is present. Regardless, in 2012 the US FDA recommended EFV dose increase to 800mg in all patients weighing over 50Kg when on concomitant RIF-based TB treatment [72]. The evidence for associating lower EFV concentrations with virological failure has been inconsistent [56, 68, 73], making blanket dose modification at

higher weights during TB co-treatment, as recommended by the US FDA, highly questionable. When the standard EFV 600mg dose was used and compared to the EFV 800mg dose, no appreciable decrease in EFV concentrations was reported with the standard dose in South African patients [64] or with Asian patients [66]. Further indirect support for the standard 600mg dose in the presence of RIF arises from successful virological and microbiological outcomes during co-treatment which have been reported consistently in several studies [60, 62, 67, 69].

To better understand the EFV-RIF interaction it is clear that differences in metabolism by race and the inter-patient variability induced in the presence of TB treatment needs to be explored further. For both EFV and RIF, pharmacogenetic variation in metabolism and uptake potentially accounts for the variability in concentrations and racial differences in drug disposition. Understanding pharmacogenetic variation is therefore critical to predicting drug concentrations.

1.1.6 Pharmacogenetic considerations for RIF and EFV

RIF hepatocellular uptake is mediated by an organic anion-transporter polypeptide 1B1 (OAT1B1) coded for by the gene SLCO1B1 [74]. RIF metabolism is mainly hepatic, with up to 24% and 50% of drug excreted in the urine and bile unchanged respectively [38]. Polymorphisms in the SLCO1B1 gene can influence RIF PK and has been implicated in low RIF exposure in some studies [75, 76]. Anti-TB activity and development of resistance can be correlated with RIF concentrations [77, 78]. Peak RIF concentrations of 8 to 24 µg /mL, attained approximately 2-3 hours after ingestion, are associated with optimal bactericidal killing and the desired post antibiotic effect [79].

EFV is metabolized to 8-hydroxyefavirenz predominately by CYP2B6 and, to a lesser extent, by CYP3A4 [33, 80]. Subsequent to hydroxylation, UGT2B7 is directly involved with

glucuronidation of EFV and its hydroxylated metabolite [81]. In addition, *in vivo* evidence has emerged that CYP2A6 also plays a role in EFV metabolism, particularly in Black patients [82, 83] and this is supported by *in vitro* data which suggest the CYP2A6 accounts for 22.5% of EFV metabolism [84]. Therefore, when CYP2B6 metabolism is impaired, it could be expected that genetic variations affecting secondary metabolic pathways would become significant for EFV metabolism.

Table 3 summarizes 33 studies conducted in HIV infected adult patients worldwide, where single nucleotide polymorphisms (SNPs) CYP2B6 516 G→T or 785A→G or 983T→C were genotyped, and are associated with EFV PK parameters. In studies where the pharmacogenetic variation could be assessed, only 11 were in TB co-infected patients on RIF (shaded grey in Table 3) and none were on the higher EFV 800mg dose. There is a paucity of data in Black, South African adult patients. South African patients are investigated in two of the 33 studies and if the two Zimbabwean studies are included to comprise the Southern African region, then four of the 33 studies would have patients similar to those in the PhD study.

The CYP2B6 516G→T and 785 A→G polymorphisms comprising the CYP2B6*6 haplotype is associated with reduced function and expression of CYP2B6 [85]. These polymorphisms can be expressed at a frequency of up to 50% [86-89] in African patients, and has been associated with higher plasma EFV exposure [90-93]. Exposure to high EFV concentrations is most likely in homozygous mutant carriers (516 TT), followed by the heterozygous 516 GT carriers [91, 93-103]. In a South African study, the 516 G→T polymorphisms are predictive of EFV concentrations >4µg/mL [104]. The 785 A→G SNP is in linkage disequilibrium with 516G→T and is associated with similar EFV concentrations to those demonstrated with 516G→T mutation [87, 105]

Table 3: Single nucleotide polymorphisms of CYP2B6 516G→T, 783A→G, 983T→C and their influence on EFV concentrations/Pharmacokinetic parameters in HIV infected adult patients*

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Haas et al, 2004 [96] (United states, Puerto Rico)	Multi-race (N=154) AfAm (n=50) W (n=89) His (n=15)	AfAm MAF 38%: GG 44% GT 36% TT 20%	NA	NA	NA	EFV AUC overall: 516GG 44 ug.h/ml, 516GT 60 ug.h/ml, 516TT 130 ug.h/ml. 516 TT higher EFV exposure and more common in AfAm (20%) than in W (3.4%)
Tsuchiya et al, 2004 [106] (Japan)	As (n=60) On EFV (n=35)	MAF17.5% GG 46.7% GT 21.7% TT 3.3%	8.3 % AA 8.3% AG 3.3% GG 1.7%	NA	NA	Mean EFV conc: 516GG 8.0 ug/mL, 516GT 9.9 ug/mL, 516 TT 25.4 ug/mL. EFV concs ↑ in CYP2B6*6 genotype (homozygous mutants)
Rodriguez-Novoa et al, 2004 [107] (Spain)	W (n=100)	MAF 26.5% GG 52% GT 43% TT 5%	NA	NA	NA	Median EFV conc: 516GG 1.7 ug/mL, 516GT 2.6 ug/mL, 516TT 3.57 ug/mL. ↑ EFV concs in hom (40%) and het (19%) carriers but WT (20%) had sub-therapeutic concs
Haas et al, 2005 [108] (Unites States, Italy)	Multi-race N=367 AfAm (n=113) His (n=70) W (n=184)	AfAm MAF 31.3% GG 46.3% GT 41.5% TT 12.2% His: 34.9%: W: 24.4%	NA	NA	NA	EFV AUC overall: 516GG 49.4 ug.h/ml, 516GT 57.9 ug.h/ml, 516TT 101.4 ug.h/ml. Similar trends for EFV exposure across race groups. Plasma exposure significantly associated with 516GT genotype.
Rotger et al, 2005 [109] (Switzerland)	N= 167 Race not defined	TT 26% Others not specified	NA	NA	NA	Mean EFV AUC for TT was 3 fold higher than GG. 516TT associated ↑ plasma and ↑ intracellular EFV exposure. CYP2B6 genotype predictive of neuropsychiatric toxicity.
Wang et al, 2006 [92] (Sweden)	N=51 Multi- race W and AF	Frequency not specified	Frequency not specified	Frequency not specified	NA	983TC linked with 785AG as novel haplotype CYP2B6*16. Common amongst Africans, steady state EFV concs ↑ in CYP2B6*16 carriers and in 516GT.
Ribaudo et al , 2006 [110] (Unites States, Puerto Rico)	152 Mixed race 32% AfAm 57% W 10% His	MAF: 29% GG 51% GT 39% TT 9% AfAm, TT 10%, 3% in W and 1% in His	NA	NA	NA	EFV $t_{1/2}$: 23, 27 and 48h for GG, GT, TT and would exceed predicted $IC_{95\%}$ for 5.8, 7 and 14 days respectively. In 29% of TT patients this could be >21 days.

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Gatanaga et al, 2007 [111] (Japan)	N=456 As (n= 450) Hs (n=2) W (n=2)	MAF 17.9% GG 46.3 % GT 22.8% TT 4.2%	MAF 7.8% AA 46.3% AG 22.8% GG 4.2%	MAF 0%	NA	CYP2B6*6/*6 carriers had extremely high EFV concs > 6000 ng/mL Genotype based dose reduction was feasible, dose dropped to 400mg in 11 patients and to 200mg in 7 patients. CNS symptoms improved in 11/14 patients.
Rotger et al, 2007[99] (Switzerland)	N=169 Multi- race W (n=141) AfAm (n=16) His (n= 7) As (n=5)	AfAm MAF 37.5% GG 31.2 % GT 62.5% TT 6.2 % As MAF 60%: GG 20% GT 40% TT 40% His MAF 21.4% GG 57.1% GT 42.9%	AfAm MAF 37.5% AA 31.25 % AG 62.5% GG 6.25% As MAF 60% AA 20% AG 40% GG 40% His 64.5 % AA 28.6% AG 71.4%	AfAm MAF 9% TT 81.25 % TC 18.75% CC 0% As 0% His 0 %	NA	EFV AUC :516 GG 44.5ugh/ml, 516GT 58.9 ugh/ml, 516TT 186.4 ugh/ml Poor metabolizer genotypes like CYP2B6*6,*18 explain ↑EFV exposure and identify individuals at risk for extremely elevated concs.
Mehlotra et al, 2007 [112] (Papua New Guinea, West Africa, United States)	N=705 PNG (n= 174 AF (n=170 NAm: (AfAm, W, His, n=361)	NA	NA	MAF: PNG=0 A=4.7% AfAm =7.5% His=1.1% W=0	NA	Africans carry the highest frequency of *16 or *18 alleles and are potentially at highest risk for adverse events from EFV but no EFV concs available.
Nyakutira et al, 2008 [93] (Zimbabwe)	AF (n=74)	MAF: 49% GG 30 % GT 44% TT 27 %	NA	NA	NA	EFV CL/F: 516GG =9.4L/h, 516GT =7.2L/h. 516TT =4.0L/h Women had higher EFV concs. EFV was >4mg/L in over 50% of patients. Simulations indicate that 35% EFV dose ↓ would maintain sufficient drug exposure. The 516TT grp could effectively receive EFV 400mg.
Kwara et al, 2008 [101] (Ghana)	AF (n=26)	MAF: 48% GG 27 % GT 50% TT 23%	AA + TT=17% (*1) AA + TC=7% (*1/*18) AG+TT=3% (*1/*4) AG+TT=43% (*1/*6) AA+TT=3% (*1/*9) AG+TC=3% (*1/*16 OR*1/*18) GG+TT=23% (*6/*6)		Yes (n=26)	EFV CL/F: 516GG =9.9L/h, 516GT =8.4L/h, 516TT =2.1L/h Median EFV Cmin ug/mL: 516GG 0.6 ug/mL, 516GT 0.5 ug/mL, 516TT 2.8 ug/mL. Variability in total EFV exposure was 110% and assoc with 516GT genotype. Inclusion of 785AG and 983TC did not improve the prediction of the EFV slow metaboliser genotype. Effect of RIF: does not reverse poor metaboliser genotype effect but effect may differ dependent on CYP2B6 genotype.

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Wyen et al, 2008 [113] (Germany)	W (n=225) AF(n=146)	W MAF 29% GG 51.3% GT 38.9% TT 9.7% AF MAF 34% GG 47.4% GT 40.4% TT 12.3%	NA	W MAF 0% AF MAF 9% TT 86% TC 10.5% CC 3.5%	NA	Overall EFV concs: 516GG 1.78 ug/mL, 516GT 2.3 ug/mL, 516TT 6.2 ug/mL 983TT 2.1 ug/mL, 983TC 2.1 ug/mL, 983CC 2.3 ug/mL 2 Black patients with 983CC withdrawn from EFV due to toxicity EFV concs sig associated with 516GT and 983TC
Ramachandra et al, 2009 [94] (India)	As (n=72)	(n= 25) MAF 44% GG 40% GT 32% TT 28%	NA	NA	Yes (n=57)	EFV CL/F: EFV only 7.35 L/h, EFV + RIF 9.27 l/h EFV C_{min}: EFV only 5.8 ug/mL, EFV+RIF 4.65 ug/mL 516GT SNP and not RIF influences EFV steady state PK TT had very high EFV concs.
Kwara et al, 2009 [82] (Ghana)	AF (n=65)	MAF 45% GG 30% GT 51% TT 19%	NA	MAF 4% TT 91% TC 9% CC 0%	Yes (46%)	Median EFV concs: 516 GG 1.3 ug/mL, 516GT 1.6 ug/mL, 516TT 8.3 ug/mL, 983 TT 1.6 ug/mL , 983TC 1.8 ug/mL CYP2B6 516TT and 516GT account for 24 and 12% total variance in EFV conc. No association between EFV concs and age, weight, gender, BMI, alcohol use or RIF containing TB therapy
Cohen et al, 2009 [95] (South Africa)	AF (n=142) 84% Black 15% Coloured	(n=122) MAF 32% GG 49% GT 38% TT 13%	NA	NA	Yes (n=40)	Median EFV concs: EFV + RIF 2.4 ug/mL, EFV alone 1.8 ug/mL Paired EFV concs in n=17, similar on and off TB treatment 516 GT strongly associated with EFV >4ug/mL: OR: 4.4 GT vs GG, OR: 31.1 TT vs GG High EFV concs associated with severe sleep disturbances Low EFV concs <1 ug/mL were associated with virological failure (OR: 12.6)
Leger et al, 2009 [105] (Haiti)	AF (n=45)	MAF 44%	MAF 42%	MAF 3%	NA	EFV median concs: 516GG 2.1 ug/mL, 516GT 3.8 ug/mL, 516TT 8.2 ug/mL, 785AA 2.2 ug/mL, 785AG 4.0 ug/mL, 785GG 8.2 ug/mL, 983CT 6.4 ug/mL, 983TT 3.2 ug/mL. Composite genotype 516/983 Slow metabolizers: conc 8228 ng/mL. CYP2B6 516GT associated with high EFV concs
Uttayamakul et al, 2010 [100] (Thailand)	As (n=124) (n=65 on EFV)	MAF: 38% GG 38.46% GT 47.69% TT 13.85%	NA	NA	Yes (n=124)	Mean EFV C_{12Hr} at weeks 6 and 12 EFV +RIF: 516 GG 2.88 and 2.45 ug/mL, 516GT 3.43 and 3.35 mg/L, 516TT 10.97 and 13.62 ug/mL. EFV alone: 516GG 2.08 mg/L, 516GT 3.21 mg/L, 516TT 8.48 mg/L. The 516TT genotype impacts on EFV concentrations and RIF co-administration only has small effects Higher % of 516TT achieved undetectable viral load than 516GG or 516GT genotypes.

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Ribaudo et al, 2010 [114] (United States)	n=831 48% W 34% AfAm 18% His	MAF W 24.5% AfAm 34.2% His 32.2%	MAF W 27.3% AfAm 34.8% His 34.7%	MAF W 0.2% AfAm 7.5% His 1.4%	NA	CYP2B 516 and 983 genotypes best predicted EFV PK but not seen in W and His patients.
Elens, et al, 2010 [115] (Brussels)	N=50 AF(n-14) W (n=31) As (n=5)	MAF 34% GG 38% GT 56% TT 6%	NA	MAF 2% TT=96% TC=4% CC=0	NA	EFV C_{min}: 983 TT 1.9 ug/mL, 983TC 12.3 ug/mL, 516GG 1.3 ug/mL , 516GT 2.6 ug/mL, 516 TT 5.5 ug/mL Always concs higher when mutations present in 983TC and 516 GT.
Kwara et al, 2011 [116] (Ghana)	AF(n =56)	MAF 40% GG 20 (35%) GT 27 (48%) TT 9 (16%)	NA	NA	Yes (n=18)	516 TT :EFV +RIF: 14.7 ug/mL, EFV alone: 6.0 ug/mL 516GT: EFV +RIF: 1.9 ug/mL, EFV alone: 1.8 ug/mL, 516GG: EFV +RIF: 1.3 ug/mL, EFV alone: 1.6 ug/mL On TB treatment, EFV concs are higher. Postulated ↑ susceptibility of CYP2B6 to inhibition by one of the anti-TB drugs or possible inhibition of CYP 2A6 by other TB drugs.
Ngaimisi et al, 2011 [103] (Tanzania)	AF (n=182)	EFV+ RIF (n=54) GG 37.5% GT 45.8% TT 16.7% EFV alone (n=128) GG 37.5% GT 47.7% TT 13.6%	NA	NA	Yes (n=54)	Effect of RIF on long term EFV auto induction is CYP2B6*1/*1 genotype dependent. EFV metabolizing enzymes are induced to maximum extent in the first 8 weeks of RIF use. Effect of RIF on EFV PK is only apparent during early stages, but has no significant long term effect.
Yimer et al, 2011 [117] (Ethiopia)	AF (n=201) DILI cases (n=41) DILI controls (n=160)	Cases GG 38.7% GT 46.8% TT 14.5% Controls GG 49.6% GT 41.0% TT9.4%	NA	NA	Yes (n=201)	Log EFV conc: DILI yes: 3.42 ug/mL, DILI no: 3.12 ug/mL DILI associated with higher EFV plasma concs
Maimbo et al, 2012 [87] (Zimbabwe)	AF (n=49)	42% GG (n=15) GT (n=27) TT (n=7)	42% AA (n=15) AG (n=27) GG (n=7)	9% TT (n= 49) TC (n=9) CC (n=0)	NA	516 GT and 785AG are in LD and assoc with EFV concs 983TC patients had fourfold higher EFV concs that wild type. CYP2B6 *6 and *18 alleles affect hepatic metabolic activity and increase systemic EFV concs

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Holzinger et al, 2012 [118] (United States)	N=856 50% W 33% Black 18% His	NA	NA	NA	NA	Median Cmin: 516TT = 3.98ug/mL (5.4 x higher than wild types 516GG, 983TT). 516GT and 983TC 5.38 ug/mL (7 .1 x higher than wild types, 516GG, 983 TT). Three polymorphisms explained 34% of inter-individual variability in EFV Cmin – 516 GT, 983TC and rs4803419
Gandhi et al, 2012 [119] (Unites States)	N=111 8% W 13% His 78% AfAm	NA	NA	NA	NA	Short term exposure AUC fold increase: 516 TT: 3.5, 983TC: 1.96. Long term exposure: 516TT:3.2, 983TC:1.96 SNPS in CYP2B6 516TT shows 3.5 fold increase on both short and long term EFV exposure – using concs in plasma and hair. Other factors that increase EFV AUC were oranges/orange juice. If ALT doubled then EFV AUC increased 1.26 fold
Mukonzo et al, 2013 [90] (Uganda)	AF (n=197)	No CNS: GG 42% GT 56% TT 2%. CNS: GG 44.5% GT 46% TT 9.5%	NA	NA	Yes (n=138)	Day 3 Median: EFV 2.48 ug/mL, EFV +RIF 1.85 ug/mL Week12 Median: EFV 2.41 ug/mL, EFV +RIF 2.04 ug/mL RIF reduced EFV concs only in week 1. CYP2B6*6 predicts EFV concs and CNS symptoms, not RIF based TB treatment
Manosuthi et al, 2013 [98] (Thailand)	As (N=138)	MAF 31.7% GG 45% GT 47% TT 8%	MAF 37.4% AA 36% AG 54% GG 10%	NA	Yes (n=101)	Median EFV: All 2.3 ug/mL, EFV + RIF: 2.1 ug/mL, EFV only: 2.7 ug/mL. 516TT, 785GG –mean EFV >7 ug/mL Low EFV conc assoc with *1/*1 haplotype and high body weight. *1/*6, *6/*6 assoc with high EFV concs. RIF has a small impact on EFV concs compared to pharmacogenetics.
Sukasem et al. 2013 [120] (Thailand)	As (n=100)	MAF 32%	MAF 36%	NA	NA	EFV concs: 516GG 1.57 ug/mL, 516GT 2.65 ug/mL, 516 TT 7.2 ug/mL
Ngaimisi et al, 2013 [88] (Ethiopia and Tanzania)	AF (n=495) Ethiopia: (n=286) Tanzania: (n=209)	Ethiopia MAF 31.4% GG 45.8% GT 45.5% TT 8.7% Tanzania MAF41.8% GG 35% GT 46.4% TT 18.6%	NA	NA	NA	EFV median concs at week 16: Ethiopia: 516GG 1.1 ug/mL, 516GT 1.4 ug/mL, 516TT 3.3 ug/mL. Tanzania: 516GG 1.2 ug/mL, 516GT 1.6 ug/mL, 516TT 3.4 ug/mL Differences in EFV PK by country, inter-ethnic variations Higher EFV concs in Tanzania even after CYP2B6 genotype

Study (N)	Race (n)	MINOR ALLELE FREQUENCY (MAF)			TB RX	Effect of SNP on EFV concentrations/pharmacokinetic parameter
		516G→T	783A→G	983T→C		
Swart et al, 2013 [104] (South Africa)	AF(n=295)	MAF 41.2%	MAF 41.2%	MAF 7%		CYP2B6 516G>T high specificity and PPV for EFV concs>4 ug/mL
Bertrand et al , 2013 [121] (Cambodia)	As (n=307)	MAF 32.2% GG 46.3% GT 42.9% TT 10.8%	NA	NA	Yes (n=307)	EFV alone CL/F: 516GG L/h=12.5, 516GT=8.8, 516TT=2.5 EFV plus TB treatment: CL/F L/h: 516GG= 11.2 NAT slow, 15.5 NAT rapid, 516GT=6.6 NAT slow, 9.9 NAT rapid, 516TT=2.1 NAT slow, 2.7 NAT rapid. NAT2 association: patients with the 516TT genotype and NAT2 poly had lowest EFV CL/F. Inducing effect of RIF is counterbalanced by a concentration dependent inhibitory effect of INH on EFV CL/F.
Sarfo et al, 2013 [122] (Ghana)	AF (n=521)	MAF 48% GG=29.5% GT=45.3% TT=25.1%	NA	MAF 4% TT=91.2% TC=8.7% CC=0.1%	NA	No differences in median EFV concs between males and females. 46% of concs were < 1.0 ug/mL and 10% above 4.0 ug/mL

Key: Rx: treatment, SNP: Single nucleotide polymorphism, As: Asian, AfAm: African American, AF=Black African, His: Hispanic, W: white, PNG: Papua New Guineans, Nam: North Americans, NA: not available, concs: concentration, Hom: homozygous mutants, Het: heterozygous mutants, WT: wild-type, OR: Odds ratio, LD: linkage disequilibrium, DILI: drug induced liver injury, PPV: positive predictive value. Grey shading depicts TB co-infection.

CYP2B6 983T→C, is a further mutation of interest as this SNP has also been shown to be associated with higher EFV exposure both in the presence [123, 124] and absence of TB treatment [87, 92, 115, 122]. Minor allele frequencies range from 7-18.7% [82, 99, 112, 122, 123]. Although this SNP is more common in Black Africans and rare in other race groups [99], variant allele homozygosity is uncommon and frequencies range between 0.1-3.5 %. Of interest is that patients with the homozygous mutant allele exhibit EFV concentrations in the toxic range [113] (Table 3).

Genome-wide sequencing conducted in 856 White, Hispanic and Black individuals found 983T→C and 516G→T to be two of three CYP2B6 variants independently associated with EFV C_{min} and also accounted for 34% of the inter-individual variability of estimated EFV C_{min} [118]. Combining these two SNPs to create composite genotypes, for example, incorporating 516GT and 983TC, have been previously associated with slow EFV metabolizer status and therefore higher EFV concentrations, in both HIV positive patients and healthy volunteers [123, 124].

Given the role of UGT2B7 in EFV metabolism, it is possible that SNPs in this gene may influence EFV concentrations. However, UGT2B7-372G→A has not been shown to be predictive of slow metabolizer status in other African cohorts [87, 88]. Studies on the usefulness of CYP2A6 for prediction of EFV exposure have produced inconsistent results and requires further study. One study of 65 African patients showed that carriers of polymorphisms account for approximately 12% of variance in EFV concentration [82] whilst others show no effect of SNPs in CYP2A6 on EFV concentrations [87, 90, 115].

Emerging evidence indicates that EFV exposure is potentially influenced by both genetic polymorphisms and TB co-treatment but reports in this regard are inconsistent. Some found that genetic polymorphisms are responsible for higher EFV exposure and not TB treatment

[82, 94, 95], whilst others show TB treatment to be contributory [98, 100] with most demonstrating evidence for a combined effect [97, 101, 121, 124].

1.2 Study rationale and objectives

Prior to 2010, the effectiveness and safety of integrating the management of drug-sensitive TB and HIV co-infection was uncertain with drug-drug interactions being one of several barriers to co-treatment. Once the survival benefits of co-treatment were established, potential drug interactions and their impact on co-treatment in our population remained unanswered research questions. Black South African patients are particularly vulnerable to TB-HIV co-infection and so would commonly receive drug regimens containing both EFV and RIF during dual treatment. However, at the time that the PhD study was designed, there were few studies describing the extent, clinical relevance and impact on patient safety of drug interactions. This led to the development of PhD study objective 1.

Prior to the PhD study, the evidence for the EFV-RIF interaction originated mainly from Caucasian and Asian patients (Table 2, Section 1.1). The general expectation was that RIF would induce CYP2B6 and lower EFV concentrations to sub-therapeutic levels during TB treatment and that a dose increase for EFV would be required. However, on closer examination, evidence of this effect was inconsistent and data from Black African patients was unavailable at the time. Hence, it remained uncertain whether the EFV dose should be increased to 800mg when RIF was present, justifying further examination of this drug interaction in our patient population. This led to the development of objectives 2 and 3. The high rates of multiple-drug resistant TB and evolving evidence for TB recurrence in HIV infected patients in South Africa formed the basis for investigating RIF concentrations. This was researched as objective 4.

Furthermore, studies showing genetic variation in drug metabolism as a significant predictor of drug exposure were also emerging. These data suggested that there may be racial

differences and led to the research questions incorporated in objectives 3 and 4, namely investigating the importance of pharmacogenetics on EFV and RIF handling.

All these research questions informed the PhD which was the PK study nested within the larger 'Starting Tuberculosis and Antiretroviral Therapy' (START) trial[1]. The PhD output aimed to complement and strengthen evidence-based recommendations for safe and effective TB-HIV treatment integration in Black, South African patients.

Accordingly the PhD study objectives were as follows:

***Objective 1*:** Assess drug safety issues that arise when combining first line HIV treatment with rifamycin-based TB treatment. This objective assessed the literature as of 2011. It identified the drugs associated with clinically relevant drug interactions and summarized guidance on dose modification during co-treatment available at that time. (Paper I, 2011)

***Objective 2*:** Develop a population pharmacokinetic non-linear mixed effects model (NONMEM) to quantify the impact of RIF based TB treatment on EFV clearance in a Black, South African population. (Paper II, 2012)

***Objective 3*:** Investigate, with the aid of trough EFV drug concentrations, the influence of pharmacogenetic polymorphisms and explore the necessity for EFV weight-based dose modification in a Black, South African population. (Paper III, 2014)

[1] More information on the START trial design can be found in Appendix B.

***Objective 4*:** Assess peak RIF concentrations achieved in a Black, South African population and investigate the presence and influence of pharmacogenetic polymorphisms in drug transporter proteins on RIF plasma exposure. (Paper IV, 2014)

Once achieved, each objective was submitted to an accredited journal as an individual publication, peer reviewed and published. These papers are presented in Chapter 2.

CHAPTER 2: PUBLICATIONS

PAPER I

Initiating antiretrovirals during tuberculosis treatment: a drug safety review

2.1 Paper I: Initiating antiretrovirals during tuberculosis treatment: a drug safety review

Expert Opinion

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healthcare

Initiating antiretrovirals during tuberculosis treatment: a drug safety review

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Introduction: Integrating HIV and tuberculosis (TB) treatment can reduce mortality substantially. Practical barriers to treatment integration still exist and include safety concerns related to concomitant drug use because of drug interactions and additive toxicities. Altered therapeutic concentrations may influence the chances of treatment success or toxicity.
Areas covered: The available data on drug-drug interactions between the rifamycin class of anti-mycobacterials and the non-nucleoside reverse transcriptase inhibitor and the protease inhibitor classes of antiretrovirals are discussed with recommendations for integrated use. Additive drug toxicities, the impact of immune reconstitution inflammatory syndrome (IRIS) and the latest data on survival benefits of integrating treatment are elucidated.
Expert opinion: Deferring treatment of HIV to avoid drug interactions with TB treatment or the occurrence of IRIS is not necessary. In the integrated management of TB-HIV co-infection, rational drug combinations aimed at reducing toxicities while effecting TB cure and suppressing HIV viral load are possible.

Keywords: drug interactions, HAART, rifamycins, safety HIV, toxicity, tuberculosis

Expert Opin. Drug Saf. (2011) 10(4):559-574

1. Introduction

In 2009, UNAIDS reported ~ 33.4 million people were infected with HIV globally. For the same year, there were 2.2. million new HIV infections and 2 million HIV-related deaths reported [1]. Developing countries, in particular, face a high burden of HIV disease that manifests as severely compromised immunity and increased susceptibility to other infectious opportunistic diseases [2]. In this regard, elevated prevalence of tuberculosis (TB)–HIV co-infection has been demonstrated, for example in South Africa, where up to 73% of patients diagnosed with active TB have been shown to be HIV-positive [2]. HIV-positive patients show a 20- to 37-fold greater risk of developing active TB than those who are HIV-negative [3]. Despite the high TB burden in HIV infected people, standard symptom, microbiological and radiologic screenings for TB are not routinely undertaken when TB is not the presenting clinical condition, resulting in unnecessary morbidity and mortality in HIV infected people [3]. In addition to being the most common co-morbidity, TB is also the leading cause of death in those who are HIV infected [2]. The emerging multidrug resistant (MDR) and extensively drug resistant (XDR) forms of the TB–HIV epidemics further complicate this situation [4].

Strategies and policies to effectively integrate the management of both diseases are, therefore, critical, but have been limited by the lack of rigorous and consistent evidence [5]. Rapid scale-up of HIV treatment in resource-constrained settings has been made possible with global solidarity and notable donor funding through the

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Article highlights.

- An overall understanding of the current state of the inter-connected tuberculosis (TB)–HIV epidemics and the potential barriers to treatment integration.
- The pharmacological basis for antiretroviral (ARV)-TB drug interactions and the influence of CYP isoenzyme effects.
- A summary of the effect of combining rifamycins and all available non-nucleoside reverse transcriptase inhibitors.
- A summary of the effect of combining rifamycins and all available protease inhibitors.
- Updated dose modifications are necessary when combining ARVs and TB drugs.
- Updated data on additive and overlapping toxicities.
- Centrality of adherence to treatment.
- Predictors and management of immune reconstitution inflammatory syndrome.
- Latest data on survival benefits when combing TB and ARV treatment agents.
- An expert opinion on future research priorities.

This box summarizes key points contained in the article.

President's Emergency Plan for AIDS Relief (PEPfAR) and the Global Fund to Fight AIDS, TB and Malaria (GFATM) that have enhanced infrastructure, skilled human resources and increased antiretroviral treatment (ART) access.

Barriers to integrated HIV and TB treatment have traditionally included a lack of rigorous empiric evidence on how to safely manage co-infected patients [6]. The obstacles include traditional separation of facilities to treat HIV and TB; the lack of clear clinical guidance on immune reconstitution inflammatory syndrome (IRIS) detection and management; fear of potential drug–drug interactions; additive drug toxicities and tolerability issues and the adherence challenges associated with high pill burdens [7,8]. The ambivalence regarding 'during TB' or 'post-TB' ART initiation based on arbitrary CD4 cell count cutoffs has also been a major factor impeding co-management of the two diseases at a programmatic level [9,10]. Although some observational studies have shown that mortality was reduced when TB and HIV therapies are combined [9,11-13], the strength of evidence for integration has been substantially advanced with the completion of the first randomized clinical trial [14].

The purpose of this review is to examine the complex drug–drug interactions that have been a known barrier to integrating antiretroviral (ARV) and TB treatment and to provide recommendations on the clinical management of co-infected patients. Available data on new classes of ARVs and drug–drug interactions with first-line TB drugs are also briefly discussed. The goals for combined treatment of HIV and TB are to reduce morbidity and mortality associated with both diseases. Attaining TB cure within 6 – 9 months of initiating anti-TB treatment and optimal HIV viral load suppression to undetectable levels by 6 months is desirable. These goals need to be attained with minimal drug-related adverse events, without compromising the TB treatment or the longevity of the first-line ART regimen. Exposure to sub-therapeutic drug concentrations which may potentially induce resistance mutations in both organisms must be avoided.

2. Review: co-administration of ARV drugs with first-line TB treatment

In 2005, Di Perri *et al.* reviewed the drug–drug interactions between anti-TB and ARV drugs in this journal [6]. This review aims to update the earlier review and include limited data on newer classes of ARVs. The potential for additive toxicities as well as updates on IRIS incidence and management and mortality data during early co-treatment are highlighted.

2.1 Pharmacological basis for potential ART–first-line TB drug interactions

Alterations in drug disposition may occur at various stages after oral administration (**Figure 1**) as a result of interacting drugs or disease influences [15].

Interactions at the level of absorption and hepatic elimination are of most significance in relation to the co-administration of TB and ARV drugs. Drugs that alter the pH of the gastrointestinal tract may alter absorption of other drugs. Variable malabsorption of TB drugs by patients with advanced HIV disease has also been reported in several studies with a potentially detrimental impact on TB treatment outcomes [16-19]. TB drug malabsorption is thought to be more likely when concurrent gastrointestinal infection or diarrhea or advanced immunodeficiency with or without diarrhea is present [17-19].

Gastrointestinal and hepatic metabolism of TB and ARV drugs have been extensively studied [20-22]. The proposed mechanisms of the TB–ARV drug interactions are related mainly to substrate activity, inhibition or induction of the hepatic CYP monooxygenase enzyme system (**Figure 2**) [21]. The *CYP450 isoforms that are most commonly associated with TB–HIV drug interactions [21,23,24] are listed in **Figure 2**. Modulation of the P-glycoprotein cellular transport system in the intestinal mucosa can increase the efflux of drugs from cells and prevent absorption of certain drugs [21]. Rifampicin (RIF) is a known inducer and several protease inhibitors (PIs) are either substrates for or inhibitors of this transport system [21,25]. The resultant effect following hepatic- or transporter-mediated pharmacokinetic interactions may impact treatment outcome in two ways depending on the potency of the effect: sub-therapeutic concentrations may result in treatment failure and higher concentrations may be associated with treatment-limiting toxicity [15,21].

The main pharmacokinetic drug–drug interactions expected between TB treatment and ART are related to hepatic elimination, involving the rifamycin class of TB antimicrobials (RIF, rifabutin (RFB) and rifapentine (RFP)), the non-nucleoside

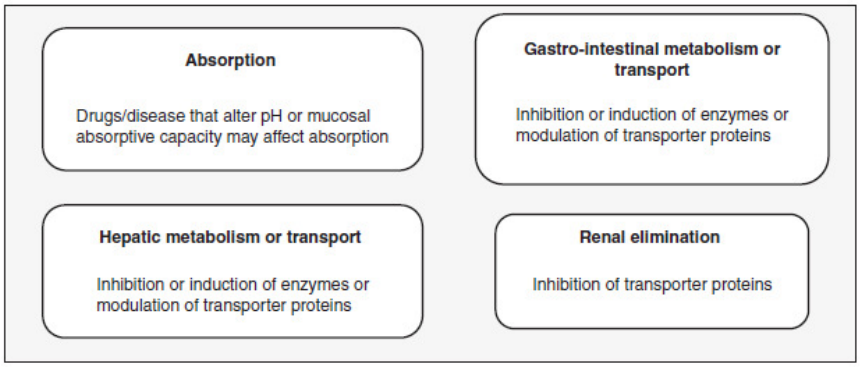

Figure 1. Drug interactions following oral administration may be mediated at four stages of disposition [15].

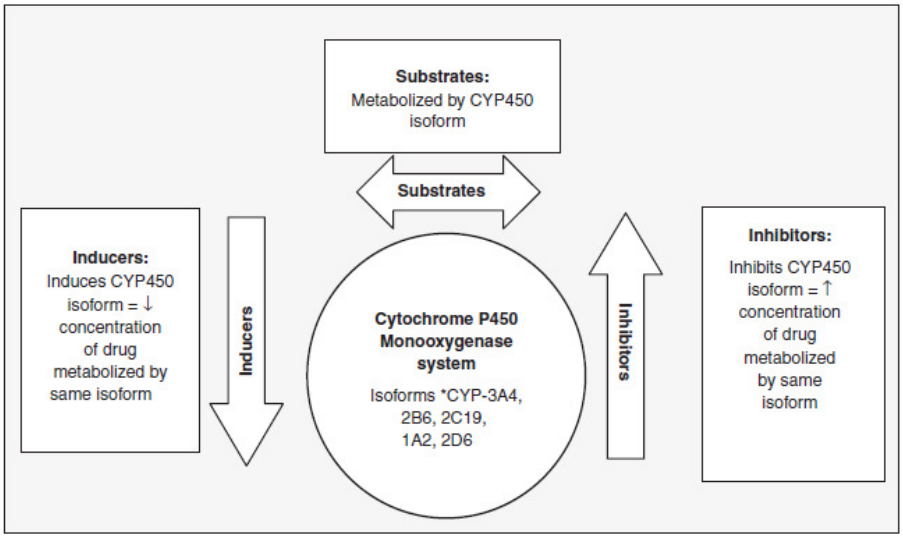

Figure 2. Possible metabolic drug interactions and the CYP system.
*Cytochrome P450 monooxygenase (CYP) system isoenzymes most commonly associated with TB-HIV drug interactions.

reverse transcriptase inhibitors (NNRTIs) and the PIs [9,21]. Other first-line TB drugs such as isoniazid, ethambutol and pyrazinamide, although metabolized hepatically, are not reported to significantly influence the CYP enzyme system in humans [26]. There is also potential for rifamycin interaction with newer ART classes, such as the CCR5-receptor antagonists and integrase inhibitors, based on current knowledge of metabolic pathways. There are no significant established drug interactions with the older nucleoside reverse transcriptase inhibitors (NRTIs), with the possible exception of zidovudine (ZDV), or the entry inhibitor class agent, enfuvirtide [27].

2.1.1 Rifamycins as the preferred backbone of first-line TB treatment

Rifamycin-based TB regimens have been used successfully to manage TB in HIV positive patients and have been found to be most effective if administered throughout TB treatment [28]. High relapse rates have been evident if rifamycins were used only in the first 2 months of treatment [29] and a minimum of 6 months of rifamycin treatment is required to effect a cure [29,30]. Some have advocated an even longer duration of 8 or more months when treating HIV co-infected patients [31,32]. Non-rifamycin-based regimens are considered less potent, as

they increase the TB treatment duration to 18 – 24 months and are associated with higher toxicity and relapse rates [23,29].

RIF is the most widely available and most commonly used of the rifamycins [31]. RFB has similar efficacy to RIF but is more expensive, neither widely available in high TB prevalence countries nor in fixed dose combinations and its use is complicated by the fact that as a substrate for CYP3A4, RFB is subject to dose modification when co-administered with ARVs [27,33]. RFP has been shown to be less effective in effecting TB cure in those with advanced disease and is not currently advocated for first-line TB treatment in HIV-positive cases [34]. There were concerns with the intermittent dosing strategies used to previously test RFP as no accompanying TB drug has a similar long half-life, which probably led to the high failure rates observed [35]. Studies are currently underway testing daily dosing of RFP [36,37]. However, due to these uncertainties and lack of data, RFP is excluded from further discussion in this review.

Standard treatment for uncomplicated pulmonary TB, particularly in developing countries, comprises of a minimum of 2 month intensive phase treatment combination consisting of RIF, isoniazid, pyrazinamide and ethambutol and a minimum 4 month continuation treatment combination of RIF and isoniazid, dosed 5 – 7 days a week. Streptomycin (aminoglycoside) is added for re-treatment cases that are still susceptible to first-line treatment [31].

Drug interactions with the rifamycins RIF or RFB, therefore, need to be anticipated and managed for the entire duration of TB treatment. The relative extent of CYP3A induction is RIF > RFP > RFB [38]. RIF is a potent inducer of CYP3A and a strong inducer of CYP2B6. RIF's concentration is not influenced by CYP3A induction, whereas RFB toxicity is influenced by its dose and the presence of CYP3A inhibitors [38].

2.2 Drug interactions between RIF/RFB and NNRTI class

NNRTIs are widely prescribed as the backbone of first-line ART, particularly in developing countries. Both efavirenz (EFV) and nevirapine (NVP) are metabolized by the CYP enzyme system. The CYP2B6 isoform is primarily responsible for EFV metabolism and the CY3A4 isoform is primarily responsible for NVP metabolism and to a less significant extent for EFV metabolism [24]. Both EFV and NVP also have the ability to induce the enzymes that are responsible for their own metabolism and may increase the clearance of co-administered drugs that share these metabolic pathways [24]. The newest NNRTI, etravirine (ETV), is similarly CYP metabolized and subject to interactions with rifamycins.

Table 1 illustrates the impact of RIF and RFB on NNRTI and PI AUC, with accompanying recommendations for dose modification.

2.2.1 *Efavirenz*

EFV has been widely studied and in clinical use for > 10 years [39]. EFV is principally metabolized by CYP2B6, with women and individuals with the 516G > T single nucleotide polymorphism appearing to have higher drug exposure [39,40].

When combined with RIF, there appears to be a 22 – 25% reduction in peak and trough EFV concentrations in caucasian populations. Accordingly, recommendations to increase the dose of EFV from 600 to 800 mg in patients weighing > 60 kg have been issued [41,42]. This reduction in concentration is less evident in Black [43,44] and Asian [45-47] adult patients, although high inter-patient variability in concentrations has been reported. In these populations, clinicians have been able to successfully co-administer standard 600 mg EFV dosing with RIF and EFV dose augmentation appears not to be necessary. If resources permit, consideration should be given for therapeutic drug monitoring of EFV and pre-emptive 516G > T genotyping, as there appears to be variability in drug handling amongst different populations [48].

2.2.2 *Nevirapine*

NVP is generally used during pregnancy or when EFV is contraindicated. NVP is thought to be associated with higher risk for symptomatic hepatic adverse events in ART-naive patients with pre-ART CD4 cell counts > 400 cells/mm^3 if male and CD4 > 250 cell/mm^3 if female [49]; however, in a 'Rapid Advice' communication in November 2009 by the WHO [50], these added risks were not confirmed.

NVP is metabolized primarily by CYP3A4 and RIF is a potent inducer of this isozyme. Co-administration of the two agents should be avoided due to reports of a 20 – 58% reduction in NVP concentration [49,51]. Options to increase the dose of NVP from 200 mg twice daily to 300 mg twice daily to counteract the RIF induction effect have been approached cautiously in studies with small numbers of patients [52]. This dose amendment is not currently recommended due to higher rates of NVP hypersensitivity [53]. An important recommendation by the WHO is that in the presence of RIF or when switching from EFV to back to NVP no lead-in dose is required [54]. This recommendation is supported by two studies which show that the omission of the NVP lead-in dose is safe [55] and ensures therapeutic drugs concentration are reached [56]. NVP has been reported in a cohort study to be less affected by the NVP–RIF interaction in its ability to suppress viral load when combined with TB treatment if NVP treatment is established prior to TB treatment initiation [57]. However, the majority of studies including the N2R randomized controlled trial have shown that EFV is more effective, has fewer adverse events, is the more durable and the preferred of the two NNRTIs when combined with TB treatment [45,57-59].

2.2.3 *Delavirdine*

Delavirdine (DLV) has a lower efficacy than other NNRTIs and needs to be administered more frequently. These factors have led the US DHHS (Department of Health and Human

Table 1. AUC changes and dose adjustment recommendations when ARV drugs and rifamycins (RIF/RFB) are co-administered.

ARV drug	*RIF effect on ARV AUC	Recommendation for dose modification and comment	‡RFB effect on ARV AUC	Recommendation for dose modification and comment
Non-nucleoside reverse transcriptase inhibitors				
EFV	↓26%	↑EFV dose to 800 mg if Caucasian and > 60 kg. TDM if possible.	↔	RFB AUC↓ 38%, RFB dose↑ to 450 – 600 mg/day or thrice weekly
DLV	↓95%	Contraindicated	↓80%	RFB AUC↑ 100%. Contraindicated
ETV	Potential for significant ↓in AUC	Avoid, no clinical experience	↓37%	RFB↓ 17%. No dose change but no clinical experience, avoid if ETV administered with boosted PI
NVP	↓50%	Use with caution, monitor viral response	↔	No change in AUC of either drug
Protease inhibitors				
ATV	PK data not available	Contraindicated	Minimal effect	RFB AUC↑ 210%, ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
DRV	PK data not available	Contraindicated	PK data not available	May inhibit RFB, ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
FPV	APV ↓82%	No study on FPV, avoid combination	↔	RFB AUC↑ 193%, ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
IDV	↓89%	Contraindicated	↓32%	RFB AUC↑ 204%, ↓RFB dose to 150 mg thrice weekly, 5monitor RFB level to adjust dose and increase unboosted IDV dose to 1000 mg every 8 h
NFV	↓82%	Contraindicated	↔	RFB AUC↑ 207% ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
LPV/r	↓75%	Avoid if possible. WHO recommends RTV super-boosting (LPV 400 mg + RTV 400 mg twice daily or LPV 800 + RTV 200 mg twice daily) [54]. Monitor LFT and viral response	↔	RFB AUC↑ 303%, ↓RFB dose to 150 mg alternate days or thrice weekly is the current CDC recommendation based on healthy volunteer data. However, RFB 150 mg thrice weekly has been found to be associated with sub-therapeutic levels in HIV co-infected [76,77]. Unadjusted dosing may be preferable
RTV	↓35%	Monitor viral response	↔	RFB AUC↑ 403%, ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose

Adapted from [27,49,71].
*RIF potent CYP3A4 and UGT1A1 inducer.
‡RFB CYP3A4 inducer and substrate.
↑: Increase; ↓: Decrease; ↔: No change/effect.
APV: Amprenavir; ARV: Antiretroviral; ATV:Atazanvir; b.i.d.: Twice a day; CDC: Centers for Disease Control; DLV: Delavirdine; DRV: Darunavir; EFV: Efavirenz; ETV: Etravirine; FPV: Fosamprenavir; IDV: Indinavir; LFT: Liver function test; LPV: Lopinavir; LPV/r: LPV with RTV; MVC: Maraviroc; NFV: Nelfinavir; NVP: Nevirapine; PI: Protease inhibitor; PK: Pharmacokinetic; RFB: Rifabutin; RGR: Raltegravir; RIF: Rifampicin; RTV: Ritonavir; SQV: Saquinavir; TDM: Therapeutic drug monitoring; TPV: Tipranavir.

