


**RESEARCH PAPER - THEMED ISSUE**

# 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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**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 4 $\beta$ -hydroxycholesterol/cholesterol (4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratios from baseline by 378% and 576% respectively. After efavirenz/rifampicin co-treatment, 4 $\beta$ -OHC/Chol ratios increased by 560% of baseline (4 weeks) and 456% of baseline (16 weeks). Neither time of ART initiation, sex,

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

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genotype nor efavirenz plasma concentration were significant predictors of 4 $\beta$ -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.

#### KEYWORDS

4 $\beta$ -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· $\mu$ <sup>-1</sup> (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

### 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.

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 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-naïve adult Ethiopian patients with newly diagnosed pulmonary TB co-infected with HIV to investigate CYP3A-mediated interactions between anti-retroviral 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<sup>-3</sup> 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 (Aмогоne et al., 2015). In parallel, treatment-naïve 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 the 16th 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 (Aмогоne 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.

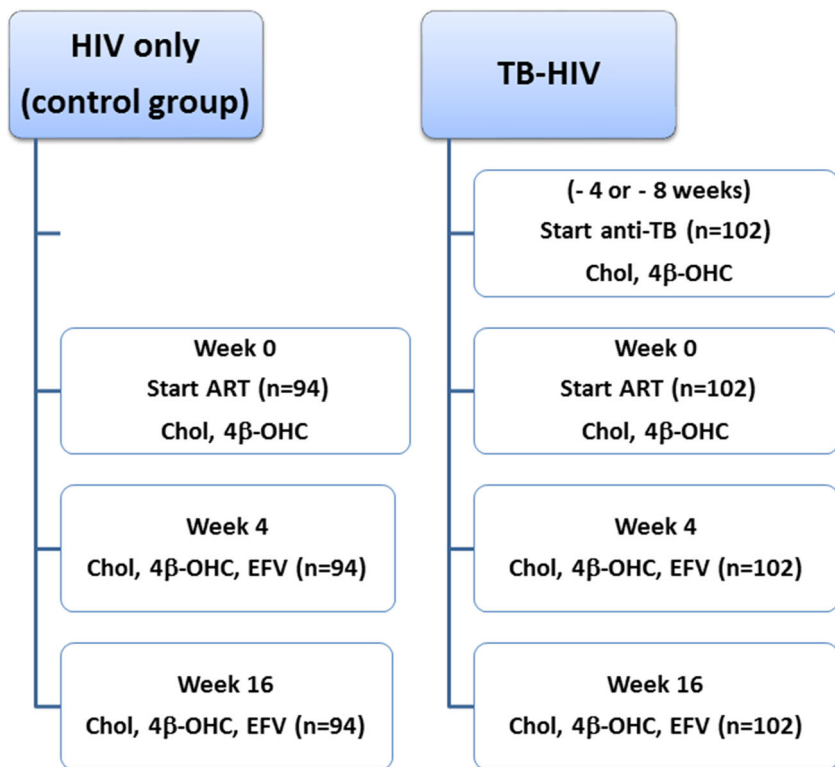
Venous blood samples were collected before starting anti-TB treatment (4 or 8 weeks before initiation of ART), at the initiation of ART (week 0) and the fourth and 16th weeks of ART. The study population, follow-up period, and study sampling time points are presented in Figure 1. Pre-treatment CD4 count, 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

4 $\beta$ -Hydroxycholesterol/cholesterol (4 $\beta$ -OHC/Chol) ratio was used as an endogenous marker for CYP3A activity (Diczfalusy et al., 2008). Blood samples for determination of cholesterol and 4 $\beta$ -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<sup>-1</sup>). Determination of 4 $\beta$ -OHC was performed by isotope-dilution gas chromatography-mass spectrometry using deuterium-labelled 4 $\beta$ -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<sup>-1</sup>).

### 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 $\times$  g for 20 min), and a plasma aliquot was stored at  $-80^{\circ}\text{C}$  for determination of efavirenz. The AUC<sub>0-24</sub> 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



**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), 4 $\beta$ -hydroxycholesterol (4 $\beta$ -OHC) and efavirenz levels are indicated with references to ART initiation in weeks

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·ml<sup>-1</sup> and the efavirenz calibration range was 10–10,000 ng·ml<sup>-1</sup>.

