Received: 14 January 2020 | Revised: 5 October 2020 | Accepted: 12 October 2020

DOI: 10.1111/bph.15309

Themed Issue: Oxysterols, Lifelong Health and Therapeutics

RESEARCH PAPER

Early or deferred initiation of efavirenz during rifampicin-based TB therapy has no significant effect on CYP3A induction in TB-HIV infected patients

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Funding information
European and Developing Countries Clinical Trials Partnership, Grant/Award Number: CG_TA.05.40204_005; Vetenskapsrådet, Grant/Award Number: 2015-03295

Background and Purpose: In TB-HIV co-infection, prompt initiation of TB therapy is recommended but anti-retroviral treatment (ART) is often delayed due to potential drug–drug interactions between rifampicin and efavirenz. In a longitudinal cohort study, we evaluated the effects of efavirenz/rifampicin co-treatment and time of ART initiation on CYP3A induction.

Experimental Approach: Treatment-naïve TB-HIV co-infected patients (n = 102) were randomized to efavirenz-based-ART after 4 (n = 69) or 8 weeks (n = 33) of commencing rifampicin-based anti-TB therapy. HIV patients without TB (n = 94) receiving efavirenz-based-ART only were enrolled as control. Plasma 4β-hydroxycholesterol/cholesterol (4β-OHC/Chol) ratio, an endogenous biomarker for CYP3A activity, was determined at baseline, at 4 and 16 weeks of ART.

Key Results: In patients treated with efavirenz only, median 4β-OHC/Chol ratios increased from baseline by 269% and 275% after 4 and 16 weeks of ART, respectively. In TB-HIV patients, rifampicin only therapy for 4 and 8 weeks increased median 4β-OHC/Chol ratios from baseline by 378% and 576% respectively. After efavirenz/rifampicin co-treatment, 4β-OHC/Chol ratios increased by 560% of baseline (4 weeks) and 456% of baseline (16 weeks). Neither time of ART initiation, sex,

Abbreviations: 4β-OHC/Chol, 4β-hydroxycholesterol/cholesterol ratio; ART, anti-retroviral therapy; cART, combination anti-retroviral therapy; HIV, human immunodeficiency virus; TB, tuberculosis; WHO, World Health Organization.

genotype nor efavirenz plasma concentration were significant predictors of 4β-OHC/Chol ratios after 4 weeks of efavirenz/rifampicin co-treatment.

Conclusion and Implications: Rifampicin induced CYP3A more potently than efavirenz, with maximum induction occurring within the first 4 weeks of rifampicin therapy. We provide pharmacological evidence that early (4 weeks) or deferred (8 weeks) ART initiation during anti-TB therapy has no significant effect on CYP3A induction.

LINKED ARTICLES: This article is part of a themed issue on Oxysterols, Lifelong Health and Therapeutics. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v178.16/issuetoc

KEYWORDS

4β-hydroxycholesterol, co-infection, CYP3A, drug–drug interaction, efavirenz, enzyme induction, HIV, rifampicin, tuberculosis

1 | INTRODUCTION

Tuberculosis (TB) remains the most common opportunistic infection and the leading cause of death in people living with HIV/AIDS (da Silva Escada et al., 2017; Floyd, Glaziou, Zumla, & Raviglione, 2018). The mortality rate is twofold to fourfold higher in TB-HIV co-infected patients than in patients with TB or HIV alone (da Silva Escada et al., 2017; WHO, 2019). According to WHO, the burden of HIV-associated TB is highest in sub-Saharan Africa, where 87% of TB patients were HIV co-infected in 2018, and a total of 477,461 TB cases of HIV-positive TB cases were reported of whom 86% were on anti-retroviral therapy (ART) (WHO, 2019). The management of TB/HIV co-infected individuals remains challenging partly due to potential pharmacokinetic drug–drug interactions, overlapping toxicities, and high pill burden conceding non-adherence to medication (Egelund, Dupree, Huesgen, & Peloquin, 2017; Tornheim & Dooley, 2018; Yimer et al., 2008; Yimer et al., 2014).

Active TB diagnosis requires prompt initiation of TB therapy, and WHO recommends initiating ART as soon as possible within the first 8 weeks of starting anti-TB treatment in case of TB-HIV co-infection ((WHO), 2016). In patients not taking ART at the time of TB diagnosis, delaying ART initiation for an extended period may result in further immune decline with increased risk of new opportunistic infections and death, especially in patients with advanced HIV disease (McHunu et al., 2016). The high case-fatality rate in TB and HIV co-infection, especially during the first two months of TB treatment necessitates early concomitant initiation of ART (Abdool Karim et al., 2011; Amogne et al., 2015; Blanc et al., 2011). ART can be delayed until after completion of 6 months of TB treatment for patients with CD4 cell counts greater than 220 cells·μl^{-1} (Mfinanga et al., 2014). Others argue that when to start ART should be based on other factors including potential drug interactions, overlapping adverse events, a high pill burden, and severity of illness rather than a pre-specified time point or CD4 cell count (Djimeu & Heard, 2019).

In sub-Saharan Africa, rifampicin-based therapy is the first-line treatment regimen for TB, and efavirenz-based anti-retroviral treatment (ART) is the recommended first-line regimens during anti-TB co-therapy ((WHO), 2016). Both rifampicin and efavirenz are inducers of cytochrome P450 3A (CYP3A) leading to potential drug–drug interactions (Habtewold et al., 2013; Mukonzo, Aklillu, Marconi, & Schinazi, 2019; Ngaimisi et al., 2014). CYP3A, the most abundant P450 enzyme in the human liver, is a major drug-metabolizing enzyme

What is already known

- Extent of anti-tuberculosis and anti-retroviral drug-interactions determines when to initiate anti-retroviral treatment during tuberculosis treatment.
- Possibility of rifampicin–efavirenz drug-interactions may delay starting anti-retroviral treatment for up to 8 weeks.

What this study adds

- Rifampicin induces CYP3A more than efavirenz, with maximum induction within 4 weeks of rifampicin treatment.
- Time of efavirenz initiation while on rifampicin therapy has no significant effect on CYP3A induction.

What is the clinical significance

- Prompt initiation of anti-retroviral treatment after commencing anti-tuberculosis therapy is recommended to reduce HIV-associated mortality.
- Anti-tuberculosis and anti-retroviral co-therapy increases risks of unpredicted CYP3A-mediated drug interactions than anti-retroviral treatment only.

responsible for the metabolism of more than 50% of clinically used drugs including protease inhibitors and non-nucleoside reverse transcriptase inhibitors. CYP3A induction by repeated doses of co-administered drugs such as rifampicin is the underlying mechanism for most clinically relevant drug interactions (Yamashita et al., 2013).

Currently, HIV treatment is life-long, whereas anti-TB treatment spans at least 6 months. Hence, concurrent use of efavirenz and rifampicin may affect long-term CYP3A induction and its activity. The extent of CYP3A enzyme induction and the effects of efavirenz pharmacokinetic and pharmacogenetic variations on rifampicin-efavirenz drug interactions require definition. Based on mortality and safety assessment, several randomized controlled trials favoured earlier ART initiation in patients with TB (Abdool Karim et al., 2011; Amogne et al., 2015; Blanc et al., 2011). However, pharmacological evidence is lacking. We performed a longitudinal cohort study of newly diagnosed TB-HIV co-infected patients to determine the effects of rifampicin co-treatment and time of ART initiation (after 4 or 8 weeks of prior anti-TB therapy) on CYP3A induction. We also explored whether efavirenz pharmacokinetics or pharmacogenetic variations influence rifampicin-efavirenz drug interactions and CYP3A induction.

2 | METHODS

2.1 | Study design and population

This was a prospective cohort study of treatment-naive adult Ethiopian patients with newly diagnosed pulmonary TB co-infected with HIV to investigate CYP3A-mediated interactions between antiretroviral and anti-TB drugs, time of ART initiation, pharmacogenetic variations, and efavirenz pharmacokinetics on the short- and long-term CYP3A enzyme activity during concomitant anti-TB and ART. TB and HIV co-infected patients with a CD4 count <200 cells·mm^{-3} were enrolled prospectively and received concomitant rifampicin based anti-TB therapy and efavirenz-based ART. The TB-HIV co-infected patients were also part of a previous open-label randomized clinical trial which investigated efficacy and safety of efavirenz-based ART, initiated at a different time point during the intensive phase of anti-TB therapy (Amogne et al., 2015). In parallel, treatment-naive HIV patients without TB co-infection were enrolled as a control group and received efavirenz-based ART alone (Habtewold et al., 2015). All study participants were followed up to the end of the16th week after the initiation of anti-retroviral therapy.

2.2 | Ethics review

The study was approved by the Institutional Review Boards (IRBs) of Karolinska Institutet, Stockholm, Sweden, Faculty of Medicine, Addis Ababa University, and Ethiopian National Ethics Review Committee and Food Drug and Health Care Administration and Control Authority of Ethiopia. All procedures were carried out according to the recommendations of the World Health Organization (WHO) and the International Conference for Harmonization (ICH) Good Clinical Practice (GCP) guidelines. All participants gave written informed consent.

2.3 | Treatment and laboratory investigations

All TB-HIV co-infected patients (n = 102) received the first line rifampicin-based anti-TB therapy and were randomized to receive efavirenz-based ART after 4 weeks (n = 69) or 8 weeks (n = 33) of starting TB treatment, as described previously (Amogne et al., 2015). TB treatment consisted of weight-adjusted fixed-dose combinations of rifampicin, isoniazid, ethambutol and pyrazinamide for 2 months (intensive phase), followed by a fixed-dose combination of isoniazid and rifampicin for 4 months (continuation phase). The ART regimen consisted of efavirenz (600 mg once daily) and lamivudine with either zidovudine or stavudine.