Table 1. AUC changes and dose adjustment recommendations when ARV drugs and rifamycins (RIF/RFB) are co-administered (continued).

ARV drug	*RIF effect on ARV AUC	Recommendation for dose modification and comment	†RFB effect on ARV AUC	Recommendation for dose modification and comment
SQV	↓84%	Avoid due to serious hepatotoxicity	↓43%	SQV must be boosted with RTV. ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
TPV	PK data not available	Contraindicated	↔	RFB AUC↑ 190%, ↓RFB dose to 150 mg thrice weekly, monitor RFB level to adjust dose
CCR5 antagonists				
MVC	↓63%	Increase dose of MVC TO 600 mg b.i.d. or avoid	No clinical experience	No clinical experience
Integrase inhibitors				
RGR	↓by 40%	↑RGR dose to 800 mg b.i.d.	No clinical experience	No clinical experience

Adapted from [27,49,71].
*RIF potent CYP3A4 and UGT1A1 inducer.
†RFB CYP3A4 inducer and substrate.
↑: Increase; ↓: Decrease; ↔: No change/effect.
APV: Amprenavir; ARV: Antiretroviral; ATV:Atazanvir; b.i.d.: Twice a day; CDC: Centers for Disease Control; DLV: Delavirdine; DRV: Darunavir; EFV: Efavirenz; ETV: Etravirine; FPV: Fosamprenavir; IDV: Indinavir; LFT: Liver function test; LPV: Lopinavir; LPV/r: LPV with RTV; MVC: Maraviroc; NFV: Nelfinavir; NVP: Nevirapine; PI: Protease inhibitor; PK: Pharmacokinetic; RFB: Rifabutin; RGR: Raltegravir; RIF: Rifampicin; RTV: Ritonavir; SQV: Saquinavir; TDM: Therapeutic drug monitoring; TPV: Tipranavir.

Services) not to recommend its use as part of first-line treatment. Cross-resistance in the NNRTI class, as well as DLV's potential for drug interactions (potent inhibitor of CYP3A4), makes the place of this agent in second-line and salvage therapy uncertain [49].

2.2.4 *Etravirine*

This new NNRTI is not widely available in high prevalence HIV–TB areas. There is little clinical experience or published data on the combination of ETV with TB treatment. However, based on its pharmacokinetic profile, ETV is a substrate of CYP3A4, CYP2C9, CYP2C19, an inducer of CYP3A and an inhibitor of CYP2C9, CYP2C19 and p-glycoprotein [60]. Co-administration of drugs that are substrates at or induce these pathways may have unknown effects on the therapeutic concentrations of ETV and vice versa [60].

2.3 Drug interactions between RIF/RFB and boosted PIs

PIs are associated with many clinically relevant drug interactions [21]. PIs are mostly substrates of CYP3A4 and P-glycoprotein, with the exception of nelfinavir (NFV) which is metabolized by CYP2C19 [20]. Ritonavir (RTV) also has the ability to potently inhibit CP3A4 and P-glycoprotein efflux pumps and this property has been used to therapeutic advantage in combination with other PIs. These combinations of low-dose RTV and PIs, commonly referred to as boosted PIs, show enhanced activity (plasma concentration) and increased likelihood of viral suppression [61]. Co-administering unboosted PIs with RIF has been shown to result in > 90% reduction in PI trough concentrations [27,33]. PI AUC reduction due to RFB is 15 – 45% [62]. Boosting with low-dose RTV may not be sufficient to overcome the RIF effect [63-65] and suggestions to add high doses of RTV (super-boosted PIs) have been made [64]. Safety concerns (hepatic adverse events) and poor tolerance curtail these treatment options [66], making individualized treatment with careful laboratory monitoring essential.

2.3.1 *Atazanavir*

The co-administration of RIF and boosted atazanavir (ATV) is contraindicated due to a combination of poor hepatic and gastrointestinal tolerability [67] as well as sub-therapeutic ATV plasma concentrations [68,69].

2.3.2 *Darunavir*

Darunavir was FDA approved in 2006 but to date there are no published studies available on co-administration with RIF. This is not surprising as the manufacturer has contraindicated its use based on the predicted effects of lowered therapeutic concentration and efficacy when combined with RIF, as is the current clinical experience with other PIs [70].

2.3.3 *Amprenavir/fosamprenavir (prodrug)*

Co-administration of RIF and amprenavir (APV) or fosamprenavir is not recommended [71]. A study done in

healthy volunteers reported that RIF induced an 82% reduction in the AUC of APV and that APV caused a significant decrease in clearance of RFB [72].

2.3.4 Indinavir

The AUC of indinavir (IDV) is reduced 89% by RIF and this combination should not be co-administered. RFB clearance is reduced in the presence of IDV and IDV concentrations are reduced by 32%. An increase in IDV dose to 1000 mg every 8 h with RFB is recommended [61,71].

2.3.5 Nelfinavir

Plasma concentrations of NFV are reduced by 82% by RIF and the two agents should not be co-administered. RFB has insignificant effect on NFV concentration, but RFB AUC increases 207%, requiring RFB dose adjustment [71].

2.3.6 Lopinavir

Lopinavir (LPV) is co-formulated with RTV (referred to as LPV/r) and is widely accessible in high TB-prevalence countries. Clinical data on dose modification when combined with RIF are limited and in some instances concerning. A study in 40 healthy volunteers had to be prematurely terminated due to high rates of grade 4 serum transaminase elevation when LPV/r was super-boosted with a higher dose of RTV or when double dose LPV/r was used to counteract the RIF effect [73]. In a retrospective analysis of observational data, 34 patients treated concomitantly with LPV/r and RIF were studied. Increased dosing of LPV/r was used in only 15% of patients and 40% of them had to prematurely stop the drug due to adverse events (nausea, vomiting, liver enzyme elevations). In the 85% of patients who were maintained on standard doses, 67% had a sub-therapeutic LPV plasma concentration and 38% had a detectable viral load [74]. The US DHHS [49] and Centers for Disease Control [71], therefore, do not recommend the combination with RIF. RFB is recommended as a possible substitute for RIF. However, in clinical practice, particularly in developing countries where the RIF + LPV/r combination is often unavoidable and RFB is unavailable, RTV super-boosting or double strength LPV/r is prescribed [33]. The WHO recommends RTV super-boosting (LPV 400 mg + RTV 400 mg twice daily) or double dose (LPV 800 + RTV 200 mg twice daily) with liver enzyme functioning and viral response monitoring [54]. These doses was tested in 32 healthy subjects and found to be of moderate tolerability with a 31% discontinuation rate in the higher-dose LPV/r arms. The 800/200 mg dose exhibited lower rates of liver function test elevation and half as many discontinuations. [64]. Additionally, in a small pharmacokinetic study conducted in 30 South African children, LPV/r pharmacokinetics was compared in 15 children taking LPV/r in a 1:1 ratio (super-boosted) with RIF to 15 children taking LPV/r in the standard 4:1 ratio without RIF. The investigators found that LPV oral clearance was 30% lower in the non-TB-infected children compared to the super-boosted LPV clearance and encouragingly the predicted C_{min} was above the recommended minimum during TB treatment [75].

Concomitant RFB and LPV/r use in healthy volunteers demonstrated a > 300% increase in RFB AUC and no effect on LPV/r AUC with a resultant RFB dose reduction recommendation [71]. However, RFB 150 mg three times weekly in combination with LPV/r resulted in inadequate RFB levels and led to acquired rifamycin resistance in patients with HIV-associated TB [76,77]. Current dosing recommendations require revision. Empiric evidence to guide safe and effective dose adjustment of LPV/r and RIF and RFB is urgently needed as treatment programs mature in developing countries and more patients move onto PI-based regimens.

2.3.7 Ritonavir

RTV is generally administered in conjunction with other PIs where its ability to inhibit CYP3A4 is exploited for therapeutic effect [61].

2.3.8 Saquinavir

Due to reports of serious hepatotoxicity in healthy volunteers, the combination of RIF and boosted saquinavir (SQV) is best avoided if possible [64,78]. RFB may be co-administered with SQV + RTV [71].

2.3.9 Tipranavir

Tipranavir (TPV) is mainly metabolized by CYP3A4 and has been boosted with low dose RTV to enhance its plasma exposure. No clinical experience is available with these combinations in TB–HIV infected individuals. However, RFB may be safe to use with dose adjustment and a predictable RIF interaction may be inferred from its metabolic pathway until substantive evidence becomes available [79].

2.4 CCR5 co-receptor antagonist

2.4.1 Maraviroc

There is very limited published clinical experience with maraviroc (MVC) and the rifamycins. MVC is metabolized by CP3A4 and is, therefore, subject to interactions with inhibitors and inducers of that isoenzyme such as the PIs (except for TPV), NVP, EFV and RIF [80,81]. TPV/r does not appear to affect the steady-state pharmacokinetics of MVC [82]. This agent is limited to use in treatment-experienced patients who are not infected with CXCR4-tropic virus. Dose reduction is advocated if MVC is administered with potent inhibitors and dose increase if administered with potent inducers of CYP3A4 [82]. No data are available for potential interactions with RFB.

2.5 Integrase inhibitor

2.5.1 Raltegravir

Raltegravir (RGR) is not a substrate of CYP enzymes and is metabolized via the UGT1A1 glucuronidation pathway [83]. RIF is a strong inducer of UGT1A1 and although there is limited clinical experience with RGR, it is recommended

that RIF be used with caution with RGR as trough concentrations of the ARV may decrease by 40 – 61%. A recent update to the package insert recommended a dose increase of RGR to 800 mg twice daily if co-administered with RIF [71,83].

2.6 Other reverse transcriptase inhibitors and rifamycins

The NRTIs do not appear to have any significant known drug interactions with the rifamycins with the exception of the limited published data with ZDV [84]. RIF may increase glucuronidation of ZDV, thereby, decreasing ZDV AUC by 47% [85]. In practice, this combination is not contraindicated so long as there is adequate laboratory monitoring of viral load.

Tenofovir disporoxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor, is now increasingly available in resource-constrained settings. TDF is advocated for use in HIV, HIV/hepatitis B (HBV) and TB–HIV co-infected patients [86]. TDF (not a substrate of CYP) is eliminated by a combination of glomerular filtration and active tubular secretion and in a study of 24 healthy volunteers receiving both TDF and RIF, no changes in the pharmacokinetic parameters of either drug were noted [87]. The nephrotoxic potential of TDF in long-term treatment has been reviewed [88]. Increased risk for nephrotoxicity exists with TB co-treatment when other agents with nephrotoxic potential such as aminoglycosides are utilized for TB re-treatment or MDR/XDR TB treatment. Extra vigilance in renal function monitoring is indicated.

3. Interactions between ARVs and anti-TB drugs used in multidrug- and extensively drug resistant TB

MDR TB is defined as TB which is resistant to both RIF and isoniazid and accounts for 5% of the global TB burden [89]. XDR TB is defined as MDR TB that is, in addition, resistant to a fluoroquinolone and at least one second-line injectable agent [90]. MDR TB and HIV co-infected patients have an exceedingly high mortality rate [4,91,92]. There is potential for ARV and TB drug interactions in the MDR and XDR TB treatment setting given the multiple drug classes exhibiting differing pharmacokinetic profiles (aminoglycosides, fluoroquinolones, thioamides, cycloserine, para-aminosalicylic acid, clofazimine, macrolides, linezolid) that will need to be safely used in combination [90]. More evidence-based, pharmacokinetic and clinical data are required when these drug classes are combined to be able to guide the field on dosing. This topic is beyond of the scope of this review but mentioned due to its importance when combining ART and drug resistant TB drugs.

4. Drug toxicities and adherence challenges when combining TB–HIV treatment

Barriers to TB–HIV treatment integration include fears about drug toxicities attributed to individual drug agents being enhanced when treatments are combined. In addition, the potential for a detrimental impact on adherence to treatment because of the high pill burden associated with HIV, TB and other opportunistic infection drugs has historically been a cause for concern.

4.1 Additive and overlapping toxicities

HIV and TB drugs are independently associated with significant toxicities. When combined, drug-related toxicities may be additive or might overlap, resulting in increased potential for morbidity, premature disruption of treatment/s and in some cases life-threatening adverse effects.

The potential for adverse events due to the interaction between ART and anti-TB drugs that have similar toxicity profile is due to shared pharmaco-metabolic pathways, the most common manifestation of which is hepatotoxicity [6]. Hepatotoxicity can occur in 5 – 10% of patients in the first year following ART initiation, and this risk is enhanced if the patient is hepatitis C and/or B co-infected. [93]. Reports indicate that HIV infected patients may be predisposed to higher rates of drug-related adverse events [94], including severe liver toxicity, when on anti-TB treatment [95,96]. High baseline bilirubin, low CD4 cell counts between 50 and 100 cell/m^3 and the use of fluconazole prescribed in the first week of TB treatment have been shown to be significant risk factors for liver toxicity [97]. A study of the risk of elevated grade 3 or 4 hepatic enzymes during RTV boosted PI use in 1161 patients revealed the following incidence: NFV, 11%; LPV/r (RTV = 200 mg/day), 9%; IDV, 13%; IDV + RTV (RTV = 200 – 400 mg/day), 12.8%; and SQV + RTV (RTV = 800 mg/day), 17.2% [98]. In a South African study of 868 HIV-positive patients, of whom 25% were receiving concomitant TB treatment during ART, episodes of severe hepatotoxicity were reported at a rate of 7.7/100 person-years, with an 8.5-, 3- and 1.9-fold increased risk if on TB treatment, HBsAg positive and possessing a nadir CD4 cells count < 100 cells/mm^3, respectively [99]. Of further importance in this study, the proportion of patients with severe hepatotoxicity on ART (4.6%) was similar to the proportion with liver enzyme elevations > 5 times the upper limit of normal before starting ART (4%) [99]. Tolerance of the NNRTIs was assessed in a cohort analysis of 2035 individuals who started ART with EFV (1074 with concurrent TB) and 1935 with NVP (209 with concurrent TB). The risk of toxicity-mediated NNRTI substitution in patients on concurrent TB treatment was increased in patients on NVP (adjusted hazard ratio (HR) 1.5; 95% CI 0.8 – 2.8), but this estimate did not reach statistical significance. In patients without concurrent TB, those on NVP were more likely to have their therapy substituted by 6 months due to toxicity compared with those on EFV (cumulative proportion: 4.9%; 95% CI 3.9 – 6.1% (NVP) vs 1.4%; 95% CI 0.8 – 2.4% (EFV)) [57]. In the SAPiT randomized clinical trial conducted in 642 HIV-TB co-infected South African patients who were randomly assigned to ART at two time points during and after TB treatment completion, 140 grade 3 or 4 adverse

events that were not regarded as immune reconstitution occurred in the TB–HIV co-treated group (30/100 person-years) and 71 in the TB treatment only group (32/100 person-years) (p = 0.69) [14].

With TB treatment alone, the reported incidence of drug-induced hepatotoxicity ranges from 2 to 28%, depending on the presence of risk factors such as advanced age, female gender, low acetylator status, malnutrition, presence of HIV infection and pre-existing liver disease and the exact mechanism is not clearly understood [26]. In a Canadian study of serious side effects (rash, hepatitis and gastrointestinal) from first-line TB treatment in 430 patients, the incidence of all major adverse events was 1.48/100 person-months, (95% CI 1.31 – 1.61) for pyrazinamide, 0.49 (95% CI 0.42 – 0.55) for isoniazid, 0.43 (95% CI 0.37 – 0.49) for RIF and 0.07 (95% CI 0.04 – 0.10) for ethambutol [96]. In this study, the adjusted HR for major adverse events was 3.8 (95% CI 1.05 – 13.4) in HIV-positive patients [96].

Peripheral neuropathy is also an important predictor of treatment-limiting toxicity in TB–HIV co-treatment. In a South African study of 7066 HIV patients initiated on stavudine as part of the national first-line ART regimen at the time, those on concurrent TB treatment were more likely to require stavudine substitution in the first 2 months of ART treatment (adjusted HR 6.6; 95% CI 30 – 14.37) [100]. Almost half (43%) of the 842 single-stavudine substitutions in this analysis were attributed to peripheral neuropathy and these patients were more likely to be on TB treatment at ART initiation or during follow-up (relative risk 1.53, 95% CI 1.33 – 1.75). Isoniazid is the TB drug most likely to be associated with peripheral neuropathy [101] as are the ARVs, stavudine [102] and didanosine [103].

Other important shared adverse events in TB–HIV co-treatment incorporating the standard use of co-trimoxazole are hypersensitivity reactions (NVP, abacavir, co-trimoxazole, TB drugs), gastrointestinal disorders (didanosine, ZDV, PIs, TB drugs), anemia (ZDV) and CNS manifestations (EFV, isoniazid), which in some cases may necessitate stopping the causative drugs, even though the preference is to manage the symptoms and maintain patients on the most efficacious combination regimens available [7,90,104].

It is recommended that all HIV-positive patients receiving TB and ART simultaneously have baseline hepatitis B [99] and hepatitis C screening, liver function test (AST, bilirubin, ALT) and full blood count with platelets, prior to initiation of either treatment. Routine safety checks of the liver and hematological parameters as well as frequent clinical symptom monitoring are recommended to ensure that hepatic flares or worsening laboratory parameters (which may be asymptomatic) are detected early. In a typical TB–HIV co-treatment scenario, several activities may occur in close succession, and optimal drug sequencing needs to be established to best manage the adverse drug reactions that may occur in order to improve tolerability to necessary drug regimens. A patient's inability to tolerate the treatment will curtail therapeutic options even though the limited treatment available may still be effective.

4.2 Adherence challenges in combination treatment

Optimal adherence to both long-term chronic ART and shorter course anti-TB treatment is critical. There is an established direct relationship among poor adherence, treatment failure and the development of resistance [105], making adherence an important public health safety concern. Adherence to TB treatment is complex and involves many facets and structural barriers that need to be identified and overcome [106]. Factors that may predispose to less than optimal adherence include high pill burden, poor drug tolerability [107], alcohol consumption and having reached the continuous phase of TB treatment [108]. Predictors of non-adherence are useful to guide caregivers. In high prevalence settings, the challenges are even more complex, encompassing economic, institutional, political and cultural factors [109]. Settings that provide comprehensive, multidisciplinary care such as adherence counseling, HIV, TB, STI treatment and provision of all chronic treatment and concomitant medication at a single facility may be a step in the right direction to enhance adherence support.

5. IRIS associated with TB and HIV treatment

IRIS is a frequent early complication in the management of TB–HIV co-infected patients initiating ART and is a result of the recovering immune system recognizing previously undetected antigens [110]. IRIS may present as unmasking of pre-existing untreated opportunistic infections (mycobacteria, herpes virus, cryptococcal meningitis) or the paradoxical clinical deterioration of appropriately treated opportunistic infections such as pulmonary TB (TB IRIS), usually shortly after ART initiation [111].

Patients develop clinical and radiographic manifestations of IRIS such as fever, worsening chest radiograph, cervical adenopathy and pleural effusion, typically developing 2 – 4 weeks after starting ART [112-114]. However, delaying ART until the continuation phase of TB treatment (2 months) does not prevent the occurrence of TB IRIS [115]. Determination of TB IRIS is challenging as there is no diagnostic test and reliance is on pre-determined clinical case definitions, clinical and laboratory data [111].

In a retrospective South African cohort study of 160 patients initiating ART whilst on TB treatment, IRIS was diagnosed in 12% (n = 19) of patients where 12/19 started ART within 2 months of TB diagnosis [116]. Low CD4 count and short interval between TB and ART initiation were predictive of IRIS occurrence. The results of the SAPiT trial were similar, with IRIS being diagnosed in 53/429 (12.4%, 95% CI 9.5 – 15.9) and 8/213 (3.8%, 95% CI 1.8 – 7.5) patients in the TB-HIV and TB-only treatment arms, respectively [14].

Mortality rates from TB IRIS have been reported in the literature and in most instances are low [113,116,117]. The results

of a pooled meta-analysis estimate that mortality from TB-associated IRIS was ~ 3.2%, whereas mortality from all types of IRIS was 4.5% [118]. However, TB IRIS is associated with higher rates of morbidity and often requires hospitalization, with Black race, low baseline CD4 count, extra-pulmonary TB and a short time interval between TB treatment and ART initiation being the most predictive of its occurrence [117,119]. In some settings, a greater rate of increase in CD4 count from baseline to 6 months offers additional predictive value [114,120].

In the clinical management of IRIS, combination ART has been continued and management has been supportive and symptomatic [104]. In a recently published randomized controlled trial, the use of prednisone as a 4 week course (1.5 mg/kg/day for 2 weeks then 0.75 mg/kg/day for 2 weeks) in suspected TB IRIS following ART and TB co-treatment reduced morbidity and the need for hospitalization [121].

6. Mortality from HIV progression and TB co-infection

The positive impact (reduction in AIDS progression and death) of combining ART and acute opportunistic infection treatment was demonstrated in the A5164 study [122]. There are also a growing number of observational studies from developing countries demonstrating reduction of mortality rates specifically in TB–HIV co-infected patients when HAART was introduced early during TB treatment [9,11,123-127]. The retrospective cohort study by Velasco *et al.* [125] showed that patients starting ART within 2 months of TB treatment start had a significantly improved survival benefit (HR 0.38; 95% CI 0.20 – 0.72) compared to those who did not. Additionally, the effect of delaying ART in 573 children by 15, 30 or 60 days after TB treatment start revealed that delays of 2 or more months are associated with less than optimal virological response and increased mortality [128].

These data are consistent with the interim results of the SAPiT randomized clinical trial [14]. The SAPiT trial was conducted in 642 ambulant HIV–TB co-infected South African patients, who were randomly assigned to ART at two time points during TB treatment (with 4 weeks of TB treatment start, within 4 weeks after 8 weeks of TB treatment was completed) and after completion of TB treatment. The integrated therapy groups were associated with a mortality rate of 5.4 deaths/100 person-years compared to 12.1/100 person-years in the sequential group (HR 0.44; 95% CI 0.25 – 0.79), which translates to a relative risk reduction of 56% when ART and TB treatment are combined [14].

In addition to the SAPiT trial results, the findings of the CAMELIA study, the second RCT in this arena, were presented at the 18th International AIDS conference in Vienna, 2010 [129]. This prospective clinical trial was conducted in 661 Cambodian patients, who were randomized to early (2 weeks post-TB treatment start) versus late (8 weeks post-TB treatment start) ART initiation and were characterized at baseline as being severely immune-compromised. In this study, the median CD4 cell count was 25 cells/m^3. The mortality rate in the early arm was 8.3 (95% CI 6.4 – 10.7) versus 13.8 (95% CI 11.2 – 16.9) in the late arm. The study demonstrated that the initiation of ART in the first 2 weeks of TB treatment significantly reduces mortality in the severely immune-compromised.

7. Conclusions

There is now more robust evidence to guide the clinical management of TB–HIV co-infected patients. Rational combination and sequencing of TB–HIV treatment, understanding the potential for significant pharmacokinetic drug interactions between rifamycins and ARVs, detecting drug-related hepatotoxicity and neuropathies associated with drugs used for each condition and managing TB IRIS after initiation of ART are key priority areas for successful treatment integration. Understanding these complexities and how to manage them diminishes the previous barriers to safe treatment integration.

Integrating ART with TB treatment is critical to improve survival outcomes. Indications from current evidence are that early integration of ART and TB treatment, as early as 2 weeks but within 2 months after TB drug initiation, is safe and effective. Combining ART and TB drugs that are compatible, have manageable and predictable drug interaction profiles and good tolerability are essential for successful treatment integration. In this regard, the NNRTIs, EFV in particular, are shown to be better able to withstand the effects of the rifamycins than the PIs. Dose adjustment of ART should be individualized according to recommendations in **Table 1** in order to maintain therapeutic concentrations with appropriate clinical monitoring where necessary. Based on current evidence, individualized laboratory monitoring and attention to clinical presentation would be most beneficial in at least the first 2 months of combined TB–HIV treatment. The use of therapeutic drug monitoring and pharmacogenetic testing if indicated may be a useful adjunct in settings where feasible and available.

8. Expert opinion

8.1 Key findings of research

It is clear that improved survival of TB–HIV co-infected patients is dependent on the early integration of treatment. Failure to integrate ART and TB treatment due to fear of potential drug–drug interactions or IRIS will result in excess mortality. The drug management of HIV and TB is entering a new era of confidence due to the emergence of substantive evidence on which to base policies and practice. Overlapping toxicities are now better understood and, although not always predictable, are found not to be treatment limiting. Data and experience with individual agents are now available for decisions to be made by caregivers as well as policymakers.

8.2 Limitations of available research

Much of the available data on drug interactions are from healthy volunteers or small clinical studies and there is a paucity of robust drug interactions studies in patients who have the diseases in question. The findings of such studies may have poor external validity when applied to HIV–TB co-infected patients [130]. There is also a general lack of information and published clinical experience on drugs used in MDR and XDR TB and their potential interactions with ARVs. Although pharmacogenetic testing for CYP polymorphisms and therapeutic drug monitoring is advocated with certain ART and TB drugs, the evidence for a clear benefit that justifies resource allocation is not yet available.

The focus of this safety review is on non-pregnant adult patients but TB co-infection in HIV-positive pregnant women in sub-Saharan African is a major non-obstetric cause of mortality [131]. Therefore, efforts need to be focused on this population. Additionally, children and young adolescent women undergoing physical development are also vulnerable to TB–HIV co-infection. Safe, rational and well-researched drug choices need to be made available with the appropriate pharmacokinetic and clinical data to guide optimal decision making in these patient groups.

8.3 Future research priorities

Pressing research priorities include assessing the safety and efficacy of double dose and super-boosted PIs with RIF-based TB regimens and assessing the hepatic safety of omitting the lowered lead-in dose of NVP, which may in part have contributed to the higher proportion of virological failure seen with NVP compared to EFV when combined with TB treatment. Pharmacokinetic studies in TB–HIV co-infected children and pregnant women are of great importance as drug handling in these groups differ from the standard 70 kg adult in which most dosing is classically derived. These data are critically needed in resource-poor high TB–HIV prevalence regions.

Opportunities for research that enhances the understanding of the mechanisms and extent of anticipated drug interactions with MDR and XDR TB drugs and ART must be acted upon. The incidence of MDR/XDR TB is on the increase and fatalities will be excessive if strategies to co-treat are not rapidly devised. Additionally, testing multiple combinations of ARVs with TB treatment in those co-infected is essential to provide more treatment options and flexibility. More potent drugs that reduce pill burden and shorten the duration of TB treatment are important to develop and continued efforts need to be made to research new compounds for both TB and HIV, as the current armamentarium is still very limited and in constant danger of being exhausted [132]. As the HIV epidemic matures and treatment becomes available in developing countries over a sustained period, the need for second-line ARV drugs that are compatible with TB drugs will become critical. Future work needs to be more attentive to these needs. Several newer classes of ARVs and newer drugs in the older classes have now become available. Well-designed drug interaction studies with rationally sequenced drugs that take into account the time to enzyme induction need to be conducted to ascertain their utility in co-infection, for example, increasing RTG dose to 800 mg or MVC to 600 mg when combined with RIF. There is limited experience with RFB use in HIV-positive patients and greater access should be available in resource poor settings in single drug and fixed dose combinations to test the efficacy of RFB as an alternate to RIF. Another RIF replacement that shows promise to possibly shorten the duration of TB treatment is moxifloxacin [133] and future research results are eagerly awaited. The anti-TB drugs TMC 207 and linezolid are also in Phase III and II testing and this testing needs to incorporate potential drug interactions with other TB treatment as well as ART.

Given the increased risk of morbidity and mortality associated with TB–HIV co-infection and the complexities of integrating treatment, strategies to prevent TB acquisition in the first place warrant further operational research in high TB prevalence settings. Granich *et al.* outline the 'Three Is for HIV–TB' which are isoniazid preventative therapy, intensified case finding and infection control for TB [134].

Finally, operational studies on integrated comprehensive care from screening to treatment are now essential to inform best practice and to impact positively on adherence, TB and HIV case finding as well as TB and HIV prevention. Opportunities also exist through the PEPfAR- and GFATM-funded ART programmes for enhanced pharmacovigilance and assimilation of these data to better inform practice.

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2.1.1 Discussion of paper I

Paper 1 is responsive to Objective 1 of the PhD thesis. This article, published in 2011, is a comprehensive review of the literature that examines expected drug interactions between the rifamycin class of anti-TB drugs and ARVs.

The review assesses the pharmacological basis for HIV and TB drug interactions, discusses drug interactions between rifamycins and ARV drug classes, and provides evidence for drug safety concerns which may arise due to additive toxicities or poor tolerability during co-administration. The article reports on the current data on expected drug toxicities (hepatotoxicity, peripheral neuropathy, hypersensitivity reactions) and associated adherence challenges when treatment is combined. Information on clinical management of IRIS during co-treatment is also presented.

A total of 134 articles were cited in the review article, and practical updated guidance on dose modifications was provided to facilitate safe TB-HIV co-treatment. In summary, the individualization of ART dosing, where possible, and individualized clinical laboratory monitoring, at least in the first two month of combined TB and HIV treatment, is recommended to ensure safe treatment. Several future research priorities are also suggested.

In conclusion, deferring treatment of HIV to avoid drug interactions with TB treatment is unwarranted because rational drug choices aimed at reducing toxicities, while maintaining efficacy in treating both diseases, is eminently achievable.

2.1.2 PhD candidates' contribution to the journal article

Student name: Tanuja Narayansamy Gengiah

Student number: 993241124

Title of the article: Initiating antiretrovirals during tuberculosis treatment: a drug safety review

Authors: **TN Gengiah**, AL Gray, K Naidoo, Q Abdool Karim

Journal: Expert Opinion on Drug Safety

Doctoral student's contribution to the journal article:

1. Formulation of the hypothesis

   Prof Q Abdool Karim was invited to submit the review for consideration to the journal and the article theme was suggested by the journal editor. I conceptualized the review article hypothesis framework, layout and content, created the title and invited co-authors with the relevant pharmacology and TB/HIV management expertise to contribute.

2. Study design

   I designed the methodology for the literature search and selected the articles for inclusion in the review.

3. Work involved in the study

   I conducted the literature search in accordance with the methodology set out, found, selected and reviewed all the articles that were included in the review article. I created the figures and tables and populated the tables after assessing various data sources.

4. Data analysis

I took responsibility to review, analyze and interpret the data from the literature review for inclusion in the text, and to create the diagrams and tables. I also searched for the latest dosing updates and updated the dosing guidance provide in the article.

5. Write up

   I took overall responsibility for the writing of the manuscript and after completion of the first full draft of the manuscript submitted the manuscript to the co-authors for review and comment. All co-authors read and approved the final version of the manuscript. I corresponded with the journal and after peer review I completed the required revisions. The final version for publication was approved by all co-authors.

I declare this to be a true reflection of my contributions to this journal article

Signature  Date 14 November 2014

PAPER II

The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis

2.2 Paper II: The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis

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PHARMACOKINETICS AND DISPOSITION

The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis

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Abstract
Purpose Drug interactions are of concern when treating patients co-infected with human immunodeficiency virus (HIV) and tuberculosis. Concomitant use of efavirenz (EFV) with the enzyme inducer rifampicin might be expected to increase EFV clearance. We investigated the influence of concomitant tuberculosis treatment on the plasma clearance of EFV.
Methods Fifty-eight patients were randomized to receive their EFV-containing antiretroviral therapy either during or after tuberculosis treatment. Steady-state EFV plasma concentrations (n=209 samples) were measured, 83 in the presence of rifampicin. Data were analyzed using a non-linear mixed effects model, and the model was evaluated using non-parametric bootstrap and visual predictive checks.
Results The patients had a median age of 32 (range 19–55) years and 43.1% were women. There was a bimodal distribution of apparent clearance, with slow EFV metabolizers accounting for 23.6% of the population and having a metabolic capacity 36.4% of that of the faster metabolizers. Apparent EFV clearance after oral administration in fast metabolizers was 12.9 L/h/70 kg whilst off tuberculosis treatment and 9.1 L/h/70 kg when on tuberculosis treatment. In slow metabolizers, the clearance estimates were 3.3 and 4.7 L/h/70 kg in the presence and absence of TB treatment, respectively. Overall there was a 29.5% reduction in EFV clearance during tuberculosis treatment.
Conclusion Unexpectedly, concomitant rifampicin-containing tuberculosis treatment reduced apparent EFV clearance with a corresponding increase in EFV exposure. While the reasons for this interaction require further investigation, cytochrome P450 2B6 polymorphisms in the population studied may provide some explanation.

Keywords Pharmacokinetics · Tuberculosis · HIV · Efavirenz · Rifampicin

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Introduction

Human immunodeficiency virus (HIV) and tuberculosis co-infection is a serious public health issue in sub-Saharan Africa. The World Health Organization has reported that in South Africa, 53% of patients diagnosed with tuberculosis are also HIV-positive [1]. When treating co-infected individuals, determination of the optimal timing of antiretroviral therapy

(ART) initiation in relation to tuberculosis treatment [2] is complicated by the potential for clinically significant drug–drug interactions [3].

Rifampicin, an important component of first-line tuberculosis treatment, induces various hepatic cytochrome P450 (CYP450) enzymes and may decrease plasma concentrations of antiretroviral (ARVs) sharing similar metabolic pathways [4]. Conversely, isoniazid, also part of first-line tuberculosis treatment, is a competitive inhibitor that specifically targets enzymes CYP2C19 and CYP3A [5]. Efavirenz (EFV) is the preferred non-nucleoside reverse transcriptase inhibitor (NNRTI) component of the first-line ARV regimen in South Africa. Metabolism of EFV entails 8-hydroxylation by CYP2B6 with subsequent glucuronidation [6, 7]. EFV is also known to induce CYP3A4 as well as its own metabolism [8]. If EFV plasma concentrations are decreased due to interaction with enzyme inducers, this may increase the potential for therapeutic failure and the development of drug resistance [9]. There are currently conflicting reports in the literature regarding the nature of the interaction, with some studies [10, 11] reporting increased metabolism of EFV in the presence of rifampicin and others reporting just the opposite [12, 13].

In order to better understand the interaction between EFV and tuberculosis treatment, we designed the present study in which a population–pharmacokinetics approach was used to describe EFV clearance in South African patients treated with rifampicin-containing first-line tuberculosis regimens and NNRTI-based ART.

Methods

This study was conducted as part of the "Starting Tuberculosis and Antiretroviral Therapy" (START) study, an open label randomized clinical trial conducted in Durban, South Africa between July 2006 and January 2008. All patients recruited to the START study were ART naive, at least 18 years of age, and then received both ART and standard first-line tuberculosis treatment in a pre-existing directly observed therapy (DOT) program. Only patients with no pre-defined laboratory abnormalities, having received at least 10 but not more than 28 days of tuberculosis treatment were enrolled. The standard tuberculosis treatment comprised rifampicin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E) dosed daily on weekdays only for 2 months, followed by R and H for a minimum of 4 additional months. Patients weighing ≥50 kg received five tablets daily of a fixed-dose combination of RHZE containing 120/60/300/200 mg respectively, followed by two tablets daily of RH 300/150 mg. Patients weighing <50 kg received four tablets of the RHZE 120/60/300/200 mg daily, followed by three tablets of RH 150/100 mg. Women recruited to the study were required to use both injectable progestogen and barrier methods of contraception. All patients received standard of care, which included multivitamins and co-trimoxazole prophylaxis. No additional drugs thought likely to interact with EFV were permitted.

Participants were randomized to receive both ART and tuberculosis treatment simultaneously (integrated arm) or to initiate ART only on completion of tuberculosis treatment (sequential arm). In both arms, ART comprised once-daily enteric-coated didanosine (400 mg for participants ≥60 kg; 250 mg for participants <60 kg), lamivudine 300 mg, and EFV. Based on the expected interaction, when EFV was administered in the presence of tuberculosis treatment, participants weighing <50 kg received 600 mg and those weighing ≥50 kg received 800 mg daily. After the tuberculosis treatment was successfully completed, all patients received EFV 600 mg. For patients in the sequential arm, ART initiation occurred a median of 7 days [interquartile range (IQR) 6–9 days] after TB treatment completion. EFV concentrations were sampled at least 28 days after the TB treatment was completed; no residual wash-out effects of the TB drugs were expected after this time due to the short half-life of the TB drugs.

At enrolment and follow-up visits, demographic, clinical, and treatment adherence data were collected. Adherence to ART was determined by means of a monthly pill count. Blood samples for trough EFV plasma concentrations were obtained at the end of months 1, 2, and 3 during tuberculosis treatment and at the same time points after tuberculosis treatment was successfully completed. The timing of blood sampling in relation to EFV and tuberculosis treatment dosing was recorded. Samples were drawn a median of 20.3 h post-dose (IQR 14.8–25.2 h). Blood was collected in heparinized tubes, which were stored on ice and separated at 3,000 rpm within 1 h. Samples were then stored at −70°C until analysis. Samples were analyzed in the Division of Clinical Pharmacology, University of Cape Town. Plasma EFV concentrations were determined using a modification of a method by Chi et al. [14] based on liquid chromatography/tandem mass spectrometry; the accuracy ranged from 97.2 to 105.6%. Intraday and interday precisions ranged from 1.3 to 4.6%. The lower limit of quantitation (LLOQ) for EFV concentrations was 0.2 mg/L. In five samples where the concentration was below the LLOQ, the actual concentration was recorded for these observations and used in the analysis. These concentrations represent less than 3% of the data and were determined to be unlikely to influence the final results.

Demographic and clinical data were analyzed using SAS ver. 9.1 (SAS Institute, Cary, NC). Baseline characteristics were compared using the Mann–Whitney, Fisher's Exact or

Student's *t* test, as appropriate. A type 1 error (α) of 0.05 was used to reject the null hypothesis.

NONMEM (ver. VI 2.0), with the first-order conditional estimation method and interaction option, was used [15]. A one-compartment model with first-order absorption and elimination was used to describe the data. The model was parameterized in terms of absorption half-life, clearance, and volume of distribution. The apparent clearance (CL/F) after oral administration, where F is the bioavailability, was estimated. As there were no EFV plasma concentrations in the absorption phase, the absorption half-life was fixed to 1 h. Because EFV has a long elimination half-life and measurements were made at steady state, there was no reliable information on the volume of distribution (V/F). Therefore, the V/F was fixed at 267 L/70 kg, as derived from Csajka et al. ([16]. The population parameter values were standardized for a body weight of 70 kg using allometric scaling [$CL/F = CL/F_{POP} \times (weight/70)^{0.75}$, $V/F = V/F_{POP} \times (weight/70)$] [17].

Sample times were classified as "occasions" (OCCs), where OCCs 1, 2, and 3 represented EFV concentrations sampled at the end of months 1, 2, and 3, respectively, when EFV and tuberculosis treatment were co-administered in the integrated arm. OCCs 4, 5, and 6 represented EFV concentrations sampled once a month in each of the first 3 months after the completion of tuberculosis treatment in both the integrated and sequential arms. The seemingly random variability (between and within subjects) was modeled as the exponent of the random effects, as pharmacokinetic parameters resemble a log normal distribution. Random residual variability was described using a combined proportional and additive error model. Using a step-wise model building process and subsequent backward deletion, we identified covariates which had a significant influence on CL/F. Mixture models with two and three sub-populations for the distribution of CL/F were evaluated. The statistical comparison of models was based on the difference in the value of the minimum objective function (ΔOBJ). ΔOBJ values are approximately χ^2 distributed, with degrees of freedom equal to the difference in the number of parameters between models. A ΔOBJ greater than minus twofold the log-likelihood of the data was considered to be significant, e.g. ΔOBJ of 3.84, α=0.05, 1*df*. The final model was evaluated using non-parametric bootstrapping and visual predictive checks.

The START study and this pharmacokinetic sub-study were approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (E183/04), South African Medicines Control Council (20040969) and were registered at ClinicalTrials.gov (NCT00091936). Written informed consent was obtained from each study participant.

Results

A total of 58 patients, randomized equally to either the integrated or sequential treatment arm, were included in the START pharmacokinetic sub-study. There were no significant differences between the groups at baseline (Table 1). Twenty-five patients received EFV 800 mg during the integrated treatment. Mean monthly adherence to ART in the integrated arm was 93.4% during and 87.1% after the tuberculosis treatment. In the sequential arm, mean monthly adherence to ART was 95.0%. There were 209 EFV plasma concentrations available for analysis across six time points, 83 having been drawn during concomitant EFV and tuberculosis treatment. Of the 29 patients in the integrated arm, 16 provided data on EFV concentrations at all six time points, four were missing data at one time point during tuberculosis treatment, seven were missing data at one time point after tuberculosis treatment, and four were unable to provide EFV data for any of the time points after tuberculosis treatment. In the sequential arm, 16 patients provided EFV data at three time points, six provided data at two time points, three provided data at one time point, and a further three patients were unable to provide EFV data at any of the time points. For one participant in the sequential arm, all three time points were classified as BLD (below the limit of detection). In total, there were six samples where the EFV concentration was BLD. During and after TB treatment, 55 and 62% of patients, respectively, had EFV concentrations in the recommended range of 1–4 mg/L, There were no serious EFV-related adverse effects, in any patient with elevated EFV levels.

Of the available covariates investigated, sex, age, serum transaminase levels, monthly EFV adherence, baseline CD4 cell count, and baseline viral load did not have a statistically significant effect on EFV concentration. There was a large improvement in the fit of the model when between-occasion variability in CL/F was included. A further improvement in model fit was achieved by assuming a bimodal distribution in CL/F, dividing patients into slow and fast metabolizers using a mixture model. There was no evidence for a third sub-population in the CL/F distribution. The only covariate that further improved the fit of the model was the use of tuberculosis treatment. Stepwise backwards deletion (Table 2) confirmed the role of the covariates in the final model.

Table 3 shows the final parameter estimates from the original data and the non-parametric bootstrap analysis (n=1,000). The bootstrap estimates are preferred because they will be less influenced by outliers. As can be seen, 23.6% of the population were slow metabolizers with a CL/F that was 36.4% that of the reference group (fast

Table 1 Patient baseline characteristics

Variable	Integrated arm (n=29)	Sequential arm (n=29)	P value
Median age in years (range)	32 (19–54)	32 (21–55)	0.45[a]
Females (%)	10 (34%)	15 (54%)	0.29[b]
Mean weight in kg (SD)	56.3 (7.8)	58.3 (9.9)	0.39[a]
Median body mass index in kg/m^2 (range)	21.0 (16.9-28.2)	22.0 (16.3–33.6)	0.42[c]
Mean baseline CD4 count in cells/μL (SD)	281 (178)	276.2 (128)	0.71[c]
Median baseline viral load in copies/mL (range)	49200(503–843000)	419,00 (685–1,750,000)	0.62[c]

SD, Standard deviation

[a] Students t test

[b] Fishers exact test

[c] Mann–Whitney test

metabolizers). The final model estimate for CL/F was 12.9 L/h/70 kg (95% confidence interval 11.1–15.2) in fast metabolizers not on tuberculosis treatment. Concomitant tuberculosis treatment reduced the overall EFV CL/F to 70.5% of the value without treatment. Based on these estimates, the CL/F for fast metabolizers and slow metabolizers on tuberculosis treatment is 9.1 and 3.3 L/h/70 kg, respectively. For slow metabolizers not on tuberculosis treatment, the CL/F was 4.7 L/h/70 kg. There was more between-occasion variability in relative bioavailability (43.5%) than between-subject variability (19.5%) in EFV CL/F. Residual error in the final model was 11.9% proportional and 0.47 mg/L additive error. The visual predictive check of the final model, as shown in Fig. 1, confirmed the adequacy of the model predictions.

Discussion

Although concomitant tuberculosis treatment was expected to decrease EFV exposure by increasing apparent clearance, the results of this study show that concomitant tuberculosis treatment actually reduced EFV CL/F by 29.5%. The initial expectation was based on the known ability of rifampicin to induce CYP2B6 and CYP3A4 and hence to increase the clearance of drugs that are substrates of these isoenzymes. This has been borne out in a number of studies. For example, in a group of 16 Italian patients, the mean CL/F of EFV was found to be significantly higher in the presence of concomitant tuberculosis treatment [11]. In a group of 24 Spanish patients, median peak and trough concentrations of EFV decreased by 24 and 18%, respectively, when tuberculosis treatment was co-administered [10]. In addition, a study of 19 Indian patients found EFV CL/F to be slightly higher in the presence of tuberculosis treatment [18]. As a result, various authors have advocated increased doses of EFV in the presence of tuberculosis treatment [19, 20]. Most recently, in silico prediction of the EFV–R interaction utilizing model input data from the literature (including weight and CYP2B6 phenotype) revealed 50 kg as the preferred weight cutoff for EFV dose increment [21].

In contrast, there are reports that have challenged this view. In a group of 20 black patients from South Africa, despite wide inter-patient variability, the geometric mean plasma concentrations of EFV were similar, whether or not rifampicin was co-administered [13]. Two other South African studies have also found that tuberculosis treatment was not an important determinant of EFV plasma concentrations [22, 23].