## 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 manufacturer's 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  $\mu$ l, consisting of 2 $\times$  TaqMan Universal PCR Master Mix® (Applied Biosystems), 20 $\times$  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 *SLCO1B1*c.521T>C (\*5) and *SLCO1B1*c.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, 4 $\beta$ -OHC concentrations, and 4 $\beta$ -OHC/Chol ratios. Between treatment, group comparison of median plasma cholesterol concentrations, 4 $\beta$ -OHC

concentrations, and 4 $\beta$ -OHC/Chol ratios at baseline and during treatment was done using the Mann–Whitney *U* test. The percent changes in plasma 4 $\beta$ -OHC/Chol ratio from baseline to the fourth and 16th weeks of ART were calculated using the following formula:

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

Plasma concentrations of EFV, 4 $\beta$ -OHC, and 4 $\beta$ -OHC/Chol ratios were not normally distributed. Therefore, concentration data were transformed into log<sub>10</sub> values for statistical analysis and the Shapiro–Wilk test for normality was applied. Log-transformed concentration data were used for graphical presentation and statistical analysis using parametric tests. Comparison of geometric means of 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratio over time

Plasma cholesterol and 4 $\beta$ -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, 4 $\beta$ -OHC and 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratio between HIV only and TB-HIV patients. The median 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratio from baseline was 378% or 576%, respectively. The median percent change in 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratios than those treated with rifampicin for 4 or 8 weeks. However, no significant difference in geometric means of the 4 $\beta$ -OHC/Chol ratio was detected, between TB-HIV patients treated with rifampicin-based therapy only for 4 or 8 weeks (Figure 2).

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

Comparison of geometric means of plasma 4 $\beta$ -OHC/Chol ratio between HIV-only patients treated with efavirenz-based ART only

**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%)		
<b>Laboratory parameters</b>	<b>Reference</b>	<b>Median (IQR)</b>	<b>Median (IQR)</b>	<b>Median (IQR)</b>
Karnofsky	Above 80%	100 (90–100)	90 (80–100)	100 (80–100)
Hb (g·L <sup>-1</sup> )	144–166	127 (116–152)	113 (100–163)	119 (100–162)
WBC count (×10 <sup>3</sup> ·μl <sup>-1</sup> )	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 (×10 <sup>3</sup> ·μl <sup>-1</sup> )	140–415	233 (164–450)	325 (233–617)	293 (250–461)
Aspartate aminotransferase (U·L <sup>-1</sup> )	0–37	33.0 (27–99)	38.5 (34–119)	52.0 (34–108)
Alanine transaminase (U·L <sup>-1</sup> )	0–55	28 (21–127)	28 (20–64)	32 (23–68)
Alkaline phosphatase (U·L <sup>-1</sup> )	40–150	106 (79–210)	115 (89–318)	114 (95–258)
Total bilirubin (μmol·L <sup>-1</sup> )	0.2–1.2	0.38 (0.3–0.6)	0.35 (0.3–0.6)	0.55 (0.3–0.9)
Direct bilirubin (μmol·L <sup>-1</sup> )	0–0.5	0.1 (0.1–0.1)	0.1 (0.1–0.22)	0.1 (0.1–0.16)
Albumin (g·L <sup>-1</sup> )	38–54	39 (35–49)	34 (30–45)	34 (29–46)
Urea (mmol·L <sup>-1</sup> )	14–56	70 (56–109)	67 (56–128)	67 (53–140)
Plasma creatinine (μmol·L <sup>-1</sup> )	53–97	71 (62–106)	80 (71–106)	80 (62–141)
CD4 counts per mm <sup>3</sup>	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·L <sup>-1</sup> )		3.14 (2.51–3.81)	2.69 (2.05–3.35)	2.72 (2.0–3.5)
4β-OHC (ng·ml <sup>-1</sup> )		29.1 (20.9–37.6)	25.7 (19.1–33.2)	27.0 (21.5–35.9)
4β OHC/cholesterol × 10 <sup>4</sup>		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.

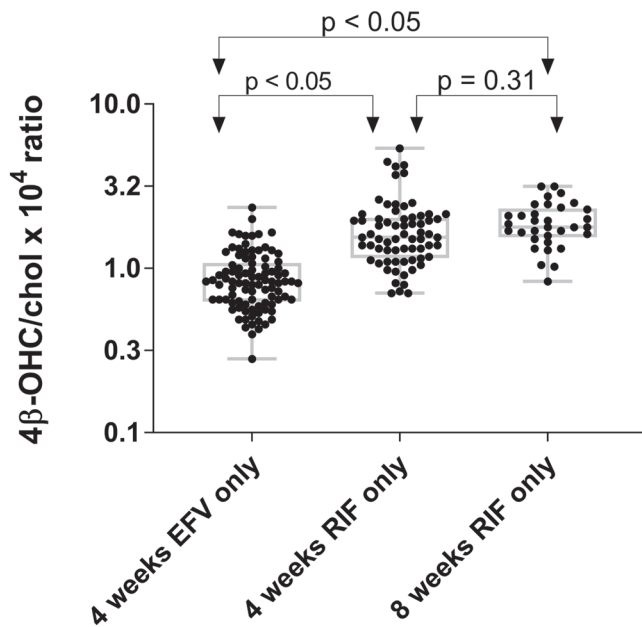
(control group) with those from the TB-HIV patients who received concomitant anti-retroviral and rifampicin-based anti-TB therapies for 4 and 16 weeks is presented in Figure 3. The TB-HIV co-infected patients initiated concomitant efavirenz-based ART after 4 or 8 weeks