Venous blood samples were collected before starting anti-TB treatment (4 or 8 weeks before initiation of ART), at the initiation of ART (week 0) and the fourth and 16th weeks of ART. The study population, follow-up period, and study sampling time points are presented in Figure 1. Pre-treatment CD4 count, HIV viral load, body mass index (BMI), plasma albumin, alanine transaminase, aspartate aminotransferase, total bilirubin, urea, hepatitis B and C virus (HBV and HCV), complete blood count (CBC) with differentials and haemoglobin (Hb) were determined. Treatment adherence was assessed by self-reporting and regularity to scheduled visits at the clinics.

2.4 | Quantification of CYP3A induction

4β-Hydroxycholesterol/cholesterol (4β-OHC/Chol) ratio was used as an endogenous marker for CYP3A activity (Diczfalusy et al., 2008). Blood samples for determination of cholesterol and 4β-OHC were collected before starting anti-TB therapy, before initiating efavirenz-based ART, and at week 4 and week 16 of ART. Cholesterol concentrations were measured by a commercial enzymic method (Cholesterol CHOD-PAPP, Roche Diagnostics GmbH, Mannheim, Germany) run on a Roche/Hitachi Modular instrument. The between-day variation was 1.3% (at 5 mmol·L^{-1}). Determination of 4β-OHC was performed by isotope-dilution gas chromatography-mass spectrometry using deuterium-labelled 4β-OHC as an internal standard as described previously (Diczfalusy, Nylen, Elander, & Bertilsson, 2011). The relative between-day variation (CV) was 4.9% (at 26.5 ng·ml^{-1}).

2.5 | Quantification of plasma efavirenz concentration

On the fourth and 16th week of efavirenz/zidovudine-based ART, a blood sample was collected, at 16 h after efavirenz dosing, from all study participants in a Vacutainer CPT (Becton Dickinson, Heidelberg, Germany), centrifuged (1700× g for 20 min), and a plasma aliquot was

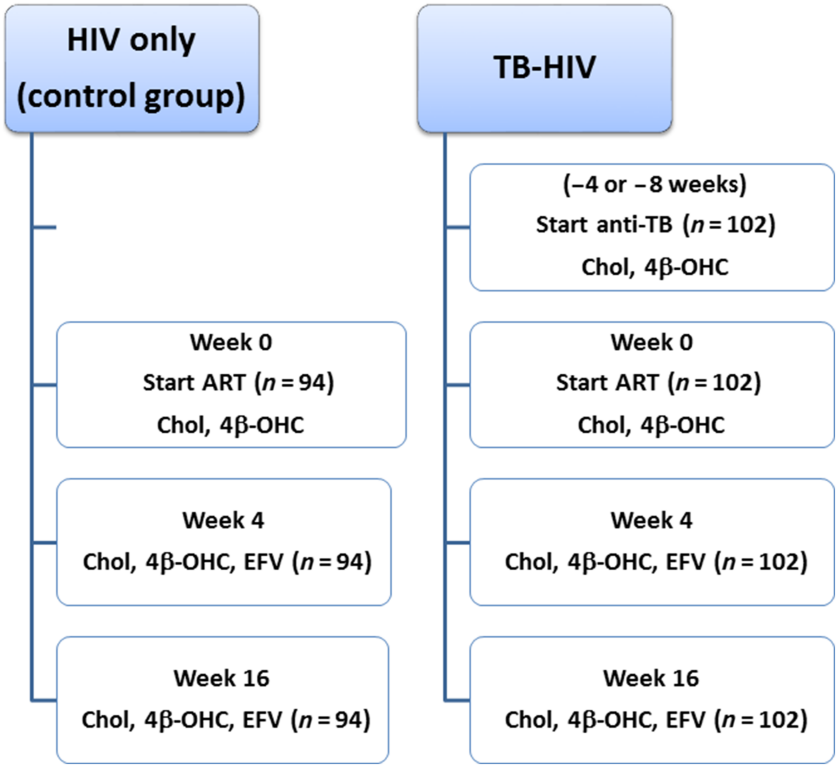

FIGURE 1 Study design and patient flow chart. HIV only = HIV patients treated with efavirenz (EFV)-based combination antiretroviral therapy (cART) alone. TB-HIV = co-infected patients treated with concomitant efavirenz-based cART plus rifampicin (RIF)-based anti-TB therapy. The study time point for the determination of plasma cholesterol (Chol), 4β-hydroxycholesterol (4β-OHC) and efavirenz levels are indicated with references to ART initiation in weeks

stored at −80°C for determination of efavirenz. The AUC_{0-24} for efavirenz can be accurately estimated from a single plasma sample obtained at 12 or 16 h post efavirenz dosing (Lopez-Cortes et al., 2005). To reduce the risk of having treatment-associated adverse events during the day, efavirenz (600 mg once a day) is recommended to be taken in the evening, preferably at bedtime. All study participants were instructed to take efavirenz at bedtime and the 16-h blood sampling time point was selected because of its convenience for the patient to come to the hospital the next morning to give a blood sample. The time of efavirenz intake was ascertained before blood withdrawal to make sure that the blood sample collection was 16 h post efavirenz dose.

The determination of plasma efavirenz by LC-MS/MS was performed as described previously (Burhenne et al., 2010; Habtewold et al., 2016; Habtewold et al., 2017). The method was validated according to the FDA validation guidelines and fulfilled all criteria concerning accuracy, precision, recovery, linearity, and stability. The lower limit of quantification of efavirenz was 10.0 $ng \cdot ml^{-1}$ and the efavirenz calibration range was 10–10,000 $ng \cdot ml^{-1}$.

2.6 | SNP selection and genotype analysis

From each study participant, 2 ml of whole blood sample was collected in an EDTA containing vacutainer tube for genotype analysis. Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Midi Kit (QIAGEN GmbH. Hilden, Germany) following the manufactures instructions. Genotyping for the common functional variant alleles in genes coding for drug-metabolizing enzymes and transporter proteins relevant for anti-TB and anti-retroviral drugs disposition was done by real-time PCR using pre-developed Taqman assay and reagents for allelic discrimination. Allelic discrimination reactions were performed using TaqMan® (Applied Biosystems, CA, USA) genotyping assays according to the manufacturer's instructions: (C__7586657_20 for *ABCB1 c.*3435C>T, C__11711730_20 for *ABCB1 c.4036A>G* (rs3842), C__7817765_60 for *CYP2B6 c.516G>T* [*CYP2B6*6*], C__30720663_20 for *UGT2B7-327G>A* [*UGT2B7*2*], C__26201809_30 for *CYP3A5* 6986A>G [*CYP3A5*3*], C__30203950_10 for *CYP3A5* 14690G>A [*CYP3A5*6*]), and C__32287188_10 for *CYP3A5* g.27131_27132insT [*CYP3A5*7*] on ABI 7500 FAST (Applied Biosystems, Foster City, CA) (Aklillu et al., 2011; Aklillu et al., 2016). The final volume for each reaction was 10 μl, consisting of 2× TaqMan Universal PCR Master Mix® (Applied Biosystems), 20× drug-metabolizing genotype assay mix, and 10 ng genomic DNA. The PCR profile consisted of an initial step at 50°C for 2 min and 50 cycles at 95°C for 10 min and 92°C for 15 s. Genotyping for *SLCO1B1c.521T>C (*5*) and *SLCO1B1c.388A>G (*1B)* was done using a Light Cycler® based method as described previously (Aklillu et al., 2011; Aklillu et al., 2016).

2.7 | Data and statistical analysis

Median (interquartile range) and proportions in percentage were used to describe baseline patient characteristics. A chi-square test was used to compare the observed and expected allele frequencies according to the Hardy–Weinberg equilibrium. Statistical analysis was undertaken only for data sets where each group size was at least $n = 5$. Group size is defined as the number of independent values, and outliers were

included in data analysis and graphic presentation. Median (interquartile range) was used to describe plasma cholesterol, 4β-OHC concentrations, and 4β-OHC/Chol ratios. Between treatment, group comparison of median plasma cholesterol concentrations, 4β-OHC concentrations, and 4β-OHC/Chol ratios at baseline and during treatment was done using the Mann–Whitney *U* test. The percent changes in plasma 4β-OHC/Chol ratio from baseline to the fourth and 16th weeks of ART were calculated using the following formula:

$$\%change\ in\ 4\beta OHC/Chol\ ratio = \left[\frac{\frac{4\beta OHC}{Chol}\text{at week x} - \frac{4\beta OHC}{Chol}\text{at baseline}}{\frac{4\beta OHC}{Chol}\text{at baseline}}\right] \times 100.$$

Plasma concentrations of EFV, 4β-OHC, and 4β-OHC/Chol ratios were not normally distributed. Therefore, concentration data were transformed into $\log_{10}$ values for statistical analysis and the Shapiro–Wilk test for normality was applied. Log-transformed concentration data ware used for graphical presentation and statistical analysis using parametric tests. Comparison of geometric means of 4β-OHC/Chol ratio between treatment groups was done using one-way ANOVA followed by Tukey's test. Pairwise comparison of data from baseline within and between treatment groups was made using paired and unpaired *t*-test, respectively. The change in mean 4β-OHC/Chol ratios over time was analysed using repeated measure ANOVA. Post hoc tests were conducted only if the measure of matching effectivity (F in ANOVA) achieved the necessary level of statistical significance ($P < 0.05$) and there was no significant variance inhomogeneity.

Univariate followed by multiple linear regression analysis was performed to identify predictors of log 4β-OHC/Chol ratios during 4 and 16 weeks of ART among TB-HIV patients. Predictor variables that resulted in a *P* value <0.2 in the univariate regression analysis were entered into a backward stepwise multivariate regression analysis to identify significant predictors in the final model. Statistical analyses were performed using Statistica version 13 (StatSoftInc, Tulsa, OK, USA). A *P* value <0.05 was considered significant. Graph Pad Prism version 8.0 for Windows (Graph Pad, La Jolla, CA, USA) was used for graphical presentations. The data and statistical analysis comply with recommendations of the British Journal of Pharmacology on experimental design and analysis (Curtis et al., 2018).