Table 2 Model characteristics showing stepwise backwards deletion

Model	Covariates included in the model	Objective function (OBJ)	Change in objective function (ΔOBJ)
1	Allometric scaling, between-occasion variability (BOV), tuberculosis treatment, bimodal metabolic capacity (fast and slow metabolizers)	439.68	-
2	Allometric scaling, BOV, tuberculosis treatment	451.94	12.26
3	Allometric scaling, BOV, bimodal metabolic capacity (fast and slow metabolizers)	479.72	40.04
4	Allometric scaling, tuberculosis treatment, bimodal metabolic capacity (fast and slow metabolizers)	1354.49	914.91

Table 3 Efavirenz original and bootstrap parameter estimates of the final model (n=1,000)

Parameter	Original estimate	Asymptotic RSE	Bootstrap average	Bootstrap RSE	Bootstrap percentile	
					2.5	97.5
Fraction with slow clearance of EFV	0.227	32.9%	0.236	33.6%	0.105	0.413
Ratio of CL/F in slow metabolizers to CL/F in fast metabolizers	0.365	11.3%	0.364	13.4%	0.291	0.464
CL/F for EFV (L/h/70 kg)	12.8	7.50%	12.9	8.05%	11.1	15.2
Ratio of CL/F when taking TB treatment to CL/F without TB treatment	0.705	9.20%	0.705	9.6%	0.574	0.836
Proportional residual error	0.120	31.8%	0.119	43.0%	0.028	0.188
Additive residual error (mg/L)	0.462	26.8%	0.466	28.8%	0.221	0.755
Between-subject variability (CL/F)	0.215	36.5%	0.197	23.8%	0.091	0.278
Between-occasion variability in F	0.453	26.6%	0.435	19.5%	0.280	0.562

EFV, Efavirenz; CL/F, apparent clearance, where F is the bioavailability; TB, tuberculosis; RSE, relative standard error

Indirectly supporting our finding of a decreased clearance is a case series describing nine patients, seven of whom developed toxicity and required an EFV dose reduction in the presence of tuberculosis treatment [12]. Of the nine patients described, eight were black patients of African origin. Data from the Liverpool Therapeutic Drug Monitoring registry also showed that the co-administration of rifampicin and being black African were important factors influencing EFV concentrations [24]. Without prior adjustment for weight and ethnicity, EFV concentrations were 48% higher when rifampicin was co-administered. It should be noted that the earlier studies, showing an increase in EFV clearance associated with rifampicin co-administration, were all conducted in Caucasian patients [10, 11, 18]

Higher EFV plasma concentrations in black Africans or those of African descent have been associated with the presence of a single nucleotide polymorphism, namely, CYP2B6 516 G > T [25, 26] and CYP2B6c.983 T > C [27, 28]. In 74 Zimbabwean patients receiving no tuberculosis treatment, EFV CL/F could be predicted by genotype, with three groups of varying metabolic capacity [29]. More recently, CYP2A6 has also been shown to independently predict EFV concentrations, a finding that requires further study [30]. Although genotype was not used as a covariate in the present model, the separation of patients into "fast" and "slow" metabolizers contributed to improving the fit of the data. The estimate that 23.6% of the population studied were slow metabolizers was similar to the slow metabolizer genotype prevalence reported in other studies in black African patients [23, 25, 29, 31]. Our model showed that the capacity of the slow metabolizers was 36.4% that of the fast metabolizers.

A Zimbabwean and West African study reported CL/F values of 9.4 and 9.9 and 4.0 and 2.1 L/h in fast and slow metabolizers, respectively [25, 29]. Our population estimate of EFV CL/F was 12.9 L/h standardized for size 70 kg in the fast metabolizers not on tuberculosis treatment. This is similar to previously reported CL/F estimates in a series of cohorts which have ranged from 9.4 to 11.7 L/h but without weight scaling. Because this value is for fast metabolizers, it is expected to be higher than other reported values which did not distinguish these groups [16, 32-34].

In conclusion, this study demonstrated that, by reducing clearance, concomitant tuberculosis treatment increased EFV exposure in black African patients.

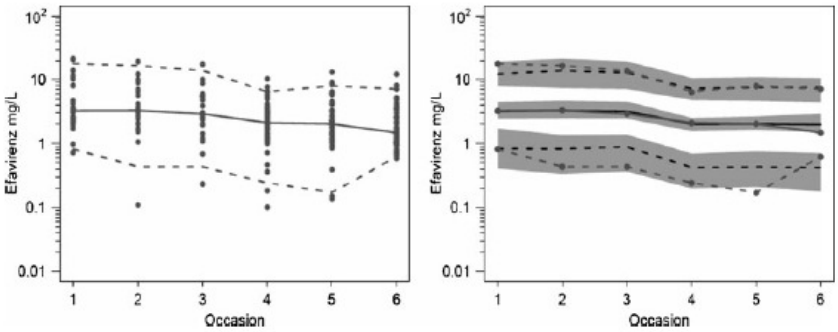

Fig. 1 Visual predictive check of model for efavirenz concentrations. **Right panel** 5, 50, and 95 percentiles of observations (*gray lines with symbols*) and predictions (*black lines*). *Gray shading* is 95% confidence interval for 5 and 95 percentile of predictions

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2.2.1 Discussion of paper II

Paper II is responsive to Objective 2 of the PhD thesis. The hypothesis driving this analysis was based on the premise that concomitant RIF based TB treatment would be expected to increase EFV clearance. Given that weight was an important determinant of EFV concentrations, the higher EFV 800mg dose was administered to all patients over 50 kg while on concomitant TB treatment, otherwise patients received EFV 600mg (integrated arm). After TB treatment completion all patients received EFV 600mg, irrespective of randomization arm. Trough EFV concentrations were measured on six occasions: at the end of months 1, 2 and 3 during TB treatment and at the end of month's 1, 2 and 3 after TB treatment completion. In total, 209 EFV steady state concentrations were sampled from 58 patients, 83 of which were collected in the presence of TB treatment. The data were then analyzed using NONMEM and the final model was suitably validated.

Unexpectedly, there was an overall 29.5% reduction in EFV clearance during TB treatment. The data also showed a bimodal distribution in apparent EFV clearance, alluding to the presence of slow and fast metabolisers in this population, with distinctly different clearance estimates both during and after TB treatment. The model showed that the capacity of the slow metabolizers was 36% that of the fast metabolisers.

To our knowledge, this was one of the first studies to model the impact of TB treatment on EFV apparent clearance (CL/F) in Black African patients exposed to the higher EFV 800mg dose. This study effectively demonstrates that the direction of the drug interaction is not as originally assumed in this population of patients, making the dose increase during TB treatment questionable.

The next step in the investigations was to assess polymorphisms, in CYP450 and other enzymes involved in EFV metabolism, to better explain these findings.

2.2.2 PhD candidates' contribution to the journal article

Student name: Tanuja N. Gengiah

Student number: 993241124

Title of the article: The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis

Authors: **TN Gengiah**, NHG Holford, JH Botha, AL Gray, K Naidoo, SS Abdool Karim

Journal: European Journal of Clinical Pharmacology

Doctoral student's contribution to the journal article:

1. Formulation of the hypothesis

I contributed to the formulation of the study hypothesis in conjunction the START study principal investigator. I wrote the PK study into the main study protocol as a secondary objective.

2. Study design

I designed the pharmacokinetic study with regards to the appropriate timing of the blood draws, and determined appropriate collection and storage of samples for assay after the study was completed. I wrote the PK section of the START protocol.

3. Work involved in the study

I was involved in the dispensing of the drug treatment and collecting patient data on case report forms, which were specifically designed for the PK study. This included verifying the recording of the timing of the EFV and RIF dose, the timing of the blood collection and documenting concomitant medication use for three days prior to the blood draw.

4. Data analysis

I extracted the data required for the NONMEM analysis from the main study database and sorted the data into the appropriate time ordered format. I assessed the literature and provided suitable EFV PK parameters for the base model. Prof Holford set up an initial basic programme to build an appropriate model. I worked with Prof Holford to refine the model and this collaboration resulted in the discovery of the final mixture model. Using a stepwise model-building process, I tested covariates of interest under Prof Holford's supervision. Once the model-building process was completed, Prof Holford evaluated the final model using non-parametric bootstrapping and generated the visual predictive checks.

5. Write up

I took overall responsibility for the writing of the manuscript and after completion of the first full draft of the manuscript submitted the manuscript to the co-authors for review and comment. All co-authors read and approved the final version of the manuscript. I corresponded with the journal and after peer review I completed the required revisions. The final version for publication was approved by all co-authors.

I declare this to be a true reflection of my contributions to this journal article

Signature  Date 14 November 2014

PAPER III

Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent TB treatment

2.3 Paper III: Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent TB treatment

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Antiviral Therapy

Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent tuberculosis treatment

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Original article

Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent tuberculosis treatment

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Abstract

Background: Rifampicin-based tuberculosis (TB) treatment alters efavirenz (EFV) clearance. Polymorphisms in important drug metabolizing enzymes and the implications for EFV dosing were investigated.

Methods: Trough (C_{min}) EFV concentrations were measured in 54 South African black patients. During TB treatment, EFV dose was 600mg in patients<50kg or 800mg if ≥50kg. Off TB treatment it was 600mg. Polymorphisms in CYP2B6, CYP2A6 and UGT2B7 enzymes were sequenced. A multivariate generalized estimating equations model was fitted to assess predictors of high median EFV C_{min}.

Results: During TB treatment, median EFV C_{min} was 3.2 (IQR: 2.6-6.3) µg/mL and 3.3 (2.4-9.5) µg/mL in the 800mg and 600mg groups respectively. After TB treatment EFV C_{min} was 2.0 (1.4 - 3.5) µg/mL. Minor allele frequencies for CYP2B6 516G→T, 785A→G, 983T→C, UGT2B7-372G→A, CYP2A6*9B and CYP2A6*17 were 0.31, 0.33, 0.23, 0.29, 0.10 and 0.02 respectively. Haplotypes CYP2B6*6 and CYP2B6*18 were found in 38.9% and 25.9% of patients respectively. Polymorphisms in all three CYP2B6 genes studied (516T-785G-983C) were present in 11.1% of patients and in this group median EFV C_{min} was 19.2 (IQR: 9.5-20) µg/mL during and 4.7 (IQR: 3.5-5.6) µg/mL after TB treatment. The presence of TB treatment and composite genotypes CYP2B6 516 GT/TT, CYP2B6 983 TC/CC and CYP2A6*9B carrier status predicted median EFV C_{min} > 4µg/mL. Adverse events due to high EFV concentrations were rare.

Conclusions: Because polymorphisms of EFV metabolizing enzymes are frequent and are associated with elevated EFV concentrations in this population, EFV dose increases are unnecessary when concomitant rifampicin-containing TB treatment is prescribed.

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Running head: Efavirenz dosing: pharmacogenetics and tuberculosis treatment

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Introduction

Concerns about an efavirenz-rifampicin (EFV-RIF) drug-drug interaction led the United States Food and Drug Administration (FDA) to approve an increase in EFV dose from 600mg to 800mg in patients weighing more than 50kg on RIF-based TB treatment [1]. The same dose increment was recommended by the British HIV Association (BHIVA) for patients above 60kg, with concomitant therapeutic drug monitoring (TDM) recommended after two weeks of treatment [2]. Evidence that RIF reduced area under the concentration time curve (AUC) of EFV had been described previously, and the 22% reduction at standard doses was corrected if EFV dose was increased to 800mg [3,4]. In contrast, more recent studies have demonstrated that an EFV dose increase may not be necessary during TB co-treatment [5] and in some instances may actually be associated with adverse events particularly in non-Caucasian patients [6,7].

There is considerable inter-patient variability in EFV metabolism and this can be attributed to genetic variation and racial differences in polymorphic frequencies found in crucial drug metabolizing enzymes. EFV is metabolized to 8-hydroxyefavirenz predominately by CYP2B6 and, to a lesser extent, by CYP3A4 [8,9]. Subsequently, UGT2B7 is directly involved with glucuronidation of EFV and its hydroxylated metabolite [10]. In addition, *in vivo* evidence has emerged that CYP2A6 also plays a role in EFV metabolism, particularly in Black patients [11,12] and this is supported by *in vitro* data which suggest the CYP2A6 accounts for 22.5% of EFV metabolism [13]. Therefore, when CYP2B6 metabolism is impaired, it could be expected that genetic variations in secondary metabolic pathways would become significant for EFV metabolism.

Having previously reported a 29.5% reduction in EFV clearance during RIF-based TB treatment [14], we now explore the influence of important drug metabolizing enzyme genotypes in CYP2B6, CYP2A6 and UGT2B7 on EFV concentrations, and report on the use of EFV 800mg in African patients on concomitant TB treatment.

Methods

Patients and study procedures:

As described in detail previously [14], adult patients were enrolled in the open label 'Starting Tuberculosis and Antiretroviral Therapy' (START) clinical trial (CAPRISA 001: NCT00091936) and were randomized to receive integrated HIV and TB treatment or sequential HIV treatment after TB treatment was successfully completed. The primary objective of that trial was to the assess the effectiveness of integrated TB and HIV care provision through a directly observed treatment (DOT) program versus sequential treatment of TB and HIV, by comparing progression to AIDS-defining illnesses/mortality during the first 18 months after enrollment in the study. Approximately 592 patients were to be enrolled.

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However, due to slow patient recruitment because of the daily weekday DOT requirement, only 58 patients were enrolled. The trial was therefore halted earlier than planned but sufficient follow-up time was allowed in order to meet the pharmacokinetic (PK) objectives. .

During RIF-based TB treatment, integrated arm patients weighing <50kg and ≥ 50kg received directly observed antiretroviral treatment comprising either 600mg or 800mg EFV respectively. After completion of TB treatment patients in both arms received EFV 600mg. Antiretroviral therapy (ART) comprised once daily EFV, lamivudine and weight appropriate enteric coated didanosine. Adherence to ART was assessed by a monthly reconciliation of the number of pills dispensed versus those returned. Although antiretroviral therapy was continued for a minimum of three months post TB treatment, many patients were not in follow up long enough to assess long term virological outcomes.

For the PK study and the current pharmacogenetic analysis, single steady state trough EFV (C_{min}) concentrations were sampled on 6 occasions at weeks 4, 8 and 12, both during and after TB treatment in the integrated arm patients, as well as after the initiation of ART in the sequential arm (3 occasions). Plasma samples were assayed retrospectively by liquid chromatography/tandem mass spectrometry, using a previously described methodology. The lower limit of quantitation of the assay was 0.2mg/L [14]. The accepted reference range for therapeutic EFV concentrations is 1-4 µg/mL [15], with concentrations less than 1 µg/mL being associated with treatment failure, and those above 4 µg/mL with toxicity [15].

Genotyping of single nucleotide polymorphisms

DNA for genotyping was extracted from stored peripheral blood monoleukocytes using the MagNa Pure LC DNA isolation kit I (Version 17.0, Roche Diagnostics®, Mannheim, Germany). All participants were genotyped for the following genetic variants: CYP2B6 516G→T, CYP2B6 785A→G, CYP 2B6 983T→C, CYP2A6*9B, CYP2A6*17 and UGT2B7 -372G→A. Allelic discrimination reactions were performed in duplicate or triplicate (if duplicate results were discordant) using TaqMan (Applied Biosystems, CA, USA) commercial genotyping assays in accordance with manufacturer's instructions for the following kits: CYP 2B6 516G→T (C__7817765_60, rs3745274), CYP2B6 785A→G (custom designed 40X, catalogue #1151580, rs2279343), CYP2B6 983T→C (C_60732328_20, rs28399499), CYP2A6*9B g.1836 G→T(C_29560333_20, rs8192726), CYP2A6*17 g.5065G→A, c.1093G→A (C_34816076_20, rs28399454) and UGT2B7 -372G→A (C_30720663_20, rs7662029) on the Roche LightCycler® 480 platform (Roche Diagnostics, Mannheim, Germany). The final volume for each reaction was 25µl, consisting of TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 20X drug metabolising genotype assay mix and 10ng genomic DNA. The thermal cycler conditions were as follows: initial step, 95°C for 10 minutes, then denature for 50 cycles at 92°C for 15 seconds and anneal/extend at 60°C for 90 seconds. For the custom designed 40X genotype assay the thermal cycler condition consisted of an initial step 95°C for 10 minutes, denature for 40 cycles at 92°C for 15 seconds and then anneal/extend at 60°C for 60 seconds. The designation of CYP2B6 haplotypes was determined using the Human CYP Allele Nomenclature Committee database (http://www.cypalleles.ki.se/). The frequency of the following previously reported haplotypes were

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assessed: *CYP2B6 *1* (516G-785A-983T), *CYP2B6 *4* (516G-785G-983T), *CYP2B6* *6 (516T-785G-983T), *CYP2B6 *9* (516T-785A-983T), *CYP2B6 *16* (516G-785G-983C), *CYP2B6 *18* (516G-785A-983C).

Adverse event monitoring

Study participants were closely monitored for signs and symptoms of drug toxicity possibly related to the antiretroviral therapy and anti-TB medications. These were graded using the 2004 *Division of AIDS (DAIDS) Table for Grading Severity of Adult and Pediatric Adverse Events* toxicity table and managed appropriately. All adverse events, irrespective of grade, were evaluated by a study physician and its relatedness to study treatment assessed. Grade 3 and 4 clinical and laboratory toxicities were captured in the study database. Central nervous system (CNS) adverse events and abnormal liver function tests were reviewed to assess association with EFV concentrations.

Statistical considerations

Baseline characteristics were compared using Mann Whitney, Fisher's Exact or Student's t test, as appropriate. EFV C_{min} concentrations on and off TB treatment were averaged for each participant and these values were used to derive the median (interquartile range) concentrations. In the integrated arm, paired concentration data comparing EFV C_{min} on and off TB treatment was tested by the Wilcoxon signed-rank sum test. The CYP2B6, CYP2A6*9B polymorphisms were tested and found to be in Hardy Weinberg equilibrium. UGT2B7-372 G→A was not in equilibrium and the Hardy-Weinberg assessment for CYP2A6*17 was therefore not possible as no homozygous variants were detected. Linkage disequilibrium was assessed for CYP2B6 and CYP2A6 genotypes located on chromosome 19 using validated bioinformatics software, Haploview (version 4.2, 29 April 2008), downloaded from http://www.broadinstitute.org/haploview.

The distribution of EFV C_{min} concentrations was examined in relation to genotype and EFV 600mg or 800mg dose whilst on and off TB treatment. Prior modeling of the EFV concentration data had indicated a bimodal distribution in EFV clearance suggesting the presence of slow and fast metabolizers in this study population [14]. Accordingly, patients who were either heterozygous or homozygous for the variant alleles were grouped together and their EFV concentrations were then compared with concentrations from wild type patients.

A multivariate generalized estimating equations (GEE) model, with a binary outcome, was fitted to assess variables associated with high EFV concentrations (>4 μg/mL). The multivariate model was adjusted for randomization arm, age, gender, presence of TB treatment (which also accounts for weight and EFV dose) and polymorphisms in drug metabolizing enzymes. There was strong correlation between the single nucleotide polymorphism (SNP) and haplotype for the CYP2B6*6 haplotype consisting of 516G→T and the 785 A→G variant allele. Because prior evidence indicated that the 516GT SNP was mostly responsible for the severely decreased function and expression associated with CYP2B6*6 [16], 785A→G genotypes were excluded from the multivariate GEE model. The genotypic polymorphisms were then coded for CYP2B6 516 G→T, CYP2B6 983T→C and CYP2A6*9B with the wild type being labelled as the reference group that was compared to the

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composite of the variant alleles. For UGT2B7-372G→A the two variant carrier alleles were compared because there was only 1 wild type individual genotyped for both groups, prohibiting the model from converging when the wild type individual was included.

All tests were two sided with a type 1 error (α= 0.05) used to reject the null hypothesis. Data analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Regulatory oversight

Regulatory oversight for this trial was provided by the University of KwaZulu-Natal Biomedical Research Ethics Committee (E116/04, BE041/11) and the South African Medicines Control Council. Patients provided written informed consent for study procedures and long term sample storage.

Results

Study population characteristics

Fifty four of the 58 enrolled patients contributed 209 EFV plasma concentrations. There were four sequential arm patients who did not contribute EFV concentrations because three of them died before ART initiation and for one patient all concentrations measured were below the limit of detection. No differences in baseline patient demographic and clinical characteristics were observed by randomization arm in the 54 patients (Table 1).

EFV concentrations on and off TB treatment

Blood was drawn a median of 20.3 hours post-dose (IQR: 14.8 -25.2). Of the 209 EFV trough concentrations quantified, 126 concentrations were from 51 patients sampled after TB treatment and 83 concentrations were from 29 patients on concomitant TB treatment. Median (IQR) EFV C_{min} was 3.1 (2.6 - 4.8) µg/mL and 2.0 (1.4 - 3.5) µg/mL, on and off TB treatment respectively. For patients receiving EFV 800mg during and 600mg after TB treatment (n=23) concentrations were 3.2 (2.6-6.3) and 2.0 (1.3-2.1) µg/mL respectively, p=0.002 while for those on EFV 600mg throughout (n=6), median EFV C_{min} was 3.3 (2.4-9.5) µg/mL on and 3.9 (1.5-5.6) µg/mL, p=1.0 off TB treatment. Individual concentrations are depicted in Figure 1.

The proportion of patients with median EFV C_{min} below 1 µg/mL was 10.3% when on TB treatment and 15.1% when off TB treatment. The proportion of patients with median EFV C_{min} above 4 µg/mL was 34.4% when on TB treatment and 11.3% when off TB treatment. There were 18 patients (33.3%) who had at least one individual concentration < 1 µg/mL resulting in a total of 26 (12.4%) concentrations that were sub-therapeutic. Of these, six (23.1%) concentrations were measured during TB treatment. For the 18 patients with sub-therapeutic concentrations, 39% had suboptimal adherence based on pill count, with adherence estimates ranging from 35 to 96%. However, all 18 patients demonstrated adequate viral load suppression (viral load <50 copies/mL) after receiving three months of ART. There were 53 (25.3%) concentrations above 4 µg/mL and these were

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contributed by 24 (44.4%) patients. Of these concentrations, 29 (54.7%) were measured during TB treatment and of these 24 concentrations (83%) were from the 800mg dose.

Outcome of genotyping, relation of EFV concentration on and off TB treatment to genotype and haplotype frequency

As shown in Table 2, the minor allele frequency (MAF) for CYP2B6 single nucleotide polymorphisms was 0.31 for 516G→T, 0.33 for 785A→G and 0.23 for 983T→C. Those for UGT2B7 -372G→A, CYP2A6*9B and CYP2A6*17 were 0.29, 0.1 and 0.02 respectively. During TB treatment heterozygous and especially homozygous variant carriers of CYP2B6 genes had higher median EFV C_{min} concentrations than wild type patients. In fact, there was a 1.5 fold increase when both were considered together. In contrast when off TB treatment median EFV C_{min} concentrations were similar between genotypes for 516GT and 785AG. However, for 983TC there was a marked increase over wild type both on and off TB treatment. All individual concentrations are shown in Figures 2a-f.

When the multivariate GEE model for predictors of EFV median concentrations >4µg/mL was fitted (Table 3), concurrent TB treatment and composite mutant genotypes of each of CYP2B6 516 GT/TT, CYP2B6 983 TC/CC and CYP2A6*9B were associated with high EFV concentrations.

Patients were categorized into 5 major haplotypes for the CYP2B6 gene with the most common being CYP2B6*6 (38.9%) followed by CYP2B6*18 (25.9%) (Table 2). Six patients (11.1%) had homozygous variant allele mutations in all three of the CYP2B6 genes studied (516T-785G-983C). In this group, median (IQR) of all occasion EFV concentrations was 7.9 (4.2-11.1) µg/mL. Genotypes 516G→T and 785A→G were in linkage disequilibrium, r^2 = 0.88 (not depicted). Median concentrations for all haplotypes on and off TB treatment are shown in Table 2.

Adverse events and EFV exposure

There were a total of 50 adverse events reported in the study from 20 (7 integrated arm and 13 sequential arm) patients, accrued over 46.12 person-years of trial follow up. Only 18 of these were considered to possibly be related to EFV. Three CNS toxicity events (2 reports of headache and 1 of dizziness) and 15 liver function laboratory abnormalities (either grade 3 or 4) were reported in nine patients. Ten of the hepatic laboratory abnormality reports were most likely associated with TB treatment as no ART was present at the time of the adverse event. The remaining five asymptomatic hepatic laboratory abnormalities occurred during EFV based treatment. Four were during combined TB treatment and one report, from a sequential arm patient, was assessed to be due to alcohol abuse. Laboratory abnormalities, when EFV was present, resolved without drug cessation. The three CNS toxicity reports were from a single patient receiving EFV 800mg whose symptoms resolved after EFV was dosed at night instead of at the clinic during DOT. In the sample closest to the time of the report, his EFV C_{min} was 21µg/mL.

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Discussion

In this study population, median trough EFV concentrations were above 3 µg/mL in patients on TB treatment. This concentration was achieved regardless of whether the patients were prescribed 600mg or 800mg daily. During HIV-TB co-treatment, 24.1% of patients exhibited individual EFV concentrations that were more than twice the upper limit of the established therapeutic range. Furthermore 89.7% of EFV C_{min} measurements were >1 µg/mL. High EFV levels (>4 µg/mL) were predicted by composite mutant genotypes of each of CYP2B6 516 GT/TT, CYP2B6 983 TC/CC and CYP2A6*9B as well as by RIF-based TB treatment.

Emerging evidence indicates that EFV exposure is potentially influenced by both genetic polymorphisms and TB co-treatment, although reports in this regard are inconsistent. Some researchers have found genetic polymorphisms rather than TB treatment to be responsible [11,17,18], while others show TB treatment contributed a small effect [19,20]. Some, with findings similar to ours, have demonstrated evidence of a combined effect [21–24] and most of these have been in black, Africans [21,22,24].

When examining individual SNPs of the highly polymorphic CYP2B6 gene our MAF was 31% for 516 G→T, consistent with the 29-50% frequency previously reported in black Africans [25]. The fact that exposure to high EFV concentrations was most likely in 516 TT carriers, followed by the heterozygous 516 GT carriers is consistent with other reports although these were from different race groups [17–21,24,26–31]. In other South African patients 516 G→T polymorphisms have also been shown to be predictive of EFV concentrations >4µg/mL [32].

Interestingly, our MAF was 23% for CYP2B6 983T→C, where others have shown much lower frequencies ranging from 7-18.7% [11,29,33–35]. Although this SNP is more frequent in blacks, and rare in other race groups [29], variant allele homozygosity has been reported to be uncommon with frequencies ranging between 0.1-1.7% in blacks while our study showed a 5.6% prevalence. This SNP has also been associated with higher EFV exposure in the presence of TB treatment in black Africans [22,35] and in the absence of TB treatment in both Africans and Caucasians [33,36–38]

Combining these two SNPs, to create composite genotypes incorporating 516GT and 983TC, has previously been associated with slow EFV metabolizer status in both HIV positive patients and in healthy volunteers [22,35]. Genome-wide sequencing conducted in 856 white, Hispanic and black individuals found 983T→C and 516G→T to be independently associated with EFV C_{min} and they were accountable for 34% of the inter-individual variability of estimated EFV C_{min} [39].

Haplotypes CYP2B6*6 or CYP2B6*18 were evident in 38.9% and 25.9% of our patients respectively, while 18.6% were CYP2A6*9B carriers and 11.1% had homozygosity for all three CYP2B6 genes studied. Our CYP2B6*6 and CYP2B6*16 haplotype frequencies of 38.9% and 3.7% were similar to those reported for other African cohorts which ranged from 36-46.9% [21,34,37,40] and around 3-10% [21,36] respectively. However, our CYP2B6*18 frequency was double that of previous reports which have indicated 4-12% haplotype prevalence in Africans [25]. Predictably, the

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CYP2B6*6 and CYP2B6*18 haplotypes show a higher EFV exposure [27,32,37] in African patients. The novel haplotype (516T-785G-983C), of which we could find no previous reports, affected 11.1% of our patients and was associated with excessively high EFV concentrations particularly whilst on TB treatment.

The usefulness of CYP2A6 *9B and CYP2A6 *17 for predicting EFV exposure requires further study. In our predictive model, only CYP2A6 *9B carriers were associated with high EFV concentrations. One study of 65 African patients showed that carriers of both account for approximately 12% of variance in EFV concentration [11] whilst others have shown no effect on EFV concentrations [37,38,41]. As in other African cohorts, our UGT2B7 -372 G→A SNP was not predictive of slow metabolizer status and was of similar frequency [37,42].

Regarding TB treatment as a predictor of high EFV concentrations, a possible explanation was provided by the 'CAMELIA' trial involving the *NAT2* enzyme responsible for the metabolism of isoniazid (INH). In Asian patients on concomitant TB treatment, EFV apparent clearance (CL/F) was highly dependent on the *NAT2* polymorphism [23]. Patients who were both CYP2B6 516 TT and *NAT2* slow acetylators had the lowest EFV CL/F. When TB treatment stopped, EFV clearance increased, suggesting an INH concentration dependent inhibitory effect. These data may contribute to a better understanding of our results but need further testing in other race groups.

Race and TB treatment both influence EFV metabolism. Many reports have documented increased EFV concentrations in black patients receiving RIF-based TB treatment irrespective of the dose used [5,18,22,43]. In the 'STRIDE' trial [43] standard doses of EFV (600mg) were associated with higher median EFV levels in black patients when they were on TB treatment (2.08 µg/mL) compared to off TB treatment (1.75 µg/mL). Due to the high EFV dose received during TB treatment in our study, median EFV concentrations were even higher (3.1 µg/mL) compared to the off TB treatment median concentration of 2.0 µg/mL. In the 'CAMELIA' trial the corresponding on and off TB treatment concentrations were 2.79 and 2.77µg/mL in Asian patients [44].

Although the FDA and BHIVA recommend an increase in EFV dose in patients weighing more than 50kg on RIF-based TB treatment, this is not necessary in our patient population. Lower EFV concentrations are not always associated with virological failure [7,45] making blanket dose modification for higher weights during TB co-treatment, questionable. Indirect support for the 600mg dose also emerges from successful virological outcomes which have been repeatedly demonstrated [43,44,46,47].

The 800mg EFV dose on concomitant RIF-based TB treatment has shown unacceptably high rates of adverse events in some patients [6], but not in others [5,47]. The low EFV adverse event rate reported in our study is consistent with these latter reports. Although high EFV concentrations may be well tolerated by some patients, there is evidence for a longer elimination half-life of EFV in patients with genetic variants in 516 G→T [48], potentially increasing the risk for selection of resistance if ART is discontinued. Consideration therefore needs to be given to actually lowering the dose and evidence from the 'ENCORE1' study, in 630 adult patients, demonstrated that dropping the dose to 400mg was

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not inferior to 600mg, at week 48 of treatment for virological suppression rates; moreover the lower dose was associated with fewer adverse events [49].

There were some limitations to the present study. The sample size was small and only trough EFV concentrations where sampled. The dose of EFV was increased to 800mg in the integrated arm if patients weighed ≥50 kg. Given that the mean weight was 56.3 kg in the integrated arm, the majority of these patients would have received the 800mg dose during TB treatment resulting in few matched concentration pairs for the EFV 600mg dose. Although the 800mg EFV dose appeared to be well tolerated, the small sample size may have accounted for the limited number of adverse event reports. Furthermore, due to the premature discontinuation of the parent trial, it was not possible to assess long term viral suppression (12 months) in both arms and thus to relate EFV concentrations and genotype to long term viral suppression.

Conclusion

Elevated EFV concentrations were common in this study population, particularly in the presence of concomitant TB treatment. The high frequency of polymorphisms of EFV metabolizing enzymes was associated with decreased metabolism of the drug in this group. Although adverse events were infrequent, increasing EFV dose to 800mg daily during TB treatment is not necessary in these patients.

Acknowledgement statement

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Disclosure statement

All authors report no conflicts of interest.

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Figures

Figure 1: Trough EFV concentrations on and off TB treatment described by EFV dose received

Figure 2a-f: EFV concentrations on and off TB treatment by CYP2B6, UGT2B7, CYP2A6 genotype and EFV dose.

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Table 1: Baseline characteristics of the study participants

Variable	All patients (n=54)	(Integrated Arm) (n=29)	(Sequential Arm) (n=25)
Race: Black	53 (98.1%)	29 (100%)	24 (96.6%)
Median age in years (range)	32 (19 - 54)	32 (19 - 54)	32 (23 - 48)
Female gender	24 (44.4%)	10 (34.5%)	14 (56.0%)
Mean weight in kg (SD)	57.4 (9.1)	56.3 (7.8)	58.8 (10.3)
Median BMI (range) kg/m^2	21.6 (16.3 - 33.6)	21.0 (16.9 - 28.2)	22.1 (16.3 - 33.6)
Mean CD4 cells/µl (SD)	280.6 (155.4)	281.7 (178.0)	279.3 (127.9)
Median VL copies/ml(range)	47 100 (503 – 1 750 000)	49 200 (503 - 843 000)	45 000 (685 - 1 750 000)
Mean haemoglobin g/dL(SD)	10.2 (1.6)	10.5 (1.7)	9.9 (1.5)
Mean AST IU/L (SD)	30.7 (16.6)	31.1 (16.6)	30.3 (16.9)
Mean ALT IU/L (SD)	25.8 (25.9)	24.7 (18.4)	27.0 (34.3)
Mean Neutrophils x10^9/L (SD)	5.5 (2.2)	5.9 (2.2)	5.1 (2.1)
Hepatitis B surface antigen positive	5 (9.3)	3 (10.3)	2 (8.0)

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Table 2: Impact of CYP2B6, UGT2B7 and CYP2A6 polymorphisms and CYP2B6 haplotypes on trough EFV concentrations on and off TB treatment

Allele (minor allele frequency)	N=54 n (%)	On TB treatment Median (IQR) EFV concentration in µg/mL	#EFV concentration fold change on TB treatment	Off TB treatment Median (IQR) EFV concentration in µg/mL	#EFV concentration fold change off TB treatment
CYP2B6 516 G→T (0.31)					
GG	27 (50)	3.0 (2.6-3.4)		2.0 (1.3-2.5)	
GT	21 (38.9)	2.8 (1.7-9.5)		1.9 (1.5-3.5)	
TT	6 (11.1)	9.6 (6.7-11.0)		2.0 (1.5-4.9)	
GT/TT	27 (50)	4.5 (1.8-10.4)	1.5	2.0 (1.5-3.9)	0
CYP2B6 785 A→G (0.33)					
AA	24 (44.4)	2.9(2.5-3.3)		2.0 (1.3-2.5)	
AG	24 (44.4)	3.1(1.7-7.1)		1.9 (1.4-3.5)	
GG	6 (11.1)	9.6 (6.7-11.0)		2.0 (1.5-4.9)	
AG/GG	30 (55.6)	4.3 (2.3-9.9)	1.5	1.9 (1.4-4.0)	0.95
CYP2B6 983 T→C (0.23)					
TT	32 (59.3)	2.7 (1.8-4.5)		1.6 (1.3-2.1)	
TC	19 (35.2)	3.9 (3.1-10.8)		3.5 (1.6-4.0)	
CC	3 (5.6)	-		6.8(3.1-9.3)	
TC/CC	22(40.7)	4.0 (3.1-10.8)	1.5	3.6 (1.8-4.7)	2.3
UGT2B7 -372 G→A (0.29)					
GG	24 (44.4)	3.7 (2.6-9.5)		2.2(2.0-3.9)	
GA	29 (53.7)	2.9 (1.7-4.5)		1.5 (1.3-3.1)	
AA	1 (1.9)	-		1.3	
GA/AA	30 (55.6%)	3.0 (1.7-4.5)	0.81	1.5 (1.3-2.6)	0.68
CYP2A6*9B 1836G→T (0.10)					
GG	44 (81.5)	3.1 (2.4-6.9)		1.9 (1.4-3.5)	
GT	9 (16.7)	3.4(3.1-4.1)		2.1 (1.4-3.6)	
TT	1 (1.9)	-		1.3	
GT/TT	10 (18.5%)	3.4 (3.1-4.2)	1.1	2.1 (1.3-3.5)	1.1

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CYP2A6*17 1093G>A (0.02)					
GG	52 (96.3)	3.1(2.4-8.9)		1.9 (1.3-3.5)	
GA	2 (3.7)	3.7 (3.3-4.2)	1.2	3.1(2.2-3.9)	1.6
CYP2B6 haplotype	**N (%) Haplotype distribution in total population**		**n Median (IQR)EFV µg/mL on TB treatment**		**n Median (IQR) EFV µg/mL off TB treatment**
CYP2B6 *1 (516G-785A-983T)	10 (18.5%)		n=6 2.5 (2.3-2.6)		n=10 1.5 (1.0-2.1)
CYP2B6 *4 (516G-785G-983T	1 (1.9%)		n=1 3.4		n=1 1.0
CYP2B6 *6 (516T-785G-983T)	21 (38.9%)		n=12 3.5 (1.7-6.9)		n=18; 1.6 (1.4-2.2)
CYP2B6 *9 (516T-785A-983T)	0		-		-
CYP2B6 *16 (516G-785G-983C)	2 (3.7%)		–		n=2 2.6 (1.3-4.0)
CYP2B6 *18 (516G-785A-983C)	14 (25.9%)		n=7 3.3 (3.1-4.2)		n=12 2.2 (1.5-4.0)
CYP2B6*?? (516T-785G-983C)	6 (11.1%)		n=3 19.2 (9.5-20)		n=6 4.7(3.5-5.6)

#Composite allelic variant concentration compared to wild type concentration.

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Table 3: Multivariate generalized estimating equations (GEE) model, assessing associations with EFV concentrations >4 µg/mL

Variable (Reference)	OR	Lower 95% Confidence Limit	Upper 95% Confidence Limit	p-value
Integrated arm (sequential arm)	0.79	0.42	1.57	0.512
Male (female)	1.43	0.74	2.78	0.287
On TB treatment (off TB treatment)	2.4	1.48	3.95	0.0004**
Age (female)	1.00	0.97	1.04	0.854
CYP 2B6 516 G→T GT/TT (GG)	2.91	1.83	4.61	<0.0001**
CYP2B6 983 T→C TC/CC (TT)	4.29	2.64	7.02	<0.0001**
UGT2B7 -372 G→A GA/AA (GG)	0.93	0.57	1.52	0.783
*CYP2A6*9B 1836G→T* GT/TT(GG)	0.53	0.28	0.99	0.047**
CYP2A6 17 1093G>A* GA (GG)	0.54	0.14	2.04	0.359

OR= odds ratio. **p<0.05 statistically significant

Figure 1: Trough EFV concentrations on and off TB treatment described by EFV dose received

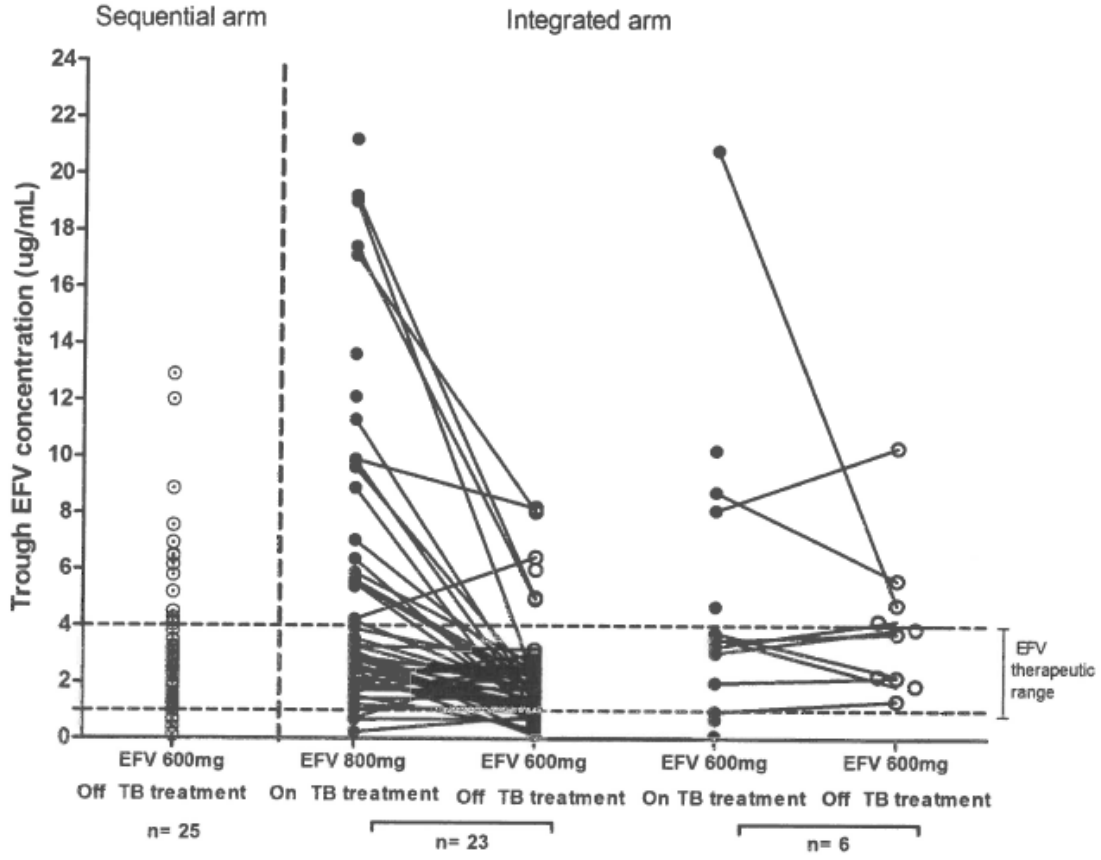
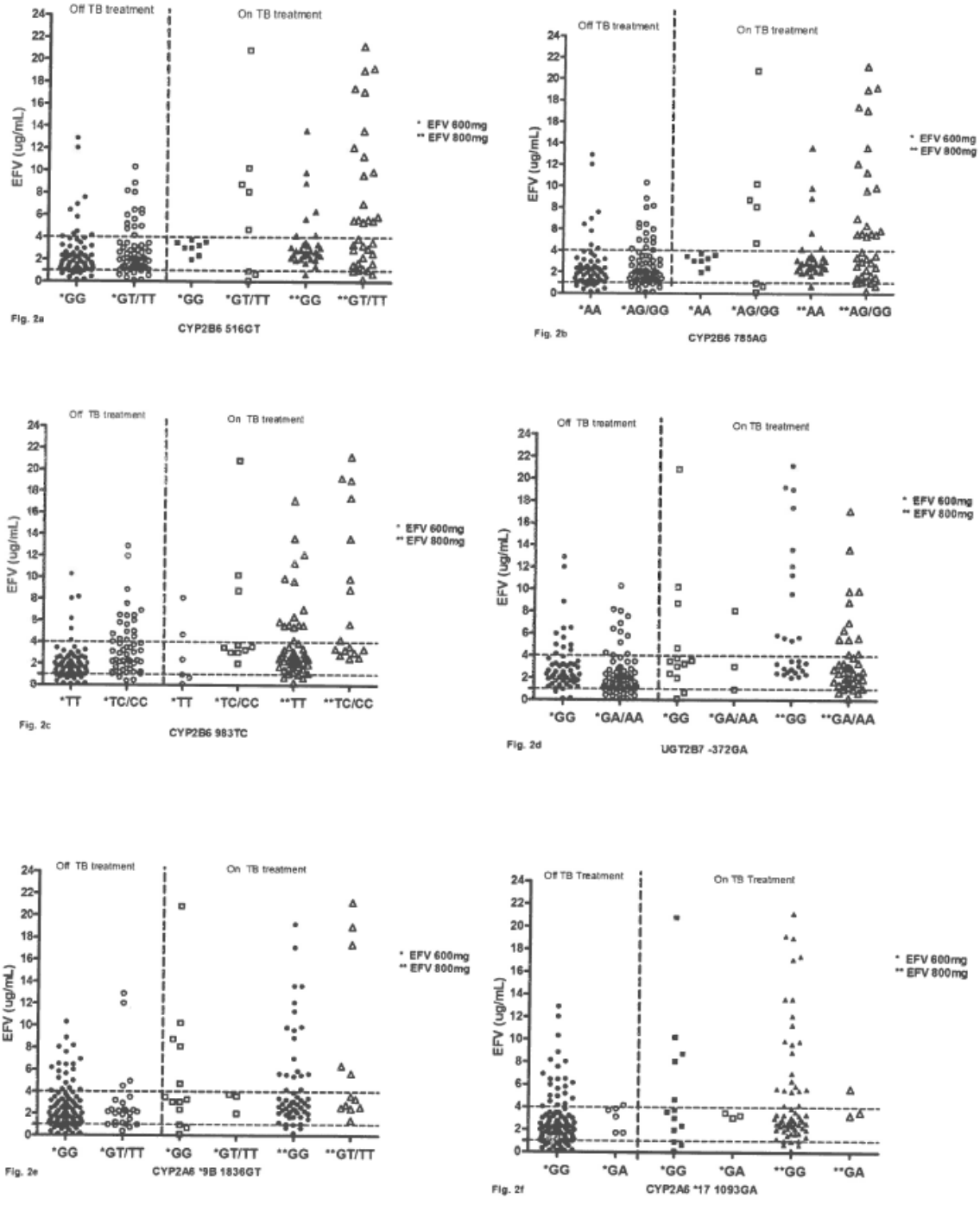


Figure 2a-f: EFV concentrations on and off TB treatment by CYP2B6, UGT2B7, CYP2A6 genotype and EFV dose.



2.3.1 Discussion of paper III

Paper III is responsive to Objective 3 of the PhD thesis. This analysis builds on the findings from Paper II by studying polymorphisms in important drug metabolizing enzymes in Black African patients, assessing the implications of these SNPs for EFV dosing and reporting on the tolerability of the higher dose. Although EFV is primarily metabolised by CYP2B6, both CYP2A6 and UGT2B7 also play a role. Therefore, polymorphisms in all three of these genes were sequenced and predictors of high median EFV C_{min} were assessed. The results from paper II indicated that the population had a bimodal distribution of clearance, with both fast and slow metabolisers evident, and this finding was investigated further in paper III.

During TB treatment, median EFV C_{min} was similar in the 800mg and 600mg groups. Minor allele frequencies (MAFs) were high for CYP2B6 516G→T, 785A→G, and 983T→C. Importantly, polymorphisms in all three CYP2B6 genes studied (516T-785G-983C) were present in 11.1% of patients and in this group, median EFV C_{min} was excessively high (19.2 (IQR: 9.5-20) µg/mL) during and high (4.7 (IQR: 3.5-5.6) µg/mL) after TB treatment. The predictors of high EFV concentrations were found to be: the presence of TB treatment, composite genotypes CYP2B6 516 GT/TT, CYP2B6 983 TC/CC and CYP2A6*9B carriers. High EFV concentrations appear to be well tolerated and adverse events related to the higher EFV concentrations were found to be rare.