2.8 | Materials

Rifampicin, isoniazid, ethambutol and pyrazinamide were given as a fixed dose combination and supplied by Lupin Limited (Chikalthana, India). Efavirenz and lamivudine with zidovudine or stavudine was the HIV treatment and supplied by Mylan Laboratories Limited (Dist-Dhar, M.P, India)

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Cidlowski et al., 2019; Alexander, Fabbro et al., 2019a, 2019b; Alexander, Kelly et al., 2019a, 2019b).

3 | RESULTS

A total of 102 TB-HIV co-infected patients and 94 HIV-infected patients were enrolled and were followed up to 16 weeks of ART (Figure 1). Sociodemographic and baseline biochemical characteristics of the study participants are presented in Table 1.

3.1 | Change in 4β-OHC/Chol ratio over time

Plasma cholesterol and 4β-OHC concentrations were monitored before the start of anti-TB therapy, at the start of anti-retroviral therapy (week 0), and the fourth and 16th week of ART. The median plasma concentrations of cholesterol, 4β-OHC and 4β-OHC/Chol ratios at baseline, at the fourth and 16th weeks of ART along with the respective median percent change from baseline by weeks 4 and 16 of ART, stratified by treatment group are presented in Table 2.

At baseline, there was no significant difference in the median 4β-OHC/Chol ratio between HIV only and TB-HIV patients. The median 4β-OHC/Chol ratio was significantly lower in HIV patients treated with efavirenz-based ART only for 4 weeks, compared to TB-HIV patients treated with rifampicin-based anti-TB therapy alone for 4 or 8 weeks, indicating that rifampicin is a more potent CYP3A enzyme inducer than efavirenz.

In the control group (treated with efavirenz based ART only), the median percent change in 4β-OHC/Chol ratio from baseline was 269% and 275% after 4 and 16 weeks of ART, respectively. In contrast, among TB-HIV patients, treatment with rifampicin based anti-TB regimen alone for 4 or 8 weeks (before starting ART) the median percent change in 4β-OHC/Chol ratio from baseline was 378% or 576%, respectively. The median percent change in 4β-OHC/Chol ratio from baseline after 4 and 16 weeks of concomitant efavirenz and rifampicin treatment was 560% and 456%, respectively (Table 2).

3.2 | Effect of efavirenz-based ART versus rifampicin-based anti-TB monotherapy

Comparison of geometric means of plasma 4β-OHC/Chol ratios among HIV-patients treated with efavirenz-based ART only for 4 weeks, with those from TB-HIV co-infected patients treated with rifampicin-based anti-TB therapy only for 4 or 8 weeks (before starting ART) is presented in Figure 2. Patients who were treated with efavirenz only had significantly lower mean 4β-OHC/Chol ratios than those treated with rifampicin for 4 or 8 weeks. However, no significant difference in geometric means of the 4β-OHC/Chol ratio was detected, between TB-HIV patients treated with rifampicin-based therapy only for 4 or 8 weeks (Figure 2).

TABLE 1 Baseline socio-demographic, clinical, and laboratory characteristics of study participants stratified by treatment group

		HIV only	TB-HIV (ART initiated 4 weeks after RIF)	TB-HIV (ART initiated 8 weeks after RIF)
		n (%)	*n* (%)	*n* (%)
Sex	Female	74 (78.7%)	36 (52%)	19 (57.6%)
	Male	20 (21.3%)	33 (48%)	14 (42.4%)
Age, median (IQR)		35 (30–55)	35 (28–52)	30 (27–53)
BMI, median (IQR)		19.5 (18.0–25.8)	18.7 (17.2–22.9)	19.0 (17.4–24.4)
HIV stage	Stage 1	1 (1.1%)		
	Stage 2	8 (8.8%)	1 (2%)	
	Stage 3	39 (42.9)	40 (61%)	16 (48.5%
	Stage 4	43 (47.3%)	25 (38%)	17 (51.5%)
Type of ART	d4T30/3TC/EFV	48 (52.7%)	26 (39%)	12 (36.4%)
	d4T40/3TC/EFV	5 (5.5%)	1 (2%)	
	TDF/3TC/EFV		21 (32%)	5 (15.2%)
	ZDV/3TC/EFV	38 (41.8%)	18 (27%)	16 (48.5%)
Hepatitis B surface antigen	Negative	85 (93.4%)	63 (95%)	30 (90.9%)
	Positive	6 (6.6%)	3 (5%)	3 (9.1%)
Hepatitis C virus antibody	Negative	90 (98.9%)	66 (100%)	33 (100%)
	Positive	1 (1.1%)		
Laboratory parameters	**Reference**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Karnofsky	Above 80%	100 (90–100)	90 (80–100)	100 (80–100)
Hb ($g \cdot L^{-1}$)	144–166	127 (116–152)	113 (100–163)	119 (100–162)
WBC count ($\times 10^3 \cdot \mu l^{-1}$)	4.5–12.1	4.35 (3.6–6.7)	5.7 (4.2–11.2)	5.6 (4.1–11.4)
Neutrophils (%)	40–74%	56 (44–78)	67 (59–83)	68 (59–84)
Platelets ($\times 10^3 \cdot \mu l^{-1}$)	140–415	233 (164–450)	325 (233–617)	293 (250–461)
Aspartate aminotransferase ($U \cdot L^{-1}$)	0–37	33.0 (27–99)	38.5 (34–119)	52.0 (34–108)
Alanine transaminase ($U \cdot L^{-1}$)	0–55	28 (21–127)	28 (20–64)	32 (23–68)
Alkaline phosphatase ($U \cdot L^{-1}$)	40–150	106 (79–210)	115 (89–318)	114 (95–258)
Total bilirubin ($\mu mol \cdot L^{-1}$)	0.2–1.2	0.38 (0.3–0.6)	0.35 (0.3–0.6)	0.55 (0.3–0.9)
Direct bilirubin ($\mu mol \cdot L^{-1}$)	0–0.5	0.1 (0.1–0.1)	0.1 (0.1–0.22)	0.1 (0.1–0.16)
Albumin ($g \cdot L^{-1}$)	38–54	39 (35–49)	34 (30–45)	34 (29–46)
Urea ($mmol \cdot L^{-1}$)	14-56	70 (56 - 109)	67 (56 - 128)	67 (53 - 140)
Plasma creatinine ($\mu mol\ L^{-1}$)	53 - 97	71 (62 - 106)	80 (71 - 106)	80 (62 -141)
CD4 counts per mm^3	500–1400	115 (68–193)	104.5 (56–192)	87 (50–173)
Log plasma HIV viral load		2.62 (2.31–2.89)	5.12 (4.43–5.66)	5.0 (4.49–5.45)
Cholesterol ($mmol \cdot L^{-1}$)		3.14 (2.51–3.81)	2.69 (2.05–3.35)	2.72 (2.0–3.5)
4β-OHC ($ng \cdot ml^{-1}$)		29.1 (20.9–37.6)	25.7 (19.1–33.2)	27.0 (21.5–35.9)
4β OHC/cholesterol $\times 10^4$		0.23 (0.18–0.33)	0.23 (0.18–0.34)	0.30 (0.18–0.35)

ZDV = zidovudine; *d4T30* (stavudine 30 mg for patients weighing < 60 kg); *d4T40* (stavudine 40 mg for patients weighing ≥ 60 Kg); 3TC = lamivudine, EFV = efavirenz, TDF = Tenofovir.

TABLE 2 Comparison of median plasma cholesterol, 4β-hydroxycholesterol (4β-OHC) and 4β-hydroxycholesterol/cholesterol ratio (4β-OHC/Chol) and median percent change from baseline in HIV only versus TB-HIV patients using Mann–Whitney *U* test

	Treatment group						
	TB-HIV (RIF + EFV)			HIV only (EFV only)			
	N	Median (IQR)	Median % change from baseline (IQR)	*N*		Median % change from baseline (IQR)	*P*
Cholesterol							
Baseline	102	2.71 (2.05 to 3.38)		94	3.14 (2.61 to 3.81)		<0.05
4 or 8 weeks of prior RIF	76	3.37 (2.85 to 3.99)	21% (7 to 50)				
Week 4	102	3.10 (2.29 to 3.87)	21% (−8 to 45)	94	2.23 (1.72 to 2.61)	−33% (−45 to −18)	<0.05
Week 16	94	2.33 (1.85 to 3.01)	−11% (−32 to 30)	93	2.28 (1.99 to 2.73)	−25% (−37 to −6)	0.91
4β-OHC							
Baseline	102	27 (19 to 36)		94	29 (21 to 38)		0.36
4 or 8 weeks of prior RIF	76	164 (117 to 200)	481% (326 to 700)				
Week 4	102	192 (144 to 267)	700% (403 to 946)	94	71 (52 to 92)	151% (68 to 256)	<0.05
Week 16	94	141 (107 to 187)	407% (220 to 596)	94	80 (53 to 115)	170% (101 to 344)	<0.05
4β OHC/cholesterol							
Baseline	102	0.26 (0.18 to 0.35)		94	0.23 (0.18 to 0.33)		0.47
4 or 8 weeks of prior RIF	76	1.13 (0.90 to 1.51)	378% (240 to 514)				
Week 4	102	1.70 (1.31 to 2.12)	560% (391 to 811)	94	0.83 (0.63 to 1.07)	269% (167 to 427)	<0.05
Week 16	94	1.44 (1.06 to 1.78)	456% (253 to 640)	93	0.88 (0.65 to 1.15)	275% (154 to 534)	<0.05

In the TB-HIV cohort, rifampicin-based anti-TB therapy (RIF) was initiated 4 or 8 weeks prior to the initiation of efavirenz-based ART. 4β-OHC in $ng \cdot ml^{-1}$. cholesterol in $mmol \cdot L^{-1}$; 4β-OHC/cholesterol molar ratio is multiplied by 10^4; OHC = hydroxycholesterol; IQR = interquartile range.

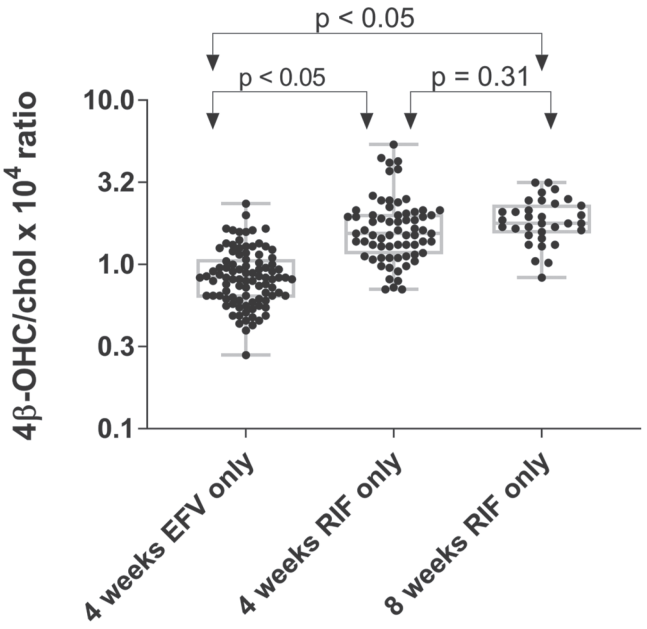

FIGURE 2 Comparison of geometric means of 4β-hydroxycholesterol/cholesterol ratio (4β-OHC/Chol × 10^4) after 4 weeks of efavirenz-based ART only (EFV) and 4 or 8 weeks of rifampicin (RIF)-based anti-TB therapy only, using one-way ANOVA. Log transformed 4β-OHC/Chol ratio was used to plot the graph. The box plots show the means ± SD, while whiskers denote the minimum and maximum values

3.3 | Effect of concomitant efavirenz-based anti-retroviral and rifampicin-based anti-TB therapies

Comparison of geometric means of plasma 4β-OHC/Chol ratio between HIV-only patients treated with efavirenz-based ART only (control group) with those from the TB-HIV patients who received concomitant anti-retroviral and rifampicin-based anti-TB therapies for 4 and 16 weeks is presented in Figure 3. The TB-HIV co-infected patients initiated concomitant efavirenz-based ART after 4 or 8 weeks of prior anti-TB therapy. Patients treated with efavirenz-based ART only had significantly lower mean plasma 4β-OHC/Chol ratios, than those in patients co-treated with rifampicin-based anti-TB therapy, regardless of the duration of anti-retroviral treatment (week 4 and week 16). However, no significant difference in the mean plasma 4β-OHC/Chol ratio among TB-HIV patients who initiated ART after 4 or 8 weeks of starting anti-TB therapy.

Figure 4 shows the pattern and extent of change in the geometric mean of plasma 4β-OHC/Chol ratios from baseline in HIV patients treated with efavirenz-based ART only, compared with values from TB-HIV patients treated with concurrent rifampicin-based anti-TB therapy and efavirenz-based ART initiated after 4 or 8 weeks of prior anti-TB therapy alone. Plasma 4β-OHC/Chol ratios remained significantly lower in HIV patients treated with efavirenz-based therapy only, compared with ratios in patients co-treated with rifampicin throughout the study period. However, after 4 weeks of efavirenz-

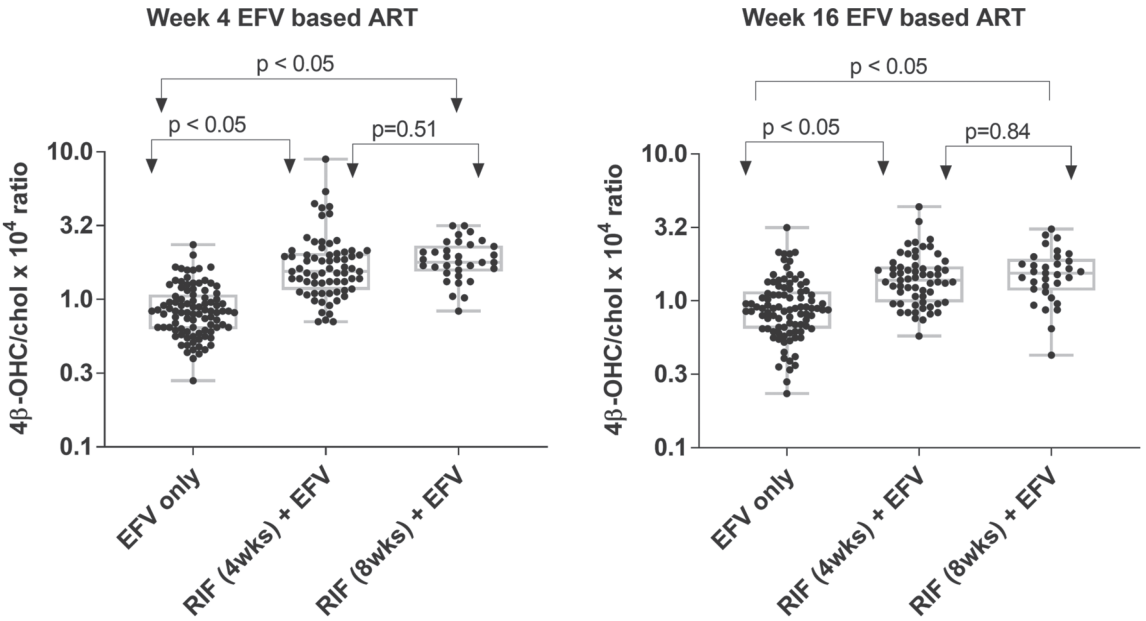

FIGURE 3 Comparison of geometric means of 4β-hydroxycholesterol/cholesterol (4β-OHC/Chol × 10^4) ratio between HIV-only patients treated with efavirenz (EFV)-based ART only and TB-HIV co-infected patients who initiated concomitant efavirenz-based ART after 4 weeks [rifampicin (RIF 4 weeks) + EFV)] or 8 weeks [rifampicin (RIF 8 weeks) + EFV] prior to anti-TB therapy. Log transformed 4β-OHC/Chol ratio was used to plot the graph. The box plots show the means ± SD, while whiskers denote the minimum and maximum values

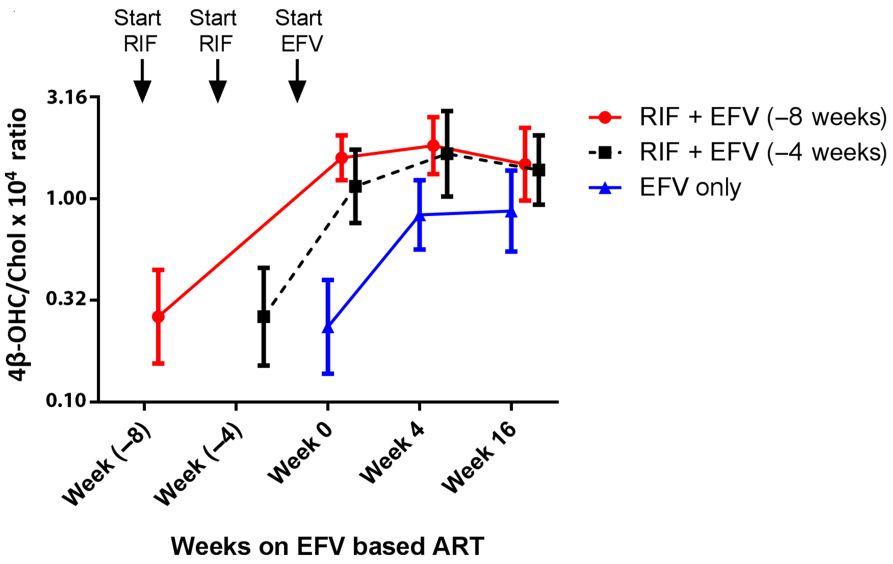

FIGURE 4 Change in plasma 4β-hydroxycholesterol/cholesterol (4β-OHC/Chol) ratio from baseline by efavirenz-based ART alone (EFV only) or with rifampicin-based anti-tuberculosis therapy (RIF) initiated 4 or 8 weeks prior to ART. Log transformed 4β-OHC/Chol ratio was used to plot the graph. Central points denote the geometric mean and the vertical bars show SD

based anti-retroviral co-treatment, there was no significant difference between TB-HIV patients who initiated ART on the 4th or 8th weeks of the intensive phase of anti-TB therapy.

3.4 | Effect of sex, *CYP2B6*, *CYP3A5*, *ABCB1*, *SLCO1B1*, and *UGT2B7* genotype

The overall allele frequencies were *CYP2B6*6* (30%), *CYP3A5*1* (19%), *CYP3A5*3* (65%), *CYP3A5*6* (15%), *ABCB1c.3435C>T* (21%), ABCB1c.4036 A>G (18%) and *UGT2B7-327G>A* (*2, 48%) *SLCO1B1c.388A>G* (*1B, 79%), and *SLCO1B1 c.521T>C* (*5, 20%). *CYP3A5*7* was absent in all subjects. Haplotype analysis indicated no linkage disequilibrium between *CYP3A5*3* and **6*. Thus, participants were stratified based on the functional *CYP3A5* allele (*CYP3A5*1*) for statistical analysis. There were no significant differences in allele frequencies between the two treatment groups as well as between the observed and expected genotype frequencies according to the Hardy–Weinberg equilibrium.

In the control group treated with efavirenz-based ART only, there were significant differences in the extent of change in plasma 4β-OHC/Chol ratio over time between the different CYP2B6 genotypes, being highest in *CYP2B6*6/*6>*1/*6>*1/*1* ($P < 0.04$). However, in TB-HIV patients, none of the genotypes including *CYP3A5* and *CYP2B6* or sex were predictors of 4β-OHC/Chol ratios at week 4. In a multivariate regression analysis, being a carrier of the *ABCB1 c.3435T* allele was a significant predictor of the 4β-OHC/Chol ratio at week 16 of ART (Table 3).