This analysis provides further evidence that EFV dose increases are unnecessary when concomitant rifampicin-containing TB treatment is prescribed in Black, African patients. The evidence implicates the influence of TB treatment itself, as well high frequency of multiple polymorphisms in key enzymes, as being responsible for EFV metabolism and the high concentrations observed.

2.3.2 PhD candidates' contribution to the journal article

Student name: Tanuja N. Gengiah

Student number: 993241124

Title of the article: Efavirenz dosing: influence of drug metabolizing enzyme polymorphisms and concurrent TB treatment

Authors: **TN Gengiah**, JH Botha, N Yende-Zuma, K Naidoo, SS Abdool Karim

Journal: Antiviral therapy

Doctoral student's contribution to the journal article:

1. Formulation of the hypothesis

I contributed to the formulation of the study hypothesis in conjunction the START study principal investigator and wrote the PK study into the main study protocol as a secondary objective. I also formulated the pharmacogenetics hypothesis for this analysis.

2. Study design

I designed the pharmacokinetic study with regards to the timing of the blood draws, and determined appropriate collection and storage of samples for assay after the study was completed. I wrote the PK section of the START protocol. I reviewed the literature and selected the appropriate drug metabolizing enzymes to sequence.

3. Work involved in the study

I was involved in the dispensing of the drug treatment and collecting patient data on case report forms, which were specifically designed for the PK study. This included verifying the recording of the timing of the EFV dose, the timing of the blood collection and documenting concomitant medication use for three days prior to the blood draw. I was involved in the DNA extraction from PBMCs, which was conducted with the aid of a qualified laboratory technician. After learning how to prepare samples and conduct endpoint genotyping on the

Roche Light Cycler®, I conducted all the sequencing for the pharmacogenetics work in duplicate and triplicate where needed.

4. Data analysis

I designed the data analysis plan. I extracted and coded the pharmacogenetics data and designated the haplotypes based on the allele frequencies. I tested the polymorphisms to check if they were in Hardy-Weinberg equilibrium. I chose the variables and references to be used in the generalized estimating equations (GEE) model and created all the tables and figures for this paper. The study statistician conducted all tests in SAS version 9.3 and I verified the SAS coding used.

5. Write up

I took overall responsibility for the writing of the manuscript and, after completion of the first full draft of the manuscript, submitted the manuscript to the co-authors for review and comment. All co-authors read and approved the final version of the manuscript. I corresponded with the journal and after peer review, I completed the required revisions, and the final version for publication was approved by all co-authors

I declare this to be a true reflection of my contributions to this journal article

Signature  Date: 14 November 2014

COMMENTARY

Should efavirenz be dosed higher when co-administered with rifampin?

2.4 Commentary: Should efavirenz be dosed higher when co-administered with rifampin?

From the publishers of The New England Journal of Medicine

JournalWATCH SPECIALTIES

Save time and stay informed. Our physician-editors offer you clinical perspectives on key research and news.

Should Efavirenz Be Dosed Higher When Coadministered with Rifampin?

The FDA recommends a dosage increase in patients weighing ≥50 kg, but data on the interaction between these drugs are inconsistent.

On January 6, 2012, the FDA announced that the package insert for efavirenz has been updated to include a new dosing recommendation for HIV-infected patients with tuberculosis (TB): When efavirenz is coadministered with rifampin in patients weighing ≥50 kg, it should be dosed at 800 mg once daily instead of the usual 600 mg daily. The evidence cited to support this recommendation is from drug–drug interaction trials and a pharmacokinetic modeling study. These studies suggest that coadministration reduces systemic exposure to efavirenz when that drug is given at 600 mg daily, but not when it is given at 800 mg daily; at the higher dose, systemic exposure is comparable to that achieved when efavirenz is dosed at 600 mg daily without rifampin.

Comment: With this new recommendation, the FDA aims to resolve a decade-long debate on how best to treat HIV/TB -coinfected patients. Efavirenz is metabolized by CYP450 enzymes, which are known to be induced by rifampin. When the two drugs are coadministered, the interaction could lead to suboptimal efavirenz levels, such that patients on efavirenz-containing triple therapy receive effective exposure to only two of the three drugs in their regimen. In a 2008 case study, FDA investigators concluded that the existing evidence on this interaction was inadequate to support a dosing change (J Clin Pharmacol 2008; 48:518 (Link to: http://dx.doi.org/10.1177/0091270008315308)), but 3 years later, they have changed their view.

Although the studies cited in the FDA's recommendation support the proposed approach, others do not. Our own work — and at least one additional pharmacokinetic study — have demonstrated that the drug–drug interaction could be in the opposite direction, thus increasing efavirenz exposure (Antivir Ther 2011; 16:527 (Link to: http://dx.doi.org/10.3851/IMP1780) , Eur J Clin Pharmacol 2011; e-pub ahead of print (Link to: http://dx.doi.org/10.1007/s00228-011-1166-5)). If this is the case, a non-individualized weight-based dosage increase could result in toxicity, possibly without any added efficacy benefit. Several studies indicate that efavirenz pharmacokinetics are influenced by CYP450 G516T and possibly other single nucleotide polymorphisms, rather than by rifampin alone. Perhaps more important, numerous studies show that patients receiving concurrent rifampin and efavirenz achieve virologic suppression even with standard efavirenz dosing (AIDS 2005; 19:1481 (Link to: http://journals.lww.com/aidsonline/Fulltext/2005/09230/Efavirenz_levels_and_24_week_efficacy_in.6.aspx) , J Antimicrob Chemother 2006; 58:1299 (Link to: http://dx.doi.org/10.1093/jac/dkl399) , Clin Infect Dis 2011; 53:716 (Link to: http://dx.doi.org/10.1093/cid/cir447)). Taken collectively, these data suggest that the rifampin–efavirenz interaction may not be consistent in all populations and that increasing the dose of efavirenz may not improve clinical outcomes.

— Tanuja N. Gengiah, M Clin Pharm, MS (Epi), and Salim S. Abdool Karim, MD, PhD (Link to: http://aids-clinical-care.jwatch.org/misc/board_about.dtl#aAbdool%20Karim)

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Food and Drug Administration. Sustiva labeling update/dosing adjustment with rifampin. Jan 6 , 2012. (http://www.natap.org/2012/newsUpdates/010612_06.htm (Link to: http://www.natap.org/2012/newsUpdates/010612_06.htm))

2.4.1 Discussion of commentary

This commentary is critical of the FDA's decision to advocate a package insert update to include a new dosing recommendation for patients over 50kg receiving RIF based TB treatment. This weight-based EFV dose increase from 600mg to 800mg was not restricted to any particular racial group despite evidence in the literature that indicated that Black Africans tend to have higher EFV exposure. Should prescribers follow the dosing directions strictly, for patients such as ours, the EFV exposure may be potentially toxic. The dose increase is possibly appropriate for Caucasian patients but is certainly not a "one size fits all" for African and Asian patients who constitute the majority of the global users of this drug.

Notably, although the FDA advocated this dosing update, global treatment guidelines, with the exception of the British HIV Association, did not amend their guidance to follow suit.

2.4.2 PhD candidates' contribution to the commentary

Student name: Tanuja N. Gengiah

Student number: 993241124

Title of the article: Should efavirenz be dosed higher when co-administered with rifampin?

Authors: **TN Gengiah**, SS Abdool Karim

Publication: Journal Watch Specialties

Doctoral student's contribution to the article:

1. Formulation of the hypothesis (not applicable)

Journal Watch contacted Prof. SS Abdool Karim as a regular contributor to comment on the United States Food and Drug Administration (FDA) of 6 January 2012. He requested that I write the contribution as it was directly relevant to the PhD topic.

2. Study design (not applicable)

3. Work involved in the study

I studied the US FDA ruling and attempted to contact the FDA for further data regarding the rationale for their dosing change recommendation.

4. Data analysis (not applicable)

5. Write up

I conducted the first draft of the response which was reviewed and edited by Prof Abdool Karim. I corresponded with the publication and after review, I completed the required revisions, and the final version for publication was approved by the co-author.

I declare this to be a true reflection of my contributions to this journal article

Signature  Date: 14 November 2014

Paper IV

Low rifampicin concentrations in tuberculosis patients with HIV infection

2.5 Paper IV: Low rifampicin concentrations in tuberculosis patients with HIV infection.

Original Article

Low rifampicin concentrations in tuberculosis patients with HIV infection

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Abstract

Introduction: The efficacy of tuberculosis (TB) treatment in Human Immunodeficiency Syndrome (HIV) co-infected patients may be compromised by genetic and pharmacokinetic variation in drug disposition. Rifampicin is a critical component of TB treatment. We investigated the influence of drug transporter gene polymorphisms on rifampicin concentrations in TB-HIV co-infected patients in Durban, South Africa.

Methodology: Rifampicin concentrations were measured 2.5 hours post-dose (approximated peak, $C_{2.5hr}$) in patients receiving either 450mg or 600mg rifampicin, randomized to either integrated or sequential antiretroviral treatment. Patients were genotyped for SLCO1B1 (rs4149032) polymorphisms. A mixed effects regression model was fitted to assess the influence of various factors on rifampicin concentrations. TB recurrence rates were also estimated.

Results: In 57 patients, median (IQR) $C_{2.5hr}$ was 3.6 (2.8-5.0) µg/mL. Polymorphism frequency in the SLCO1B1 (rs4149032) drug transporter gene was high (0.76) and was associated with low median rifampicin $C_{2.5hr}$, 3.7 (2.8-5.0) µg/mL in the heterozygous and 3.4 (2.7-4.7) µg/mL in the homozygous variant carriers. Concentrations were also low in males ($p < 0.0001$) and those with low haemoglobin ($p = 0.02$). Although reinfection could not be distinguished from reactivation for the 43 patients followed post trial, the incidence of TB recurrence was 7.1 per 100 person-years. Of the eight patients in whom TB recurred, seven had the polymorphism.

Conclusion: Approximated peak rifampicin concentrations were well below the recommended target range of 8 to 24 µg/mL in this patient population with its high frequency of the SLCO1B1 (rs4149032) polymorphism. Increased rifampicin dosage may be warranted in African, HIV- TB co-infected patients.

Key words: drug interactions; pharmacogenetics; TB recurrence; co-infection.

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Introduction

In 2011 it was estimated that globally 8.7 million people were newly infected with TB [1]. Of these, 1.2 million were also co-infected with HIV and most of these patients resided in sub-Saharan Africa [1]. In co-infected patients, integrated treatment with anti-TB drugs and antiretrovirals has been shown to improve survival [2]. However, drug interactions, drug tolerability and sub-optimal tuberculosis drug bioavailability remain a concern [3]. In addition, the pharmacokinetics of anti-TB drugs are influenced by other factors including genetics and disease states such as those that compromise immunity [4-6].

Rifampicin is a critical and potent component of first-line, multi-drug TB therapy because of its early sterilizing activity against *Mycobacterium tuberculosis* in the intensive phase and sustained activity against persistent bacilli throughout the continuation phase of TB treatment [7,8]. Metabolism is mainly hepatic, with up to 24% and 50% of drug excreted in the urine and bile unchanged respectively [9]. Rifampicin induces several cytochrome P450 enzymes [10] and hepatocellular uptake is mediated by an organic anion-transporter polypeptide 1B1 (OAT1B1) coded for by the gene SLCO1B1 [11]. Polymorphisms in this gene influence rifampicin pharmacokinetics significantly and are implicated in low rifampicin exposure [6,12]. Anti-TB activity and development of resistance are linked to rifampicin concentrations [13,14] and rifampicin peak concentrations of 8 to 24 µg /mL are generally considered to be associated with optimal bactericidal killing and post antibiotic effect [15].

The purpose of this study was to assess rifampicin concentrations in TB-HIV co-infected patients and to

investigate the phenotypic and genotypic attributes that may influence these concentrations.

Methodology

In the 'Starting Tuberculosis and Antiretroviral Therapy' (START) trial (CAPRISA 001: NCT00091936) described previously, patients (n = 58) who had no prior history of TB, were randomized equally to receive integrated TB and HIV treatment (n = 29) or HIV treatment following the completion of TB treatment (n = 29) [16]. In both arms, ART comprised of once daily enteric-coated didanosine (400 mg for participants > 60 kg; 250 mg for participants < 60 kg), lamivudine 300mg and efavirenz 600mg, but if > 50kg and on TB treatment, then efavirenz 800mg was prescribed. TB treatment was provided using fixed dose combination (FDC) TB drugs, usually for 6 months, in a directly observed therapy (DOT) program. In the intensive phase of TB treatment each FDC tablet contained: rifampicin 120mg / isoniazid 60mg / pyrazinamide 300mg / ethambutol 200mg. In the continuation phase of TB treatment FDCs comprised rifampicin 300mg / isoniazid 150mg or rifampicin 150mg / isoniazid 100mg. FDCs were dosed according to weight bands detailed in the TB control programme guidelines in effect at the time of the study. Accordingly, rifampicin dose was 450mg daily for five days a week in patients weighing < 50 kilograms (kg) or 600mg dosed daily five times a week in patients weighing 50 kg or more [16].

Blood samples were collected from 57 patients, at weeks 4, 8 and 12 of TB treatment at 2.5 hours post-dose in order to approximate peak rifampicin concentrations. Serum rifampicin concentrations were measured by tandem HPLC mass spectrometry using a methodology described previously [17]. The assay had a lower limit of quantitation of 0.1 µg/mL and inter- and intra-day coefficients of variation below 10%. DNA for the genotyping of the drug transporter gene coded by SLCO1B1 (rs4149032), previously shown to be associated with low rifampicin concentrations [12], was extracted from stored peripheral blood mononuclear cells using the Roche MagNA Pure LC DNA kit I (Version 17.0, Roche Diagnostics, Mannheim, Germany). Allelic discrimination reactions were performed in duplicate using a TaqMan (Applied Biosystems, Foster City, USA) commercial genotyping assay (C_1901709_10, rs4149032). Real Time polymerase chain reaction (RT-PCR) was performed on the Roche Light Cycler 480 platform (Roche Diagnostics, Mannheim, Germany). The final volume for each reaction was 25µl, consisting of TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 40X drug metabolising genotype assay mix and 10ng of genomic DNA. The thermal cycler conditions were as follows: initial step: 95°C for 10 minutes, then 50 cycles consisting of denature at 92°C for 15 seconds and anneal/extend at 60°C for 90 seconds.

Data analysis was performed with SAS version 9.3 (SAS Institute Inc., Cary, NC). A Mann- Whitney test was used to compare median rifampicin concentrations. A mixed effects regression model, suitable for repeated measures, was used to test the influence of variables of interest on rifampicin concentrations. Post-trial TB recurrence was assessed by determining the period of risk for TB acquisition as the time in follow-up from the initial successful TB treatment completion date up to the re-treatment date or the last clinical contact date available. Poisson approximations were used to calculate confidence intervals for TB recurrence rates. SLCO1B1 (rs4049032) mutations were tested and found to be in Hardy-Weinberg equilibrium. A type 1 error, $\alpha = 0.05$, was used to reject the null hypothesis.

Ethics approval was obtained from the University of KwaZulu-Natal biomedical research ethics committee (E116/04) and all patients provided written informed consent.

Results

The median age of the 57 patients in this analysis was 33 years (range: 19-54). All were Black African and 56% were men. At baseline, mean (SD) weight was 57.3 (9.0) kg, BMI was 21.8 (3.6) Kg/m^2, CD4 count was 282 (153) cells/mm^3 and haemoglobin was 10.3 (1.6) g/dL.

Median duration of TB treatment was 219 days (range: 181-291 days) and was similar in both the integrated and sequential arms. One hundred and fifty six rifampicin concentrations were available for analysis from the 57 patients. Of these, 44 patients contributed concentrations at three time points, 11 at two and two at one time point. Median (IQR) rifampicin concentrations were 3.6 (2.8-5.0) µg/mL overall and 3.5 (2.8-4.7) µg/mL and 3.7 (2.8-5.2) µg/mL in the integrated and sequential treatment arms respectively (p = 0.8). Relevant concentrations for the 450mg and 600mg dose of rifampicin were 3.29 (1.25-5.25) µg/mL and 4.06 (2.91-5.76) µg/mL, respectively (p = 0.06). Taking weight into account the rifampicin dosages ranged from 6.4mg/kg -13.2 mg/kg (Figure 1) and were similar in both arms.

Figure 1. Graph of approximated peak rifampicin concentrations in relation to dose (mg/kg) for the integrated and sequential arms.

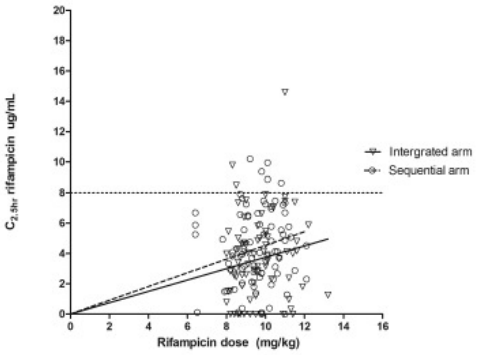


Figure 2. Graph of approximated peak rifampicin concentrations and gender. The median concentration is denoted by the bar.

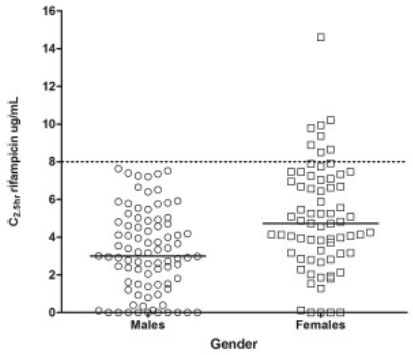


Figure 3. Graph of approximated peak rifampicin concentrations and SLCO1B1 (rs4149032) allele frequencies. The median and interquartile range are represented by the bar and whiskers.

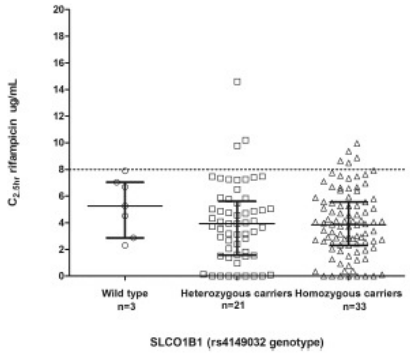


Figure 4. Graph of approximated peak rifampicin concentrations, SLCO1B1 (rs4149032) genotype and gender. The median concentration is denoted by the bar.

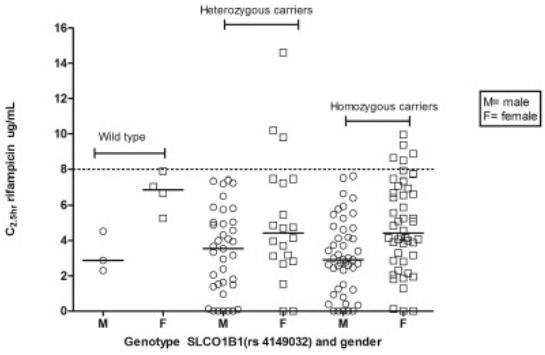


Table 1. Mixed effect model estimating the influence of selected variables on $C_{2.5hr}$ rifampicin concentrations

Variables (N = 57)	Univariate Model			Multivariate model		
	β estimate	*SE*	P value	β estimate	*SE*	P value
Weight (kg)	0.01	0.02	0.84	0.01	0.02	0.56
Female vs. Male	1.78	0.39	<0.0001*	2.64	0.48	<0.0001*
Age (years)	-0.01	0.03	0.65	0.05	0.03	0.09
Non –Smoker vs. Smoker (status at baseline)	1.14	0.62	0.07	0.63	0.52	0.23
Dose/Weight (mg/kg)	0.09	0.16	0.56	0.33	0.16	0.04*
SLCO1B1 (rs4149032) allele						
Heterozygous vs. Wild type	-1.66	0.97	0.09	-1.45	0.83	0.09
Homozygous vs. Wild type	-1.63	0.93	0.09	-1.71	0.79	0.04*
Haemoglobin (g/dL)	-0.12	0.13	0.34	0.32	0.13	0.02*
CD4 Count (per 50 cell/mm^3 increase)	-0.04	0.07	0.59	0.019	0.07	0.76
TB recurrence vs. sustained cure	-0.10	0.67	0.88	0.10	0.56	0.86

*p<0.05, statistically significant

Women had a median rifampicin concentration of 4.1 (3.3-5.3) µg/mL compared to 3.2 (2.6-4.1) µg/mL in men (p = 0.006) as shown in Figure 2.

Allele frequency for SLCO1B1 (rs4149032) polymorphism was 0.76. Fifty seven percent (n = 33) of patients were homozygous for the variant allele, while 38% (n = 21) were heterozygous and 5% (n = 3) were homozygous for the common allele of the gene (wild type). The median (IQR) rifampicin concentrations were 3.4 (2.7-4.7) µg/mL, 3.7 (2.8-5.0) µg/mL and 5.3 (3.8-6.7) µg/mL respectively (Figure 3). Figure 4 shows the relationship between rifampicin concentrations, genotype and gender.

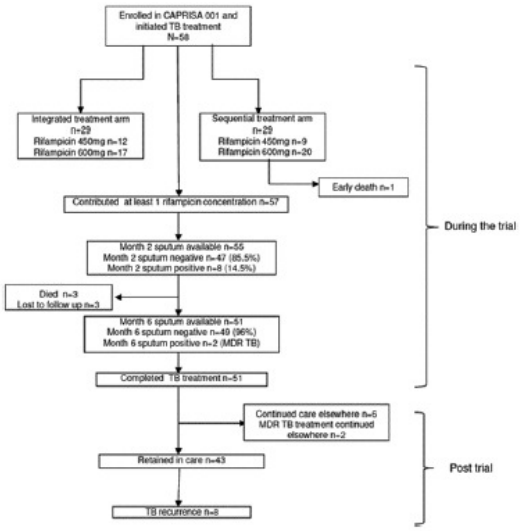
Predictors thought to influence rifampicin concentrations were tested in both univariate and multivariate models for repeated measures (Table 1). Male gender ($p < 0.0001$), haemoglobin per unit increase ($p = 0.02$) and homozygous variant allele carriers were associated with lower rifampicin concentrations. Higher dose per kilogram of body weight was associated with a higher rifampicin concentration ($p = 0.04$), but weight alone, smoking status, CD4 count and later TB recurrence were not predictive of lower rifampicin concentrations.

Patient outcomes, during and after the trial, are summarized in Figure 5. Sputum cultures were available at month two for only 55 of the 57 patients and 47 (85.4%) of these were found to be negative. Among the eight patients who were sputum positive, two had primary MDR TB and remained sputum positive throughout the study. Three subsequently developed recurrent TB, after completion of treatment, and the remaining three went on to become sputum negative at 6 months.

Fifty one of the 57 patients completed TB treatment and 49 of them were sputum culture negative at 6 months. The two with MDR TB who remained sputum positive are mentioned above. Of the 58 patients originally enrolled, four had died (one of unknown cause prior to contributing a drug level, one suicide, one embolism and one death was TB-related) and three had been lost to follow up.

Forty three patients continued receiving antiretroviral treatment at our CAPRISA clinic; eight (18.6%) of them developed recurrent TB during 113.1 person years of follow-up. The TB recurrence rate was 7.1 (95% CI: 3.1-13.9) cases per 100 person-years. The median time from completion of TB treatment to the post-trial diagnosis of recurrent TB was 639 days (range 56 – 1832 days). Of the eight patients in whom TB recurred, seven had the polymorphism and of these six were homozygous carriers of the variant allele.

Figure 5. Patient outcomes post enrollment.



TB treatment interruptions were investigated and durations of greater than 14 days occurred in two participants, both during the continuation phase of TB treatment. One patient, who was incarcerated and missed 19 days of treatment, was among the eight who had recurrent TB after treatment was completed. The other, the patient who died of TB, had missed a cumulative 43 days of treatment.

Discussion

The bactericidal activity of rifampicin is concentration dependent [18]. Ratios of both C_{max} to MIC and AUC to MIC are important [5, 8], and a C_{max} target range of 8 to 24 µg /mL is recommended for optimal bactericidal activity and post-antibiotic effect [15]. Accordingly, the fact that our 57 patients had a median $C_{2.5hr}$ of 3.6 µg/ml, with none being > 8 µg/ml, is a cause for concern. As expected, median rifampicin concentrations where similar when HIV treatment was integrated or sequential.

Various factors could have contributed to our low rifampicin concentrations in both arms, one being the routine use of FDCs in South Africa, where formulation factors could affect bioavailability [19]. Consistent with the findings of earlier studies, male gender was associated with lower concentrations [20, 21] as was low haemoglobin, which has previously been linked to poorer TB outcomes [22]. HIV co-infection may also be a factor; in a group of 34 co-infected patients receiving 600mg of rifampicin daily,

where reported C_{max} concentrations of < 8µg/ml occurred in 77% of cases and 35% of these concentrations were < 4µg/ml. Overall the median was 5.4 µg/ml [5]. Similarly, in 155 co-infected patients from Botswana, median C_{max} values (at 2 hours) were 4.4 and 5.7 µg/ml in those with CD4 counts of less than and greater than 200 cells/µL respectively [23]. We found no association between CD4 count at baseline and rifampicin concentrations sampled.

We found the polymorphism frequency for the drug transporter gene SLCO1B1 (rs4149032) to be high in our patients (0.76) and mutations in this gene were associated with low rifampicin concentrations. Our allelic frequencies and related associations with low concentrations are very similar to those reported in Cape Town by Chigutsa *et al*. who found an allelic frequency of 0.70 and rifampicin C_{max} < 8µ/ml in 69% of their patients [12]. Evidence for the importance of the SLCO1B1 drug transporter gene in determining rifampicin exposure, also comes from a study by Weiner *et al*. These authors studied SLCO1B1 463C→A and found that patients with this polymorphism had a 36% decrease in AUC_{0-24}. Their African patients had the highest frequency of this polymorphism [15] in contrast to the Cape Town study where the 463C→A polymorphism was infrequent and did not appear to associated with rs4149032 [12].

Limitations of the current analysis include the small sample size and the inability to assess for delayed absorption due to the single time point sampling, although from the literature, sampling 2-3 hours post administration is generally when peak rifampicin concentrations are reached. Moreover, in more than one occasion the same patient was sampled.

We were also unable to distinguish between reinfection and reactivation, as this was not part of the study design. However, TB recurred in 18.6% of patients who had been confirmed sputum negative at the end of TB treatment. Our recurrence rate of 7.1 per 100 person-years was comparable with the figure of 8.4 per 100 person-years in similar patients in a Ugandan study [24], although rifampicin concentrations were not measured in that study. While it was not possible to directly attribute recurrence in our study to low rifampicin concentrations, lower TB drug concentrations have previously been postulated to be associated with poorer treatment outcomes [8,15,23]. Of the 8 patients in whom TB recurred after treatment was completed, 87.5% had the polymorphism and 75% were homozygous for the variant allele. Furthermore, the TB related death was also in a patient homozygous for the variant allele in whom low rifampicin concentrations had been measured. The two cases of MDR TB were primary and only diagnosed retrospectively and hence were unrelated to treatment and rifampicin concentrations.

Chigutsa *et al*., who considered the SLOC1B1 (rs4149032) polymorphism an important determinant of the low rifampicin concentrations in African patients, recommended an increase to the current standard daily dose. After model simulations, they demonstrated that a daily dose increment of 150mg would produce concentrations similar to those achieved in wild type individuals and would reduce the percentage of patients with concentrations below < 8µ/ml by 50% [12]. In support of a dose increase is a recent 14 day study where rifampicin doses of 10, 20, 25 and 30mg/kg produced mean C_{max} concentrations of 7.4, 21.6, 25.1 and 33.1 mg/L in adult smear positive TB patients [25]. Evidence from this trial suggested that higher doses of rifampicin, even up to 35mg/kg, appear to be well tolerated, safe and exhibit optimal drug exposure [25].

Conclusion

Our patient cohort of TB-HIV co-infected Black South African patients exhibited both extremely low rifampicin concentrations and a high frequency of polymorphisms in the SLCO1B1 (rs4149032) drug transporter gene. Further research on the possible need for increased rifampicin dosage and a more comprehensive exploration of the role of polymorphisms in the SLCO1B1 drug transporter gene is warranted.

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Conflict of interests: No conflicts of interest are declared.

2.5.1 Discussion of paper IV

Paper IV is responsive to Objective 4 of the PhD thesis. TB and HIV drug interactions usually implicate RIF as having an impact on ARV concentrations without a pharmacologic basis for any effect on TB drug concentrations. However, in co-infected patients HIV disease or other host factors may influence TB drug absorption and affect TB treatment outcomes. In this analysis, we measured approximated peak RIF concentrations in the START study and investigated the presence and influence of polymorphisms in drug transporter proteins on RIF plasma exposure.

We were able to assess 156 peak RIF concentrations, sampled at 2.5 hours post dose administration. The recommended peak concentration should range between 8-24µg/mL, however, the median $C_{2.5h}$ in this study was 3.6 µg/mL. A polymorphism in the SLCO1B1 gene was common, with a MAF of 76%. In addition to this SNP, male gender and presence of anaemia were associated with low RIF concentrations. Incidence rates for TB recurrence in 43 of the 58 patients who could be followed long-term was 7.1 per 100 person years.

Although this is a small study, the findings are a cause for concern and are of importance for the TB control programme. Extremely low RIF peak concentrations diminish optimal treatment outcomes and a revision of the current dose of RIF for this population is therefore strongly recommended. The majority of our patients had the drug transport polymorphism under study and, in conjunction with HIV infection, may be receiving sub-therapeutic RIF doses. These sub-therapeutic RIF doses could impact on TB recurrence, or worse, could be fueling the MDR TB epidemic.

2.5.2 PhD candidates' contribution to the journal article

Student name: Tanuja N. Gengiah

Student number: 993241124

Title of the article: Low rifampicin concentrations in tuberculosis patients with HIV infection.

Authors: **TN Gengiah**, JH Botha, D Soowamber, K Naidoo, SS Abdool Karim

Journal: Journal of Infection in Developing Countries

Doctoral student's contribution to the journal article:

1. Formulation of the hypothesis

I contributed to the formulation of the study hypothesis in conjunction with the START study principal investigator and wrote the PK study into the main study protocol as a secondary objective. I formulated the pharmacogenetic hypothesis for the influence of polymorphisms in drug transporter proteins on RIF exposure.

2. Study design

I designed the pharmacokinetic study with regards to the timing of the blood draws, and determined appropriate collection and storage of samples for assay after the study was completed. I wrote the PK section of the START protocol. I selected the drug transporter polymorphism for further study in this analysis.

3. Work involved in the study

I was involved in the dispensing of the drug treatment and collecting patient data on case report forms, which were specifically designed for the PK study. This included verifying the recording of the timing of the RIF dose, the timing of the blood collection and documenting concomitant medication use for three days prior to the blood draw. I was involved in the DNA

extraction from PBMCs, which was conducted with the aid of a qualified laboratory technician. After learning how to prepare samples and conduct endpoint genotyping on the Roche Light Cycler®, I conducted all the sequencing for the pharmacogenetics work in duplicate and triplicate where needed.

4. Data analysis

I designed the data analysis plan. I extracted and coded the pharmacogenetics data. I tested the polymorphisms to check if they were in Hardy-Weinberg equilibrium. I chose the variables and references to be used in the mixed effects model and created all the tables and figures for this paper. The statistician conducted all tests in SAS version 9.3 and I verified the coding used.

5. Write up

I took overall responsibility for the writing of the manuscript and after completion of the first full draft of the manuscript submitted the manuscript to the co-authors for review and comment. All co-authors read and approved the final version of the manuscript. I corresponded with the journal and after peer review, I completed the required revisions, and the final version for publication was approved by all co-authors

I declare this to be a true reflection of my contributions to this journal article

Signature  Date 14 November 2014

CHAPTER 3: OVERALL CONCLUSIONS AND RECOMMENDATIONS

3.1 Discussion of major findings

The purpose of the PhD study (START PK sub-study) was to assess the EFV-RIF drug interaction with the aim of determining the need for the higher EFV 800mg dose when TB and HIV treatment are combined. Subsequently, the work was expanded to study the influence of pharmacogenetic variation on EFV trough and RIF peak concentrations, along with determinants influencing RIF concentrations and long-term TB recurrence in TB-HIV co-infected Black, South African patients. The novel contribution of this work was two-fold; firstly, the EFV dose was weight adjusted to 800mg for integrated arm participants above 50kg and secondly, this was the first clinical trial to evaluate EFV PK and RIF PK in Black, South African patients from KwaZulu-Natal, South Africa, an area regarded as the epicenter of the HIV epidemic in sub-Saharan Africa.

Using a NONMEM population PK approach, the impact of RIF-based TB treatment on EFV clearance was assessed by modelling the EFV concentration-time data from the START trial. We anticipated that CYP450 enzyme induction by RIF would have resulted in lowered EFV concentrations. Therefore, the overall 29.5% reduction in EFV clearance during TB treatment that was observed was unexpected. Further, a bimodal distribution of EFV apparent clearance was evident from the model and indicated that slow EFV metabolisers accounted for 21.9% of the population. EFV clearance in fast metabolisers was found to be 11.5 L/h/70kg off TB treatment and 7.6 L/h/70kg when on TB treatment, while in slow metabolisers the clearance estimates were 2.9 and 4.3 L/h/70kg in the presence and absence of TB treatment, respectively. These results indicated that apart from metabolic differences amongst patients, TB treatment collectively, appears to play a role in the higher EFV exposure observed in our patients [125].

These finding are in contrast to those reported by others where either mean EFV CL/F was shown to be raised [58, 94] or EFV concentration was reduced [57] in the presence of RIF based TB treatment. These studies in Caucasian and Asian patients laid the basis for the traditional understanding of the direction of this interaction. However, other studies in African patients have found similar results to those observed in our study [61, 64, 93], indicating that racial differences affect drug disposition. Specifically, Nyakutira et al. 2008, showed that approximately 50% of TB uninfected Zimbabwean patients (n=74) had EFV concentrations greater than 4µg/mL after taking a 600mg dose of EFV [93]. In South African patients undergoing TB treatment, EFV concentrations were significantly raised when RIF was present compared to EFV alone [64].

The TB treatment effect on EFV clearance has also been previously described, with reports of high inter-subject variability whilst on TB treatment in different populations. In South African patients it was shown that the coefficient of variation (CV) =157% while on TB treatment and CV=58% off TB treatment [60]. In Italian patients, the CV was 93% on TB treatment and 62% off TB treatment, whilst in Thai patients, the CV on TB treatment was 107% [49]. At the time that these studies were conducted it was not clear why RIF-based TB treatment would increase variability in EFV concentrations. However, possible explanations arose later when the pharmacogenetics aspects of the study were assessed. Most recently, in the large multi-centre STRIDE trial of 780 patients, EFV 600mg was dosed during concomitant TB treatment and median EFV concentrations on TB treatment were higher than off TB treatment concentrations, with this effect being more pronounced in Black patients [69].

Despite these contradictions in EFV exposure when on TB treatment, in the same year that our NONMEM results were published (2012), the United States Food and Drug

Administration (FDA) announced that due to concerns about the EFV-RIF drug-drug interaction they had approved an increase in EFV dose from 600mg to 800mg in patients weighing more than 50kg on RIF-based TB treatment [72]. No consideration was taken of race or the influence of pharmacogenetic polymorphisms on EFV concentrations. Our objection to this recommendation was raised in a commentary in Journal Watch (Chapter 2, section 2.4) and confirmed our impression that it was necessary to further explore this interaction in our patients.

After the completion of the NONMEM analysis, it was clear that there was a reduction in EFV clearance attributed to TB treatment and there were also population differences in metabolic capacity. We then decided to sequence polymorphisms of interest in CYP2B6, CYP2A and UGT2B7 genes, all known to contribute to EFV metabolism, to assess their association with EFV concentrations both during and after TB treatment [126]. After careful consideration of the literature we selected the CYP2B6 516G→T, CYP2B6 785A→G, CYP 2B6 983T→C, CYP2A6*9B, CYP2A6*17 and UGT2B7 -372G→A polymorphisms for assessment.

The minor allele frequencies in our population for CYP2B6 516G→T and 785A→G were comparable with those reported in other African cohorts [89, 95]. CYP2B6 516G→T has been studied extensively and polymorphisms have been shown to be associated with higher EFV exposure in different races [82, 86, 98, 101, 116]. Our results are supportive of this.

Where our results differed from literature reports was with the CYP2B6 983T→C mutation. This SNP is thought to be rare in Caucasian populations and more likely to be detected in African patients. However, lower frequencies ranging from 7-18.7% [82, 99, 112, 113, 123, 127] have been reported previously. We demonstrated a MAF of 23%. Nevertheless, variant allele homozygosity, even in African patients, has been reported to be even more uncommon,

with frequencies ranging between 0.1- 3.5% [113, 122], while our study showed a 5.6% prevalence.

The CYP2B6 983T→C SNP has also been associated with higher EFV exposure in the presence of TB treatment in Black Africans [123, 124] and in the absence of TB treatment in both Africans and Caucasians [87, 92, 113, 115, 127]. This SNP has been shown to be an important determinant of EFV concentrations by other researchers [113, 118, 124] and possibly explains the high EFV concentrations in our study.

Several CYP2B6 haplotypes were also identified and over two thirds of our population were either CYP2B6 *6 (38.9%) or *18 (25.9%) [126]. These haplotypes have also been shown to be associated higher EFV concentrations in other studies [87, 98, 99, 101, 104], thus supporting our findings. Unexpectedly, polymorphisms in all three CYP2B6 genes studied (516T-785G-983C) were present in 11.1% of our patients. Median (IQR) EFV concentrations in this group were 19.2 (9.5-20) µg/mL and 4.7 (3.5-5.6) µg/mL when on and off TB treatment. This group's concentrations were exceptionally different from the results in the overall study population. During TB treatment, median EFV Cmin was 3.2 (IQR: 2.6-6.3) µg/mL in the EFV 800mg group and 3.3 (IQR: 2.4-9.5) µg/mL for the EFV 600mg group. After TB treatment, all patients received EFV 600mg and the Cmin was 2.0 (1.4 - 3.5) µg/mL[126].

Using a generalized estimating equation (GEE) model we were able to show that TB treatment, composite genotypes CYP2B6 516 GT/TT, CYP2B6 983 TC/CC or being a CYP2A6*9B carrier were predictors of a median EFV Cmin> 4 µg/mL [126]. These polymorphisms are also supported by others as specifically predictive for higher EFV exposure [82, 104, 124]. As seen in other studies of African patients, UGBT2B7 was not associated with EFV exposure [87, 88], while being a carrier for CYP2A6*17 was predictive of the higher EFV concentrations in some [82] but not in others [90, 115].

Although we were not able to assess in our study how TB drugs other than RIF influence EFV concentrations, the 'CAMELIA' trial in Thai patients possibly provides a clue as to why TB treatment is associated with higher EFV exposure and higher inter-patient variability [121]. In this important study, the possible role of N-acetyltransferase (NAT) 2 genetic polymorphisms and change in EFV clearance in 307 Asian patients on concomitant TB treatment is investigated. The change in EFV CL/F was highly dependent on the *NAT*2 polymorphism; patients who were both CYP2B6 516 TT and NAT2 slow acetylators had the lowest EFV CL/F of 2.1L/h compared to the wild type 516 GG where EFV CL/F was 11.2 L/h in slow acetylators and 15.5L/h in rapid acetylators [121]. It has been well documented that isoniazid is metabolized by NAT2 and that acetylator status predicts individual isoniazid exposure which can be predicted by NAT2 genotype [128]. When TB treatment stopped in 'CAMELIA', EFV clearance increased, suggesting an isoniazid concentration dependent inhibitory effect, which possibly counterbalances any RIF induction effect during TB treatment [121]. Furthermore, *in vitro* evidence shows that INH mediates the CYP2B6*6 genotype dependent interaction with EFV and TB medication by potent time-inhibition of EFV 7-hydroxylation in human liver microsomes and mechanism based inactivation of CYP2A6. [129]. Collectively, these data may explain some of our results, implicating an isoniazid effect, but it would need to be tested in other racial groups.

Although few adverse effects were reported with the higher EFV 800mg dose, we do not advocate the dose increase during TB treatment. This is due to the presence of multiple pharmacogenetic polymorphisms and the unique influence of TB treatment in our patients, pre-disposing them to higher exposures. There is also evidence that high EFV exposure with certain poor metaboliser genotypes (like 516TT) or a prolonged elimination half-life may predict an increased risk for EFV resistance in patients who discontinue EFV regimens [110]. Further, in one Ethiopian study, drug induced liver injury (DILI) risk was shown to be

associated with higher EFV concentrations and 516TT genotype [117]. Conversely, despite some early evidence demonstrating that low EFV concentrations are associated with virological failure [56], more recent studies fail to show the correlation with viral suppression and sub-therapeutic concentrations [68, 73].

Regarding RIF PK, the median RIF (IQR) $C_{2.5hr}$ was found to be extremely low at 3.6 (2.8-5.0) µg/mL. Having studied reports in the literature on the role of human drug transporter genes on RIF concentrations [75, 76] we sequenced our patients for SLCO1B1 (rs4149032). We showed an extremely high MAF of 76% for this polymorphism, similar to that seen in Zimbabwean patients [75]. Using a mixed effects model, SLCO1B1 (rs4149032) drug transporter gene polymorphism was associated with low RIF concentrations as was male gender and having a low baseline haemoglobin. We also showed, from longitudinal data, that TB recurrence incidence was 7.1 per 100 person years [130].

These results are of concern for several reasons. Firstly, ratios of both C_{max} and AUC/MIC are crucial for the optimal bactericidal effect of RIF [19, 131]. Our results were, however, far below the accepted C_{max} of 8-24µg/mL [79]. Secondly, although we could not distinguish between TB reinfection and reactivation, it is plausible that the extremely low treatment concentrations observed in this study may have played a role in TB reactivation. Lastly, because the SLCO1B1 polymorphism frequency was high in our patients, who were also HIV co-infected and prone to suboptimal absorption, they are theoretically at higher risk for TB treatment failure and development of MDR TB. Studies have shown that HIV infected patients are also at higher risk for recurrent TB disease [132-134]. The potential for the contribution of lower RIF concentrations to further increasing the risk TB relapse or reinfection in HIV infected patients will have negative consequences at the patient, TB control program and population levels.

In conclusion, this PhD study has shown that in a population with a high frequency of polymorphisms in drug metabolizing enzymes, concomitant TB treatment reduces EFV CL/F with corresponding elevations in EFV concentrations. Increasing the dose of EFV during RIF co-treatment is therefore not supported in these patients. The low peak RIF concentrations demonstrated may put these patients at high risk for TB recurrence, possibly warranting an increase in dose.

3.2 Study limitations

The eventual sample size of the START trial was small (n=58) and only trough EFV and approximated peak RIF concentrations post DOT where sampled. Therefore, it was not possible to obtain full PK profiles. However, trough concentrations were sampled at steady state for EFV and the NONMEM approach was applied to the analysis. With RIF, due to the single time point sampling, 2.5 hours post dose administration, we were unable to account for patients with particularly early or delayed absorption. However, sampling 2-3 hours post administration has previously been shown to be an acceptable time to estimate peak RIF concentrations. Also, each patient had plasma sampled for this peak measurement on more than one occasion.

With regards to the dose of EFV, this was increased to 800mg in the integrated arm only if patients weighed $\geq$50 kg. However, given that the mean weight of participants was 56.3 kg in the integrated arm, the majority of these patients would have received the 800mg dose during TB treatment resulting in few matched concentration pairs for comparison with the EFV 600mg dose off TB treatment. Although the 800mg EFV dose appeared to be well tolerated,

the small sample size may have accounted for the limited number of adverse events reported.

Due to the premature discontinuation of the parent trial, it was not possible to assess long-term viral suppression (12 months) in both arms and thus to relate EFV concentrations and genotype to long-term viral suppression. This would have been a valuable analysis to conduct.

Lastly, due to the study design, it was not possible to distinguish between TB reinfection and reactivation when the RIF concentrations and TB recurrence was assessed.

3.3 Recommendations for clinical practice

Selected recommendations for clinical practice in South African patients are proposed:

- Simultaneous co-administration of EFV and RIF containing HIV and TB treatment is feasible without EFV dose increase.
- In patients with poor or delayed TB treatment response, investigate RIF peak concentrations if possible.
- As RIF concentrations were shown to be low on standard doses, careful attention should be paid to weight gains in patient so that they get appropriate dosage increments to TB treatment without delay.
- If pharmacogenetic screening is available then SNPs of value in this population in predicting EFV concentrations are CYP2B6 516G→T and 983T→C, while SLCO1B1 (rs4149032) is useful when assessing RIF concentrations.

3.4 Recommendations for future research

During the preparation of the review article (Chapter 2, Paper I), it was recognized that much of the available data on drug interactions were obtained from healthy volunteers or small clinical studies. There is a paucity of robust drug interaction studies in patients who have the diseases in question and the findings of healthy volunteer studies may have poor external validity when applied to TB-HIV co-infected patients [135].

Additionally, there is limited data from African patients where the need for co-treatment is most prevalent and most of the data that is available are for adult patients. Pharmacokinetic studies in TB/HIV co-infected children and pregnant women are needed as drug handling in these groups differ from the standard 70kg adult from whom most dosing is traditionally derived.

In general, all ARV drug combinations, both first and second-line should be assessed for drug interactions with TB treatment in co-infected patients as it is critical to provide more treatment options and provider flexibility in clinical management. Well-designed drug interaction studies with rationally sequenced drugs need to be conducted to ascertain their safe use in co-infection.

Specifically, related to our findings and on assessment of the literature the following future research is recommended:

- The 'CAMELIA' trial findings of a potential INH concentration dependent inhibitory effect on EFV CL/F when patients have the NAT2 genetic polymorphisms and CYP516TT genotype have only been demonstrated in Thai patients [121]. These data may explain some of our results but needs to be urgently tested in other ethnic

groups. We are planning to assess the influence of NAT2 acetylator status in our patients in the near future.