3.5 | Correlation between plasma efavirenz and 4β-hydroxycholesterol concentrations

In all study participants, plasma efavirenz concentration was quantified at week 4 and week 16 of cART. In the HIV only arm treated with efavirenz-based ART only (control group), there were significant positive correlations between plasma efavirenz and 4β-OHC/Chol ratios at week 4 and at week 16 (Habtewold et al., 2013). In the control group, linear regression analysis indicated plasma efavirenz concentration as a significant predictor of Log 4β-OHC/Chol ratio at week 4 (beta coefficient, with 95% CI: 0.215, 0.003 to 0.226) and at week 16 (beta coefficient with 95% CI: 0.296, 0.060 to 0.351). In TB-HIV patients receiving rifampicin-based anti-TB and efavirenz-based ART co-treatment, a similar tendency was observed at week 4, which became significant at week 16 (Figure 5). Results and the correlations between plasma efavirenz and 4β-OHC/Chol ratios among the TB-HIV treatment group are presented in Figure 5.

4 | DISCUSSION

To our knowledge, this is the first longitudinal cohort study of newly diagnosed TB-HIV co-infected patients comparing short and long-term CYP3A4 induction by efavirenz alone, rifampicin alone (before starting ART) and concomitant ART initiated at different time points during anti-TB therapy. Increase in plasma metabolite concentrations following treatment with an inducer drug is indicative of CYP enzyme induction and metabolism-based drug–drug interactions (DDI) (Gu et al., 2018; Tang, Lin, & Lu, 2005). The plasma 4β-OHC/Chol ratio is the preferred non-invasive endogenous biomarker for CYP3A-mediated clinical DDIs, where CYP3A activity is altered by long-term treatment, and administration of exogenous probe drugs such as midazolam in patients on multiple drug therapy is technically and/or ethically not feasible (Bjorkhem-Bergman et al., 2013; Gravel, Chiasson, Gaudette, Turgeon, & Michaud, 2019; Penzak & Rojas-Fernandez, 2019). In clinical DDI studies involving rifampicin, monitoring change in 4β-OHC/Chol ratio is a more reliable and better predictor of CYP3A4 activity in such patients (Dutreix, Lorenzo, & Wang, 2014; Gravel, Chiasson, Gaudette, Turgeon, & Michaud, 2019; Penzak & Rojas-Fernandez, 2019). It is noticeable that regardless of

TABLE 3 Predictors of 4β-hydroxycholesterol/cholesterol (4β-OHC/Chol) ratio using linear regression at week 4 and week 16 of ART in TB-HIV co-infected patients receiving concomitant efavirenz-based antiretroviral therapy and rifampicin based anti-TB therapy

	Log 4β-OHC/Chol ratio at week 4 of ART				Log 4β-OHC/Chol ratio at week 16 of ART			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	Beta coefficient (95% CI)	*P*	Beta coefficient (95% CI)	*P*	Beta coefficient (95% CI)	*P*	Beta coefficient (95% CI)	*P*
Time of ART initiation (4 or r 8 weeks prior rifampicin therapy)	0. 104 (–0.038 to 0.122)	0.30			0. 064 (–0.052 to 0.099)	0.54		
Sex (reference = female)	–0.189 (–0.149 to 0.003)	0.06	–0.020 (–0.103 to 0.089)	0.81	–0.202 (–0.145 to 0.001)	0.06	–0.13 (–0.14 to 0.05)	0.31
Age in years	–0.088 (–0.006 to 0.002)	0.38						
*CYP2B6*6* carriers	–0.001 (–0.076 to 0.075)	0.99			0.104 (–0.035 to 0.108)	0.32		
ABCB1 c.3435C>T carriers	–0.019 (–0.085 to 0.070)	0.85			0.148 (–0.021 to 0.128)	0.15	0.262 (0.01 to 0.18)	< 0.05
ABCB1c.4036A>G carriers	–0.128 (–0.129 to 0.028)	0.20	–0.151 (–0.166 to 0.045)	0.23	–0.092 (–0.035 to 0.060)	0.68		
*CYP3A5*1*carriers	0.063 (–0.054 to 0.104)	0.53			0.130 (–0.028 to 0.122)	0.21	0.099 (–0.06 to 0.13)	0.44
SLCO1B1 c.388 A>G (*1B)	–0.159 (–0.153 to 0.016)	0.11	–0.205 (–0.174 to 0.022)	0.12	–0.113 (–0.127 to 0.037)	0.28		
SLCO1B1 c.521 T>C (*5) carrier	–0.061 (–0.100 to 0.053)	0.54			0.082 (–0.105 to 0.045)	0.43		
UGT2B7-327G>A (*2) carrier	0.045 (–0.066 to 0.105)	0.66			0.001 (–0.082 to 0.082)	0.91		
Log plasma efavirenz concentration at week 4 ($ng \cdot ml^{-1}$)	0.204 (–0.030 to 0.236)	0.13	0.195 (–0.034 to 0.230)	0.14				
Log plasma efavirenz concentration at week 16 ($ng \cdot ml^{-1}$)					0.397 (0.061 to 0.288)	< 0.05	0.399 (0.066 to 0.286)	< 0.05

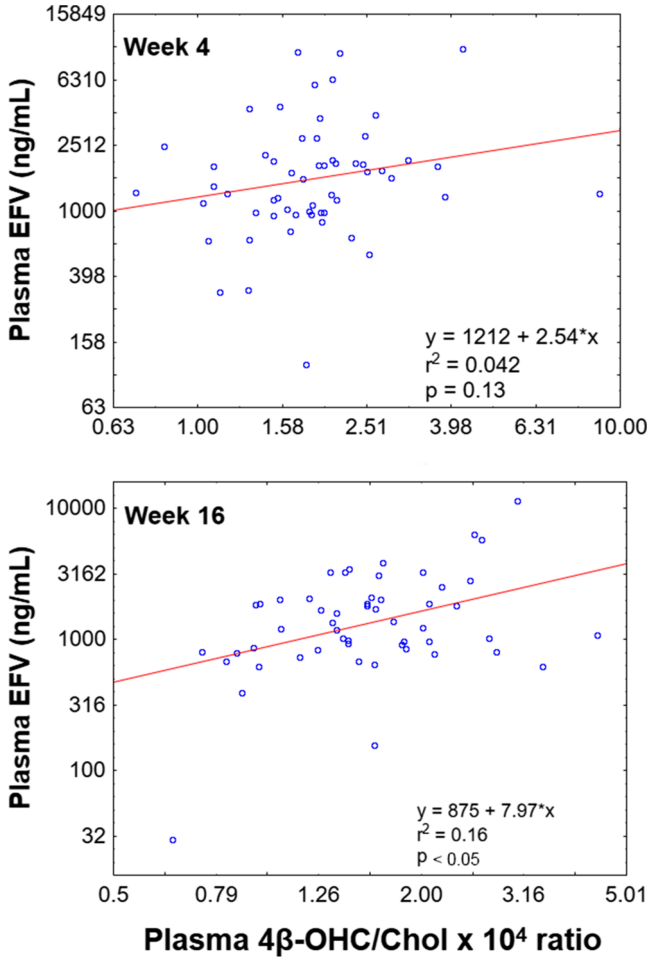

FIGURE 5 Correlation between plasma efavirenz (EFV) concentration and the 4-β hydroxycholesterol/cholesterol ratio (4β-OHC/Chol) at weeks 4 and 16 of concomitant rifampicin based anti-TB and efavirenz-based ART in TB-HIV co-infected patients. The correlation was significant only at 16 weeks

TB co-infection, no significant differences in pre-treatment plasma 4β-OHC concentrations and 4β-OHC/Chol ratios before starting treatment was observed between the different treatment groups (Table 2). The change in plasma, cholesterol, 4β-OHC concentrations and 4β-OHC/Chol ratios from baseline was prospectively monitored at different time points while on therapy, thereby each study participant effectively functioning as his/her own control, with consequent lowering of the level of unexplained variance.

There are four main findings from our study: (i) Initiating ART after 4 or 8 weeks of anti-TB therapy initiation results in no significant differences in the extent of CYP3A induction, or CYP3A-mediated rifampicin-efavirenz drug interactions; (ii). rifampicin is a more potent inducer of CYP3A than efavirenz; (iii) efavirenz and rifampicin co-therapy result in higher CYP3A induction than efavirenz alone, and (iv) high efavirenz plasma concentration and *ABCB1 c.3435C>T* genotype appear to be predictors of long-term CYP3A induction by rifampicin/efavirenz co-treatment.

In TB-HIV co-infected patients, early ART initiation results in rapid immune recovery, reduces the risk of other opportunistic infections and all-cause mortality (Lawn, Torok, & Wood, 2011). However paradoxical worsening or recurring of preexisting tuberculous lesions (TB-IRIS) in some patients is a concern (Abay et al., 2015). Findings from a number of randomized clinical trials concluded that initiation of ART during the first 2 weeks of treatment for serious opportunistic infections is associated with improved survival, except for patients with tuberculous meningitis and cryptococcal meningitis (Lawn, Torok, & Wood, 2011), and early initiation of ART does not increase the incidence of IRIS (Grant et al., 2010). Overall, since ART is key to the recovery of immune function, the benefits of early ART initiation in patients with active TB and very low CD4 counts are likely to outweigh the risks for morbidity associated with TB-IRIS (2014; Lawn, Torok, & Wood, 2011).

The potential risk of drug interaction between anti-TB and antiretroviral drugs is one of the reasons to delay ART initiation until the completion of the intensive phase of TB-therapy (Lawn, Torok, & Wood, 2011). Our results indicate that the CYP3A4 enzyme is maximally induced by rifampicin within the first 4 weeks of starting anti-TB therapy, and the contribution of efavirenz-based ART initiated after 4 or 8 weeks of prior rifampicin based anti-TB therapy is minor and insignificant for the total CYP3A induction (Figure 3). The magnitude of long-term CYP3A enzyme induction was not influenced by the time of ART initiation during the intensive phase of anti-TB therapy. There were no significant differences in the mean plasma 4β-OHC/Chol ratios at 4 or 16 weeks of efavirenz co-treatment between patients who initiated efavirenz-based ART in the middle (4 weeks) or at the end (8 weeks) of the intensive phase of TB therapy (Figure 3). Accordingly, early or late efavirenz based ART initiation while on rifampicin therapy has no significant effect on the extent of CYP3A4 enzyme induction or CYP3A-mediated rifampicin-efavirenz drug interactions.

Having the same duration of therapy (4 weeks), rifampicin was a more potent CYP3A inducer than efavirenz (Figure 2). This is in line with a previous study in primary hepatocytes where a sixfold CYP3A induction by rifampin but only threefold to fourfold induction by efavirenz was reported (Hariparsad et al., 2004). We observed a different CYP3A induction profile by efavirenz in the presence or absence of rifampicin. Among the TB-HIV co-infected patients, there was a sharp increase in the 4β-OHC/Chol ratio after 4 weeks of starting rifampicin which increased further slightly, on adding efavirenz co-therapy, to reach a maximum after 4 weeks of concomitant therapy but no significant difference was observed after 16 weeks of efavirenz co-treatment. Our results are in line with a previous study in Tanzanian TB-HIV patients, where a pharmacokinetic model predicted full induction of CYP3A4 to be achieved within 1–2 weeks after commencing treatment with rifampicin, a potent CYP3A inducer (Ngaimisi et al., 2014).

Pattern recognition of concentration-time data revealed graphically is a key element in pharmacokinetic data analyses to investigate drug–drug interactions (Duan, 2010; Gabrielsson, Meibohm, & Weiner, 2016). Interestingly CYP3A enzyme activity remained higher throughout the study period in patients receiving rifampicin and

efavirenz than those treated with efavirenz only (Figure 4) although maximum CYP3A induction by rifampicin and efavirenz was achieved quite early after treatment initiation and was maintained high throughout the rifampicin-based therapy. Efavirenz and rifampicin co-treatment result in higher CYP3A enzyme induction than efavirenz alone and patients on concomitant efavirenz- rifampicin therapy are theoretically at a higher risk of drug interaction involving CYP3A than patients taking efavirenz alone.

Efavirenz induces CYP3A mainly via the **constitutive androstane receptor** (Faucette et al., 2006; Faucette et al., 2007; Mouly et al., 2002). CYP3A induction by efavirenz is concentration- and time-dependent in humans (Hariparsad et al., 2004; Mouly et al., 2002), and higher systemic efavirenz exposure results in higher CYP3A induction as reflected by a higher 4β OHC/Chol ratio (Habtewold et al., 2013; Ngaimisi et al., 2014). In patients with HIV infection without TB, a high efavirenz plasma concentration and *CYP2B6*6* genotype were significant predictors of CYP3A4 induction (Habtewold et al., 2013). However, in TB-HIV patients receiving efavirenz with rifampicin, no such association was found during early treatment (4 weeks of ART). This may indicate that in the presence of a potent CYP3A4 inducer (rifampicin), *CYP2B6* genotype and efavirenz plasma exposure play minor roles in modulating CYP3A4 induction. However, at week 16 of ART, high efavirenz plasma concentration and *ABCB1 c.3435C>T* genotype were significant predictors of CYP3A4 induction (Table 3). This may be due to a continued contribution from efavirenz in inducing CYP3A, which becomes apparent in the long-term. This is in line with our previous finding that CYP3A induction continues to increase up to 48 weeks of ART in HIV only infected patients treated with efavirenz-based ART only (Habtewold et al., 2013).

Rifampicin induces **P-glycoprotein 1 (P-gp)** and CYP3A enzymes mainly via the **pregnane xenobiotic receptor** (Faucette et al., 2006; Faucette et al., 2007). Previous studies reported that P-gp, encoded by the polymorphic *ABCB1* gene is a major determinant of rifampicin-inducible expression of CYP3A in humans (Schuetz, Schinkel, Relling, & Schuetz, 1996). Being an efflux transporter, P-gp limits cellular uptake of substrate drugs from blood circulation and reduce their systemic exposure (Lin & Yamazaki, 2003). Rifampicin is a potent inducer of P-gp, resulting in an average reduction in substrate exposure ranging between 20 and 67% (Elmeliegy, Vourvahis, Guo, & Wang, 2020). *ABCB1 c.3435C>T* is associated with decreased expression of P-gp and increased the inducer drug exposure (Hitzl et al., 2001). Our result indicates that *ABCB1c.3435C>T* is associated with high plasma 4β-OHC/Chol ratio and CYP3A induction. This could be due to altered P-gp expression/activity resulting in high exposure of CYP3A inducer drugs. The importance of the *ABCB1* genotype for variability in plasma efavirenz exposure has been reported previously (Mukonzo et al., 2009; Ngaimisi et al., 2013). In general, although efavirenz is a weaker inducer than rifampicin, its long-term CYP3A induction in *ABCB1 c.3435C>T* genotypes may result in rapid metabolism of concomitant drugs whose clearance is mainly dependent on CYP3A activity.

Our study found no significant effect of *SLCO1B1*, *CYP3A5*, *UGT2B7*2* genotype or sex on plasma 4β-OHC/Chol ratio. Due to wide sequence similarity and overlapping substrate specificity, both **CYP3A4** and **CYP3A5** contribute to CYP3A-mediated drug metabolism in humans (Kuehl et al., 2001). CYP3A5 is not expressed in most white populations due to the high frequency of a defective variant allele, *CYP3A5*3*. In contrast, about 70% of the black African populations are *CYP3A5* expressors as they carry the functional *CYP3A5*1* allele (Kuehl et al., 2001; Mutagonda et al., 2019; Quaranta et al., 2006). Although *CYP3A4* is genetically polymorphic, no clear link between genotype and variability in enzyme activity has been reported yet. On the other hand, *CYP3A5* genotype is the most important contributor to inter-individual differences in CYP3A-dependent drug disposition particularly in black African populations (Diczfalusy et al., 2008; Gebeyehu et al., 2011; Mukonzo, Waako, Ogwal-Okeng, Gustafsson, & Aklillu, 2010; Mutagonda et al., 2019). We previously reported a significant influence of sex on CYP3A activity in healthy Ethiopian volunteers using the 4β-OHC/Chol ratio as a marker (Gebeyehu et al., 2011) and also in healthy Ugandans using **quinine** as a CYP3A probe drug (Mukonzo, Waako, Ogwal-Okeng, Gustafsson, & Aklillu, 2010). However, no significant effect of sex or *CYP3A5* genotype on 4β-OHC/Chol ratio during efavirenz-based ART alone was reported previously (Habtewold et al., 2011). Likewise, no such association was found in the presence of rifampicin co-treatment. Previous studies reported approximately 50% lower CYP3A activity in HIV-infected patients compared to healthy volunteers (Jetter et al., 2010) and the need for cautious extrapolation of pharmacokinetic data from healthy volunteers to HIV patients (Mukonzo et al., 2011). In our study, the median 4β-OHC/Chol ratios before starting efavirenz therapy in HIV patients were comparable to that reported in healthy Ethiopians previously (Gebeyehu et al., 2011). Our study now provides pharmacological evidence that early (4 weeks) or deferred (8 weeks) ART initiation during anti-TB therapy has no significant long term-effect on CYP3A-mediated drug interactions. ART could be initiated concomitantly with the start of TB therapy.

In conclusion, rifampicin is a more potent CYP3A inducer than efavirenz, and maximum induction occurs during the first 4 weeks of rifampicin therapy. High efavirenz plasma exposure and *ABCB1* genotype predict increased long-term CYP3A activity during concomitant rifampicin and efavirenz co-treatment. Efavirenz and rifampicin co-treatment results in higher CYP3A induction than efavirenz alone. Patients receiving concomitant rifampicin based anti-TB therapy are at a higher risk of unpredicted drug interactions with CYP3A substrates than HIV patients on efavirenz-based ART only. However, CYP3A induction is not affected by the time of efavirenz initiation while on rifampicin therapy. Our study indicates that there is a low risk of CYP3A-mediated drug interaction between rifampicin and efavirenz. Thus, concomitant initiation of both TB drugs and ART in newly diagnosed TB/HIV-co-infected is prudent to reduce the risk of all-cause of mortality particularly (Amogne et al., 2015).

ACKNOWLEDGEMENTS

The study was financially supported by grants from European and Developing Countries Clinical Trials Partnership (NL) (Grant number: CG_TA.05.40204_005) and from Swedish Research Council

(Vetenskapsrådet, Grant number: 2015-03295). AZ holds an NIHR senior investigator award and acknowledges support from the NIHR Biomedical Research Centre at UCL hospitals.