- Given the high EFV concentrations that we demonstrated in both the presence and absence of TB treatment at *'standard'* dosing, small clinical studies are needed to show that in the South African population EFV dose decrease is safe and effective and potentially cost saving. The 'ENCORE 1' trial conducted in 630 patients, of which 37% were African, suggest that a 400mg EFV dose is non-inferior to 600mg[136], and dose simulation by Nyakutira et al. 2008 showed that dose reduction from 600mg to 400mg would give adequate exposure in poor metabolisers [93].

- There is some evidence for drug-induced liver injury (DILI) when EFV concentrations are high in poor metaboliser genotypes [117]. When patients present with abnormal liver function, genotype may play a role but this needs to be confirmed in clinical studies in South Africans.

- Our finding of extremely low peak RIF concentrations needs to be confirmed. RIF peak concentrations could be measured either during routine care in a large number of patients or in smaller PK studies with more frequent sampling. Concentrations assayed at single time points in many patients during routine care would need to be modelled with NONMEM to generate PK parameters. If our findings are validated then studies should be designed to assess the safety and efficacy of higher doses of RIF in South African patients to decrease the risk of TB treatment failure and mitigate TB transmission risk to the population at large. There are already some groups proposing that up to 35mg/kg may be safe and more effective than the standard 10mg/kg that is currently used [137].

3.5 Concluding statements

The following statements conclude the various analyses that were conducted, as part of, or in association with the PhD study:

- Sufficient evidence is available to demonstrate major survival benefits of integrating HIV and TB treatment.
- Deferring treatment of HIV to avoid drug interactions with TB treatment is not beneficial to patients and may actually be harmful.
- RIF containing TB treatment does not lower EFV concentrations.
- Efavirenz clearance is reduced by 29.5% in the presence of TB treatment.
- CYP2B6 516GT, 785AG and 983TC minor allele frequencies are 31%, 33% and 23% respectively and are associated with impaired EFV metabolism.
- The presence of TB treatment and composite genotypes CYP2B6 516 GT/TT, CYP2B6 983 TC/CC and CYP2A6*9B carrier status predicts high median EFV $C_{min} > 4\mu g/mL$.
- Patients who have triple mutations (516T-785G-983C) have extremely high EFV concentrations.
- Few adverse events are experienced, even when high EFV concentrations are present.
- Increasing EFV dose to 800mg during RIF-based TB treatment is unnecessary in African patients with the polymorphisms identified and dose reduction needs to be explored further.
- Median peak RIF concentrations are extremely low in this patient population.
- Increased RIF dosage warrants urgent consideration in African, TB-HIV co-infected patients.

- Men and anaemic patients appear to be at higher risk for having sub-therapeutic RIF concentrations.

  These findings support the use of standard EFV doses during TB co-treatment and a possible increase in RIF dose during TB treatment to achieve safe and effective integration of HIV and TB treatment in adult, Black patients in South Africa.

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APPENDICES

APPENDIX A:

Co-authored publications relevant to the PhD topic

A. Co-authored publications relevant to the PhD topic

This section contains five co-authored publications that are related to safe and effective integration of HIV and TB treatment.

A brief discussion follows each publication and describes why the paper is regarded as important to the integration of TB-HIV treatment and its contribution to generating new knowledge to the field.

A1: Paper V

Timing of initiation of antiretroviral drugs during tuberculosis therapy

A1. Paper V: Timing of initiation of antiretroviral drugs during tuberculosis therapy

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Timing of Initiation of Antiretroviral Drugs during Tuberculosis Therapy

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ABSTRACT

BACKGROUND

The rates of death are high among patients with coinfection with tuberculosis and the human immunodeficiency virus (HIV). The optimal timing for the initiation of antiretroviral therapy in relation to tuberculosis therapy remains controversial.

METHODS

In an open-label, randomized, controlled trial in Durban, South Africa, we assigned 642 patients with both tuberculosis and HIV infection to start antiretroviral therapy either during tuberculosis therapy (in two integrated-therapy groups) or after the completion of such treatment (in one sequential-therapy group). The diagnosis of tuberculosis was based on a positive sputum smear for acid-fast bacilli. Only patients with HIV infection and a CD4+ cell count of less than 500 per cubic millimeter were included. All patients received standard tuberculosis therapy, prophylaxis with trimethoprim–sulfamethoxazole, and a once-daily antiretroviral regimen of didanosine, lamivudine, and efavirenz. The primary end point was death from any cause.

RESULTS

This analysis compares data from the sequential-therapy group and the combined integrated-therapy groups up to September 1, 2008, when the data and safety monitoring committee recommended that all patients receive integrated antiretroviral therapy. There was a reduction in the rate of death among the 429 patients in the combined integrated-therapy groups (5.4 deaths per 100 person-years, or 25 deaths), as compared with the 213 patients in the sequential-therapy group (12.1 per 100 person-years, or 27 deaths); a relative reduction of 56% (hazard ratio in the combined integrated-therapy groups, 0.44; 95% confidence interval, 0.25 to 0.79; P=0.003). Mortality was lower in the combined integrated-therapy groups in all CD4+ count strata. Rates of adverse events during follow-up were similar in the two study groups.

CONCLUSIONS

The initiation of antiretroviral therapy during tuberculosis therapy significantly improved survival and provides further impetus for the integration of tuberculosis and HIV services. (ClinicalTrials.gov number, NCT00398996.)

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In 2007, it was estimated that there were about 33 million persons living with human immunodeficiency virus (HIV) infection[1] and 9.2 million persons with newly diagnosed tuberculosis worldwide.[2] The two diseases are closely intertwined, and the number of patients with coinfection continues to grow rapidly.[3] Tuberculosis is the most common opportunistic disease[4] and the most common cause of death in patients with HIV infection in developing countries.[5] Notwithstanding effective tuberculosis chemotherapy, in the presence of HIV infection, tuberculosis is associated with substantially increased case fatality rates[6] and is also the most commonly reported cause of death in South Africa.[7] In 2007 in South Africa, an estimated 5.3 million people were infected with HIV and 341,165 with tuberculosis, of whom approximately 73% were coinfected with HIV.[8]

The optimal timing for the initiation of antiretroviral therapy in patients with HIV and tuberculosis coinfection remains unclear. Current guidelines are based on observational studies and expert opinion.[9] Despite World Health Organization (WHO) guidelines supporting concomitant treatment of the two diseases and urging more aggressive management,[10] the initiation of antiretroviral therapy is often deferred until completion of tuberculosis therapy because of concern about potential drug interactions between rifampin and some classes of antiretroviral drugs,[11] the immune reconstitution inflammatory syndrome,[12,13] overlapping side effects,[14] a high pill burden, and programmatic challenges.[15] This study, called the Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPIT) trial, was designed to determine the optimal time to initiate antiretroviral therapy in patients with HIV and tuberculosis coinfection who were receiving tuberculosis therapy.

METHODS

STUDY DESIGN

The study was an open-label, randomized, controlled trial conducted at the eThekwini HIV–tuberculosis clinic, which is operated by the Centre for the AIDS Programme of Research in South Africa (CAPRISA) in Durban, South Africa. This clinic adjoins one of the largest outpatient tuberculosis facilities in South Africa, the Prince Cyril Zulu Communicable Disease Centre.

Guidelines of the South African National Tuberculosis Control Programme[16] stipulate that a first episode of tuberculosis be treated with a 2-month intensive combination-drug regimen of rifampin, isoniazid, ethambutol, and pyrazinamide, with doses determined according to pretreatment weight. Thereafter, patients receive a 4-month continuation regimen of isoniazid and rifampin. Patients with a history of tuberculosis receive a 3-month intensive regimen (including the addition of streptomycin for the first 2 months), followed by a 5-month continuation phase. In our study, patients were routinely offered therapy that was directly observed by clinic-based nurses. Some patients selected community-based supervisors, heads of households, and treatment supporters in workplaces who supervised and recorded the taking of medication.

PATIENTS

From June 28, 2005, to July 11, 2008, we recruited patients who were at least 18 years of age and who had confirmed HIV infection (on the basis of two rapid HIV tests) and a positive smear for tuberculosis acid-fast bacilli (with the use of auramine and Ziehl–Neelsen staining methods). Inclusion in the study required independent confirmation of positive tuberculosis status at the Department of Medical Microbiology at the Nelson R. Mandela School of Medicine, initiation of treatment with the standard tuberculosis regimen at the Communicable Disease Centre, a CD4+ cell count of less than 500 per cubic millimeter at screening, and an absence of clinical contraindications to the initiation of antiretroviral therapy. Female patients were required to agree to use contraception while receiving efavirenz.

STUDY PROCEDURES

After providing written informed consent, patients with confirmed HIV and tuberculosis coinfection were randomly assigned in a 1:1:1 ratio (with the use of sealed envelopes) to one of three study groups in permuted blocks of six or nine with no stratification. In the first group, antiretroviral therapy was to be initiated within 4 weeks after the start of tuberculosis therapy (early integrated-therapy group). In the second group, antiretroviral therapy was to be initiated within 4 weeks after the completion of the intensive phase of tuberculosis therapy (late integrated-therapy group). In the third group, antiretroviral therapy was to be initiated within 4 weeks after the completion of tuberculosis therapy (sequential-therapy group).

All patients received adherence counseling, prophylaxis with trimethoprim–sulfamethoxazole against HIV-related opportunistic infections, and the same once-daily three-drug antiretroviral therapy regimen, consisting of didanosine (250 mg for a body weight of <60 kg and 400 mg for a weight ≥60 kg), lamivudine (300 mg), and efavirenz (600 mg). Adherence to the antiretroviral regimen was assessed monthly according to pill counts (pills issued minus pills returned as a percentage of anticipated pill consumption). Regardless of the study-group assignment, patients could be started on antiretroviral therapy at any time by clinicians at the Communicable Disease Centre, by study clinicians, or by personal physicians at their discretion.

Follow-up visits for the monitoring of safety and clinical status were scheduled monthly for 24 months. Adverse events were graded with the use of the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0, as recommended by the National Institute of Allergy and Infectious Diseases (December 28, 2004). Measurements of CD4+ cell counts with the use of flow cytometry (FACSCalibur, Becton Dickinson) and HIV RNA (Cobas Amplicor HIV-1 Monitor, version 1.5, Roche) were performed at the time of screening, at randomization, and every 6 months thereafter. Monitoring for radiologic changes and sputum conversion was performed at the time of screening, at the end of the intensive phase of tuberculosis therapy, 1 month before the end of tuberculosis therapy, and whenever clinically indicated.

END POINTS

The primary end point was death from any cause. Secondary end points included discontinuation because of side effects, toxic effects, HIV RNA levels, tuberculosis outcomes, and the occurrence of the immune reconstitution inflammatory syndrome. Discontinuation because of side effects was documented as study-initiated treatment interruptions in the pharmacy records. Toxic effects were assessed by means of a clinical checklist and standard laboratory tests for hematologic, hepatic, and renal abnormalities. The immune reconstitution inflammatory syndrome was defined as a paradoxical deterioration in clinical status or laboratory findings after the initiation of antiretroviral or antituberculosis therapy without another attributable cause.

INTERIM MONITORING

After a planned interim analysis, on September 1, 2008, almost 2 months after completion of enrollment, the data and safety monitoring committee recommended that all patients in the sequential-therapy group be started on antiretroviral therapy as soon as possible but continue in follow-up until study completion. The committee also recommended continuation of the two integrated-therapy groups with no changes. The patients in the sequential-therapy group were contacted within a week after the committee's meeting, and almost all of them started antiretroviral therapy within a month. We present data up to September 1, 2008, comparing the sequential-therapy group with the combined early and late integrated-therapy groups, which are hereafter referred to as the integrated-therapy group.

STUDY OVERSIGHT

The trial was approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal and the South African government's Medicines Control Council.

STATISTICAL ANALYSIS

We estimated that we would need to enroll 649 patients (factoring in an anticipated loss to follow-up) in order to have a power of 80% and an alpha level of 0.05 to detect a 60% reduction in mortality on the basis of a predicted death rate of 10% in the study group with the worst outcome. All analyses were performed according to the intention-to-treat principle. The primary outcome was analyzed with the use of Kaplan–Meier curves and the log-rank test. The duration of time in the study was calculated as the time from randomization to death, withdrawal from the study, or the cutoff date of September 1, 2008, whichever occurred first. Poisson approximations were used to calculate confidence intervals for the rate of death. Proportional-hazards regression models were used to adjust for confounding variables. Fisher's exact test was used for the analysis of categorical data, and unpaired t-tests or the Wilcoxon two-sample test for the analysis of continuous data.

RESULTS

PATIENTS

A total of 642 patients with HIV and tuberculosis coinfection were enrolled: 429 in the combined

integrated-therapy group and 213 in the sequential-therapy group (Fig. 1). At baseline, patients in the two groups had similar demographic and clinical characteristics, including age, CD4+ cell counts, and HIV RNA levels (Table 1).

FOLLOW-UP

At the time of the data cutoff, on September 1, 2008, a total of 338 of the 642 patients (52.6%) were still in active follow-up, 52 (8.1%) had died during follow-up, 134 (20.9%) had completed follow-up, and 56 (8.7%) had withdrawn before study completion. Of the 62 patients (9.7%) who were regarded as lost to follow-up (9.6% in the integrated-therapy group and 9.9% in the sequential-therapy group), 35 were known to be alive, and the clinical status of the remaining 27 was unknown. (Patients were considered to be lost to follow-up if they went 4 months without a visit.) The median duration of follow-up in the trial was 12.1 months (interquartile range, 6.1 to 21.6).

INITIATION OF ANTIRETROVIRAL THERAPY

The median duration of tuberculosis therapy was similar among patients who completed such therapy: 210 days for 271 patients in the integrated-therapy group and 207 days for 137 patients in the sequential-therapy group. At the time of this analysis, 102 patients in the integrated-therapy group and 48 patients in the sequential-therapy group were still receiving tuberculosis therapy.

Of the 350 patients in the integrated-therapy group who started antiretroviral therapy, 338 did so while they were receiving tuberculosis therapy. Patients in this group started antiretroviral therapy at a mean (±SD) of 70±72 days after the start of tuberculosis therapy. Of the 100 patients in the sequential-therapy group who started antiretroviral therapy, 7 did so while they were receiving tuberculosis therapy. In this group, antiretroviral therapy was initiated a mean of 260±71 days after the initiation of tuberculosis therapy. Thus, patients in the sequential-therapy group started antiretroviral therapy, on average, 190 days later than those in the integrated-therapy group.

PRIMARY END POINT

There were 25 deaths in the integrated-therapy group, for a death rate of 5.4 per 100 person-years, as compared with 27 deaths in the sequential-therapy group, for a death rate of 12.1 per 100 person-years (hazard ratio in the integrated-therapy group, 0.44; 95% confidence interval [CI], 0.25 to 0.79; P=0.003) (Table 2 and Fig. 2). After adjustment for baseline WHO status of HIV infection (stage 4 vs. stage 3), CD4+ cell count, age, sex, the presence or absence of a history of tuberculosis, the presence or absence of extrapulmonary tuberculosis, and baseline HIV RNA level, the hazard ratio was 0.43 (95% CI, 0.25 to 0.77; P=0.004).

Information on the 52 deaths was based on hospital chart notes for 28 patients, a death certificate for 1 patient, and two independent oral reports of death for 23 patients. On the basis of the chart notes and death certificate for 29 patients, causes of death in the integrated-therapy group were tuberculosis (including tuberculous meningitis) for 2 patients, respiratory distress or *Pneumocystis jiroveci* pneumonia for 6 patients, and metabolic acidosis, cardiomyopathy, and a motor-vehicle accident for 1 patient each; causes of death in the sequential-therapy group were tuberculosis (including tuberculous meningitis) for 6 patients, respiratory distress or *P. jiroveci* pneumonia for 3 patients, and nontuberculous meningitis, gastroenteritis, renal failure, hepatic failure, and glioma for 1 patient each. The cause of death was unclear in the chart notes of four patients.

The baseline CD4+ cell count independently predicted mortality in the two study groups. Mortality was lower in the integrated-therapy group in all CD4+ count strata (Table 2). The median baseline CD4+ count was similar in the two study groups. There was no interaction between the CD4+ count and the study groups (P=0.57).

TREATMENT OUTCOMES

The rate of adherence to antiretroviral therapy according to pill counts was 97.2% in the integrated-therapy group and 97.6% in the sequential-therapy group. Outcomes with respect to tuberculosis therapy were similar in the two study groups, regardless of whether patients were receiving first-episode therapy or repeated therapy (Table 3). At 12 months after randomization, the proportion of patients with a suppressed HIV RNA level was higher in the integrated-therapy group than in the sequential-therapy group (90.0% vs. 77.8%, P=0.006). However, the proportion of patients with a suppressed HIV RNA level 6 months after the initiation of antiretroviral therapy was similar in the two groups (Table 4).

ADVERSE EVENTS

The immune reconstitution inflammatory syndrome was diagnosed in 53 of 429 patients (12.4%;

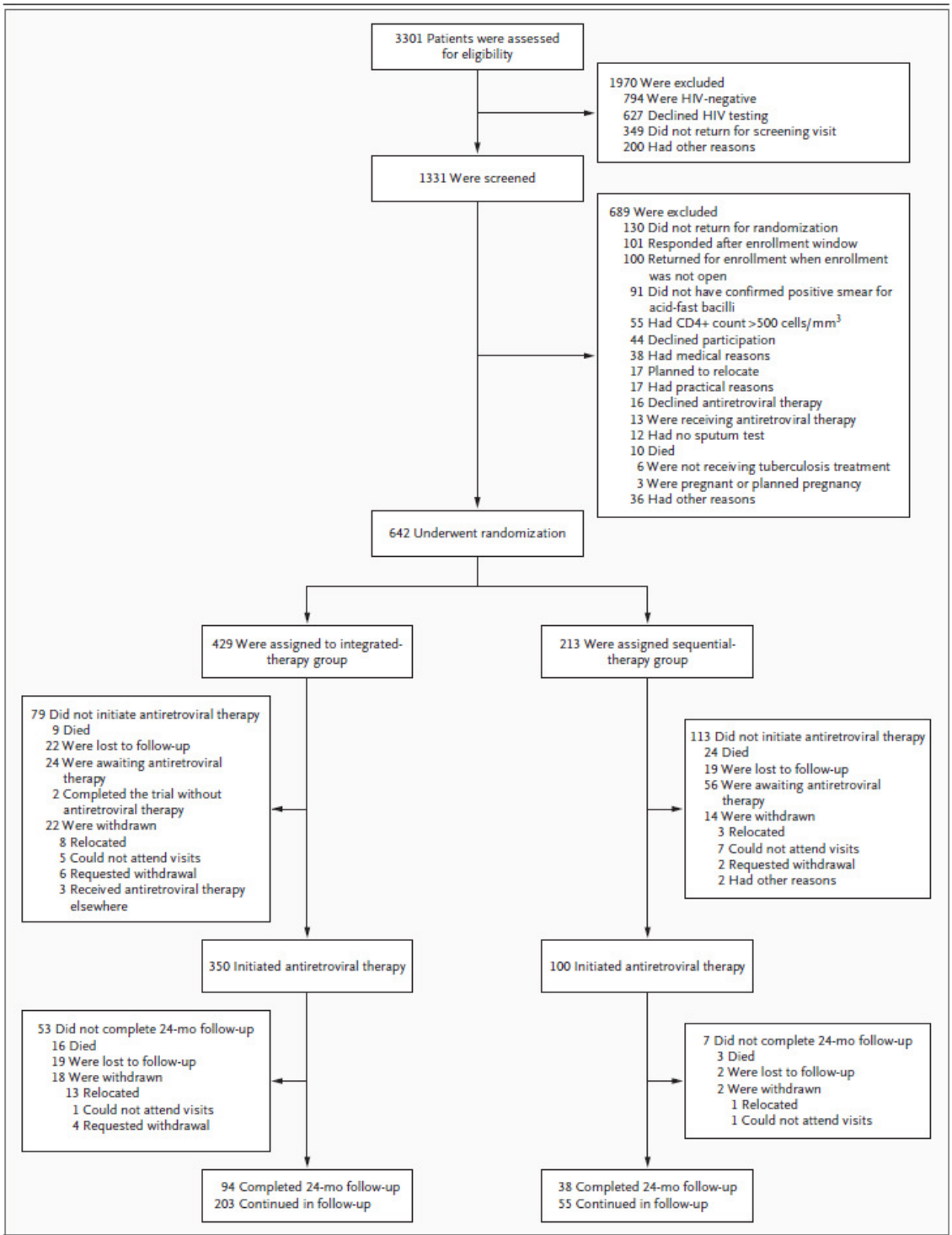

Figure 1. Enrollment and Outcomes.

Table 1. Baseline Characteristics of the Patients.*

Variable	Integrated Therapy (N=429)	Sequential Therapy (N=213)	P Value
Age — yr			0.48
Mean	34.4±8.38	33.9±8.18	
Range	19–72	19–60	
Male sex — %	48.7	52.1	0.45
Educational level — no./total no. (%)			0.03
Primary school or less	92/427 (21.5)	48 (22.5)	
Some secondary school	205/427 (48.0)	80 (37.6)	
Completed secondary school	130/427 (30.4)	85 (39.9)	
Employed — no./total no. (%)	251/428 (58.6)	116 (54.5)	0.35
History of tuberculosis — no. (%)	144 (33.6)	64 (30.0)	0.42
Karnofsky score — no./total no. (%)			0.44
90 or 100	251/425 (59.1)	132/209 (63.2)	
70 or 80	165/425 (38.8)	75/209 (35.9)	
<70	9/425 (2.1)	2/209 (1.0)	
Median CD4+ count (interquartile range) — cells/mm³†	150 (77–254)	140 (69–247)	0.32
Median log viral load (interquartile range) — copies/ml‡	5.2 (4.5–5.6)	5.2 (4.7–5.6)	0.22
WHO stage 4 HIV infection — no. (%)§	21 (4.9)	10 (4.7)	1.00
Presence of extrapulmonary tuberculosis — no. (%)	24 (5.6)	10 (4.7)	0.71
Median no. of days of tuberculosis therapy at randomization (interquartile range)	9 (1–14)	9 (7–16)	0.15

* Plus–minus values are means ±SD.
† Patients underwent randomization on the basis of the CD4+ count at screening (criterion for study enrollment, <500 cells per cubic millimeter). However, for 16 patients, the CD4+ count at enrollment was more than 500 cells per cubic millimeter.
‡ The viral load at baseline was measured in 397 patients in the integrated-therapy group and in 201 patients in the sequential-therapy group.
§ The remainder of patients had stage 3 infection, according to criteria of the World Health Organization (WHO).

95% CI, 9.5 to 15.9) in the integrated-therapy group and in 8 of 213 patients (3.8%; 95% CI, 1.8 to 7.5) in the sequential-therapy group (P<0.001). Six patients required the use of corticosteroids (five in the integrated-therapy group and one in the sequential-therapy group). No changes in the antiretroviral regimen were needed because of immune-reconstitution events. None of the deaths were determined to be related to the immune reconstitution inflammatory syndrome. Among grade 3 or 4 adverse events that were not regarded as immune reconstitution, 140 occurred in the integrated-therapy group (30 per 100 person-years) and 71 in the sequential-therapy group (32 per 100 person-years) (P=0.69) (see the table in the Supplementary Appendix, available with the full text of this article at NEJM.org).

DISCUSSION

This trial showed that the initiation of antiretroviral therapy during tuberculosis therapy in patients with confirmed tuberculosis and HIV coinfection reduced mortality by 56% (95% CI, 21 to 75). The death rate rose from 5.4 per 100 person-years to 12.1 per 100 person-years when initiation of antiretroviral therapy was delayed until the completion of tuberculosis therapy. The interval between the completion of tuberculosis therapy and the initiation of antiretroviral therapy is important; a considerable number of deaths in the sequential-therapy group occurred during this time (Fig. 2). Once antiretroviral therapy was initiated, however, it was associated with similarly high levels of viral suppression in the two study

Table 2. Death Rates and Hazard Ratios, Stratified According to CD4+ Cell Count.

CD4+ Count	Integrated Therapy				Sequential Therapy				Hazard Ratio (95% CI)*	P Value
	No. of Patients	No. of Person-Yr	No. of Deaths	Death Rate/ 100 Person-Yr (95% CI)	No. of Patients	No. of Person-Yr	No. of Deaths	Death Rate/ 100 Person-Yr (95% CI)		
All patients	429	467	25	5.4 (3.5–7.9)	213	223	27	12.1 (8.0–17.7)	0.44 (0.25–0.79)	0.003
≤200 cells/mm³	273	281	23	8.2 (5.2–12.3)	138	137	21	15.3 (9.6–23.5)	0.54 (0.30–0.98)	0.04
>200 cells/mm³	156	186	2	1.1 (0.1–3.9)	75	86	6	7.0 (2.6–15.3)	0.16 (0.03–0.79)	0.02

* Hazard ratios are for the integrated-therapy group, as compared with the sequential-therapy group.

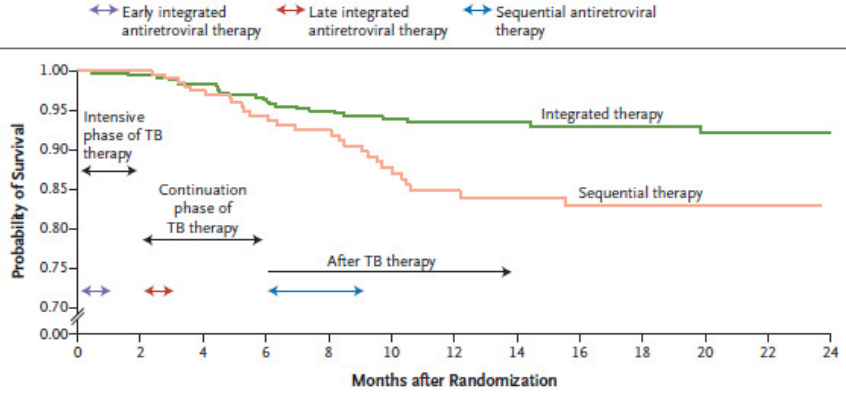

Figure 2. Kaplan–Meier Survival Curves.
TB denotes tuberculosis.

groups, findings that are similar to those observed in other HIV treatment programs in South Africa.[17]

Mortality among patients with HIV and tuberculosis coinfection is known to be high despite the use of effective tuberculosis therapy.[5] Observational studies have indicated that the initiation of antiretroviral therapy during tuberculosis therapy improves treatment outcomes in such patients. A meta-analysis of studies involving 6934 patients at five hospitals in Madrid showed a significant improvement in survival (63% increase) among patients who began antiretroviral therapy while they were receiving tuberculosis therapy.[18] In Thailand, an analysis of 1003 patients showed an increase by a factor of 20 in the rate of death among patients who did not receive simultaneous antiretroviral and tuberculosis therapies, as compared with those who did receive the two therapies.[19] A Thai review of studies involving 626 patients showed a hazard ratio for death of 0.17 for patients who started antiretroviral therapy during tuberculosis treatment, as compared with patients who did not receive antiretroviral therapy.[20] Although the selection of patients for integrated treatment by clinicians may have led to bias in these studies, the trials show a consistent association between antiretroviral therapy and survival in coinfected patients. The randomized design of our trial validates and extends the findings from these retrospective observational data.

Among patients with CD4+ counts of less than 200 cells per cubic millimeter, the rate of death was 46% lower in the integrated-therapy group than in the sequential-therapy group (P=0.04). Although the number of deaths was small in the subgroup of patients who had CD4+ counts between 200 and 500 cells per cubic millimeter, there was a trend toward lower mortality in the

Table 3. Clinical Outcomes of Tuberculosis Therapy.*

Outcome	Integrated Therapy (N=343)		Sequential Therapy (N=171)		P Value
	Repeated Therapy of Tuberculosis (N=116)	First Episode of Tuberculosis (N=227)	Repeated Therapy of Tuberculosis (N=58)	First Episode of Tuberculosis (N=113)	
	number (percent)				
Tuberculosis cure†	67 (57.8)	131 (57.7)	31 (53.4)	67 (59.3)	1.00
Successful completion‡	16 (13.8)	42 (18.5)	5 (8.6)	16 (14.2)	0.20
Therapy success (cure plus successful completion)	83 (71.6)	173 (76.2)	36 (62.1)	83 (73.5)	0.07
Died before completion of therapy	7 (6.0)	12 (5.3)	6 (10.3)	9 (8.0)	0.19
Therapy interruption	2 (1.7)	3 (1.3)	3 (5.2)	7 (6.2)	0.01
Therapy failure§	0	0	0	1 (0.9)	0.33
Loss to follow-up	13 (11.2)	13 (5.7)	6 (10.3)	6 (5.3)	1.00
Therapy outcome unknown because of transfer to another clinic	1 (0.9)	5 (2.2)	2 (3.4)	1 (0.9)	1.00
Other outcome	1 (0.9)	1 (0.4)	0	0	
Outcome pending (still receiving therapy at time of analysis)	9 (7.8)	20 (8.8)	5 (8.6)	6 (5.3)	0.49

* Only patients who were enrolled in the study at least 7 months before the date of the analysis are included. Percentages may not total 100% because of rounding.
† Tuberculosis cure was defined as a sputum smear that was negative for acid-fast bacilli close to the time of therapy completion.
‡ Successful completion of therapy was defined as the use of more than 85% of the prescribed medication.
§ Therapy failure was defined as the presence of a positive smear or culture for *Mycobacterium tuberculosis* obtained at least 5 months after the initiation of tuberculosis therapy.

integrated-therapy group. This finding has implications for treatment guidelines and policies. Current WHO guidelines for the treatment of patients with HIV and tuberculosis coinfection recommend the deferment of antiretroviral therapy until the completion of tuberculosis therapy in patients with WHO stage 3 HIV infection and CD4+ counts of more than 200 cells per cubic millimeter.[3,10] Our findings suggest that this guideline should be expanded to include cotreatment of HIV infection and tuberculosis in patients with CD4+ counts of less than 500 cells per cubic millimeter.

There is increasing evidence that even among patients with HIV infection who do not have tuberculosis, earlier initiation of antiretroviral therapy is associated with improved outcomes.[21-23] In a study involving 8362 asymptomatic patients with HIV infection in the United States and Canada,[22] mortality was 69% lower among patients in whom antiretroviral therapy was initiated when the CD4+ count was between 350 and 500 cells per cubic millimeter than in those in whom such therapy was deferred until the CD4+ count was less than 350 cells per cubic millimeter. Similarly, data from 18 prospective cohort studies have shown that deferring antiretroviral therapy was associated with higher rates of the acquired immunodeficiency syndrome (AIDS) and death than starting therapy when the CD4+ count was more than 350 cells per cubic millimeter.[23]

However, there are major concerns regarding the early initiation of antiretroviral therapy during tuberculosis treatment, including the increased risk of the immune reconstitution inflammatory syndrome, additive toxic effects, and the potential adverse effect on outcomes of tuberculosis therapy. We found similar rates of grade 3 and 4 adverse events in the two study groups and similar outcomes of tuberculosis therapy. Since many of the deaths occurred after the completion of tuberculosis therapy, the providers of such therapy were unaware of the benefits of cotherapy of tuberculosis and HIV infection. Although the incidence of immune-reconstitution events was significantly higher in the integrated-therapy group, this finding was not unexpected, since such events have been associated with the early initiation of antiretroviral therapy in patients with tuberculosis.[24,25] The incidence of such events in the integrated-therapy group was similar to that observed in studies of other cohorts

Table 4. Clinical Outcomes of HIV Therapy.

Outcome	Integrated Therapy	Sequential Therapy	P Value
Viral load <400 copies/ml			
At 12 mo after randomization			0.006
No./total no.	199/221	70/90	
Percent (95% CI)	90.0 (85.1–93.5)	77.8 (67.6–85.6)	
At 6 mo after initiation of antiretroviral therapy			0.40
No./total no.	174/191	39/45	
Percent (95% CI)	91.1 (85.9–94.6)	86.7 (72.5–94.5)	
Mean increase in CD4+ count from baseline			
At 12 mo after randomization			0.004
No. of patients	207	84	
No. of cells/mm^3 (95% CI)	148.7 (130.5–166.9)	100.7 (77.5–124.0)	
At 6 mo after initiation of antiretroviral therapy			0.71
No. of patients	187	41	
No. of cells/mm^3 (95% CI)	124.2 (105.4–143.1)	116.3 (88.0 to 144.6)	

in developing countries,[24,26] including a retrospective analysis of hospitalized Thai patients receiving both antiretroviral and tuberculosis therapies, which showed that 21 of 167 patients (12.6%) had an immune-reconstitution event.[26] However, none of the deaths in our trial, in which data regarding the cause of death were available, were considered attributable to the immune reconstitution inflammatory syndrome. It is reassuring that recent studies of tuberculosis-associated immune reconstitution inflammatory syndrome indicate that this complication is rarely fatal and that severe episodes can be successfully managed with corticosteroids.[27] Thus, the concern about increasing the likelihood of such episodes must be tempered by the survival benefit shown in our study. Nevertheless, the paradoxical deterioration in the clinical status is sufficiently common to warrant close clinical monitoring in the first few months after the initiation of antiretroviral therapy in patients coinfected with tuberculosis.

We acknowledge several limitations of our study. The use of death from any cause as the primary end point might underestimate the potential effect of integrated HIV–tuberculosis treatment on the rates of death specifically from tuberculosis or HIV infection. Since we were not able to obtain reliable information on the causes of all deaths in the trial, we were not able to estimate the effect on the rate of deaths that were related only to tuberculosis or HIV infection. Since our trial included only patients who had a positive sputum smear for acid-fast bacilli and whose disease was diagnosed and treated in an outpatient tuberculosis clinic, the results may not be directly generalizable to all forms and severity levels of tuberculosis. Since a retrospective analysis of 549 patients with AIDS and extrapulmonary tuberculosis showed that the introduction of highly active antiretroviral therapy significantly improved survival,[28] the early initiation of antiretroviral therapy may have similar benefits in patients with extrapulmonary tuberculosis. Although we have no reason to believe that our findings do not apply to sputum-smear–negative tuberculosis, our findings require empirical confirmation in this group. It should be noted that the judgment of study and nonstudy care providers took precedence over protocol-defined timing of the initiation of antiretroviral therapy, which led to early initiation of such therapy in some patients in the sequential-therapy group and delayed initiation in some patients in the integrated-therapy group. Another limitation was the delay in the initiation of antiretroviral therapy after the completion of tuberculosis therapy in the sequential-therapy group because of clinical issues (e.g., elevated levels of liver enzymes) or missed visits. Furthermore, the question of when antiretroviral therapy should be initiated during tuberculosis therapy awaits completion of the study.

In summary, our findings provide compelling

evidence of the benefit of initiating antiretroviral therapy during tuberculosis therapy in patients with HIV coinfection. The findings also support recommendations by the WHO and others for the integration of tuberculosis and HIV care.

Supported by the U.S. President's Emergency Plan for AIDS Relief for the care of patients, the Global Fund to fight AIDS, Tuberculosis and Malaria for drugs used in the trial, and the Comprehensive International Program of Research on AIDS for the research infrastructure (including data management and laboratory and pharmacy facilities) to conduct the trial.

An overview of the results was presented at the 16th Conference on Retroviruses and Opportunistic Infections, Montreal, February 9, 2009.

Dr. S. Abdool Karim reports being listed as a coinventor on two patents (2000/3437 and PCT/IB02/04550) that are part of the development of clade C HIV vaccines; and Mr. Gray, receiving lecture fees from AstraZeneca, Aspen Pharmacare, and Fresenius Kabi. No other potential conflict of interest relevant to this article was reported.

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Discussion of Paper V: Paper V is a seminal article that assesses the impact of integrated HIV and TB treatment on survival, adverse events and safety.

This important study was an open label RCT that assigned 642 Black, South African patients to start ART during TB treatment or to complete TB treatment and then initiate ART. This RCT showed a 56% relative reduction in risk of death amongst those receiving integrated treatment, across all CD4 strata, compared to those who initiated their HIV treatment after completing TB treatment.

The trial was halted prematurely by the data safety monitoring board to enable all patients to benefit from integrated treatment. In terms of adverse events, IRIS was diagnosed in 12% and 3.8% of integrated vs. sequential arm participants, however, amongst all other grade 3 or 4 adverse events there was no statistical difference in frequency between groups. Integrated treatment was therefore regarded as safe and effective.

Importance for TB/HIV treatment integration: This was the first randomized trial to unequivocally show that delaying ART initiation during TB co-infection results in higher mortality. In addition, the study identifies a critical period between TB treatment completion and the subsequent start of ART as a high risk period for death. The findings were rapidly incorporated into global and local HIV and treatment guidelines and policy and lent support to the recommendations of others to urgently integrate TB and HIV care.

A2: PAPER VI

Ritonavir/saquinavir safety concerns curtail antiretroviral therapy options for tuberculosis-HIV-co-infected patients in resource-constrained settings

A2. Paper VI: Ritonavir/saquinavir safety concerns curtail antiretroviral therapy options for tuberculosis-HIV-co-infected patients in resource-constrained settings

Ritonavir/saquinavir safety concerns curtail antiretroviral therapy options for tuberculosis–HIV-co-infected patients in resource-constrained settings

Tuberculosis is the most common serious opportunistic infection associated with HIV infection in sub-Saharan Africa, and a strong case has been made for integrating tuberculosis and HIV care [1]. In those settings where the tuberculosis and HIV epidemics converge, existing tuberculosis programmes provide an opportunity for efficiently identifying those HIV-infected patients who are eligible for antiretroviral therapy (ART), as well as for initiating this therapy in order to utilize the existing tuberculosis directly observed therapy infrastructure. This approach is, however, dependent on the availability of effective, safe and affordable antiretroviral regimens that are compatible with the standard treatments for tuberculosis, including rifampicin. As a result of cost considerations, the widespread use of alternative rifamycins, such as rifapentine or rifabutin, is not feasible in resource-constrained settings.

Current guidelines, such as those from the US Department of Health and Human Services, suggest that regimens based on the non-nucleoside reverse transcriptase inhibitor efavirenz be used as first-line choices in patients receiving concomitant rifampicin [2]. Therapy choices become far more difficult in the face of treatment-limiting toxicities, virological failure or pregnancy. In these cases, a protease inhibitor-based regimen is needed as nevirapine may not be an appropriate option because of its interaction with rifampicin and additive hepatic toxicity. The guidelines suggested the use of ritonavir-boosted saquinavir [2]. Although the ability of ritonavir to boost plasma concentrations of saquinavir is well described, there is only limited evidence that this combination is adequate to counteract the enzyme induction caused by rifampicin and is clinically effective [3,4]. The issue of a 'Dear Health Care Provider' letter by Roche Pharmaceuticals on 7 February 2005 has now caused considerable additional uncertainty [5]. The manufacturers have informed the US Food and Drug Administration of problems experienced in a phase I, randomized, open-label, multiple-dose clinical pharmacology study in

healthy volunteers. Of 28 patients given rifampicin 600 mg once a day together with ritonavir 100 mg and saquinavir 1000 mg twice a day, 11 (39.3%) had developed significant hepatocellular toxicity during the 28-day study period. In the light of this evidence, the continued use of this combination cannot be supported. As a consequence, the US Food and Drug Administration Advisory has now removed the only protease inhibitor-containing regimen recommended for use concurrently with rifampicin containing tuberculosis treatment, and has thereby reduced the therapeutic options available for those requiring ART beyond standard first-line therapy options. In the absence of direct advice to the contrary, two options are being explored that we feel to be questionable. Some have argued that the exact doses of ritonavir and saquinavir used in the pharmacokinetic study (100 mg and 1000 mg, respectively) were different from those used in practice (400 mg and 400 mg, given twice a day), thus justifying thte continued use of this combination. Others have argued that adding additional ritonavir to co-formulated lopinavir–ritonavir would be sufficient to overcome the hepatic enzyme induction caused by concomitant rifampicin. However, in an open-label, randomized trial of two such dosing regimens in health volunteers, 12 out of 32 subjects withdrew from the study [6]. For nine of these, the co-administration of lopinavir–ritonavir and rifampicin was associated with elevations in liver enzyme levels. The similarity to the outcome of the saquinavir–ritonavir pharmacokinetic study cannot be ignored. The remaining options are, thus, either to switch to a triple nucleoside regimen or to cease further antiretroviral therapy until the completion of the rifampicin-containing tuberculosis treatment.

Until recently, the multitude of studies on alternative treatment regimens has focused on the needs of the developed world where ART has been widely available and switching options are increasingly important. The Global Fund Against AIDS, TB and Malaria, the WHO's 3-by-5 programme, the US President's Emergency Program For AIDS Relief (PEPFAR) and local efforts are slowly increasing access to ART in resource-constrained regions such as sub-Saharan Africa. The newly described toxicity of the rifampin, saquinavir, ritonavir regimen thus vividly highlights the great need for research, particularly pharmacokinetic studies, also to address the ART options appropriate for resource-limited settings, and in particular, in this instance, for co-administration with rifampicin-containing tuberculosis treatment.

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Appendix

The Starting Tuberculosis and Anti-Retroviral Therapy (START) project team includes: Salim Abdool Karim, Quarraisha Abdool Karim, Susan Brobst, Gavin Churchyard, Wafaa El-Sadr, Gerald Friedland, Clive Gray, Andy Gray, Rene Gonin, Richard Hafner, Scott Hammer, Rodney Hoff, Munira Khan, Mark Lurie, Terence Moodley, Marita Muurman, Kogieleum Naidoo, Gonasagrie Nair, Nesri Padayatchi, Diane Pizzano, Moussa Sarr, Aarthi Singh and Douglas Wilson.

Care should be taken when promoting microbicide use among sex workers who are able to use condoms consistently: response to Smith *et al.* (2005)

In a recent paper in *AIDS*, Smith *et al.* [1] used mathematical modelling to explore the issue of condom migration, or 'condom replacement', on the potential impact of microbicide use by female sex workers. Similar to an earlier paper that we published [2], they developed a static model of the risk of HIV acquisition, and used this to

Discussion of Paper VI: This article was published eight years ago, in response to a notice issued by Roche pharmaceuticals, when the US FDA was informed of results of a phase I open-label RCT in healthy individuals. When the volunteers were administered RIF 600mg with saquinavir/ritonavir 1000/100mg twice a day, 39% developed significant hepatotoxicity during the 28 day study period. The US FDA then disallowed the use of this combination during TB treatment.

At that time, guidelines advocated use of ritonavir-boosted saquinavir as the only available PIs in South Africa. The consequences were therefore severe at a time when not all ARV drugs were available in resource-constrained settings for situations where treatment limiting toxicity existed, or virological failure had developed or were required for use in pregnant women.

Importance for TB/HIV treatment integration: The article was timely in that it highlighted the need for more drug options in resource-limited settings, and the need for more PK studies in sick individuals, to better understand the drug interactions and to offer safer treatment options for HIV infected patients requiring TB treatment. It was fortunate that the lopinavir/ritonavir registration was filed in South Africa in that same year and became more widely available in subsequent years. Elevations in liver enzymes, of the lopinavir/ritonavir combination seen in healthy volunteers, did not preclude effective and safe treatment in the HIV and TB co-infected and the drug was used during TB co-treatment with appropriate laboratory monitoring.

A3: PAPER VII

The SAPIT trial provides essential evidence on risks and benefits of integrated and sequential treatment of HIV and TB

A3. Paper VII: The SAPIT trial provides essential evidence on risks and benefits of integrated and sequential treatment of HIV and TB

ISSUES IN RESEARCH

The SAPIT trial provides essential evidence on risks and benefits of integrated and sequential treatment of HIV and tuberculosis

Quarraisha Abdool Karim, Salim S Abdool Karim, Cheryl Baxter, Gerald Friedland, Tanuja Gengiah, Andrew Gray, Anneke Grobler, Kogieleum Naidoo, Nesri Padayatchi, Wafaa El-Sadr

Boulle *et al.*[1] queried whether a clinical trial was needed to provide the evidence for the mortality benefits of antiretroviral therapy (ART) initiation during tuberculosis (TB) treatment. While several experts, including foremost TB-HIV scientists from South Africa[2] and the USA,[3] senior World Health Organization (WHO)[4] and UNAIDS[5] officials at the time the study was initiated, the 2003 WHO AIDS Treatment Guidelines Committee Chair[3], the Chair of the Ethics Committee[6] and the researchers,[7] have previously addressed the points raised, the SAPIT (Starting Antiretroviral Therapy at Three Points in Tuberculosis) research team welcomes the opportunity also to address the comments. We hold Boulle and his colleagues in high regard and appreciate their contributions to the field of HIV and tuberculosis co-infection. More importantly, we share with them the common goal of rigorously and relentlessly seeking answers to critically important research questions as we confront the devastating dual AIDS and tuberculosis epidemics.

The SAPIT trial,[8] which was developed in 2004, set out to assess whether integrating tuberculosis and AIDS treatment would lead to improved outcomes compared with the widely practised approach of treating them sequentially. The trial's Safety Monitoring Committee halted the sequential treatment arm in September 2008 because of a 56% lower mortality rate in the integrated treatment arm. We systematically address the queries on equipoise and standard of care.

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Did the SAPIT study have clinical equipoise?

Yes, the optimal timing of ART initiation in patients with tuberculosis was not known at the time the trial was planned and conducted.