AUTHOR CONTRIBUTIONS

E.A., U.D., A.Z., and E.M. conceived and designed the study; E.A., A.H., W.A., E.M., G.Y., J.B., and U.D. conducted the study. E.A., A.Z., and U.D. analysed the data and wrote the manuscript. All authors contributed to scientific design and methodology and reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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REFERENCES

Abay, S. M., Deribe, K., Reda, A. A., Biadgilign, S., Datiko, D., Assefa, T., … Deribew, A. (2015). The effect of early initiation of antiretroviral therapy in TB/HIV-coinfected patients: A systematic review and meta-analysis. *Journal of the International Association of Providers of AIDS Care (JIAPAC)*, *14*, 560–570. https://doi.org/10.1177/2325957415599210

Abdool Karim, S. S., Naidoo, K., Grobler, A., Padayatchi, N., Baxter, C., Gray, A. L., … Abdool Karim, Q. (2011). Integration of antiretroviral therapy with tuberculosis treatment. *The New England Journal of Medicine*, *365*, 1492–1501. https://doi.org/10.1056/NEJMoa1014181

Aklillu, E., Habtewold, A., Ngaimisi, E., Yimer, G., Mugusi, S., Amogne, W., … Weiss, J. (2016). SLCO1B1 gene variations among Tanzanians, Ethiopians, and Europeans: Relevance for African and worldwide precision medicine. *Omics*, *20*, 538–545. https://doi.org/10.1089/omi.2016.0119

Aklillu, E., Mugusi, S., Ngaimisi, E., Hoffmann, M. M., Konig, S., Ziesenitz, V., … Weiss, J. (2011). Frequency of the SLCO1B1 388A>G and the 521T>C polymorphism in Tanzania genotyped by a new LightCycler(R)-based method. *European Journal of Clinical Pharmacology*, *67*, 1139–1145. https://doi.org/10.1007/s00228-011-1065-9

Alexander, S. P. H., Cidlowski, J. A., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., … CGTP Collaborators. (2019). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Nuclear hormone receptors. *British Journal of Pharmacology*, *176*, S229–S246. https://doi.org/10.1111/bph.14750

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., … CGTP Collaborators. (2019a). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Catalytic receptors. *British Journal of Pharmacology*, *176*, S247–S296. https://doi.org/10.1111/bph.14751

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., … CGTP Collaborators. (2019b). The concise guide to pharmacology 2019/20: Enzymes. *British Journal of Pharmacology*, *176*, S297–S396.

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Faccenda, E., … CGTP Collaborators. (2019a). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Other Protein Targets. *British Journal of Pharmacology*, *176*, S1–S20. https://doi.org/10.1111/bph.14747

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., … CGTP Collaborators. (2019b). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Transporters. *British Journal of Pharmacology*, *176*, S397–S493. https://doi.org/10.1111/bph.14753

Amogne, W., Aderaye, G., Habtewold, A., Yimer, G., Makonnen, E., Worku, A., … Lindquist, L. (2015). Efficacy and safety of antiretroviral therapy initiated one week after tuberculosis therapy in patients with CD4 counts < 200 cells/μL: TB-HAART study, a randomized clinical trial. *PLoS ONE*, *10*, e0122587. https://doi.org/10.1371/journal.pone.0122587

Bjorkhem-Bergman, L., Backstrom, T., Nylen, H., Ronquist-Nii, Y., Bredberg, E., Andersson, T. B., … Diczfalusy, U. (2013). Comparison of endogenous 4β-hydroxycholesterol with midazolam as markers for CYP3A4 induction by rifampicin. *Drug Metabolism and Disposition*, *41*, 1488–1493. https://doi.org/10.1124/dmd.113.052316

Blanc, F. X., Sok, T., Laureillard, D., Borand, L., Rekacewicz, C., Nerrienet, E., … CAMELIA (ANRS 1295–CIPRA KH001) Study Team. (2011). Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *The New England Journal of Medicine*, *365*, 1471–1481. https://doi.org/10.1056/NEJMoa1013911

Burhenne, J., Matthee, A. K., Pasakova, I., Roder, C., Heinrich, T., Haefeli, W. E., … Weiss, J. (2010). No evidence for induction of ABC transporters in peripheral blood mononuclear cells in humans after 14 days of efavirenz treatment. *Antimicrobial Agents and Chemotherapy*, *54*, 4185–4191. https://doi.org/10.1128/AAC.00283-10

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., … Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: Updated and simplified guidance for authors and peer reviewers. *British Journal of Pharmacology*, *175*, 987–993. https://doi.org/10.1111/bph.14153

da Silva Escada, R. O., Velasque, L., Ribeiro, S. R., Cardoso, S. W., Marins, L. M. S., Grinsztejn, E., … Veloso, V. G. (2017). Mortality in patients with HIV-1 and tuberculosis co-infection in Rio de Janeiro, Brazil—Associated factors and causes of death. *BMC Infectious Diseases*, *17*, 373–383. https://doi.org/10.1186/s12879-017-2473-y

Diczfalusy, U., Miura, J., Roh, H. K., Mirghani, R. A., Sayi, J., Larsson, H., … Bertilsson, L. (2008). 4β-hydroxycholesterol is a new endogenous CYP3A marker: Relationship to CYP3A5 genotype, quinine 3-hydroxylation and sex in Koreans, Swedes and Tanzanians. *Pharmacogenetics and Genomics*, *18*, 201–208. https://doi.org/10.1097/FPC.0b013e3282f50ee9

Diczfalusy, U., Nylen, H., Elander, P., & Bertilsson, L. (2011). 4β-hydroxycholesterol, an endogenous marker of CYP3A4/5 activity in humans. *British Journal of Clinical Pharmacology*, *71*, 183–189.

Djimeu, E. W., & Heard, A. C. (2019). Treatment of HIV among tuberculosis patients: A replication study of timing of antiretroviral therapy for HIV-1-associated tuberculosis. *PLoS ONE*, *14*, e0210327. https://doi.org/10.1371/journal.pone.0210327

Duan, J. Z. (2010). Drug–drug interaction pattern recognition. *Drugs in R&D*, *10*, 9–24. https://doi.org/10.2165/11537440-000000000-00000

Dutreix, C., Lorenzo, S., & Wang, Y. (2014). Comparison of two endogenous biomarkers of CYP3A4 activity in a drug–drug interaction study between midostaurin and rifampicin. *European Journal of Clinical Pharmacology*, *70*, 915–920. https://doi.org/10.1007/s00228-014-1675-0

Egelund, E. F., Dupree, L., Huesgen, E., & Peloquin, C. A. (2017). The pharmacological challenges of treating tuberculosis and HIV coinfections. *Expert Review of Clinical Pharmacology*, *10*, 213–223. https://doi.org/10.1080/17512433.2017.1259066

Elmeliegy, M., Vourvahis, M., Guo, C., & Wang, D. D. (2020). Effect of P-glycoprotein (P-gp) inducers on exposure of P-gp substrates: Review of clinical drug–drug interaction studies. *Clinical Pharmacokinetics*, *59*, 699–714. https://doi.org/10.1007/s40262-020-00867-1

Faucette, S. R., Sueyoshi, T., Smith, C. M., Negishi, M., Lecluyse, E. L., & Wang, H. (2006). Differential regulation of hepatic CYP2B6 and CYP3A4 genes by constitutive androstane receptor but not pregnane X receptor. *The Journal of Pharmacology and Experimental Therapeutics*, *317*, 1200–1209. https://doi.org/10.1124/jpet.105.098160

Faucette, S. R., Zhang, T. C., Moore, R., Sueyoshi, T., Omiecinski, C. J., LeCluyse, E. L., … Wang, H. (2007). Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *The Journal of Pharmacology and Experimental Therapeutics*, *320*, 72–80. https://doi.org/10.1124/jpet.106.112136

Floyd, K., Glaziou, P., Zumla, A., & Raviglione, M. (2018). The global tuberculosis epidemic and progress in care, prevention, and research: An overview in year 3 of the end TB era. *The Lancet Respiratory Medicine*, *6*, 299–314. https://doi.org/10.1016/S2213-2600(18)30057-2

Gabrielsson, J., Meibohm, B., & Weiner, D. (2016). Pattern recognition in pharmacokinetic data analysis. *The AAPS Journal*, *18*, 47–63. https://doi.org/10.1208/s12248-015-9817-6

Gebeyehu, E., Engidawork, E., Bijnsdorp, A., Aminy, A., Diczfalusy, U., & Aklillu, E. (2011). Sex and CYP3A5 genotype influence total CYP3A activity: High CYP3A activity and a unique distribution of CYP3A5 variant alleles in Ethiopians. *The Pharmacogenomics Journal*, *11*, 130–137. https://doi.org/10.1038/tpj.2010.16

Grant, P. M., Komarow, L., Andersen, J., Sereti, I., Pahwa, S., Lederman, M. M., … Zolopa, A. (2010). Risk factor analyses for immune reconstitution inflammatory syndrome in a randomized study of early vs. deferred ART during an opportunistic infection. *PLoS ONE*, *5*, e11416. https://doi.org/10.1371/journal.pone.0011416

Gravel, S., Chiasson, J. L., Gaudette, F., Turgeon, J., & Michaud, V. (2019). Use of 4β-hydroxycholesterol plasma concentrations as an endogenous biomarker of CYP3A activity: Clinical validation in individuals with type 2 diabetes. *Clinical Pharmacology and Therapeutics*, *106*, 831–840. https://doi.org/10.1002/cpt.1472

Gu, H., Dutreix, C., Rebello, S., Ouatas, T., Wang, L., Chun, D. Y., … He, H. (2018). Simultaneous physiologically based pharmacokinetic (PBPK) modeling of parent and active metabolites to investigate complex CYP3A4 drug–drug interaction potential: A case example of midostaurin. *Drug Metabolism and Disposition*, *46*, 109–121. https://doi.org/10.1124/dmd.117.078006

Habtewold, A., Aklillu, E., Makonnen, E., Amogne, W., Yimer, G., Aderaye, G., … Burhenne, J. (2016). Long-term effect of rifampicin-based anti-TB regimen coadministration on the pharmacokinetic parameters of efavirenz and 8-hydroxy-efavirenz in Ethiopian patients. *Journal of Clinical Pharmacology*, *56*, 1538–1549. https://doi.org/10.1002/jcph.756

Habtewold, A., Aklillu, E., Makonnen, E., Yimer, G., Bertilsson, L., Burhenne, J., & Owen, J. S. (2017). Population pharmacokinetic model linking plasma and peripheral blood mononuclear cell concentrations of efavirenz and its metabolite, 8-hydroxy-efavirenz, in HIV patients. *Antimicrobial Agents and Chemotherapy*, *61*(8), e00207-17. https://doi.org/10.1128/AAC.00207-17