Availability of, and experience in providing, ART in developing countries, including South Africa, was limited before 2004, and even less was known about the timing of ART initiation in tuberculosis-HIV co-infected patients. Treatment guidelines were either silent on this issue or contained tentative and provisional guidance largely based on expert opinion, due to the lack of reliable and compelling evidence. The 2003 WHO guidelines specifically mention this limitation and that their recommendations are 'Pending ongoing studies ...'[9]

The SAPIT trial had clinical equipoise because the balance between the potential increase in morbidity and mortality due to combined antiretroviral-tuberculosis drug intolerance, drug-drug interactions and immune reconstitution inflammatory syndrome (IRIS) on the one hand and the potential improved morbidity and mortality from early antiretroviral initiation on the other were unknown.[9] Published data on IRIS-associated morbidity and mortality at the time were limited, not least owing to substantial under-reporting resulting from the lack of a consistent case definition of what constituted IRIS. A WHO consultation[10] in 2005 highlighted the problem of inadequate data on IRIS and recommended that 'validating the definition of IRIS' be regarded as a research priority. When the standardised case definition of IRIS was published in 2008, the authors pointed out that this definition will help clinicians by providing insight into the incidence, clinical manifestations and impact of TB-associated IRIS.[11]

Boulle *et al.* cite two retrospective chart review studies[12-13] undertaken in the UK to support their argument that the beneficial effect of ART during TB therapy was already known. The first included 159 patients with TB and HIV who were not on ART at presentation, 45% of whom were subsequently initiated on ART by the treating clinicians.[12] Just over a third of the latter patients had either TB or HIV treatment discontinued because of adverse events, making interpretations of safety and effectiveness difficult. The second compared outcomes in 36 patients with TB and HIV in a pre-antiretroviral era with 60 patients in the antiretroviral era.[13] Both studies provide useful initial descriptive information on experiences in co-treatment, but do not provide the quality of evidence essential for the development of clinical guidelines and treatment policy. Some co-treatment challenges are epitomised in the statement: 'More doubts than certainties are available on which basing the decision on how to cope therapeutically with active tuberculosis developing in a patient also requiring antiretroviral treatment.'[14]

The existence of equipoise was confirmed by several levels of scientific, regulatory and ethical review, including independent approval from the Medicines Control Council and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (a US Office for Human Research Protections-accredited ethics committee), which also oversees research conducted by several of

the co-authors of the critique.[1] The SAPIT trial was undertaken after widespread consultation in scientific and community forums, including presentations and dedicated sessions at the South African AIDS conferences in 2003 and 2005. Importantly, a rationale for the trial was published in the peer-reviewed literature before its initiation.[15]

Were the patients in the SAPIT trial provided the best standard of care?

Yes, the SAPIT trial provided the prevailing best standard of care for antiretroviral initiation, viz. clinician judgement. Clinicians with prior experience in treating TB-HIV co-infected patients were in a position throughout the study to initiate patients (who were seen daily during the week in the directly observed treatment programme) on ART at any time, based on their clinical judgement.

On their point of adequacy of the standard of care provided to patients in the SAPIT trial, Boulle *et al.*[1] quote from the World Medical Association's Declaration of Helsinki:[16] '… "the best current" intervention should be provided as the standard of care to patients in studies …'. In doing so, they omit the crucial word '*proven*'; paragraph 32 of the current Declaration (quoted and referenced in their article) stated in full is: 'The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current *proven* intervention ...' (our italics).

While it was well recognised that HIV and TB co-infected patients with low CD4+ counts had a higher mortality, it was *not* proven that early antiretroviral initiation in TB co-infected patients improved morbidity and mortality. The 2003 WHO AIDS treatment guidelines, which informed the 2004 South African AIDS treatment guidelines, stated clearly that recommendations on the initiation of ART in TB co-infected patients were 'provisional', since the 'optimal time to initiate [antiretroviral agents] in patients with [tuberculosis] is not known'.[17] Such tentative guidance cannot be considered a proven intervention. To further confirm that that this was not proven, a WHO consultation in 2005 on management of patients with HIV and TB concluded that the optimal time for initiating ART in HIV and TB co-infected patients is 'the major research priority'.[10]

Was this trial needed for tuberculosis and HIV treatment?

Yes, the SAPIT trial informed the new WHO treatment guidelines.

Retrospective chart reviews, such as cited by Boulle *et al.* in support of initiation of ART during TB treatment, are seldom regarded as sufficient evidence for authoritative treatment guidelines, as they are prone to many biases and are rarely effective in influencing clinician practices. In 2009, the WHO guidelines[18] were updated to provide definitive advice on the timing of antiretroviral initiation in patients with TB and HIV, drawing upon the results of the SAPIT trial. On World AIDS Day in 2009, the South African government also announced[19] a change in TB-HIV co-treatment guidelines, drawing on the SAPIT trial results.

The SAPIT trial provided the essential evidence on the risks and benefits on integrated HIV and TB treatment to guide clinicians and inform both global and local policy on HIV and TB treatment. We now have the collective opportunity and the responsibility to ensure that patients with TB and HIV are rapidly diagnosed and are initiated on ART during TB treatment.

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Discussion of Paper VII: This article was written in response to another publication where the authors' debate that that the SAPIT study was unnecessary and that the conclusions reached could have been surmised from observational research. Their major concern was that because of its design SAPIT, which could allow patients with CD4 counts less than 200 cells/$mm^3$to have ART deferred and that mortality experienced in the study could have been avoided[138]. The response by the authors in Paper VII was that at the time the trial was designed and ran there was clinical equipoise regarding the optimal timing of ART initiation in patients with TB. Patients were also provided with best standard of care which at the time was regarded to be clinician assessment for when to start treatment, and most importantly although it is acknowledged that HIV patients with TB and low CD4 counts had a higher mortality, it was not beyond doubt, at that time, that co-treatment in this group improved morbidity and mortality.

Importance for TB/HIV treatment integration: Despite the evidence provided by observational study designs, clear guidance from local and international guidelines, and therefore clinical practice at a provider level, was ambivalent in 2005 when the study was conceptualized. The study had a DSMB in place to monitor the safety of participants and in addition the trial results directly informed new WHO guidelines. This was, however, a useful publication to have in the public domain because the controversy also raised awareness amongst providers about the ethical issues and may have indirectly resulted in providers taking more effort to integrate TB and HIV care.

A4: PAPER VIII

Integration of antiretroviral therapy with tuberculosis treatment

A4: Paper VIII: Integration of antiretroviral therapy with tuberculosis treatment

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Integration of Antiretroviral Therapy with Tuberculosis Treatment

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ABSTRACT

BACKGROUND

We previously reported that integrating antiretroviral therapy (ART) with tuberculosis treatment reduces mortality. However, the timing for the initiation of ART during tuberculosis treatment remains unresolved.

METHODS

We conducted a three-group, open-label, randomized, controlled trial in South Africa involving 642 ambulatory patients, all with tuberculosis (confirmed by a positive sputum smear for acid-fast bacilli), human immunodeficiency virus infection, and a CD4+ T-cell count of less than 500 per cubic millimeter. Findings in the earlier-ART group (ART initiated within 4 weeks after the start of tuberculosis treatment, 214 patients) and later-ART group (ART initiated during the first 4 weeks of the continuation phase of tuberculosis treatment, 215 patients) are presented here.

RESULTS

At baseline, the median CD4+ T-cell count was 150 per cubic millimeter, and the median viral load was 161,000 copies per milliliter, with no significant differences between the two groups. The incidence rate of the acquired immunodeficiency syndrome (AIDS) or death was 6.9 cases per 100 person-years in the earlier-ART group (18 cases) as compared with 7.8 per 100 person-years in the later-ART group (19 cases) (incidence-rate ratio, 0.89; 95% confidence interval [CI], 0.44 to 1.79; P=0.73). However, among patients with CD4+ T-cell counts of less than 50 per cubic millimeter, the incidence rates of AIDS or death were 8.5 and 26.3 cases per 100 person-years, respectively (incidence-rate ratio, 0.32; 95% CI, 0.07 to 1.13; P=0.06). The incidence rates of the immune reconstitution inflammatory syndrome (IRIS) were 20.1 and 7.7 cases per 100 person-years, respectively (incidence-rate ratio, 2.62; 95% CI, 1.48 to 4.82; P<0.001). Adverse events requiring a switching of antiretroviral drugs occurred in 10 patients in the earlier-ART group and 1 patient in the later-ART group (P=0.006).

CONCLUSIONS

Early initiation of ART in patients with CD4+ T-cell counts of less than 50 per cubic millimeter increased AIDS-free survival. Deferral of the initiation of ART to the first 4 weeks of the continuation phase of tuberculosis therapy in those with higher CD4+ T-cell counts reduced the risks of IRIS and other adverse events related to ART without increasing the risk of AIDS or death. (Funded by the U.S. President's Emergency Plan for AIDS Relief and others; SAPIT ClinicalTrials.gov number, NCT00398996.)

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In patients who have infection with the human immunodeficiency virus (HIV) and tuberculosis, antiretroviral therapy (ART) may be initiated at the same time as or soon after the initiation of tuberculosis treatment. However, antiretroviral agents are often deferred until after the intensive phase of tuberculosis treatment because of concern about the immune reconstitution inflammatory syndrome (IRIS),[1,2] a high pill burden, and overlapping side effects[3] when three antiretroviral agents are added to the standard four antituberculosis drugs. These challenges may result in interruption or discontinuation of treatment for the acquired immunodeficiency syndrome (AIDS) or tuberculosis, which can lead to drug resistance and potentially limit future therapeutic options,[4,5] but the disadvantages must be weighed against the risk of increased mortality early in the treatment of tuberculosis.

The Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPIT) trial[6] was designed to determine the clinical consequences of the time to the start of ART in patients with HIV infection and tuberculosis. We previously reported that integrating ART with tuberculosis treatment reduces mortality.[6] Here, we report on the initiation of ART at two points during tuberculosis treatment.

METHODS

STUDY DESIGN, PATIENTS, AND PROCEDURES

We conducted a prospective, open-label, randomized trial in South Africa. A total of 642 ambulatory patients with both pulmonary tuberculosis and HIV infection, 18 years of age or older, were enrolled after providing written informed consent.

The diagnosis of pulmonary tuberculosis was confirmed by a positive sputum smear for acid-fast bacilli. HIV infection was confirmed by two rapid screening tests for HIV. All patients had a CD4+ T-cell count of less than 500 per cubic millimeter at screening and were started on a standard tuberculosis treatment regimen.[7] All patients with a first episode of tuberculosis were treated with a fixed combination of rifampin, isoniazid, ethambutol, and pyrazinamide, with doses determined according to pretreatment weight, for 2 months (intensive phase) and a subsequent fixed combination of isoniazid and rifampicin for 4 months (continuation phase). Patients who had previously received treatment for tuberculosis received a 60-day intensive regimen that included streptomycin, followed by a 100-day continuation regimen.

The once-daily ART regimen consisted of enteric-coated didanosine (250 mg if the patient's weight was <60 kg and 400 mg if the weight was ≥60 kg), lamivudine (300 mg), and efavirenz (600 mg). Adherence to ART was assessed monthly by means of pill counts. Notwithstanding the study-group assignments, patients could be started on ART at any time at the discretion of the study clinicians or the patients' primary care physicians. Details of the study design and procedures have been described previously[6] and are provided in the protocol and Supplementary Appendix, available with the full text of this article at NEJM.org.

The outcome of the sequential-therapy group (ART initiated after the completion of tuberculosis treatment) has been reported previously.[6] The analysis reported here includes complete follow-up data on the 214 patients in the early integrated-therapy group (ART initiated within 4 weeks after the start of tuberculosis treatment) and the 215 patients in the late integrated-therapy group (ART initiated within 4 weeks after completion of the intensive phase of tuberculosis treatment).

STUDY OVERSIGHT

The trial was approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal and by the Medicines Control Council of the South African government. Study data were reviewed periodically by a data and safety monitoring committee. All authors vouch for the completeness and accuracy of the data and analyses presented.

STATISTICAL ANALYSIS

All analyses were performed in the intention-to-treat population. The primary outcome, the incidence rate of AIDS or death, was analyzed with the use of Kaplan–Meier curves. The duration of time in the study was calculated as the time from randomization to death or AIDS-defining illness, withdrawal from the study, or 18 months after randomization, whichever occurred first. Poisson approximations were used to calculate confidence intervals for the incidence-rate ratios. Cox proportional-hazards regression was used to adjust for confounding variables. Fisher's exact test was used for the analysis of categorical data, and unpaired t-tests or the Wilcoxon two-sample test was used

for the analysis of continuous data. Interactions between therapy group and CD4+ T-cell count were evaluated by fitting a proportional-hazards model with therapy group, CD4+ T-cell count, and the interaction between therapy group and CD4+ T-cell count.

RESULTS

STUDY PARTICIPANTS

A total of 429 patients were enrolled in the two integrated-therapy groups: 214 were randomly assigned to receive early integrated therapy (hereafter referred to as the earlier-ART group), and 215 were assigned to receive late integrated therapy (hereafter referred to as the later-ART group). At baseline, the two groups had similar demographic and clinical characteristics (Table 1). The median CD4+ T-cell count was 150 per cubic millimeter, and the median viral load was 161,000 copies per milliliter. The median duration of follow-up in the trial was 17.7 months (interquartile range, 14.0 to 17.8). At study completion, the retention rates were 76.9% and 71.5% in the earlier-ART and later-ART groups, respectively (information on retention and causes of death is provided in the Supplementary Appendix).

INITIATION OF ART

Among patients who completed tuberculosis therapy, the median treatment duration was 210 days in the earlier-ART group (207 participants) and 203 days in the later-ART group (210 participants). A total of 92.5% of the patients in the earlier-ART group (198 of 214) and 76.3% in the later-ART group (164 of 215) started ART during the study (P<0.001). The longer period from randomization to the initiation of ART in the later-ART group meant that more patients in this group were lost to follow-up, withdrew, or died before the start of ART, as compared with the earlier-ART group (Fig. 1). However, there were no significant differences between the earlier-ART and later-ART groups in the overall rates of loss to follow-up (12.1% [26 of 214] and 15.8% [34 of 215], P=0.33) and withdrawal (9.3% [20 of 214] and 10.7% [23 of 215], P=0.75).

The 198 patients in the earlier-ART group who started ART did so at a median of 21 days (interquartile range, 15 to 29) after the initiation of tuberculosis therapy. Of the 33 patients who started ART after the 4-week window, 9 missed the study-clinic visit for the initiation of ART, 8 had abnormal liver function, 2 had other laboratory abnormalities, 4 declined ART, and 10 had clinical conditions that precluded ART initiation.

The 164 patients in the later-ART group who started ART did so at a median of 97 days (interquartile range, 77 to 126) after the initiation of tuberculosis therapy. One patient started ART during the intensive phase of tuberculosis treatment. Of the 47 patients who started ART more than 4 weeks after completion of the intensive phase of tuberculosis treatment, 29 missed the study-clinic visit for the initiation of ART, 1 had abnormal liver function, 6 declined ART, and 11 had clinical conditions that precluded ART initiation.

INCIDENCE RATES OF AIDS OR DEATH

The incidence rate of AIDS or death was 6.9 cases per 100 person-years in the earlier-ART group (18 cases) as compared with 7.8 per 100 person-years in the later-ART group (19 cases) (incidence-rate ratio, 0.89; 95% confidence interval [CI], 0.44 to 1.79; P=0.73). After adjustment for baseline World Health Organization (WHO) disease stage (stage 4 vs. stage 3), age, sex, history of tuberculosis (yes or no), presence or absence of extrapulmonary tuberculosis, and baseline CD4+ T-cell count and HIV RNA level, the hazard ratio with earlier ART was 0.86 (95% CI, 0.42 to 1.85; P=0.72). The probability of observing 18 AIDS cases or deaths in the earlier-ART group and 19 cases in the later-ART group was 5.6%, 1.9%, and 0.4%, if the true difference in AIDS cases or deaths between the groups was 40%, 50%, and 60%, respectively. In a sensitivity analysis in which all participants lost to follow-up were classified as having died, the incidence was 17.0 cases per 100 person-years (95% CI, 12.3 to 22.8) in the earlier-ART group and 21.7 per 100 person years (95% CI, 16.3 to 28.4) in the later-ART group (incidence-rate ratio, 0.78; 95% CI, 0.51 to 1.19; P=0.23).

INCIDENCE RATES OF AIDS OR DEATH ACCORDING TO CD4+ T-CELL COUNT

A significant interaction between therapy group and CD4+ T-cell count was observed for AIDS or death (P=0.03), indicating heterogeneity across the two CD4+ strata in the effect of time to initiation of ART on the incidence of AIDS or death. The incidence rates of AIDS or death among the 72 patients with CD4+ T-cell counts of less than 50 per cubic millimeter were 8.5 cases per 100 person-years (95% CI, 2.3 to 21.9) in the earlier-ART group as compared with 26.3 per 100 person-years (95% CI, 12.6 to 48.4) in the later-ART group (incidence-rate ratio, 0.32; 95% CI, 0.07 to 1.13; P=0.06) (Table 2

Table 1. Baseline Characteristics of the Patients.*

Variable	Earlier ART (N=214)	Later ART (N=215)	Total (N=429)	P Value
Age — yr				0.75
Mean	34.3±8.0	34.5±8.7	34.4±8.4	
Range	19–63	21–72	19–72	
Male sex — no. (%)	97 (45.3)	112 (52.1)	209 (48.7)	0.18
Educational level — no. (%)†				0.23
Primary school or less	43 (20.2)	49 (22.9)	92 (21.5)	
Some secondary school	97 (45.5)	108 (50.5)	205 (48.0)	
Completed secondary school	73 (34.3)	57 (26.6)	130 (30.4)	
Employed — no. (%)	135 (63.1)	117 (54.4)	252 (58.7)	0.08
History of tuberculosis — no. (%)	80 (37.4)	68 (31.6)	148 (34.5)	0.22
Karnofsky performance score — no. (%)‡				0.84
90 or 100	123 (57.5)	128 (59.5)	251 (58.5)	
70 or 80	86 (40.2)	81 (37.7)	167 (38.9)	
<70	5 (2.3)	6 (2.8)	11 (2.6)	
WHO stage 4 HIV infection — no. (%)§	14 (6.5)	11 (5.1)	25 (5.8)	0.54
Presence of extrapulmonary tuberculosis — no. (%)	10 (4.7)	9 (4.2)	19 (4.4)	0.82
Resistance to tuberculosis drugs — no./total no. (%)				
Isoniazid	13/102 (12.7)	5/101 (5.0)	18/203 (8.9)	0.08
Rifampin	8/102 (7.8)	4/101 (4.0)	12/203 (5.9)	0.37
Ethambutol	1/101 (1.0)	0/100 (0.0)	1/201 (0.5)	1.00
Multidrug resistance	6/102 (5.9)	3/101 (3.0)	9/203 (4.4)	0.50
CD4+ T-cell count — cells/mm^3¶				0.93
Median	154	149	150	
Interquartile range	75–261	77–244	77–254	
Viral load — $\log_{10}$ copies/ml‖				0.53
Median	5.1	5.2	5.2	
Interquartile range	4.5–5.6	4.5–5.6	4.5–5.6	
No. of days of tuberculosis therapy at randomization				0.49
Median	9	9	9	
Interquartile range	7–13	7–14	7–14	

* Plus–minus values are means ±SD.
† Data on educational level were not available for one patient in each group.
‡ The Karnofsky performance score is a measure of the patient's general condition and degree of autonomy on a scale ranging from 0 to 100, with lower numbers indicating poorer function.
§ The remainder of patients had stage 3 infection, according to criteria of the World Health Organization (WHO).
¶ Patients underwent randomization on the basis of the CD4+ T-cell count at screening (criterion for study enrollment, <500 per cubic millimeter). However, for 16 patients, the CD4+ T-cell count at enrollment was 500 per cubic millimeter or higher.
‖ Data on the viral load at baseline were not available for 16 patients in each group.

and Fig. 2). Among the 357 patients with baseline CD4+ T-cell counts of 50 per cubic millimeter or higher, the incidence rates of AIDS or death were 6.6 cases per 100 person-years (95% CI, 3.6 to 11.0) and 4.4 per 100 person-years (95% CI, 2.0 to 8.3) in the earlier-ART and later-ART groups, respectively (incidence-rate ratio, 1.51; 95% CI, 0.61 to 3.95; P=0.34).

IRIS

Among patients with a CD4+ T-cell count of less than 50 per cubic millimeter, the incidence of IRIS was 4.7 times as high in the earlier-ART group as in the later-ART group (P=0.01) (Table 2). Among patients with a CD4+ T-cell count of 50 per cubic millimeter or higher, the incidence of IRIS was 2.2 times as high in the earlier-ART group as in the

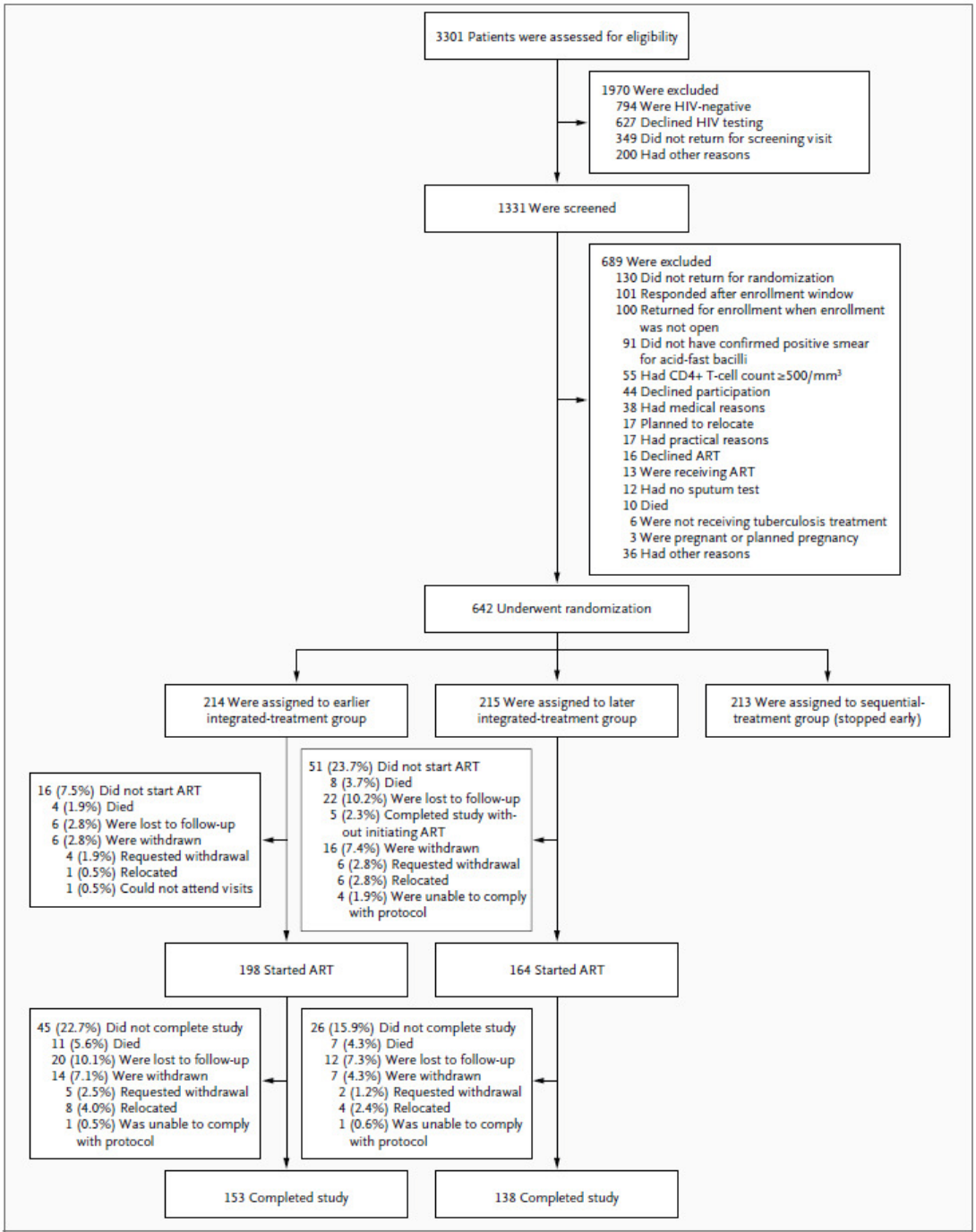

Figure 1. Enrollment and Outcomes.

Loss to follow-up was defined as no visit during the previous 4 months. ART denotes antiretroviral therapy.

later-ART group (P=0.02) (Table 2, and Table 1 in the Supplementary Appendix). No significant interaction was observed that would indicate a lack of heterogeneity across the two CD4+ strata in the effect of time to the initiation of ART on the incidence of IRIS. The median time from the initiation of ART to the development of IRIS was 15.0 days (interquartile range, 7 to 30) in the earlier-ART group and 15.5 days (interquartile range, 14 to 28) in the later-ART group.

ADHERENCE TO THERAPY AND SWITCHING OF DRUGS

Nineteen patients in each of the two study groups were considered to have defaulted tuberculosis therapy (8.9% and 8.8% in the earlier-ART and later-ART groups, respectively), either if they chose to interrupt therapy or if they did not attend the clinic for any further scheduled study visits before treatment completion. According to monthly pill counts, patients in the earlier-ART and later-ART groups took 98.0% and 98.8% of their assigned antiretroviral tablets, respectively, during the trial.

Ten patients in the earlier-ART group and one patient in the later-ART group needed to switch antiretroviral drugs because of adverse events (P=0.006). Among patients with CD4+ T-cell counts of 50 per cubic millimeter or higher, seven patients in the earlier-ART group and one patient in the later-ART group switched antiretroviral drugs (P=0.04).

A total of 15 patients (6 in the earlier-ART group and 9 in the later-ART group) changed their ART regimens because of virologic failure (defined as a viral load >1000 copies per milliliter on two occasions at least 4 weeks apart). The instances of drug switching occurred an average of 9.0 months (95% CI, 5.9 to 12.2) and 11.9 months (95% CI, 9.1 to 14.6) after the initiation of ART in the earlier-ART and later-ART groups, respectively (P=0.18).

OUTCOMES OF TUBERCULOSIS AND HIV TREATMENT

There was no significant difference between the study groups in resistance to tuberculosis drugs at baseline (Table 1). Outcomes of tuberculosis treatment did not differ significantly between the groups (Table 3, and Table 2 in the Supplementary Appendix); this finding did not change after adjustment for the presence or absence of multidrug resistance. At 6 and 12 months after randomization, the proportions of participants with a suppressed HIV RNA level did not differ significantly between

Table 2. Rates of Death, AIDS-Defining Illness or Death, and IRIS, According to Baseline CD4+ T-Cell Count.*

Outcome and CD4+ T-Cell Count	Earlier ART				Later ART				Incidence-Rate Ratio (95% CI)	P Value
	no. of patients	*no. of person-yr*	*no. of events*	*event rate/ 100 person-yr (95% CI)*	*no. of patients*	*no. of person-yr*	*no. of events*	*event rate/ 100 person-yr (95% CI)*		
Death										
All patients	214	261.7	15	5.7 (3.2–9.5)	215	250.9	15	6.0 (3.3–9.9)	0.96 (0.44–2.10)	0.91
CD4+ count <50/mm³	37	47.5	3	6.3 (1.3–18.5)	35	43.1	7	16.3 (6.5–33.5)	0.39 (0.06–1.70)	0.17
CD4+ count ≥50/mm³	177	214.2	12	5.6 (2.9–9.8)	180	207.8	8	3.8 (1.7–7.6)	1.46 (0.55–4.10)	0.41
AIDS-defining illness or death										
All patients	214	259.4	18	6.9 (4.1–11.0)	215	244.2	19	7.8 (4.7–12.2)	0.89 (0.44–1.79)	0.73
CD4+ count <50/mm³	37	46.8	4	8.5 (2.3–21.9)	35	38.0	10	26.3 (12.6–48.4)	0.32 (0.07–1.13)	0.06
CD4+ count ≥50/mm³	177	212.6	14	6.6 (3.6–11.0)	180	206.2	9	4.4 (2.0–8.3)	1.51 (0.61–3.95)	0.34
IRIS										
All patients	214	213.4	43	20.1 (14.6–27.1)	215	233.6	18	7.7 (4.6–12.2)	2.62 (1.48–4.82)	<0.001
CD4+ count <50/mm³	37	29.9	14	46.8 (25.6–78.4)	35	40.3	4	9.9 (2.7–25.4)	4.71 (1.48–19.64)	0.01
CD4+ count ≥50/mm³	177	183.4	29	15.8 (10.6–22.7)	180	193.3	14	7.2 (4.0–12.1)	2.18 (1.12–4.47)	0.02

* Incidence-rate ratios are for the earlier-ART group, as compared with the later-ART group. IRIS denotes immune reconstitution inflammatory syndrome.

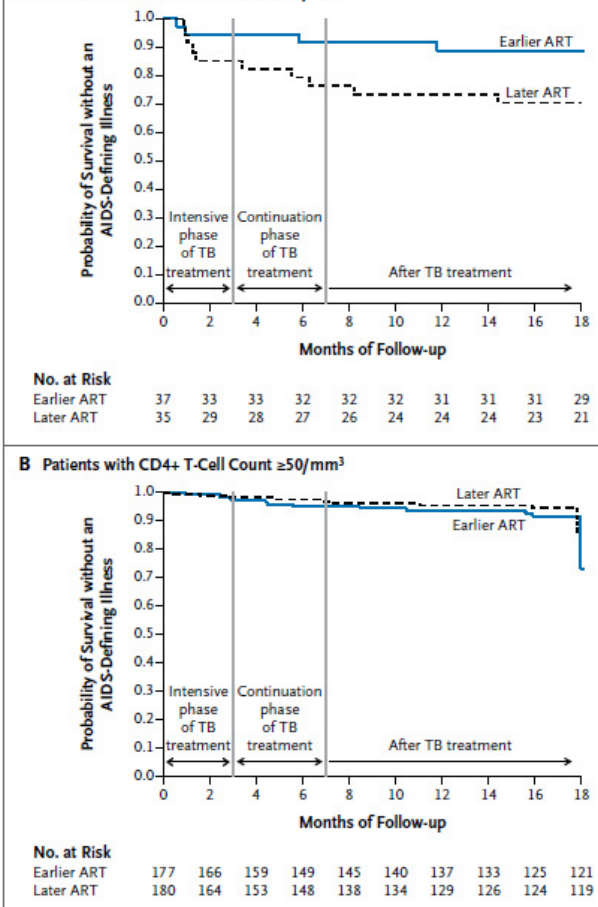

Figure 2. Kaplan–Meier Curves for Survival without an AIDS-Defining Illness.
Panel A shows the data for patients with a CD4+ T-cell count of less than 50 per cubic millimeter, and Panel B shows the data for patients with higher CD4+ T-cell counts. TB denotes tuberculosis.

the earlier-ART and later-ART groups. However, the mean increases from baseline in the CD4+ T-cell count at 12 and 18 months were significantly higher in the earlier-ART group than in the later-ART group (Table 4).

ADVERSE EVENTS

Grade 3 or 4 non-IRIS adverse events occurred in 112 patients in the earlier-ART group and in 107 patients in the later-ART group (42.8 and 42.6 events per 100 person-years, respectively; P=0.98); serious adverse events occurred in 56 and 50 patients in the respective groups. Table 3 in the Supplementary Appendix provides details of the adverse events.

DISCUSSION

Overall, the rates of AIDS or death did not differ significantly between the patients who received early integrated ART and those who received late integrated ART, but the earlier-ART group had higher rates of IRIS and switching of antiretroviral drugs because of adverse events. However, the findings in severely immunocompromised patients differed. In the subgroup of patients with CD4+ T-cell counts of less than 50 per cubic millimeter, earlier ART was associated with a rate of AIDS or death that was about two thirds lower than the rate with later ART; this benefit outweighs the significantly higher rates of IRIS (incidence-rate ratio, 4.7) and of switching of antiretroviral drugs associated with earlier ART. For patients with CD4+ T-cell counts of less than 50 per cubic millimeter, our findings support the 2009 WHO recommendation[8] to start ART as soon as possible after the initiation of tuberculosis treatment.

Our findings suggest a different approach for patients with tuberculosis and HIV who have a CD4+ T-cell count of 50 per cubic millimeter or higher. The initiation of ART during the first 4 weeks of the continuation phase of tuberculosis treatment versus initiation during the first 4 weeks of the intensive phase of tuberculosis treatment was not associated with an increased risk of AIDS or death but was associated with about half the risk of IRIS and a significantly lower risk of the need to switch antiretroviral drugs because of adverse events. Thus, for this subgroup of patients, ART can be deferred until the start of the continuation phase of tuberculosis treatment. However, a longer delay should be avoided, in light of our previous finding that sequential ART (after the completion of tuberculosis treatment) was associated with 56% higher mortality, as compared with its initiation during tuberculosis treatment.[6]

Some limitations of our study need to be considered. First, the observed 68% lower rate of AIDS or death among severely immunocompromised patients as compared with the rate among other patients (incidence-rate ratio, 0.32), although substantial, was not significant (P=0.06). However, it is unlikely that this finding was due to chance, because a survival benefit in severely immunocompromised patients was also observed in the Cam-

Table 3. Clinical Outcomes of Tuberculosis Treatment.

Outcome	Baseline CD4+ T-Cell Count <50/mm³		Baseline CD4+ T-Cell Count ≥50/mm³	
	Earlier ART (N=37)	Later ART (N=35)	Earlier ART (N=177)	Later ART (N=180)
	number of patients (percent)			
Tuberculosis cured*	23 (62)	24 (69)	108 (61)	114 (63)
Tuberculosis treatment successfully completed†	8 (22)	4 (11)	32 (18)	34 (19)
Treatment successful‡	31 (84)	28 (80)	140 (79)	148 (82)
Patient died before tuberculosis treatment completed	3 (8)	4 (11)	11 (6)	7 (4)
Treatment interruption	0	1 (3)	5 (3)	3 (2)
Treatment failure with first-line regimen§	1 (3)	0	5 (3)	2 (1)
Patient lost to follow-up before tuberculosis treatment completed	1 (3)	1 (3)	12 (7)	15 (8)
Patient transferred to other clinic, tuberculosis treatment outcome not known	1 (3)	1 (3)	4 (2)	5 (3)

* Tuberculosis cure was defined in accordance with the *South African National Tuberculosis Control Programme Practical Guidelines 2004*, which states, "Patient who is smear-negative at, or one month prior to, the completion of treatment and also on at least one previous occasion." Most study patients were unable to produce sputum after the first few months of tuberculosis treatment, making demonstration of a cure difficult.
† Successful completion of treatment was defined as the use of more than 85% of the prescribed medication.
‡ Treatment success was defined as tuberculosis cure and successful completion of tuberculosis treatment.
§ Treatment failure was defined as a positive smear or culture for *Mycobacterium tuberculosis* that was obtained at least 5 months after the initiation of tuberculosis therapy.

bodian Early versus Late Introduction of Antiretrovirals study (CAMELIA; ClinicalTrials.gov number, NCT01300481). In the Cambodian study, among patients coinfected with tuberculosis and HIV who had a median CD4+ T-cell count of 25 per cubic millimeter, those who started ART 2 weeks after the initiation of tuberculosis treatment had 38% lower mortality than those who waited 8 weeks to start ART (P=0.006).[9] Among patients with CD4+ T-cell counts below 50 per cubic millimeter in the AIDS Clinical Trials Group Study A5221 (NCT00108862), 15.5% of patients in the earlier-ART group versus 26.6% in the later-ART group had an AIDS-defining illness or died (95% CI, 1.5 to 20.5; P=0.02).[10]

Second, the lack of a survival benefit in patients with CD4+ T-cell counts of 50 per cubic millimeter or higher may be due to the sample size (357 patients) and the small number of deaths observed. There would be only a 9.2% probability of observing these rates of death if the true difference in mortality between the earlier-ART and later-ART groups was 34% or greater. Furthermore, the limited observational data available show similar trends. In a pilot study of the initiation of ART involving 70 patients with a median CD4+ T-cell count of 103 per cubic millimeter, there were 2 deaths in the early-therapy group (within 2 weeks after the start of tuberculosis treatment) versus 1 death in the delayed-therapy group (8 weeks after the start of tuberculosis treatment) (P=0.601).[11]

Third, inaccuracies in the diagnosis of IRIS, and therefore in the reported incidence of the syndrome, may have affected the study outcome. The incidence rate of 14.2% observed in this study is consistent with findings from other South African studies. In one study of patients coinfected with tuberculosis and HIV, the incidence of IRIS was 12% overall, yet 32% of patients who started ART within 2 months after receiving a diagnosis of tuberculosis had an IRIS event.[12] The risk of IRIS remained elevated if ART was started within 3 months after the initiation of tuberculosis treatment, but it was highest during the first month of tuberculosis treatment. A retrospective analysis of 627 patients from India showed that 7.6% of patients with tuberculosis (18 of 237) had paradoxical tuberculosis-associated IRIS, and 3.1% of patients without tuberculosis (12 of 390) had IRIS associated with ART.[13] A low CD4+ T-cell count at baseline and early initiation of ART

Table 4. Clinical Outcomes of ART.

Outcome and Baseline CD4+ T-Cell Count	Earlier ART		Later ART		P Value
	no./total no.	*% (95% CI)*	*no./total no.*	*% (95% CI)*	
Viral load <400 copies/ml					
At 6 mo after initiation of ART					
Overall	161/179	89.9 (84.3–93.8)	166/179	92.7 (87.6–95.9)	0.45
CD4+ count <50/mm³	30/34	88.2 (71.6–96.2)	32/35	91.4 (75.8–97.8)	0.71
CD4+ count ≥50/mm³	131/145	90.3 (84.0–94.4)	134/144	93.1 (87.3–96.4)	0.52
At 12 mo after randomization					
Overall	147/159	92.5 (86.9–95.9)	130/147	88.4 (81.9–92.9)	0.25
CD4+ count <50/mm³	30/32	93.8 (77.8–98.9)	23/27	85.2 (65.4–95.1)	0.40
CD4+ count ≥50/mm³	117/127	92.1 (85.6–96.0)	107/120	89.2 (81.9–93.9)	0.51
At 18 mo after randomization					
Overall	144/153	94.1 (88.8–97.1)	135/143	94.4 (88.9–97.4)	1.00
CD4+ count <50/mm³	28/30	93.3 (76.5–98.8)	25/26	96.2 (78.4–99.8)	1.00
CD4+ count ≥50/mm³	116/123	94.3 (88.2–97.5)	110/117	94.0 (87.6–97.4)	1.00
	no. of patients	*mean increase (95% CI)*	*no. of patients*	*mean increase (95% CI)*	
Mean increase in CD4+ count					
At 6 mo after initiation of ART					
Overall	178	132 (113–152)	179	132 (111–152)	0.95
CD4+ count <50/mm³	34	124 (94–154)	35	104 (83–124)	0.25
CD4+ count ≥50/mm³	144	134 (111–157)	144	138 (113–163)	0.82
At 12 mo after randomization					
Overall	159	183 (162–204)	147	125 (105–145)	0.009
CD4+ count <50/mm³	32	170 (127–213)	27	111 (81–141)	0.03
CD4+ count ≥50/mm³	127	186 (163–210)	120	128 (104–152)	0.001
At 18 mo after randomization					
Overall	152	217 (192–243)	142	172 (150–194)	0.009
CD4+ count <50/mm³	30	207 (166–248)	26	173 (134–212)	0.22
CD4+ count ≥50/mm³	122	220 (189–251)	116	172 (146–198)	0.02

were significantly associated with paradoxical tuberculosis-associated IRIS.

The rates of adverse events in the earlier-ART and later-ART groups were not substantially different. Published data on additive treatment-related toxic effects in patients receiving treatment for both tuberculosis and HIV infection are limited. A retrospective study in India showed that concomitant use of ART and tuberculosis treatment was a predictor of adverse events (odds ratio, 1.88).[14] Furthermore, a study from Thailand showed that 44.6% of patients receiving treatment for tuberculosis and HIV had adverse events due to antituberculosis drugs or ART.[15] Of these patients, 66% had adverse events within the first 2 months after the start of tuberculosis treatment, and 76.8% had to stop or change either antituberculosis or antiretroviral drugs. In contrast, a retrospective study from South Africa showed that the occurrence of serious adverse events was unrelated to the use of antiretroviral drugs in patients with tuberculosis.[16]

In our study, there were no significant differences between the earlier-ART and later-ART groups in the outcomes of tuberculosis treatment

or in the proportion of participants with a suppressed viral load. However, significant increases in the CD4+ T-cell count at 12 and 18 months were observed in the earlier-ART group, probably as a result of the longer duration of ART as compared with the duration in the later-ART group. This finding may have implications for longer-term survival, for which long-term follow-up would be needed.

These results of the SAPIT study further support the integration of treatment for tuberculosis and HIV infection. The current WHO recommendation to initiate ART as soon as possible after the start of tuberculosis treatment, regardless of the CD4+ T-cell count, may need to be revisited in view of the findings of this study. We found that early initiation of ART in patients with CD4+ T-cell counts of less than 50 per cubic millimeter increased AIDS-free survival, whereas deferral of the initiation of ART to the first 4 weeks of the continuation phase of tuberculosis therapy in those with higher CD4+ T-cell counts reduced the risks of IRIS and other adverse events related to ART without increasing the risk of AIDS or death.

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Discussion of Paper VIII: This article was published from the SAPIT study and gives clear guidance on the timing of ART initiation during TB treatment, using the patients CD4 count as a guide to determine when during the TB treatment phases ART should be initiated. Early initiation of ART in this trial, was regarded as four weeks after the start of TB therapy (within the intensive phase of treatment), and in patients with baseline CD4 counts less than 50 mm^3 was associated with increased AIDS-free survival. While deferral of ART to the first four weeks of the continuation phase of TB treatment, in patients with higher CD4 counts, reduced the risk for IRIS and other adverse events without increasing the risk for HIV disease progression or death

Importance for TB/HIV treatment integration: This analysis was important in that it provided practical guidance to providers with extremely ill patients with extremely low CD4 counts requiring co-treatment. One of the major concerns for co-treatment was management of IRIS and this result gave providers more confidence in co-treating those very ill early during TB treatment and deferring less ill patients ART initiation to the continuation phase of TB treatment without being concerned about increased risk for mortality.

A5: PAPER IX

Changes to antiretroviral drug regimens during integrated TB-HIV treatment: results of the SAPiT trial

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Original article

Changes to antiretroviral drug regimens during integrated TB–HIV treatment: results of the SAPiT trial

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Background: Frequency of drug changes in combination antiretroviral therapy among patients starting both tuberculosis (TB) and HIV therapy, as a result of treatment-limiting toxicity or virological failure, is not well established.

Methods: Patients in the Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPiT) trial were randomized to initiate antiretroviral therapy (ART) either early or late during TB treatment or after completion of TB treatment. Drug changes due to toxicity (defined as due to grade 3 or 4 adverse events) or virological failure (defined as viral load >1,000 copies/ml on two occasions, taken ≥4 weeks apart) were assessed in these patients.

Results: A total of 501 TB–HIV-coinfected patients were followed for a mean of 16.0 months (95% CI 15.5, 16.6) after ART initiation. The standard first-line antiretrovirals used were efavirenz, lamivudine and didanosine. Individual drug switches for toxicity occurred in 14 patients (incidence rate 2.1 per 100 person-years, 95% CI 1.1, 3.5), and complete regimen changes due to virological failure in 25 patients (incidence rate 3.7 per 100 person-years, 95% CI 2.4, 5.5). The most common treatment limiting toxicities were neuropsychiatric effects (n=4, 0.8%), elevated transaminase levels and hyperlactataemia (n=3, 0.6%), and peripheral neuropathy (n=2, 0.4%). Complete regimen change due to treatment failure was more common in patients with CD4$^+$ T-cell count <50 cells/mm^3 (P<0.001) at ART initiation and body mass index >25 kg/m^2 (P=0.01) at entry into the study.

Conclusions: Both drug switches and complete regimen change were uncommon in patients cotreated for TB–HIV with the chosen regimen. Patients with severe immunosuppression need to be monitored carefully, as they were most at risk for treatment failure requiring regimen change.

Introduction

There were an estimated 8.6 million cases of tuberculosis (TB) in 2012, approximately 1.1 million of which were coinfected with HIV [1]. Sub-Saharan Africa accounted for 80% of the global burden of TB–HIV coinfections [1]. Cotreatment of these diseases presents several management challenges. Treatment-limiting toxicity is an important concern when integrating TB–HIV treatment. Other concerns include drug interactions between rifampicin and some classes of antiretrovirals [2], immune reconstitution inflammatory syndrome and high pill burden [3,4].

These clinical challenges potentially undermine the success of both HIV and TB control programmes, contribute to the poor tolerability of combined antiretroviral therapy (ART) and TB therapy, and impact on treatment adherence. There is now evidence that initiating ART during TB therapy in coinfected patients significantly reduces mortality, and improves outcomes in both conditions [5–8]. However, these benefits need to be weighed against the risks of morbidity due to treatment interruptions, toxicity or treatment failure.

There are limited prospective data from randomized controlled trials available to inform clinical guidelines. In this paper we report the incidence, predictors of, and reasons for ART changes, in a cohort of TB–HIV-coinfected patients enrolled in a randomized controlled trial

designed to determine the optimal time to initiate ART in TB treatment.

Methods

Study design and participants

The Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPiT) trial was an open label three-arm randomized-controlled trial, which enrolled 642 patients between June 2005 and July 2008, to determine the optimal timing of ART initiation in TB–HIV-coinfected patients. Details of the study design and procedures and the primary outcomes of the study have been described previously [5,6]. In brief, TB–HIV-coinfected patients, aged ≥18 years (screening $CD4^+$ T-cell count <500 cells/mm^3), were enrolled at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) eThekwini clinical research site, which adjoins the Prince Cyril Zulu Communicable Disease Centre (PCZCDC), in Durban, South Africa. HIV infection was confirmed by two rapid HIV tests and pulmonary TB (PTB) was confirmed by acid fast bacilli smear positivity.

Study procedures

Patients were randomized to initiate ART within 4 weeks of TB treatment initiation (early integrated treatment arm), within 4 weeks after completion of intensive phase of TB treatment (late integrated treatment arm), or within 4 weeks after TB therapy completion (sequential treatment arm). Patients were initiated on a once-daily ART regimen consisting of efavirenz 600 mg, lamivudine 300 mg and enteric-coated didanosine 250 mg (weight <60 kg) or 400 mg (weight ≥60 kg). All first episode PTB was treated with a fixed–dose combination of rifampicin, isoniazid, ethambutol and pyrazinamide according to pretreatment weight for 2 months (intensive phase), with subsequent fixed-dose combination of isoniazid and rifampicin for 4 months (continuation phase). Patients with re-treatment PTB received a 60-day intensive phase, which included streptomycin, followed by a 100-day continuation phase, in accordance with the national policy. Patients were offered community- or clinic-based directly observed therapy. All patients received a standard package of care which included adherence counselling and cotrimoxazole prophylaxis. Additionally, female patients were required to use hormonal contraception while on efavirenz.