Habtewold, A., Amogne, W., Makonnen, E., Yimer, G., Nylen, H., Riedel, K. D., … Aklillu, E. (2013). Pharmacogenetic and pharmacokinetic aspects of CYP3A induction by efavirenz in HIV patients. *The Pharmacogenomics Journal*, *13*, 484–489. https://doi.org/10.1038/tpj.2012.46

Habtewold, A., Amogne, W., Makonnen, E., Yimer, G., Riedel, K. D., Ueda, N., … Aklillu, E. (2011). Long-term effect of efavirenz autoinduction on plasma/peripheral blood mononuclear cell drug exposure and CD4 count is influenced by UGT2B7 and CYP2B6 genotypes among HIV patients. *The Journal of Antimicrobial Chemotherapy*, *66*, 2350–2361. https://doi.org/10.1093/jac/dkr304

Habtewold, A., Makonnen, E., Amogne, W., Yimer, G., Aderaye, G., Bertilsson, L., … Aklillu, E. (2015). Is there a need to increase the dose of efavirenz during concomitant rifampicin-based antituberculosis therapy in sub-Saharan Africa? The HIV-TB pharmagene study. *Pharmacogenomics*, *16*, 1047–1064. https://doi.org/10.2217/pgs.15.35

Hariparsad, N., Nallani, S. C., Sane, R. S., Buckley, D. J., Buckley, A. R., & Desai, P. B. (2004). Induction of CYP3A4 by efavirenz in primary human hepatocytes: Comparison with rifampin and phenobarbital. *Journal of Clinical Pharmacology*, *44*, 1273–1281. https://doi.org/10.1177/0091270004269142

Hitzl, M., Drescher, S., van der Kuip, H., Schaffeler, E., Fischer, J., Schwab, M., … Fromm, M. F. (2001). The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics*, *11*, 293–298. https://doi.org/10.1097/00008571-200106000-00003

Jetter, A., Fatkenheuer, G., Frank, D., Klaassen, T., Seeringer, A., Doroshyenko, O., … Wyen, C. (2010). Do activities of cytochrome P450 (CYP)3A, CYP2D6 and P-glycoprotein differ between healthy volunteers and HIV-infected patients? *Antiviral Therapy*, *15*, 975–983. https://doi.org/10.3851/IMP1648

Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., … Schuetz, E. (2001). Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nature Genetics*, *27*, 383–391. https://doi.org/10.1038/86882

Lawn, S. D., Torok, M. E., & Wood, R. (2011). Optimum time to start antiretroviral therapy during HIV-associated opportunistic infections. *Current Opinion in Infectious Diseases*, *24*, 34–42. https://doi.org/10.1097/QCO.0b013e3283420f76

Lin, J. H., & Yamazaki, M. (2003). Role of P-glycoprotein in pharmacokinetics: Clinical implications. *Clinical Pharmacokinetics*, *42*, 59–98.

Lopez-Cortes, L. F., Ruiz-Valderas, R., Marin-Niebla, A., Pascual-Carrasco, R., Rodriguez-Diez, M., & Lucero-Munoz, M. J. (2005). Therapeutic drug monitoring of efavirenz: Trough levels cannot be estimated on the basis of earlier plasma determinations. *Journal of Acquired Immune Deficiency Syndromes*, *39*, 551–556.

McHunu, G., van Griensven, J., Hinderaker, S. G., Kizito, W., Sikhondze, W., Manzi, M., … Harries, A. D. (2016). High mortality in tuberculosis patients despite HIV interventions in Swaziland. *Public Health Action*, *6*, 105–110. https://doi.org/10.5588/pha.15.0081

Mfinanga, S. G., Kirenga, B. J., Chanda, D. M., Mutayoba, B., Mthiyane, T., Yimer, G., … Onyebujoh, P. (2014). Early versus delayed initiation of highly active antiretroviral therapy for HIV-positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): A prospective, international, randomised, placebo-controlled trial. *The Lancet Infectious Diseases*, *14*, 563–571. https://doi.org/10.1016/S1473-3099(14)70733-9

Mouly, S., Lown, K. S., Kornhauser, D., Joseph, J. L., Fiske, W. D., Benedek, I. H., & Watkins, P. B. (2002). Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clinical Pharmacology and Therapeutics*, *72*, 1–9. https://doi.org/10.1067/mcp.2002.124519

Mukonzo, J., Aklillu, E., Marconi, V., & Schinazi, R. F. (2019). Potential drug–drug interactions between antiretroviral therapy and treatment regimens for multi-drug resistant tuberculosis: Implications for HIV

care of MDR-TB co-infected individuals. *International Journal of Infectious Diseases*, *83*, 98–101.

Mukonzo, J., Nanzigu, S., Rekic, D., Waako, P., Roshammar, D., Ashton, M., … Aklillu, E. (2011). HIV/AIDS patients display lower relative bioavailability of efavirenz than healthy subjects (vol 50, pg 531, 2011). *Clinical Pharmacokinetics*, *50*, 624–624.

Mukonzo, J., Waako, P., Ogwal-Okeng, J., Gustafsson, L., & Aklillu, E. (2010). Genetic variations in ABCB1 and CYP3A5 as well as sex influence quinine disposition among Ugandans. *Therapeutic Drug Monitoring*, *32*, 346–352. https://doi.org/10.1097/FTD.0b013e3181da79d6

Mukonzo, J. K., Roshammar, D., Waako, P., Andersson, M., Fukasawa, T., Milani, L., … Aklillu, E. (2009). A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans. *British Journal of Clinical Pharmacology*, *68*, 690–699. https://doi.org/10.1111/j.1365-2125.2009.03516.x

Mutagonda, R. F., Minzi, O. M. S., Massawe, S. N., Asghar, M., Farnert, A., Kamuhabwa, A. A. R., & Aklillu, E. (2019). Pregnancy and CYP3A5 genotype affect day 7 plasma lumefantrine concentrations. *Drug Metabolism and Disposition*, *47*, 1415–1424. https://doi.org/10.1124/dmd.119.088062

Ngaimisi, E., Habtewold, A., Minzi, O., Makonnen, E., Mugusi, S., Amogne, W., … Burhenne, J. (2013). Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: A parallel-group prospective cohort study in two sub-Saharan Africa populations. *PLoS ONE*, *8*, e67946. https://doi.org/10.1371/journal.pone.0067946

Ngaimisi, E., Minzi, O., Mugusi, S., Sasi, P., Riedel, K. D., Suda, A., … Diczfalusy, U. (2014). Pharmacokinetic and pharmacogenomic modelling of the CYP3A activity marker 4β-hydroxycholesterol during efavirenz treatment and efavirenz/rifampicin co-treatment. *The Journal of Antimicrobial Chemotherapy*, *69*, 3311–3319. https://doi.org/10.1093/jac/dku286

Penzak, S. R., & Rojas-Fernandez, C. (2019). 4β-hydroxycholesterol as an endogenous biomarker for CYP3A activity: Literature review and critical evaluation. *Journal of Clinical Pharmacology*, *59*, 611–624.

Quaranta, S., Chevalier, D., Allorge, D., Lo-Guidice, J. M., Migot-Nabias, F., Kenani, A., … Lhermitte, M. (2006). Ethnic differences in the distribution of CYP3A5 gene polymorphisms. *Xenobiotica*, *36*, 1191–1200. https://doi.org/10.1080/00498250600944300

Schuetz, E. G., Schinkel, A. H., Relling, M. V., & Schuetz, J. D. (1996). P-glycoprotein: A major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 4001–4005. https://doi.org/10.1073/pnas.93.9.4001

Tang, C., Lin, J. H., & Lu, A. Y. (2005). Metabolism-based drug–drug interactions: What determines individual variability in cytochrome P450 induction? *Drug Metabolism and Disposition*, *33*, 603–613.

Tornheim, J. A., & Dooley, K. E. (2018). Challenges of TB and HIV co-treatment: Updates and insights. *Current Opinion in HIV and AIDS*, *13*, 486–491. https://doi.org/10.1097/COH.0000000000000495

World Health Organization. (2016). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach - Second edition. Available from http://apps.who.int/iris/bitstream/10665/208825/1/9789241549684_eng.pdf?ua=1 Accessed January 2019.

World Health Organization. (2019). *Global tuberculosis report 2019*. Geneva: World Health Organization. 2019. Licence: CC BY-NC-SA 3.0 IGO. Geneva. Available at: https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1 Accessed 20 April 2020

Yamashita, F., Sasa, Y., Yoshida, S., Hisaka, A., Asai, Y., Kitano, H., … Suzuki, H. (2013). Modeling of rifampicin-induced CYP3A4 activation dynamics for the prediction of clinical drug–drug interactions from in vitro data. *PLoS ONE*, *8*, e70330. https://doi.org/10.1371/journal.pone.0070330

Yimer, G., Aderaye, G., Amogne, W., Makonnen, E., Aklillu, E., Lindquist, L., … Aseffa, A. (2008). Anti-tuberculosis therapy-induced hepatotoxicity among Ethiopian HIV-positive and negative patients. *PLoS ONE*, *3*, e1809. https://doi.org/10.1371/journal.pone.0001809

Yimer, G., Gry, M., Amogne, W., Makonnen, E., Habtewold, A., Petros, Z., … Aklillu, E. (2014). Evaluation of patterns of liver toxicity in patients on antiretroviral and anti-tuberculosis drugs: A prospective four arm observational study in ethiopian patients. *PLoS ONE*, *9*, e94271. https://doi.org/10.1371/journal.pone.0094271

How to cite this article: Aklillu E, Zumla A, Habtewold A, et al. Early or deferred initiation of efavirenz during rifampicin-based TB therapy has no significant effect on CYP3A induction in TB-HIV infected patients. *Br J Pharmacol*. 2021; 178:3294–3308. https://doi.org/10.1111/bph.15309