Follow-up visits for the monitoring of safety, clinical status and adherence to ART were scheduled monthly for 24 months. Laboratory investigations included baseline (at screening and enrolment) $CD4^+$ T-cell count using a FACS flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA), viral load by HIV RNA PCR (Roche Cobas Amplicor HIV-1 Monitor, version 1.5, Roche Molecular Systems, Inc., Branchburg, NJ, USA; lower limit of detection 400 copies/ml), full blood counts, urea, electrolytes, creatinine, liver function, hepatitis B surface antigen tests, and syphilis serology. These investigations were done at baseline and repeated every 6 months or earlier, if clinically indicated. ART adherence was assessed monthly using pharmacy pill counts. Pill counts were assessed based on the number of pills dispensed and physically returned. In addition we took into account lost doses and remaining doses reported on previously that may have been returned at a subsequent visit.

Adverse events were graded with the use of the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.0) [9]. A toxicity grading of 3 or 4 was used as indication for discontinuation or substitution of specific antiretroviral drugs, and referred to as drug switch due to toxicity. Drug switch could also occur as a result of contraindication or drug interaction.

Virological failure, defined as a viral load >1,000 copies/ml on two occasions, taken ≥4 weeks apart, resulted in discontinuation or complete regimen change of all first-line ART drugs. Viral suppression or undetectable viral load was defined as a viral load of <400 copies/ml. Drug changes therefore referred to both individual drug switches, as a result of toxicity, and to complete regimen changes, as a result of virological failure. Although second-line regimen choices in patients requiring complete regimen change were individualized, the most commonly utilised (72%) regimen consisted of lopinavir/ritonavir, tenofovir and zidovudine.

Study oversight

Ethical approval for the study was provided by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (E107/05), and the Medicines Control Council of South Africa (reference 20060157).

Statistical analyses

This analysis was based on a 24-month post randomization follow-up time period to allow for the patients in the sequential treatment arm to have sufficient time on ART to be comparable to the other two arms.

Time at risk was calculated from ART initiation to the date on which drugs were stopped, death, withdrawal or termination from the study. For patients who changed drugs more than once, only the first change was included in the incidence rate calculation. CIs for incidence and incidence rate ratios (IRR) assumed a Poisson distribution.

Multivariate Cox proportional hazards regression models were used separately for drug switch and

complete regimen change to assess the risk factors for regimen changes. Tests for interactions between selected risk factors were performed. Insignificant interaction terms were removed from the final model.

Data published in 2010 [5] provided interim results following the September 2008 safety monitoring committee review. Results presented in 2011 were based on the complete set of trial data [6]. The data presented in this paper, in addition, cover the full 24-month follow-up period post-randomization. All statistical tests were two-sided. Fisher's exact test or the Fisher-Freeman-Halton test was used for the analysis of categorical data. The Student's *t*-test for independent samples, Wilcoxon two-sample, one-way ANOVA or the Kruskal-Wallis tests were used for the analysis of continuous data. Statistical analysis was done using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA).

Results

Of 1,331 patients screened for eligibility, 642 were enrolled and randomized into the study, with 501 initiating ART: 198 (92.5%), 164 (76.3%) and 139 (65.3%) in the early integrated, late integrated and sequential arms respectively (Figure 1). Patients were followed for an average of 17.6 months (95% CI 16.6, 18.6), 16.8 months (95% CI 16.0, 17.6) and 13.0 months (95% CI 12.3, 13.7) after ART initiation, with a retention rate at 24 months post randomization of 76.6%, 71.2% and 71.3%, in the early integrated, late integrated and sequential arms respectively.

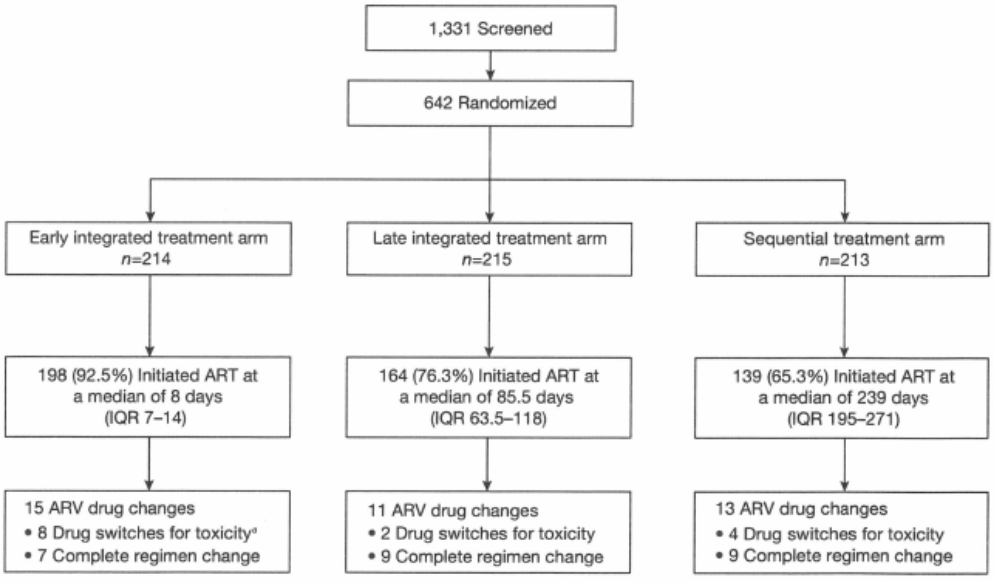
Baseline results

There were differences only for weight (P=0.01) and haemoglobin (P<0.001) across the three treatment arms, and CD4$^+$ T-cell counts were lower in patients who had drug changes (P=0.01; Table 1). At baseline, the proportion of patients with hepatitis B surface antigenaemia, peripheral neuropathy and raised transaminases (≥5× the upper limit of normal) were similar across the three treatment arms (Table 1).

Incidence of complete regimen change and drug switch across the three treatment arms

ART changes occurred in 39/501 patients, with an incidence rate of 5.8 per 100 person-years (py; 95%

Figure 1. SAPiT trial: screening, randomization and follow-up of study participants, demonstrating distribution of patients with drug switches due to toxicity and complete regimen change due to virological failure



[a]One patient experienced two individual drug switches due to toxicity for different reasons in the early integrated arm (9 drug switches and 16 antiretroviral [ARV] drug changes in total), but only the initial drug switch is illustrated in this figure and used in the incidence rate calculation. ART, antiretroviral therapy.

Table 1. Baseline characteristics for patients initiated on antiretroviral therapy

Characteristic	Early integrated treatment arm (*n*=198)	Late integrated treatment arm (*n*=164)	Sequential treatment arm (*n*=139)	*P*-value	Participants without ART changes (*n*=462)	Participants with ART changes (*n*=39)	*P*-value
Mean age, years (SD)	34.6 (8.1)	35.1 (9.1)	34.8 (9.5)	0.86	34.9 (8.7)	35.1 (6.8))	0.81
Male, *n* (%)	90 (45.5)	79 (48.2)	68 (48.9)	0.79	221 (47.9)	16 (41.0)	0.50
WHO stage 4, *n* (%)	13 (6.6)	11 (6.7)	8 (5.8)	0.95	29 (6.3)	3 (7.7)	0.73
Past history of tuberculosis, *n* (%)	73 (36.9)	49 (29.9)	43 (30.9)	0.31	154 (33.4)	11 (28.2)	0.50
Median CD4$^+$ T-cell count, cells/mm^3 (IQR)[a][b]	145.5 (73–267)	141 (57.5–247)	146 (74–260)	0.60	150 (82–253)	65 (27–215)	0.01
Mean HIV RNA, $\log_{10}$ copies/ml (SD)[b][c]	5.0 (0.9)	5.0 (0.9)	5.0 (0.8)	0.98	5.0 (0.9)	5.2 (0.9)	0.27
Mean haemoglobin, g/dl (SD)[b][d]	10.6 (2.0)	11.5 (1.8)	12.0 (2.0)	<0.001	11.3 (2.0)	11.3 (1.8)	0.87
Mean weight, kg (SD)[b][e]	59.6 (10.5)	61.7 (10.7)	62.9 (11.5)	0.01	61.0 (10.9)	62.2 (10.8)	0.51
Body mass index[e]				0.45			0.06
<18.5 kg/m^2, *n* (%)	18 (9.1)	10 (6.1)	7 (5.1)		32 (7.0)	3 (7.7)	
18.5–25 kg/m^2, *n* (%)	132 (66.7)	105 (64.0)	88 (64.2)		306 (66.5)	19 (48.7)	
>25 kg/m^2, *n* (%)	48 (24.4)	49 (29.9)	42 (30.7)		122 (26.5)	17 (43.6)	
Past history of alcohol use[f]				0.81			0.44
Never, *n* (%)	159 (84.6)	138 (85.7)	110 (82.1)		374 (83.9)	32 (88.9)	
Occasionally, *n* (%)	23 (12.3)	16 (9.9)	17 (12.7)		54 (12.1)	2 (5.6)	
Frequently, *n* (%)	6 (3.2)	7 (4.4)	7 (5.2)		18 (4.0)	2 (5.6)	
First-line regimen[g]							
EFV, 3TC, ddI, *n* (%)	195 (98.5)	162 (98.8)	134 (97.1)	0.59	455 (98.7)	36 (92.3)	0.03
NVP, 3TC, ddI/EFV, 3TC, AZT, *n* (%)	3 (1.5)	2 (1.2)	4 (2.9)		6 (1.3)	3 (7.7)	
Presence of peripheral neuropathy before ART initiation, *n* (%)	6 (3.0)	6 (3.7)	2 (1.4)	0.52	13 (2.8)	1 (2.6)	1.00
Hepatitis B surface antigen[b][h]				0.80			1.00
Positive, *n* (%)	15 (9.4)	11 (7.3)	11 (8.3)		34 (8.4)	3 (8.3)	
Negative, *n* (%)	144 (90.6)	139 (92.7)	122 (91.7)		372 (91.6)	33 (91.7)	
ALT≥5×ULN, *n* (%)	1 (0.5)[i]	0	0[i]		1 (0.2)[k]	0	0
AST≥5×ULN, *n* (%)	2 (1.0)[i]	2 (1.2)	0[i]	0.57	3 (0.7)[k]	1 (2.6)	0.28

[a]One participant had missing CD4$^+$ T-cell count in the sequential arm. [b]At antiretroviral therapy (ART) initiation. [c]Six participants in the early integrated treatment arm, one in the late integrated treatment arm and two in the sequential treatment arm had missing viral load. [d]Two participants in the early integrated treatment arm, two in the late integrated treatment arm and three in the sequential treatment arm had missing haemoglobin. [e]Two participants in the late integrated treatment arm did not have weight measurements; missing data was not included in the percentage calculation. [f]A total of 10 participants in the early integrated treatment arm, 3 in the late integrated treatment arm and 5 in the sequential treatment arm had missing past history of alcohol use. [g]One participant in the sequential arm had no regimen data. [h]A total of 39 participants in the early integrated treatment arm, 14 in the late integrated treatment arm and 6 in the sequential treatment arm had missing hepatitis data. [i]*n*=197. [j]*n*=137. [k]*n*=459. ALT, alanine transaminase; AST, aspartate aminotransferase; AZT, zidovudine; ddI, didanosine; EFV, efavirenz; NVP, nevirapine; ULN, upper limit of normal; 3TC, lamivudine.

CI 4.1, 8.0). One participant experienced two individual drug switches for different reasons. Among 14/501 (2.8%) patients, drug switches due to toxicity occurred at a median time of 3.6 months (IQR 2.5–6.9) post ART initiation, with an incidence rate of 2.1 (95% CI 1.1, 3.5). Complete regimen change occurred in 25/501 (5.0%), with an incidence rate of 3.7 per 100 py (95% CI 2.4, 5.5).There was no significant difference in the incidence of individual drug switches or complete regimen changes between the three arms (*P*=0.25). There were no differences in median time to single drug switches (*P*=0.64) and complete regimen changes (*P*=0.86) across the three treatment arms. Incidence of complete regimen changes in the early integrated treatment arm was 2.3 per 100 py (95% CI 0.9, 4.8) compared to 3.9 per 100 py (95% CI 1.8, 7.4) in the late integrated treatment arm (IRR 0.6, 95% CI 0.2, 1.8; *P*=0.37) and 5.9 per 100 py (95% CI 2.7, 11.1) in the sequential treatment arm (IRR 0.4, 95% CI 0.1, 1.2; *P*=0.19; Table 2).

In patients with CD4$^+$ T-cell counts <50 cells/mm^3, the incidence of complete regimen change was 5.5 (95%

Table 2. Incidence rate of drug switches and compete regimen change across the three treatment arms

Treatment or drug change	Early integrated treatment arm			Late integrated treatment arm			Sequential treatment arm						
	Total py (*n*)	Number of switches	IR, per 100 py (95% CI)	Total py (*n*)	Number of switches	IR, per 100 py (95% CI)	Total py (*n*)	Number of switches	IR, per 100 py (95% CI)	*P*-value	Early versus late[a]	Early versus sequential[a]	Late versus sequential[a]
Drug change[b]	290.4 (198)	15	5.2 (2.9, 8.5)	229.3 (164)	11	4.8 (2.4, 8.6)	150.0 (139)	13	8.7 (4.6, 14.8)	0.54	1.1 (1.5, 2.6); 0.70	0.6 (0.3, 1.4); 0.55	0.6 (0.2, 1.3); 0.21
Drug switch	290.4 (198)	8	2.8 (1.2, 5.4)	229.3 (164)	2	0.9 (0.1, 3.2)	150.0 (139)	4	2.7 (0.7, 6.8)	0.25	3.2 (0.6, 30.5); 0.09	1.03 (0.3, 4.7); 0.56	0.3 (0.03, 2.3); 0.30
Complete regimen change	300.1 (198)	7	2.3 (0.9, 4.8)	230.6 (164)	9	3.9 (1.8, 7.4)	153.3 (139)	9	5.9 (2.7, 11.1)	0.32	0.6 (0.2, 1.8); 0.37	0.4 (0.1, 1.2); 0.19	0.7 (0.2, 1.9); 0.44

[a]Data are presented as incidence rate ratio (95% CI); *P*-value. [b]Combination of drug switches and complete regimen change. IR, incidence rate; py, person-years

CI 1.1, 16.1), 12.9 (95% CI 4.7, 28.2) and 13.4 (95% CI 2.8, 39.3) per 100 py in the early integrated, late integrated and sequential treatment arms respectively (P=0.53). In patients with CD4$^+$ T-cell count ≥50 cells/mm^3, the incidence of complete regimen change was 1.6 (95% CI 0.4, 4.2), 1.6 (95% CI 0.3, 4.8) and 4.6 (95% CI 1.7, 10.0) per 100 py in the early integrated, late integrated and sequential treatment arm, respectively (P=0.17).

Reasons for drug switches

The reasons for and time to individual drug switches from ART initiation are shown in Table 3. We found the most common treatment-limiting toxicities to be neuropsychiatric effects (n=4, 0.8%) elevated transaminases and hyperlactataemia (n= 3, 0.6%), and peripheral neuropathy (n=2, 0.4%). Overall, 11 of the 15 drug switches occurred within the first 6 months after ART initiation. Among the 14 patients with drug switches, 5 were on concurrent TB–HIV treatment. Five patients in each of the early and late treatments arms and one in the sequential treatment arm experienced ART treatment interruptions due to toxicity, but these toxicities did not lead to any drug switches.

Complete regimen change

The median time to complete regimen change from ART initiation was 9.9 months (IQR 6.4–13.0), 10.4 months (IQR 9.7–11.0) and 9.5 months (IQR 8.1–10.9) with no significant difference between the arms (P=0.64). Among the patients with complete regimen change, none were on concurrent TB therapy at the time of regimen change. The median viral load before complete regimen change was 4.9 log copies/ml (IQR 4.3–5.6). Virological suppression rates were high in all treatment arms after 18 months of follow-up [5,6].

Adherence

The overall adherence at 24 months post ART initiation, based on pill count data, was similar across the three treatment arms (P=0.64). Among patients with drug changes, the adherence rates were 90.4%, 86.2% and 93.6% (P=0.58), whereas among patients with no drug changes, the adherence rates were 95%, 96% and 97.4% in the early, late and sequential treatment arms, respectively (P=0.48).

Risk factors associated with drug switches and complete regimen change in cotreated patients

The treatment arm was not associated with drug switches and complete regimen changes. Baseline CD4$^+$ T-cell count <50 cells/mm^3 was significantly associated with complete regimen change (HR 4.7, 95% CI 1.6, 14.0; P=0.005) compared to CD4$^+$ T-cell count ≥50 cells/mm^3. Additionally, patients with a body mass index (BMI)>25 kg/m^2 were more likely to experience complete regimen change (HR 3.3; 95% CI 1.4, 7.8; P=0.01) compared to patients with BMI 18.5–25 kg/m^2 (Table 4). There were no significant interactions between the risk factors.

Discussion

We demonstrated similar incidence of ART drug switches irrespective of the timing of ART initiation relative to the start of TB treatment, providing evidence that potentiated drug toxicity may be of limited concern in TB–HIV-cotreatment. Low rates of drug switching due to toxicity was observed in all three arms, with no significant difference in the incidence of drug switching between the treatment arms, although the number of drug switches was higher in the early and late integrated compared to the sequential treatment arm. The

Table 3. Summary of antiretroviral therapy changes

Drug switch/reason for switch and regimen change	Early integrated treatment arm	Late integrated treatment arm	Sequential treatment arm	Total
Drug switches[a]				
ddI (*n*=5)				
Peripheral neuropathy	2 (10.9 and 1.0)	0	0	2 (10.9 and 1.0)
Hyperlactataemia	1 (12.3)	0	0	1 (12.3)
Pancreatitis, hepatomegaly	0	1 (3.1)	0	1 (3.1)
Acute viral hepatitis	0	0	1 (3.2)	1 (3.2)
EFV (*n*=5)				
Neuropsychiatric	3 (0.5, 2.5 and 3.6)	0	1 (5.6)	4 (0.5, 2.5, 3.6 and 5.6)
Rash	0	0	1 (0.5)	1 (0.5)
AZT (*n*=2)				
Anaemia	0	1 (8.3)	0	1 (8.3)
Hyperlactataemia	1 (2.4)	0	0	1 (2.4)
NVP (*n*=2)				
Hypersensitivity	1 (6.9)	0	0	1 (6.9)
Recurrent tuberculosis	0	0	1 (5.3)	1 (5.3)
ddI and 3TC (*n*=1)				
Severe anaemia	1 (3.7)	0	0	1 (3.7)
Complete regimen change				
EFV/3TC/ddI (*n*=25)				
Virological failure	7 (9.9 [6.4–13.0])[b]	9 (10.4 [9.7–11.0])[b]	9 (9.5 [8.1–10.9])[b]	25 (10.2 [8.1–11.0])[b]

Data are number of switches (months to switch), unless indicated otherwise. [a]Overall, there were 15 switches (occurring in 14 patients). [b]These data are *n* (median [IQR]) changes to second line drugs (complete regimen change) due to virological failure (*n*=25). AZT, zidovudine; ddI, didanosine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine.

Table 4. Risk factors for drug switch and complete regimen change

	Drug switch for toxicity				Complete regimen change for virological failure			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Randomization arm								
Sequential treatment arm	Reference		Reference		Reference		Reference	
Early integrated treatment arm	1.4 (0.4, 4.8)	0.55	0.9 (0.2, 3.3)	0.83	0.5 (0.2, 1.3)	0.14	0.5 (0.2, 1.5)	0.24
Late integrated treatment arm	0.4 (0.1, 2.6)	0.31	0.4 (0.1, 2.1)	0.25	0.7 (0.3, 1.9)	0.51	0.6 (0.2, 1.6)	0.35
Sex								
Male	Reference		Reference		Reference		Reference	
Female	2.3 (0.7, 7.2)	0.17	2.0 (0.5, 7.9)	0.32	0.9 (0.4, 2.1)	0.89	0.8 (0.4, 2.0)	0.71
Age (per 1 year increase)	0.99 (0.9, 1.1)	0.67	0.97 (0.9, 1.1)	0.48	1.01 (0.97, 1.1)	0.61	1.0 (0.9, 1.0)	0.79
CD4$^+$ T-cell count[a]								
<50 cells/mm^3	1.2 (0.3, 4.4)	0.77	1.3 (0.3, 6.5)	0.74	4.4 (2.0, 9.6)	0.0002	5.1 (2.2, 11.5)	<0.001
≥50 cells/mm^3	Reference		Reference		Reference		Reference	
Body mass Index								
18.5–25 kg/m^2	Reference		Reference		Reference		Reference	
<18.5 kg/m^2	2.2 (0.5, 10.0)	0.33	1.2 (0.1, 10.2)	0.86	1.0 (0.1, 8.1)	0.96	0.8 (0.1, 6.6)	0.87
>25 kg/m^2	0.8 (0.2, 2.9)	0.72	0.5 (0.1, 2.5)	0.38	3.5 (1.5, 7.9)	0.003	3.3 (1.4, 7.8)	0.01
WHO stage								
Stage 3	Reference				Reference			
Stage 4	1.1 (0.1, 8.5)	0.92	–	–	1.3 (0.3, 5.4)	0.73	1.0 (0.2, 4.3)	0.99
Hepatitis B surface antigen								
Negative	Reference		Reference		Reference		Reference	
Positive	2.3 (0.5, 10.9)	0.28	2.9 (0.6, 14)	0.20	0.4 (0.1, 3.1)	0.39	0.3 (0, 2.5)	0.29

[a]At antiretroviral therapy initiation. HR, hazard ratio

regimen chosen for this study provided a once-daily option at a time before the availability of tenofovir, to be taken with once-daily TB treatment. Reports from two other randomized controlled trials also show similar rates of toxicity in patients who start ART early (within 2 weeks), or later (within 8 weeks), in the course of TB treatment. In the STRIDE study, 44% and 47% of patients experienced grade 3 and 4 adverse events, with 14/405 patients in the early group and 7/401 patients in the late-ART group switching ART regimen for toxicity, respectively [7]. Likewise, the CAMELIA study found similar incidences of drug-related adverse events: 2.93 (95% CI, 2.58 to 3.32) and 3.21 (95% CI, 2.83 to 3.63) events per 100 person-months in the earlier and later ART groups respectively [8]. The first-line ART regimen used in the STRIDE and CAMELIA studies included once-daily efavirenz, emtricitabine and tenofovir, and twice-daily efavirenz, lamivudine and stavudine, respectively.

Data on rates of adverse events and drug switches due to toxicity in patients receiving therapy for both HIV and tuberculosis are limited. Observational studies in TB–HIV cotreatment demonstrate conflicting rates of drug-related adverse events, compared to evidence to the contrary from the three relatively large randomized controlled trials [5,7,8]. A retrospective study from South Africa showed that the occurrence of serious adverse events was unrelated to the use of antiretroviral drugs in patients with TB [10]. However, retrospective studies conducted in Thailand and India, among patients with CD4$^+$ T-cell count <100 cells/mm^3, found drug-related adverse events occurred in 66.1% of cotreated patients in the first 2 months of TB treatment [11], and that concomitant use of ART and TB treatment was a predictor of adverse events (OR 1.88) [12]. Notably, in these two relatively small studies (<150 patients), two-thirds of all patients received a nevirapine-containing ART regimen, whereas almost all of our patients were initiated on an efavirenz-based ART regimen.

Previous studies have shown that peripheral neuropathy (43%) and hepatoxocity (5–10%) are the most common toxicities in patients receiving TB–HIV cotreatment [7,13–15]. The most common cause of drug switching in our study was neuropsychiatric toxicity, most likely related to the use of an efavirenz-based first-line regimen. Contrary to other studies, describing the increased risk of hepatotoxicity when ART is introduced during the intensive phase of TB therapy [15], there were no drug switches for treatment limiting hepatotoxicity among patients in the early integrated treatment arm. However, drug switch for hepatotoxicity was observed in the late integrated and sequential treatment arms. This may be as a result of patients having lower CD4$^+$ T-cell counts due to delay in initiation of ART [15]. While studies that report an increased risk of hepatotoxicity in TB–HIV cotreatment cite baseline elevated transaminases and hepatitis B antigenaemia as likely risk factors [16–25], the prevalence of both conditions was low in our study, which may account for the small number of drug switches resulting from hepatotoxicity that were observed.

It is likely that the profile of toxicities presenting in this cohort is linked to our choice of first-line ART regimen, which was chosen for its suitability to be coadministered with directly observed TB treatment and once-daily dosing. The absence of clinically significant alteration of efavirenz plasma concentration when coadministered with rifampicin has been demonstrated [26,27]. Efavirenz has also been shown to have a lower risk of hepatotoxicity than nevirapine [25]. Enteric coated didanosine has a lower risk of peripheral neuropathy and gastro-intestinal toxicities than stavudine and buffered didanosine [14], the available nucleoside reverse transcriptase inhibitors at the time of study conduct.

In addition, this study was conducted in ambulant, relatively clinically stable patients with TB disease mainly confined to the lungs. Although other nucleoside/nucleotide reverse transcriptase options, included in fixed-dose combinations, have now eclipsed didanosine-containing regimens as the first-line options, enteric-coated didanosine may still provide a useful alternative in patients unable to tolerate the alternative once-daily options [28].

Despite the additional pill burden when TB and ART therapy was coadministered in the early and late integrated treatment arms, the adherence was similar across the three treatment arms. The incidence of ART complete regimen change from virological failure was low and did not differ by treatment arm, despite the addition of three antiretrovirals to the four-drug intensive phase of TB or to the two-drug maintenance phase of TB therapy. Similar high rates of virological suppression were achieved and sustained, through to 18 months of follow-up after ART initiation across all study arms. These rates were similar to rates achieved at 48 and 50 weeks in the STRIDE and CAMELIA studies respectively [7,8], and better than reports of virological suppression rates (76%) achieved in treatment programmes from sub-Saharan Africa at 12 months [29].

The higher incidence of complete regimen change in patients with CD4$^+$ T-cell count <50 cells/mm^3 observed in this study has also been reported in other TB and non-TB settings [30–33]. Several studies have shown that low CD4$^+$ T-cell counts are a predictor for complete regimen change due to virological failure [34–36]. The drug switches in patients with low CD4$^+$ T-cell counts may not be directly associated with TB–HIV cotreatment, but may instead be due to

the presence of other comorbidities in patients with advanced HIV disease.

Low BMI has been shown in previous studies to be associated with poor treatment outcomes and a potential predictor of treatment failure in resource-constrained settings [34–36]. By contrast, our study found higher BMI (>25 kg/m^2) to be associated with a higher risk of complete regimen change. This may, in part, be explained by findings from other studies which found sub-therapeutic drug levels [37] and BMI>25 kg/m^2 to be an independent risk factor for virological failure [38]

The following study limitations need to be considered. We included ambulant patients with CD4$^+$ T-cell count up to 500 cells/mm^3, which is higher than the CD4$^+$ T-cell threshold for ART in current WHO and South African ART treatment guidelines. The inclusion of patients less advanced in the course of their HIV disease may have led to an under-estimation of the true effect of additive toxicity when cotreating TB–HIV. In this study, ART drug switches were triggered by grade 3 or 4 toxicities. However, grade 1 and 2 toxicities may affect adherence to therapy or patients' quality of life. Interruptions to TB drug therapy are also not included in this analysis. Pill count as a measure of adherence reported in this study, may have over-estimated the adherence reported.

In conclusion, both drug switches and complete regimen changes were uncommon in patients cotreated for TB–HIV using a didanosine, lamivudine and efavirenz first-line regimen. Manageable treatment-limiting toxicities occurred early, and affected a small percentage of the trial participants. The survival benefit from early initiation of ART in TB–HIV-coinfected patients outweighed the concerns of treatment-limiting toxicities. Low CD4$^+$ T-cell count and higher BMI (>25 kg/m^2) at baseline increased the risk of treatment failure and complete regimen change. The association with higher BMI may need further validation.

Patients with severe immunosuppression need to be monitored carefully, using viral load determinations, as they were most at risk for treatment failure requiring regimen change. The additional pill burden with combined TB–HIV treatment did not have a significant effect on adherence to ART in this study. These data further strengthen the available evidence of the benefits of integrating TB–HIV treatment and underline the continued usefulness of alternative once-daily regimens in such settings.

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The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to data, commented on drafts, and approved the final report. The corresponding author had final responsibility for the decision to submit for publication.

Disclosure statement

The authors declare no competing interests.

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Discussion of Paper IX: Drug tolerability has always been a concern for TB-HIV co-treatment. This sub-analysis from the SAPiT trial data assessed the data in 501 patients over 16 months, analyzing individual drug switches for toxicity and complete regimen changes due to virological failure. In this study the frequency of drug switches and complete regimen changes was extremely low. Treatment limiting toxicities per category investigated (neuropsychiatric effects, elevated transaminases and lactic acid, peripheral neuropathy) affected less than 1% of participants in all instances. Virological failure occurred in 25 patients and it appears that those who were severely immunocompromised at ART start (CD4 < 50 cells/mm^3) were most likely to fail treatment.

Importance for TB-HIV treatment integration: This article's strength is the large number of patients assessed (n=500). The authors show that co-treatment was very well tolerated by the majority of patients, dispelling the concern for drug toxicity and showing that in practice this was not frequently encountered in either treatment arm. Also, the study demonstrates that the severely immunocompromised require careful monitoring and support to maintain adherence and frequent assessment of treatment success.

APPENDIX B:

The START trial

B. The START Trial

The CAPRISA 001 START trial in Durban, South Africa was an open-label-randomized clinical trial in adult patients attending the Prince Cyril Zulu Communicable Diseases Centre, for directly observed TB treatment, and the adjoining CAPRISA eThekwini Clinical Research site, for HIV care and clinical trial procedures. Participants were randomized equally to receive HIV and TB treatment concurrently (integrated treatment) or HIV treatment after TB treatment was successfully completed (sequential treatment). The primary objective of that trial was to assess the effectiveness of integrated TB and HIV care provision through a directly observed treatment (DOT) program versus sequential treatment of TB and HIV, by comparing progression to AIDS-defining illnesses/mortality during the first 18 months after enrollment in the study.

Approximately 592 patients were to be studied. Due to slow accrual, only 58 patients were enrolled and the trial duration was tailored so that the PhD PK sub-study objectives could be met. Participants in the sequential arm were therefore exited from the study after successful completion of at least six months of TB treatment and having received at least three months of ARV treatment after TB cure. The integrated arm participants received at minimum 6 months of combined TB and HIV treatment plus three months of ARVS after TB treatment completion.

During RIF-based TB treatment, integrated arm patients weighing <50kg and $\geq$ 50kg received directly observed ART comprising either 600mg or 800mg EFV respectively. For the PK study, single steady state trough EFV (C_{min}) concentrations were sampled on 6 occasions at weeks 4, 8 and 12, both during and after TB treatment in the integrated arm patients, as well as after the initiation of ART in the sequential arm (3 occasions). After completion of TB

treatment, patients in both arms received EFV 600mg. RIF peak concentrations were assayed at 2.5h after the dose was administered on three occasions, at weeks 4, 8 and 12 during TB treatment in both arms. PBMCs were stored for post-trial assessment of pharmacogenetics.

The information contained in sections B1-B4 are transcribed directly from the START study protocol, version 1.0.

B1: START TRIAL

Study schema

B1. Study schema

START: Starting Tuberculosis and Anti-Retroviral Therapy

Design: This is a two-armed, randomized, open-label clinical trial evaluating whether the integration of HIV care into existing TB care services is feasible and practical in resource-poor settings. The primary objective is to assess the effectiveness of integrated TB and HIV care provision enhanced with an adherence support program (ASP) versus sequential treatment of TB and HIV, by comparing the progression to AIDS-defining illnesses/mortality in participants with pulmonary TB co-infected with HIV during the first 18 months after enrollment in the study. The study is conducted in two phases. The first phase represents the duration of TB therapy. The second phase represents the period after completion of TB therapy. Study participants will be randomized to one of the following arms stratified by CD4+ cell count, 50-200 cells/μL vs. > 200 cells/μL. Participants randomized into the integrated arm will receive anti-retroviral therapy (ART) consisting of didanosine (ddI)/ didanosine enteric coated (ddI-EC), lamivudine (3TC), and efavirenz (EFV) in conjunction with TB therapy upon randomization. Participants randomized to the sequential arm will complete TB treatment and then start ART consisting of ddI/ddI-EC, 3TC, and EFV. In instances where ddI/ddI-EC, 3TC, and EFV are contraindicated, an alternative regimen will be used.

Duration: Study duration is 24 months after randomization.

Sample Size: 592 participants will be enrolled.

Population: Men and women ≥ 18 years of age with documented HIV infection and smear-positive pulmonary TB.

Regimen: At entry, participants will be randomized (1:1) to one of the following treatment arms:

Integrated arm: (ddI/ddI-EC) + 3TC + EFV once daily concurrently with standard TB treatment upon randomization.

Sequential arm: (ddI/ddI-EC) + 3TC + EFV once daily initiated after completion of TB therapy.

ART substitution options will be available for participants who become pregnant, experience toxicities, or have treatment failure.

B2: START TRIAL

Inclusion criteria

B2: Inclusion criteria

- Males or females age ≥18 years.
- At least one positive acid-fast sputum smear for TB by microscopy with clinical symptoms of TB or two positive smears by microscopy. This is the diagnostic criteria for TB as defined by the Prince Cyril Zulu CDC and the South African TB Clinical and Diagnostic Treatment Guidelines.
- Receiving standard regimen anti-TB therapy (isoniazid, rifampicin, ethambutol, pyrazinamide).
- Participating in the Prince Cyril Zulu CDC DOT program and receiving supervised treatment daily at the Prince Cyril Zulu CDC.
- HIV infection, as documented by two positive rapid HIV tests (e.g., OraQuick or Smart Check or other tests approved by the US FDA or the South African Department of Health) and confirmed by HIV-1 RNA polymerase chain reaction (PCR).
- Ability and willingness of participant or legally authorized representative to provide written informed consent to take part in the study.
- Karnofsky score ≥ 70 within 14 days prior to entry.
- The following laboratory parameters from samples obtained within 14 days prior to study randomization:
- AST ≤ 2.5 x the upper limit of normal (ULN).
- ALT ≤ 2.5 x ULN.
- Creatinine ≤ 1.5 x ULN.
- Total bilirubin ≤ 2.5 x ULN.
- Absolute neutrophil count (ANC) ≥1000.

- Hemoglobin ≥ 7.0 g/dL.
- Not intending to relocate out of the current geographical area for the duration of study participation.
- Willingness of participant to adhere to study follow-up schedule.
- Women must agree to undergo serum or urine β-HCG pregnancy testing at Day 0 and during regularly scheduled monthly visits during ART therapy. Female study volunteers of reproductive potential must have a negative serum or urine pregnancy test performed within 48 hours before initiating EFV.
- Negative serum or urine β-HCG pregnancy test obtained within 14 days prior to study entry for women with reproductive potential (defined below). The urine test must have a sensitivity of **≤** 50 mIU/mL.
- "Female participants without reproductive potential" are defined as women who have reached menopause or undergone hysterectomy, bilateral oophorectomy, or tubal ligation or female participants whose male partner has undergone successful vasectomy with documented azoospermia or has documented azoospermia for any other reason.
- "Female participants of reproductive potential" are defined as girls who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months (i.e., who have had menses within the preceding 24 months) or have not undergone sterilization (e.g., hysterectomy, bilateral oophorectomy, or salpingotomy).
- All participants must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, donate sperm, in vitro fertilization).

- Female participants who are participating in sexual activity that could lead to pregnancy and who are receiving EFV, must agree to use two reliable methods of contraception: a barrier method of contraception (male or female condoms or diaphragm with spermicide or cervical

cap with spermicide) together with either an intrauterine device (IUD) or hormonal-based contraception while receiving the protocol-specified drugs and for 6 weeks after stopping the drugs. Another ART drug will be substituted for EFV if participants are not able, or willing, to use two forms of contraception simultaneously.

  - Note: Female participants who are taking rifampicin, but not taking EFV, must agree to use a barrier method of contraception or an IUD while receiving rifampicin.

- Female participants who are participating in sexual activity that could lead to pregnancy, but who are not receiving EFV, must use at least one barrier method of contraception or an IUD while receiving the protocol-specified drugs.

- Female participants who are not of reproductive potential, as defined above, or whose male partner(s) have undergone successful vasectomy or have documented azoospermia for any other reason, are eligible without requiring the use of contraception. . Written or oral documentation communicated by clinician or clinician's staff of one of the following is required for participants receiving EFV: physician report/letter, discharge summary, FSH measurement elevated into the menopausal range as established by the reporting laboratory.

  - NOTE: If the female study volunteer reports a history of infertility based on one of the above categories but written documentation is not obtainable, or she states that her partner has had a vasectomy, the female study volunteer must agree to use at least one barrier method of contraception with a possible second method required at the discretion of the site study physician.

B3: START TRIAL

Exclusion criteria

B3. Exclusion criteria

- ≥ 28 days of cumulative ART prior to study entry.
    - NOTE: Past Mother to Child Transmission (MTCT) and Post Exposure Prophylaxis (PEP) prevention treatments are allowed.
- < 10 days or > 28 days since the initiation of TB treatment.
- Temperature > 38.5°C, ≥ Grade 3 rash, ≥ Grade 3 nausea, or ≥ Grade 3 vomiting at time of screening or enrollment.
- Hospitalized or referred for hospitalization for care and treatment of opportunistic infections, TB, or other causes at time of screening or enrollment.
- CD4+ cell count < 50 cells/µL within 28 days of study entry.
- Active TB meningitis or miliary TB.
- History of prior TB treatment or any prior active TB episode.
- History of current or prior AIDS-defining condition(s) as described in the modified WHO Stage IV clinical staging system.
- Previous or current acute or chronic pancreatitis.
- ≥ Grade 2 peripheral neuropathy.

- Currently taking allopurinol, zalcitabine, astemizole, terfenadine, ergotamine or ergot derivatives, midazolam, triazolam, cisapride, phenytoin, phenobarbitone, carbamazepine, voriconazole, ribavirin, Echinacea-containing complementary medicines or supplements, St. John's Wort-containing complementary medicines or supplements.

- Pregnant at the time of study entry. Breastfeeding mothers are not excluded.

- Suspected MDR-TB, defined as "participant's awareness of contact with someone diagnosed with MDR-TB at home or in the workplace."

- Any other condition that, based on the opinion of the participant's study clinician, would preclude provision of informed consent or result in the participant being unable to fully participate in required study procedures.

- Participation in any other trial or study with objectives and intervention(s) that may interfere with the START study.

B4: START TRIAL

Pharmacokinetic (PhD) study design

B4: START Pharmacokinetic study design (PhD study)

Dose/plasma concentration data will be obtained over time from participants in each arm of the study. Pharmacokinetic testing will be conducted on frozen samples by the Division of Pharmacology at the University of Cape Town.

Integrated Arm:

Phase I: To obtain sufficient data points, EFV trough levels (samples taken immediately prior to dosing) and rifampicin peak levels (approximately 2.5 hours post dose) will be measured at the end of months 1, 2 and 3. This is designed to show the extent of the interaction over time.

Phase II: Additional trough EFV levels will be measured at the end of the first, second, and third month after TB treatment is completed (Phase II) to show how this interaction resolves over time.

Sequential Arm:

Phase I: Peak rifampicin levels will be analysed at the end of months 1, 2 and 3.

Phase II: After completion of TB treatment, when ART is initiated in Phase II, EFV trough levels will be assessed at the end of months 1, 2 and 3.

The choice of sampling times is determined by the long half-life of EFV, estimated to be between 52-76 hours, making the measurement of trough levels feasible. Rifampicin peak levels were selected based on the relatively short half-life of 2-5 hours of this agent. Previous studies have also shown the median Tmax after dosing to be 2.5 hours [139-141]. Levels taken 8-12 hours post dosing are low to undetectable in the average participant.

The exact time of blood draw and time of last efavirenz and rifampicin dose will be recorded on CRFs.

APPENDIX C:

Regulatory approvals for study conduct

C1: Regulatory approvals

South African Medicines Control Council

C. Regulatory approvals for study conduct

C1. South African Medicines Control Council Approval

MEDISYNEBEHEERRAAD

Republiek van Suid-Afrika

MEDICINES CONTROL COUNCIL

Republic of South Africa

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Dear Prof Abdool-Karim,

AUTHORISATION FOR THE IMPORTATION OF UNREGISTERED MEDICINE IN TERMS OF SECTION 21 OF THE MEDICINES AND RELATED SUBSTANCES CONTROL ACT, 1965 (ACT 101 OF 1965)

PRODUCT: TENOFOVIR

Your application letter dated **06 Sep 2004** refers

1. **RESOLUTION AND APPROVAL**

It was recently resolved by the Medicines Control Council that; the clinical trial application according to the following Protocol be approved :-

CAPRISA 001 protocol version Final version 1.1 dated 08-Nov-04

The START (Starting Tuberculosis and Anti-Retroviral Therapy) Trial: A Randomised Controlled Trial to Assess the Effect of Integrated Tuberculosis and HIV Care on the Incidence of AIDS-Defining Conditions or Mortality in participants Co-Infected with Tuberculosis and HIV.

1.1 **BEFORE COMMENCEMENT OF TRIAL**

Please Note: Copies of written Ethics Committee approval/(s) to be submitted to MCC before the study commences.

2. **AUTHORISATION**

Authorisation is hereby granted for the importation and administration of a sufficient quantity, for the duration of the trial, of the unregistered medicine:

TENOFOVIR

solely for the purpose of a clinical trial to be conducted by:

Prof SS Abdool -Karim	Prince Cyril Zulu Communicable Disease Centre/King Edward	Principal
Prof Q Abdool-Karim	Prince Cyril Zulu Communicable Disease Centre/King Edward	
Dr G Churchyard	Prince Cyril Zulu Communicable Disease Centre/King Edward	

mcc MEDICINES CONTROL COUNCIL

Page 1 of 2

Dr M Khan	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr T Moodley	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr K Naidoo	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr G Nair	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr N Padayatchi	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr A Singh	Prince Cyril Zulu Communicable Disease Centre/King Edward
Dr D Wilson	Prince Cyril Zulu Communicable Disease Centre/King Edward

3. **PLEASE FORWARD**

It is a requirement that a copy of this letter be forwarded to all the relevant Trialist/(s), including the approving Ethics Committee(s).

4. **THIS AUTHORISATION IS SUBJECT TO THE FOLLOWING PROVISOS:**

(a) The Council shall be informed immediately of any toxic effects or death, which may occur during the Clinical Trial and of any data received which, might cast doubt on the validity of the continuation of the Clinical Trial.

(b) The Council shall be notified of any decision to discontinue the Clinical Trial. The reason for such cancellation shal be stated.

(c) The Clinical Trial shall be conducted in accordance with the Protocol submitted to the Council. Any Amendment/(s) to the Protocol shall first be submitted to the Council for approval. All Clinical Trials be conducted in accordance with ICH GCP Guidelines, and the South African Clinical Trials Guidelines.

(d) The medicine shall be administered by or under the direction of the authorised Trialist. In the case where the Trialist permits another Medical Practitioner to administer a medicine, which is exempted from the registration for the purpose of the Trial, the Trialist shall remain responsible for any eventuality arising from such usage.

(e) Where a Trialist who is not authorised in the initial Authorisation, is requested to participate in the Clinical Trial, the Council requests that the relevant MCC Curriculum Vitae Format be completed detailing their Full Names, Address and Qualifications of the proposed Trialist (Practitioner) concerned, and be submitted to the Council for Approval.

(f) In the event of the authorised Trialist ceasing to participate in the Clinical Trial, the Council shall be informed and the reason for such cessation shall be given.

5. **PROGRESS REPORTS**

The Council must be furnished with signed six-monthly Progress Report from each Trialist including a report of the Final Results.

6. **INFORMED CONSENT**

It is a Council requirement that in all Clinical Trials the 'Principles of Informed Consent' should be adhered to. This applies to Trial Volunteers, as well as Participants (Patients). (Reference: Section 4.8 of ICH GCP Guidelines and Section 3.5 of SACT Guidelines).

PLEASE NOTE: : Dr A Singh may commence participation the study after proof of GCP training has been forwarded to MCC.

Yours faithfully,

DR BAJEN MISRA

FOR AND ON BEHALF OF REGISTRAR OF MEDICINES

MCC TRIAL REFERENCE NO: 20040969

MEDICINES CONTROL COUNCIL

C2: Regulatory approvals

University of KwaZulu-Natal Biomedical Research Ethics Committee

C2. University of KwaZulu-Natal Biomedical Research Ethics Committee Approvals

UNIVERSITY OF
KWAZULU-NATAL

14 February 2006

Professor S S Abdool Karim
CAPRISA
Nelson R Mandela School of Medicine

Dear Professor Abdool Karim

PROTOCOL : CAPRISA 001 – START - Starting tuberculosis and anti-retroviral therapy. A randomized controlled trial to assess the effect of integrated tuberculosis and HIV care on the incidence of AIDS-defining conditions or mortality in subjects co-infected with tuberculosis and HIV. S S Abdool Karim, CAPRISA. Ref.: E183/04

I wish to confirm that at a full sitting of the Biomedical Research Ethics Committee held on 14 February 2006, the Committee concurred with the decision of the sub-Committee that the application for recertification of the above study dated 18 October 2005 be approved.

Yours sincerely

Cheryl Borresen
Medical Research Administration

Nelson R Mandela School of Medicine, Faculty of Health Sciences, Medical Research Administration

Postal Address: Private Bag 7, Congella 4013, South Africa

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ng Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

23 January 2012

Ms. TN Gengiah
CAPRISA, Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Ms Gengiah

PROTOCOL: Integrating human immunodeficiency virus (HIV) and tuberculosis (TB) drug treatment. REF: BE041/11

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a quorate meeting of BREC on 13 September 2011 pending appropriate responses to queries raised. Your responses dated 21 December 2011 to queries raised on 28 August 2011 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 23 January 2012

This approval is valid for one year from 23 January 2012. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.
BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The following Committee members were present at the meeting that took place on 13 September 2011:

Professor D Wassenaar - Chair
Professor D J Pudifin - Medicine
Dr T Hardcastle - Surgery - Trauma
Professor A Coutsoudis - Paediatrics & Child Health
Professor C Rout - Anaesthetics
Dr D Singh - Dentistry
Dr S paruk - Psychiatry
Dr H Dawood - Medical School
Dr R Green-Thompson - Obstetrics & Gynaecology
Dr A Sathar - Medicine
Professor TE Madiba - General Surgery
Professor S Collings - Psychology
Mrs T Makhanya - Community Representative

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

C3: Regulatory approvals

Postgraduate education committee

C3. Postgraduate education committee approval

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

20 December 2011

Professor J Botha
Department of Therapeutics and Medicines
Medical School NRMSM

Dear Professor Botha

PROTOCOL:"Integrating human immunodeficiency virus (HIV) and Tuberculosis (TB) drug treatment"
TN Gengiah 993241124 – PhD Therapeutics and Medicines Management.

The Postgraduate Education Committee ratified the approval of the abovementioned study on 13 December 2011.

Please note:

- The Postgraduate Education Committee must review any changes made to this study.
- The study may not begin without the approval of the Biomedical Research Ethics Committee.

May I take this opportunity to wish the student every success with the study.

Yours sincerely

Professor SJ Botha
Chair: Postgraduate Education Committee

CC. Dr TN Gengiah

Biomedical Research Ethics Committee
Westville Campus

Postgraduate Education Administration
Medical School Campus
Postal Address: Private Bag 7, Congella, 4013, South Africa
Telephone: +27 (0) 31 260 4327 Facsimile: +27 (0)31 260 4401 Email: heslopd@ukzn.ac.za Website: www.ukzn.ac.za

C4: Regulatory approvals

Permission from study Principal Investigator to access stored samples

C4. Permission from study Principal Investigator to access stored samples

CAPRISA

CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA

UNAIDS

CAPRISA IS A UNAIDS COLLABORATING CENTRE FOR HIV PREVENTION RESEARCH

Permission to access stored samples for the purposes of PhD postgraduate work

Permission is granted to PhD candidate, Tanuja Gengiah, student#993241124 to access stored samples for participants from the START (CAPRISA 001) and SAPiT (CAPRISA 003) studies.

The specimen samples accessed will be limited to those essentially required to meet the pharmacology objectives of the PhD, specifically the access of samples for the assay of ARV and TB drug concentrations and sequencing of samples for the purposes of detecting pharmacogenetic polymorphisms.

Tanuja Gengiah shall treat all information relating to the sample access , analysis and associated participant data as confidential. Publications arising from the PhD will be subject to the CAPRISA Publication policy.

Prof Salim S Abdool Karim Signature: _____

Principal Investigator: START and SAPiT studies

Date: 11 July 2011

Dr Kogieleum Naidoo Signature: _____

Project Director: START and SAPiT studies

Date: 11 July 2011

C5: Regulatory approvals

Sample informed consent forms

C5. Sample Informed Consent Forms

STUDY INFORMED CONSENT FORM

(ENGLISH)

CAPRISA 001 - START: Starting Tuberculosis and Anti-Retroviral Therapy. Implementing Anti-Retroviral Therapy in Resource-Constrained Settings: A Randomized Controlled Trial to Assess the Effect of Integrated Tuberculosis and HIV Care on the Incidence of AIDS-Defining Conditions or Mortality in Subjects Co-Infected with Tuberculosis and HIV.

Short Title: START: Starting Tuberculosis and Anti-Retroviral Therapy

Sponsored by: The National Institute of Allergy and Infectious Diseases
Division of AIDS (DAIDS)
6700B Rockledge Drive
Bethesda, Maryland 20852
United States

Principal Investigator: Professor Salim Abdool Karim, MBChB, PhD

INTRODUCTION

You are being asked to take part in this research study because you are infected with the HIV virus and tuberculosis (TB). This study is sponsored by the US National Institutes of Health (NIH). The doctor in charge of this study at this site (CAPRISA) is Prof. Salim S. Abdool Karim, MBChB, PhD. Before you decide if you want to be a part of this study, we want you to know about the study.

This consent form gives you information about this study, which will be done at Prince Cyril Zulu Communicable Disease Centre (CDC) and King Edward VIII Hospital (KEH). The research staff will talk to you about this information. Ask questions and discuss any concerns you may have with the research staff. If you agree to take part in this study, you will be asked to sign this consent form. You will be given a copy of this consent form to keep.

Please note that:

- Your participation in this study is entirely voluntary. You do not have to participate in the START study. You may decide to obtain your HIV care through your own medical provider.

- You may stop taking part in the study at any time without losing your standard health care.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to learn whether it is safe and effective to give TB and HIV medicines at the same time to people who have active TB and HIV. The study will also see how well people who take TB and HIV medicines at the same time are able to stay on the medicines without serious side effects.

The best time to start HIV medicines, called antiretroviral therapy or ART, in people who have TB and HIV infection is not known. There is little information about the best way to treat people who are infected with both HIV and TB.

All of the drugs used in this study are approved by the US Food and Drug Administration (FDA) for treating HIV/AIDS. These drugs do not cure HIV/AIDS. These are the same drugs that you would receive from the South African government, although not all of the study drugs may be available.

OVERVIEW OF THE STUDY

If you agree to participate in this study and tests show that you can enter the study, you will be randomized (assigned by chance, like flipping a coin) to one of two treatment groups. This means you will have an equal chance of being in one of the two groups listed below. You will not be able to choose which group you are in, but both you and your doctor will know which group you are in.

EARLY group: This group will start ART about 2 to 4 weeks after starting TB treatment.

LATER group: This group will begin ART soon after the TB treatment is finished (about 6 to 9 months later).

Both groups will receive the standard TB medicines as part of the Tuberculosis Control Programme. TB treatment usually lasts for about 6 to 9 months. You will come to the Prince Cyril Zulu Communicable Disease Centre (CDC) to receive and be observed while taking your TB medicines. If you are in the EARLY group you will receive ART at the same time at the CDC and be observed and on weekends take ART on your own until your TB treatment is finished. After you finish TB treatment, you will take ART on your own. If you are in the LATER group, you will start taking ART on your own soon after you have completed your TB treatment. Both groups will be seen at KEH for all study visits after TB treatment is finished. You will receive ART there until the end of the study (2 years after you enter the study).

WHAT DO I HAVE TO DO IF I PARTICIPATE IN THIS STUDY?

If you enroll in this study, you will need to come into the clinic for examinations, interviews, and laboratory tests frequently; at least 28 times during the 2 years of the study depending on when you complete your TB treatment. Weekly visits will take about 30 minutes and most other visits will take about 1 to 1½ hours. Three visits will take up to 3 hours to measure the amount of TB and HIV drugs in your blood. Site staff will pay close attention to whether you have kept appointments since they want to keep track of your health. If you miss appointments, the persons you named will be contacted or field workers will be sent to your home.

Any time that results of exams and laboratory tests such as viral load (which measures how much HIV virus is in your blood), CD4+ T cell counts (immune cells that help fight infection such as HIV), and safety tests are known, they will be given to you. There may be times that you must come for additional visits if these exams or tests are abnormal. Some of the blood drawn throughout the study can be stored. You will be asked for your permission to store the blood for future research and asked to sign a separate consent form if you agree to do this. You may still participate in the study if you do not agree to have blood stored.

Study Entry Visit

If the results from screening showed you can be in the study, you will return to the clinic to enter the study within 28 days after starting TB medicines. This visit will take about 2 hours.

You will be asked questions about your medical history and any medicines that you have taken. You will be asked how to be contacted in case you miss a visit or there are ever problems with your lab results. You will be given a physical exam and have about 60 mL of blood drawn for routine tests, CD4 + cell counts, and to check for hepatitis (infection of the liver). You will be told your test results throughout the study. Some of your blood will be stored (with usual protectors of identity) for future HIV-related testing including a test for HIV resistance (to see if the HIV is able to respond to the ART). If you are a woman and able to become pregnant, you will be asked to provide some urine for a pregnancy test. You will be asked questions about your sexual activities. Some of these questions may cause you to be embarrassed. You do not have to answer these questions in order to be in this study.

After you complete the entry tests, you will be assigned to one of the two study groups as described above. If you are in the EARLY group, you will meet with a counselor for about 30 minutes to discuss the ART and how to take the medicines properly. The ART will then be started.

Follow up Visits

Both groups will have weekly visits for a month and then monthly visits at the CDC until TB treatment is complete (usually 6 months but up to 1 year for people who need more treatment).

After completion of TB therapy, all your visits will be at the KEH in Phase II. If you are in the LATER group, you will be seen weekly for the first month of ART and then monthly until the end of the study. At the first visit, you will have tests to see if you can start ART safely at that time. If you can, ART will begin at the next visit. If you cannot safely start ART then, these tests will be repeated at later visits until you can start ART. The EARLY group will continue ART and will have visits monthly until the end of the study.

During the Follow Up visits, you will be asked about any medicines you are taking, be given a physical exam, and at some of these visits will have about 30 to 40 mL of blood drawn for routine tests, CD4 counts, and HIV viral load. If you are a woman and able to become pregnant, at every visit while you are taking HIV drugs you be asked to provide urine for a pregnancy test. At some monthly visits, you will be asked about your health, receive your next study drug supply, and women will be asked to provide urine for a pregnancy test. At 6 visits, about 10 mL of blood will be taken to measure the amount of TB or HIV drugs in your blood. At 3 of these visits, the blood to measure your TB medicines will be drawn about 2 ½ hours after you take your TB medicine. If you agree and sign a separate consent form, at some visits about 35 mL of blood will be drawn, stored, and used for research in the future. You may still participate in the study if you do not agree to have blood stored.

Sputum will be collected at least twice during the study. Sputum will be collected and you will have a chest x-ray at 2 months and any time the doctors think you have TB after finishing TB treatment.

Once you start ART, you will complete a questionnaire about taking your study medicines. This will take about 30 minutes. In addition, if you are in the EARLY group, you will meet with a counselor three times for about 15 to 30 minutes to discuss ART and how to continue the medicine after TB treatment.

Four times during the study, you will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. This will take about 30 minutes to one hour.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 592 people will take part in this study.

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 2 years after you enter the study. If you need to stop study drugs permanently for any reason, you will still be asked to continue with study visits. Once you have finished the study, the study will no longer provide you with ART drugs. After the study is over, we anticipate that you will receive ART drugs from the South African rollout program for antiretroviral therapy or another HIV treatment or research program.

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- the study is canceled by the US NIH, the Medicines Control Council, or the Ethics Committee (EC). An EC is a committee that watches over the safety and rights of research participants.
- a Data Safety Monitoring Board (DSMB) recommends that the study be stopped early. A DSMB is a group of experts who are not involved in the study that monitors the results of the study.

If you decide that you do not want to participate in the study anymore, you may leave the study at any time.

The study doctor may also need to take you off the study drugs without your permission if:

- continuing the study drugs may be harmful to you,
- you need a treatment that you may not take while on the study;
- you are not able to take the study drugs as required by the study or you are not able to attend the study visits as required by the study; or
- these medicines are not working well enough against the HIV virus and no other effective drugs are available.

If you must stop taking the study drug(s) before the study is over, the study doctor may ask you to continue to be part of the study and return for your regularly scheduled study visits.

WHAT ARE THE RISKS OF THE STUDY?

Risk of Early versus Later HIV Treatment for People with TB

The South African government is starting an ART rollout program to provide HIV treatment. Current guidelines suggest that people with HIV who have a very weak immune system (CD4+ cell count of less than 200 cells/μL) should begin taking ART.

On this study, even if your immune system is weak (CD4+ cell count from 50 to 199 cells/μL), you may have to wait 6 to 12 months before you start ART if you are randomized to the LATER group. If you have advanced HIV disease, you are at risk for developing HIV-related infections until after you start ART. While treatments are available for most of them, some of these infections can be very serious and can result in death. During this time the study staff will watch your health very carefully. If you are becoming increasingly sick from HIV, the study doctors may decide to begin ART before you finish your TB treatment. It is also possible that if you do not start taking ART until AFTER TB treatment is completed (6 months or more), you may not respond as well to treatment for TB as if you started ART within the next few weeks.

If you do not want to take a chance with the study, the study staff will try to help you find other ways to get ART, including treatment from the government or other research programs. People who start HIV treatment at the government rollout sites need to meet certain requirements. The study staff will tell you if you meet these requirements.
The HIV drug combination that we use in this study, is different from the one used by the government hospitals. All the drugs are accepted worldwide to treat HIV but the SA government uses them in a different order from what we will. There is a possibility that if you develop resistance or problems with any of our drugs during the study period and require alternate drug regimens that your choices of drugs on transitioning to the government ARV roll-out programme will be limited

Possible Risks of Starting ART During TB Treatment

The risks of ART while receiving TB treatment may include added side effects from the medications, either from the drug used against HIV or from the TB drugs. Interactions between these two kinds of drugs may also cause trouble. There may also be an increase in the chance of getting IRIS. (See below)

Risk of Immune Reconstitution Inflammatory Syndrome (IRIS)

While being treated with ART, your improving immune system's strong response to TB or other HIV/AIDS-related infections may also cause illness. This is called Immune Reconstitution Inflammatory Syndrome (IRIS). Usually, it causes a return or worsening of at least some of the symptoms.

Some examples of what could happen are that your lymph nodes (small organs in your body that help filter disease germs from the blood) could swell up, you could get a high fever, and you might have worsened cough and shortness of breath. While some of these reactions can be serious, they usually last for a short time and can be treated without stopping the ART. If this reaction happens to you, you will be treated for the problem and asked to have some additional blood drawn for testing of your immune system at that time.

DRUG RISKS

Anti-HIV Drugs:

There are many drugs available to treat HIV and AIDS. The study doctor will determine the best combination of these drugs to treat you. It is possible that the study drugs will make you feel sick or will affect your blood tests, in which case the study doctor may either switch you to different drugs, or stop them all together. It is very important for you to return to the clinic whenever you feel sick. Feeling sick may be due to the drugs or it may be due to a sickness caused by your HIV infection. Either way, we want to see you when you feel sick so we can take care of you.

All anti-HIV drugs can cause side effects, which can be more serious or severe with long-term use. Some of these side effects are mild and may go away after you have taken the drugs for a few weeks. Examples of these types of side effects include upset stomach, vomiting, headache, and changes in your mood, sleep, or concentration. Other side effects are severe and may require treatment or hospitalization. Examples of these types of side effects include rash, liver problems, severe depression or psychosis, and pancreas problems. Rarely, some people taking HIV drugs can develop a condition called "lactic acidosis." Some symptoms that might be caused by lactic acidosis include: unexplained weight loss, stomach upset, nausea, vomiting, fatigue, weakness, and shortness of breath. Lactic acidosis, along with an enlarged and fatty liver, may result in problems such as liver failure. In some cases, the condition results in death. The liver problems and death have been seen more in women on these drug regimens.

The use of anti-HIV drug combinations may be associated with an abnormal placement of body fat and wasting. Some of the body changes include: increase in fat around the waist and stomach area; increase in fat on the back of the neck; thinning of the face, legs, and arms; and breast enlargement. The use of anti-HIV drug combinations may also be associated with altered fat metabolism including elevated triglycerides and/or elevated cholesterol.

Most people who agree to participate in this study will receive the medicines listed below to begin their HIV treatment: Efavirenz (EFV) + didanosine (ddI/ddI-EC) + lamivudine (3TC) once a day.

Below are the risks for the initial anti-HIV drug combination that you could be given.

Risks of Didanosine (Videx®, ddI/ddI-EC)

- Pancreatitis (inflammation of the pancreas), which may cause death. If you develop pancreatitis, you may have one or more of the following: stomach pain, nausea, and vomiting.
- Deaths from liver failure have been reported in pregnant women receiving the combination of didanosine and stavudine with other anti-HIV drugs.

- Numbness, tingling, and pain in the hands or feet
- Abnormal vision changes
- Upset stomach, vomiting and loose or watery stools
- Headache
- Abnormal pancreatic function blood tests or abnormal liver function blood tests
- Increase in uric acid in the bloodstream

When didanosine is used with other medicines with similar side effects, these side effects may be seen more often and may be more severe than when didanosine is used alone. People who take didanosine together with stavudine, with or without hydroxyurea, may be at greater risk for pancreatitis or liver problems or both. These conditions may result in death.

Risks of Efavirenz (Sustiva®, Stocrin, EFV)
Effects on mental function include:
- Dizziness
- Trouble sleeping such as inability to sleep, abnormal dreams, and drowsiness
- Confusion
- Difficulty concentrating
- Hallucinations
- A feeling of strangeness and losing touch with reality
- An exaggerated feeling of well-being
- Agitation or anxiety

If alcohol or mind- or mood-altering drugs are used with efavirenz, it is possible that the above symptoms could become worse.

Serious psychiatric problems include:
- Depression, which may be severe
- Suicidal thoughts or attempts (rarely)
- Aggressive behavior
- Psychosis-like symptoms, such as abnormal thinking, paranoia, and delusions

People with a history of psychiatric problems may be at greater risk for these serious psychiatric problems.

Other risks include:
- Rash
- Upset stomach
- Loose or watery stools
- Headache

Increases in substance in the blood which can mean problems with the pancreas, such as inflammation or swelling of the pancreas with abdominal pain

- Increase in cholesterol
- Increase in triglycerides
- Abnormal liver function tests and inflammation of the liver (hepatitis)
- Abnormal vision
- Fever
- An abnormal or unusual distribution of body fat

Studies using efavirenz in pregnant monkeys have shown newborn monkeys with birth defects. Three out of twenty monkeys had birth defects. One monkey had a defect in the roof of the mouth, another had small eyes, and another was missing a brain and missing one eye. The monkeys in this study received doses of efavirenz similar to those that are being studied in humans. It is not known what this information means or whether this could happen in humans; therefore, you should not become pregnant while taking efavirenz.

A false-positive urine screening test for marijuana has been seen with one particular test brand and has not been seen when using other screening tests or with tests used to confirm results for marijuana.

Risks of Lamivudine (Epivir®, 3TC)

- Headache
- Feeling of vague overall discomfort
- Feeling tired
- Dizziness
- Depression
- Upset stomach
- Vomiting
- Loose or watery stools
- Decrease in appetite
- Abdominal cramps
- Sleeplessness
- Rash
- Numbness, tingling, and pain in the hands or feet
- Decrease in the number of white blood cells that help fight infection
- An increase in a substance in the blood (a type of a pancreatic enzyme) which could mean a problem with the pancreas
- Increased liver function tests, which could mean liver damage

Participants who are infected with both Hepatitis B and HIV should be warned that their liver function tests may increase, and symptoms associated with hepatitis (an acute inflammation of the liver) may worsen after lamivudine has been stopped. Although most of these cases have resolved without treatment, some deaths have been reported.

At the end of this consent form, there is a table that describes the side effects for other anti-HIV drugs that may be provided to you by the study if you need to switch anti-

HIV drugs. These drugs include nevirapine (NVP), tenofovir (TDF), abacavir (ABC), saquinavir and ritonavir (SQV + RTV), or lopinavir and ritonavir (LPV/r). When the study doctor gives you the study drugs, he or she will discuss the possible side effects with you. Throughout the study, these side effects will be told to you, particularly if you receive a new anti-HIV drug. If the study doctor gives you an anti-HIV drug that is not listed in the table, he or she will make sure that you understand the side effects of the drug. If you have questions concerning study drug side effects, please ask the study staff.

After you begin taking the anti-HIV drugs, do not stop taking any of them unless you discuss it with the study doctor. Suddenly stopping your treatment can cause an increase in the amount of HIV in your blood, and the virus can become resistant to HIV, which means that the drugs will no longer work.

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with study drugs. For your safety, you must tell the study doctor or nurse about all medications, herbs, or home remedies you are taking before you start the study and also before you take any treatment. You must also tell the study doctor or nurse before taking any nonstudy medications, herbs, or home remedies while you are on the study. In addition, you must tell the study doctor or nurse before enrolling in any other clinical trials while on the study.

Risks of Blood Draws

You may feel some discomfort, pain or lightheadedness during the blood sample collection. Some people get a bruise or swelling where the needle is put in your arm to draw blood. In rare cases, the blood drawing procedure can cause fainting or infection.

ARE THERE RISKS RELATED TO PREGNANCY?

It is not known if the drug or drug combinations in this study harm unborn babies. Tests in pregnant animals do show some risks for some drugs. If you are having sex that could lead to pregnancy, you must agree not to become pregnant or make a woman pregnant. Some drugs in this study and some of the TB drugs make some hormonal birth control methods less effective. Breastfeeding mothers are allowed in the study.

- If you are on a drug combination that includes EFV, you and your partner must use two methods of birth control that you discuss with the study staff. You must continue to use both methods until 6 weeks after stopping EFV. (If you are a woman and are unable to use two methods, your doctor will talk with you about taking another ART drug rather than EFV.) You may choose two of the birth control methods listed below:
  1. Birth control drugs that prevent pregnancy given by pills, shots, or placed on or under the skin.

2. Male or female condoms with or without a cream or gel that kills sperm
3. Diaphragm or cervical cap with a cream or gel that kills sperm
4. Intrauterine device (IUD)

- If you are not taking EFV or rifampicin, you must use one method of birth control listed below that you discuss with the study staff:
  1. Male or female condoms with or without a cream or gel that kills sperm
  2. Diaphragm or cervical cap with a cream or gel that kills sperm
  3. Intrauterine device (IUD)

ARE THERE SPECIAL RISKS RELATED TO BREASTFEEDING?

A mother who is infected with HIV may infect her baby through breast milk. HIV-infected mothers who are able to obtain baby formula and clean water should not breast feed their babies. It is unknown whether the study medicines pass through the breast milk and cause harm to your infant. It is also unknown whether study drugs may reduce the chances that HIV can pass to your baby through your breast milk.

WHAT HAPPENS IF I BECOME PREGNANT?

If you think you may be pregnant at any time during the study, tell your study staff right away. The study staff will talk to you about your choices and refer you to a provider of prenatal care at the King Edward VIII Hospital clinics. If you decide to continue in the START study, you will be asked to sign another consent form. This study will not provide or pay for care related to your pregnancy or the delivery of your baby.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you participate in this study, there may be a direct benefit to you, but no guarantee can be made. Your health will be followed more closely than usual while you are on the study, which may help you to feel better. Laboratory tests to monitor the effects of these drugs will be provided by the study. It is also possible that you may receive no benefit from being in this study.

Information learned from this study may help others who have HIV/AIDS by identifying whether it is safe and effective to start HIV medications while being treated for TB.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- treatment with ART through the South African national rollout program once this program begins (which is expected to begin in 2005), if you qualify
- treatment with experimental drugs, if you qualify
- no treatment

Antiretroviral medications, laboratory tests to monitor the effectiveness of these medications, and quality medical care for HIV/AIDS may or may not be available to you outside the study. The clinic staff will discuss with you other treatment choices in your area and the risks and the benefits of all the choices.

If you choose not to join this study, you can still receive TB treatment through the Tuberculosis Control Programme at CDC.

WHAT ABOUT CONFIDENTIALITY?

Your medical records, personal information, and the results of your HIV tests and other medical and laboratory evaluations will be kept strictly confidential within the extent of South African law. Only your doctor and/or nurse will know the results of your tests. Your records will be identified by a study code. Any publication of this study will not use your name or identify you personally.

Every effort will be made to protect your confidentiality but we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your records may also be reviewed by regulatory authorities, such as the Medicines Control Council (MCC), the Ethics Committees, the US National Institutes of Health (NIH), study staff, and study monitors.

WILL I RECEIVE ANY PAYMENT?

You will receive reimbursement for transportation and one meal when you attend study visits.

WHAT ARE THE COSTS TO ME?

The HIV treatment (ddI/ddI-EC, EFV, 3TC, TDF, NVP, ABC, SQV + RTV, LPV/r) will be provided free of charge while you are on study. If you require HIV treatment that does not include these drugs, you will receive this care from a local authority and/or provincial health facility. Provincial hospitals may ask you to pay a fee, depending on your income.

The TB treatment is provided free of charge to you by the Tuberculosis Control Programme at the CDC.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries at KEH. The study will pay for your medical management at the hospital. There is no program for compensation for research-related injuries through the U.S. National Institutes of Health (NIH). You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A VOLUNTEER IN A RESEARCH STUDY?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. You will be treated the same no matter what you decide.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact any of the following persons:

Clinic Manager at Prince Cyril Zulu Communicable Disease Clinic:
Dr. Nesri Padayatchi
Tel: (031) 260-4574

Clinic Manager at King Edward VIII Clinic:
Dr. Kogieleum Naidoo
Tel: (031) 260-4687

Project Director:
Dr. Kogieleum Naidoo
University of KwaZulu-Natal
King George V Avenue
Durban 4001
Tel: (031) 260-4687

Principal Investigator:
Prof. Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001
Tel: (031) 260-2381

For questions about your rights as a research participant, you may contact:

Ms. Cheryl Borresen
Postgraduate Administration Office
University of KwaZulu-Natal
Tel: (031) 260 4495

Prof. Ames Dhai
Chairperson of the Research Ethics Committee
University of KwaZulu-Natal
Tel: (031) 260-4604

STUDY INFORMED CONSENT SIGNATURES PAGE

I have read this form, or had it read to me, and voluntarily agree to take part in the study. The purpose of the study, the procedures, and the risks and benefits have been explained to my satisfaction. My signature, thumbprint or mark indicates that I consent to take part in the study, have received a copy of this consent form, and that I understand the consequences of taking part in the study.

Participant's Name (print)	Participant's Signature and Date
Participant's Legal Guardian (print) (As appropriate)	Legal Guardian's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date

For illiterate participants:

Mark or thumbprint:_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

Here is a table that describes the side effects for other anti-HIV drugs that may be provided to you by the study if you need to switch anti-HIV drugs. When the study doctor gives you the study drugs, he or she will discuss the possible side effects with you. Throughout the study, these side effects will be told to you, particularly if you receive a new anti-HIV drug. If the study doctor gives you an anti-HIV drug that is not listed in the table, he or she will make sure that you understand the side effects of the drug. If you have questions concerning study drug side effects, please ask the study staff.

Anti-HIV Drug	Side Effects
Abacavir [Ziagen®, ABC]	• An allergic reaction which may include many different symptoms, such as: fever, rash, feeling tired, upset stomach, vomiting, loose or watery stools, abdominal pain, cough, sore throat, shortness of breath, achiness, or a general feeling of illness. • These symptoms usually appear within the first six weeks after starting this drug but can occur at any time during treatment. This reaction can be severe and could even lead to death if abacavir is not stopped. The severe form of allergic reaction can also recur if abacavir is restarted after it has been stopped and can even lead to death. IF YOU THINK YOU MIGHT BE DEVELOPING A REACTION TO ABACAVIR, DO NOT TAKE ANY MORE DOSES AND CONTACT THE DOCTOR AT THE CLINIC IMMEDIATELY. NOTE: Severe or fatal allergic-type reactions can occur within hours after abacavir is restarted in participants who have interrupted abacavir therapy. Allergic-type reactions to abacavir can occur in patients who have had no prior identified history or whose symptoms were previously unrecognized. If you interrupt abacavir for any reason, immediately contact the medical staff at the site. If your doctor decides to restart, you may need to be monitored more closely in the clinic or in the hospital. • Upset stomach • Vomiting • Vague overall feeling of discomfort • Feeling tired • Decrease in appetite • Loose or watery stools • Inflammation or swelling of the pancreas with abdominal pain • Headache • Increased triglycerides • Lactic acidosis. The signs of lactic acidosis that you may notice are: unexplained weight loss, stomach ache, upset stomach,

Anti-HIV Drug	Side Effects
	throwing up, tiredness, weakness, and shortness of breath. Lactic acidosis can cause death
Lopinavir/ritonavir [Kaletra®, LPV/r]	• Pancreatitis (inflammation of the pancreas), which may cause death. If you develop pancreatitis, you may have one or more of the following: stomach pain, nausea, vomiting or abnormal pancreatic function blood tests • Abnormal bowel movements (stools), including loose or watery stools, upset stomach and stomach pain • Large increases in triglycerides and cholesterol in the blood • Liver problems and worsening liver disease, which may result in death. People with these conditions may have abnormal liver function blood tests • Feeling weak and tired • Headache • Rash (seen in children) • The use of protease inhibitors may be associated with the development of or the worsening of elevations in blood sugar and diabetes • There have been reports of increased bleeding in HIV-infected persons with hemophilia who were treated with protease inhibitors. It is not known if protease inhibitors were the cause of these bleeding episodes.
Nevirapine [Viramune®, NVP]	• Severe liver damage that can cause death may occur. People with higher CD4 cell counts are at increased risk for developing liver damage, which is often associated with a rash. Women with CD4 cell counts greater than 250 cells/μL, including pregnant women receiving chronic nevirapine therapy, are at even higher risk for developing liver damage. People who have abnormal liver function tests before starting nevirapine and people with active Hepatitis B or C infection are also at higher risk for liver damage • If you are developing liver damage, you may have one or more of the following: Tiredness, general feeling of illness, loss of appetite, nausea, pale stools, dark urine, yellowing of the skin or whites of your eyes, liver tenderness or abnormal liver function tests • Rash is the most common side effect associated with nevirapine. Rash occurs more often in women. Most rashes occur early during treatment. The rash may be severe and rarely may cause death. One of the risk factors for developing serious skin reactions includes failure to take nevirapine properly during the first 14 days of treatment. • Hypersensitivity reactions (HSR), which can rarely be fatal, may occur. Symptoms associated with an HSR include rash, fever, fatigue, muscle or joint aches, blisters, mouth sores, facial swelling, red eyes and irritation of the eyes, general feeling of discomfort, hepatitis, kidney problems, and/or changes in white blood cell levels. • The risk of people developing any of the serious side effects listed above is greatest during the first few months of treatment, but these side effects also can occur later. If you develop any of the side effects listed above, no matter how long you have been receiving nevirapine, you must contact your health care provider right away and try to be seen by the medical staff at your site before your next dose. If you and your doctor then decide to stop your treatment because of symptomatic hepatitis,

Anti-HIV Drug	Side Effects
	hypersensitivity or severe skin reactions, you should never take Nevirapine again. • Other risks include: Fever, headache, upset stomach

Ritonavir [Norvir®, RTV]	• Stomach and bowel problems including abdominal pain, upset stomach, vomiting, abnormal stools, and loose or watery stools • An increase in triglycerides • Numbness and tingling in the arms, legs and around the mouth • Rash • Abnormal liver function tests • Fever • A change in the sense of taste • The use of protease inhibitors may be associated with the development of or the worsening of elevations in blood sugar and diabetes • There have been reports of increased bleeding in HIV-infected persons with hemophilia who were treated with protease inhibitors. It is not known if protease inhibitors were the cause of these bleeding episodes.
Saquinavir [Fortovase®, SQV]	• Loose or watery stools • Upset stomach • Abdominal discomfort/pain • Heartburn • Gas • Feeling tired • Headache • Increased CPK (an enzyme found in the heart) • Abnormal liver function tests • Low blood sugar • Decrease in the number of white blood cells that help fight infection • The use of protease inhibitors may be associated with the development of or the worsening of elevations in blood sugar and diabetes • There have been reports of increased bleeding in HIV-infected persons with hemophilia who were treated with protease inhibitors. It is not known if protease inhibitors were the cause of these bleeding episodes

Tenofovir [Viread®, TDF]	• Upset stomach, vomiting, gas, loose or watery stools • Dizziness • Abdominal pain • Lack of energy • Kidney damage or failure • Inflammation or swelling and possible damage to the pancreas • Shortness of breath • Rash • Low phosphate, a chemical in the blood • Increase of liver functions tests in children • Allergic reaction, which may include fever, rash, upset stomach, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath or a general feeling of illness • Changes in bone growth and strength were seen in study animals given tenofovir. It is unknown if taking tenofovir for a long time will cause bone abnormalities in adults. In children, some decrease in bone thickness (density) has been seen. • If you are infected with both Hepatitis B and HIV, you should be aware that your liver function tests may increase, and symptoms associated with hepatitis (an acute inflammation of the liver) may worsen if tenofovir is stopped. • Because there is only a small amount of information on tenofovir in pregnant women, tenofovir should be used during pregnancy only if clearly needed. • Lactic acidosis. The signs of lactic acidosis that you may notice are: unexplained weight loss, stomach ache, upset stomach, throwing up, tiredness, weakness, and shortness of breath. Lactic acidosis can cause death.

INFORMED CONSENT FORM FOR SPECIMEN STORAGE

(ENGLISH)

CAPRISA 001 - START: Starting Tuberculosis and Anti-Retroviral Therapy.
Implementing Anti-Retroviral Therapy in Resource-Constrained Settings: A Randomized Controlled Trial to Assess the Effect of Integrated Tuberculosis and HIV Care on the Incidence of AIDS-Defining Conditions or Mortality in Subjects Co-Infected with Tuberculosis and HIV.

Short Title: START: Starting Anti-Retroviral Therapy

Sponsored by: The National Institute of Allergy and Infectious Diseases
Division of AIDS (DAIDS)
6700B Rockledge Drive
Bethesda, Maryland 20852
United States

Principal Investigator: Professor Salim Abdool Karim, MBChB, PhD

INTRODUCTION

If you decide to participate in the START research study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This consent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to sign this consent form. You will be given a copy of this form to keep.

BLOOD AND BIOLOGICAL SAMPLES

At each of your clinic visits, blood and other biological samples (sputum, urine) will be taken from you. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a number, not your name, will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES

The stored samples may be used for future research, to confirm test results, or to do additional testing. Your samples will not be sold or used in products that make money for the researchers. Any studies that use your samples will be reviewed by the Ethics Committee of the Nelson R. Mandela School of Medicine.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

STORAGE OF SAMPLES

Your samples will be stored at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is no time limit on how long your samples may be stored.

BENEFITS

There is no direct benefit to you through having your samples stored and tested later. Information learned from stored samples may help others who have HIV/AIDS.

RISKS

There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a study number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

CONFIDENTIALITY

The results of future tests of your samples will not go into your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your records may also be reviewed by regulatory authorities, such as the Medicines Control Council (MCC), the Ethics Committees, the U. S. National Institutes of Health (NIH), study staff and study monitors.

PARTICIPANT RIGHTS

The decision to allow your samples to be stored is completely voluntary. If you do not allow your samples to be stored, you may still participate in the main study. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time and still participate in the main study. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you want all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, "Withdrawal of Consent" so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, contact any of the following:

Clinic Manager at Prince Cyril Zulu Communicable Disease Clinic:
Dr. Nesri Padayatchi
Tel: (031) 260-4574

Clinic Manager at King Edward VIII Clinic:
Dr. Kogieleum Naidoo
Tel: (031) 260-4687

Project Director:
Dr. Kogieleum Naidoo
University of KwaZulu-Natal
King George V Avenue
Durban 4001
Tel: (031) 260-4687

Principal Investigator:
Prof. Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001
Tel: (031) 260-2381

For questions about your rights as a research [redacted], you may contact:

Ms. Cheryl Borresen
Postgraduate Administration Office
University of KwaZulu-Natal
Tel: (031) 260 4495

Prof. Ames Dhai
Chairperson of the Research Ethics Committee
University of KwaZulu-Natal
Tel: (031) 260-4604

SPECIMEN STORAGE CONSENT FORM SIGNATURES PAGE

Please read the statement below and think about your choice. No matter what you decide, it will not affect your care.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_____ Yes

_____ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:_____ Date:_____
Participant/Guardian

Signed:_____ Date:_____
Witness

Signed:_____ Date:_____
Researcher

For illiterate participants:

Mark or thumbprint:_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

Withdrawal of Consent

I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed:_____ Date:_____
Participant/Parent/Guardian

Signed:_____ Date:_____
Witness

Signed:_____ Date:_____
Researcher

For illiterate participants:

Mark or thumbprint':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

APPENDIX D

Assay and NONMEM Information

D1: Assay and NONMEM information

EFV LCMS/MS

D. Assay and NONMEM information

D1. EFV LCMS/MS

Blood was collected in heparinized tubes, which were stored on ice and separated at 3000rpm within one hour. Aliquoted samples were then stored at -80 ℃ until analysis. Samples were analysed at the Division of Clinical Pharmacology, University of Cape Town. Plasma efavirenz concentrations were determined by liquid chromatography/tandem mass spectrometry using a modification of a method by Chi et al [142] using liquid chromatography/tandem mass spectrometry.

Accuracy ranged from 97.2% to 105.6%. Intraday and interday precisions ranged from 1.3% to 4.6% and 3.6% -7.2% respectively. The lower limit of quantitation of the assay was 0.2mg/L [142].

D2: Assay and NONMEM information

RIF HPLC

D2. RIF HPLC

Blood was collected in heparinized tubes, which were stored on ice and separated at 3000rpm within one hour. Aliquoted samples were then stored at -80 ℃ until analysis. Samples were analysed in the Division of Clinical Pharmacology, University of Cape Town. Serum rifampicin concentrations were measured using tandem HPLC mass spectrometry [143] with a lower limit of quantitation of 0.1 µg/mL and inter- and intra-day coefficients of variation below 10%.

D3: Assay and NONMEM information

TAQMAN® Drug Metabolizing Enzyme Genotyping Assays

D3. TAQMAN® Drug Metabolizing Enzyme Genotyping Assays

DNA for genotyping was extracted from stored peripheral blood monoleukocytes using the MagNa Pure LC DNA isolation kit I (Version 17.0, Roche Diagnostics®, Mannheim, Germany).

All participants were genotyped for CYP2B6 516G→T, CYP2B6 785A→G, CYP 2B6 983T→C, CYP2A6*9B, CYP2A6*17, UGT2B7 -372G→A and SLCO1B1, rs4149032 genetic variants. Allelic discrimination reactions were performed in duplicate or triplicate (if duplicate results were discordant) using TaqMan (Applied Biosystems, CA, USA) commercial genotyping assays in accordance with manufacturer's instructions for the following kits: CYP 2B6 516G→T (C__7817765_60, rs3745274), CYP2B6 785A→G (custom designed 40X, catalogue #1151580, rs2279343), CYP2B6 983T→C (C_60732328_20, rs28399499), CYP2A6*9B g.1836 G→T(C_29560333_20, rs8192726), CYP2A6*17 g.5065G→A, c.1093G→A (C_34816076_20, rs28399454), UGT2B7 -372G→A (C_30720663_20, rs7662029) and (40X SLCO1B1, C_1901709_10, rs4149032)on the Roche LightCycler® 480 platform (Roche Diagnostics, Mannheim, Germany).

The final volume for each reaction was 25µl, consisting of TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 20X or 40X drug metabolising genotype assay mix and 10ng genomic DNA. The thermal cycler conditions were as follows: initial step, 95℃ for 10 minutes, then denature for 50 cycles at 92℃ for 15 seconds and anneal/extend at 60℃ for 90 seconds.

For the custom designed 40X genotype assay the thermal cycler condition consisted of an initial step 95℃ for 10 minutes, denature for 40 cycles at 92℃ for 15 seconds and then anneal/extend at 60℃ for 60 seconds.

D4: Assay and NONMEM information

NONMEM coding and model building

D4. NONMEM coding and model building

```
110_ef_bov_RFCL_CLEFmix_b.smr                                   2010/03/10 04:43 PM

 1  THETA:       PROB         FSLOW        POP_CL_EF    POP_V_EF     POP_TABS_EF FCLEF_RF     FCLEF_RF_FA:
 2  ETA:         PPV_CL_EF    PPV_V_EF     PPV_TABS_EF  PPV_RUV_EF   BOV_CL_EF1
 3  ERR:         EPS1
 4  110_ef_bov_RFCL_CLEFmix_b.lst    439.681 eval=132 sig=4.1 sub=55 obs=209 CCIL=YNYN NVI2.0 PV2.0
 5  THETA     = 0.211        0.374        11.5         168          1            0.664        1
 6  ETASD     = 0.213776     0c           0c           0c           0.34641      0.34641      0.34641
 7  ERRSD     = 1
 8
 9  MINIMIZATION SUCCESSFUL
10
11
12     user 0:16.63 real 0:16.63     tcl 0:4.53
13
14  $PROB EFAVIRENZ
15  $INPUT ID PID ARM OCC DATE=DROP TIME CMT AMT RATX=DROP SS II
16  DVID DV LLOQ MDV EVID
17  WT HT BMI AGE SEX
18  SMER=DROP AST=DROP ALT=DROP HB=DROP ANC=DROP
19  XRAY=DROP HEPB-DROP AIDS=DROP SMOK=DROP CONM=DROP VCOD=DROP
20
21  $DATA ..\..\..\Data\efrfcd4vl.csv
22  IGNORE (DVID.EQ.2,DVID.EQ.4,DVID.EQ.5,DVID.EQ.6)
23
24  $ESTIM MAX=9990 SIG=3 NOABORT PRINT=1
25  METHOD=CONDITIONAL INTERACTION
26  MSFO=110_ef_bov_rfcl_clefmix_b.msf
27  ;$COV
28
29  $THETA
30  (0,0.239,1) ; PROB SLOW CLEF
31  (0,0.433,) ; FSLOW
32
33  ;EFAVIRENZ
34  $THETA
35  (0.1,11.4,) ; POP_CL_EF L/H/70KG
36  (0.1,168.,) FIX ; POP_V_EF L/70KG
37  (0.1,1.,3) FIX ; POP_TABS_EF H
38  (0,1,)  ; FCLEF_RF
39  (0,1,) FIX ; FCLEF_RF_FAST
40  (0,1,) FIX ; FCLEF_RF_SLOW
41  (0,0.0987,) FIX ; RUV_CV_EF
42  (0,0.329,) FIX ; RUV_SD_EF MG/L
43
44  $OMEGA
45  0.0518 ; PPV_CL_EF
46  0 FIX ; PPV_V_EF
47  0 FIX ; PPV_TABS_EF
48  0 FIX ; PPV_RUV_EF
49  $OMEGA BLOCK(1)
50  0.112 ; BOV_CL_EF1
51  $OMEGA BLOCK(1) SAME
52  ;; BOV_CL_EF2
53  $OMEGA BLOCK(1) SAME
54  ;; BOV_CL_EF3
55  $OMEGA BLOCK(1) SAME
56  ;; BOV_CL_EF4
57  $OMEGA BLOCK(1) SAME
58  ;; BOV_CL_EF5
59  $OMEGA BLOCK(1) SAME
60  ;; BOV_CL_EF6
61
62  $SIGMA 1. FIX ; EPS1
63
64  $SUBR ADVAN2 TRANS2
65
66  $PK
67     IF (NEWIND.LE.1) THEN ; INITIALIZE EACH SUBJECT
68        LN2=LOG(2)
69        H2YR=1/(24*365)
70     ENDIF
71

                                Page 1 of 3
```

```
72      IF (WT.GT.0) THEN
73         FSZV=WT/70
74         FSZCL=(WT/70)**0.75
75      ELSE
76         FSZV=1
77         FSZCL=1
78      ENDIF
79
80      IF (OCC.EQ.1) THEN
81         BOVCLE=BOV_CL_EF1
82      ENDIF
83      IF (OCC.EQ.2) THEN
84         BOVCLE=BOV_CL_EF2
85      ENDIF
86      IF (OCC.EQ.3) THEN
87         BOVCLE=BOV_CL_EF3
88      ENDIF
89      IF (OCC.EQ.4) THEN
90         BOVCLE=BOV_CL_EF4
91      ENDIF
92      IF (OCC.EQ.5) THEN
93         BOVCLE=BOV_CL_EF5
94      ENDIF
95      IF (OCC.EQ.6) THEN
96         BOVCLE=BOV_CL_EF6
97      ENDIF
98
99      IF (MIXNUM.EQ.1) THEN ; SLOW
100        FMIX=FSLOW
101     ELSE
102        FMIX=1 ; FAST
103     ENDIF
104     IF (ARM.EQ.1.AND.OCC.LE.3) THEN
105        IF (MIXNUM.EQ.1) THEN ; SLOW
106           FCLERF=FCLEF_RF
107        ELSE
108           FCLERF=FCLEF_RF
109        ENDIF
110     ELSE
111        FCLERF=1
112     ENDIF
113
114
115     GCLEF=POP_CL_EF*FSZCL*FCLERF*FMIX
116     GVEF=POP_V_EF*FSZV
117     GTAEF=POP_TABS_EF
118
119
120     CLEF=GCLEF*EXP(PPV_CL_EF+BOVCLE)
121     VEF=GVEF*EXP(PPV_V_EF)
122     TAEF=GTAEF*EXP(PPV_TABS_EF)
123     KAEF=LN2/TAEF
124
125     CL=CLEF
126     V=VEF
127     KA=KAEF
128     S2=VEF
129
130  $MIX
131     NSPOP=2 ; 2 SUB POPULATIONS FOR CLEF
132     P(1)=THETA(1)
133     P(2)=1-THETA(1)
134
135  $ERROR
136
137     CEF=A(2)/VEF
138
139     PROPEF=CEF*RUV_CV_EF
140     ADDEF=RUV_SD_EF
141     SDEF=SQRT(PROPEF*PROPEF + ADDEF*ADDEF)*EXP(PPV_RUV_EF)
142     Y=CEF + SDEF*EPS1
```

```
143
144  $TABLE ID TIME OCC AMT
145  CLEF VEF TAEF
146  DVID Y
147  ONEHEADER NOPRINT FILE=110_ef_bov_rfcl_clefmix_b.fit
148
149
```