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

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

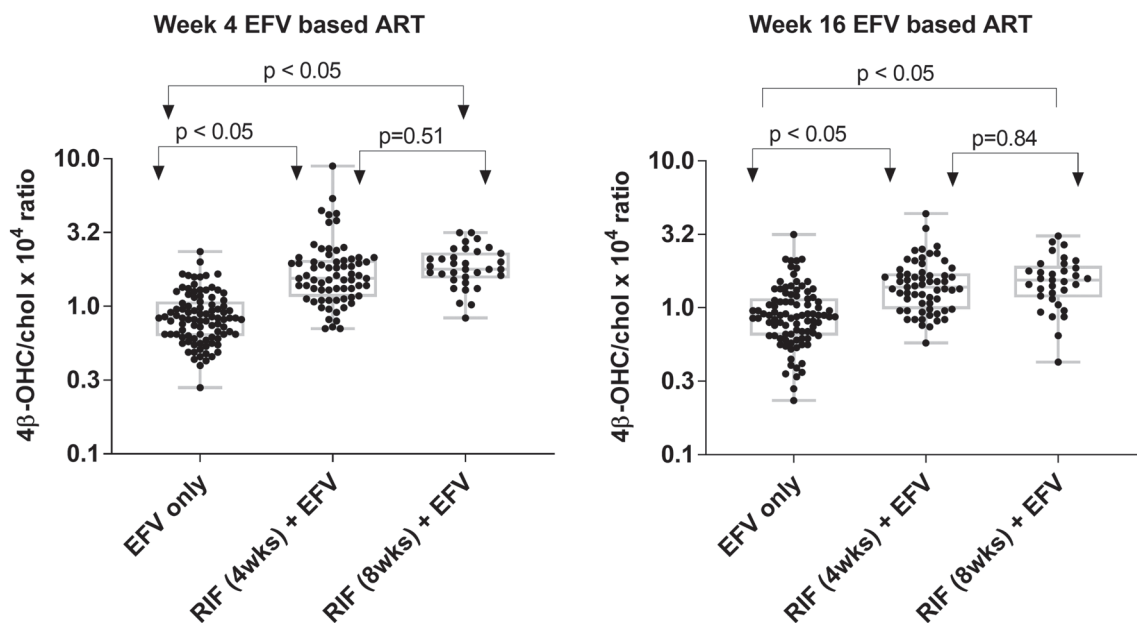
	Treatment group						P
	TB-HIV (RIF + EFV)			HIV only (EFV only)			
	N	Median (IQR)	Median % change from baseline (IQR)	N	Median % change from baseline (IQR)	Median % change from baseline (IQR)	
<b>Cholesterol</b>							
Baseline	102	2.71 (2.05 to 3.38)		94	3.14 (2.61 to 3.81)		<0.05
4 or 8 weeks of 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
<b>4<math>\beta</math>-OHC</b>							
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
<b>4<math>\beta</math> OHC/cholesterol</b>							
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. 4 $\beta$ -OHC in ng·ml<sup>-1</sup>, cholesterol in mmol·L<sup>-1</sup>, 4 $\beta$ -OHC/cholesterol molar ratio is multiplied by 10<sup>4</sup>, OHC = hydroxycholesterol; IQR = interquartile range.



**FIGURE 2** Comparison of geometric means of 4 $\beta$ -hydroxycholesterol/cholesterol ratio (4 $\beta$ -OHC/Chol  $\times 10^4$ ) 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 4 $\beta$ -OHC/Chol ratio was used to plot the graph. The box plots show the means  $\pm$  SD, while whiskers denote the minimum and maximum values

week 16). However, no significant difference in the mean plasma 4 $\beta$ -OHC/Chol ratio among TB-HIV patients who initiated ART after 4 or 8 weeks of starting anti-TB therapy.



**FIGURE 3** Comparison of geometric means of 4 $\beta$ -hydroxycholesterol/cholesterol (4 $\beta$ -OHC/Chol  $\times 10^4$ ) 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 4 $\beta$ -OHC/Chol ratio was used to plot the graph. The box plots show the means  $\pm$  SD, while whiskers denote the minimum and maximum values

Figure 4 shows the pattern and extent of change in the geometric mean of plasma 4 $\beta$ -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 4 $\beta$ -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-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.

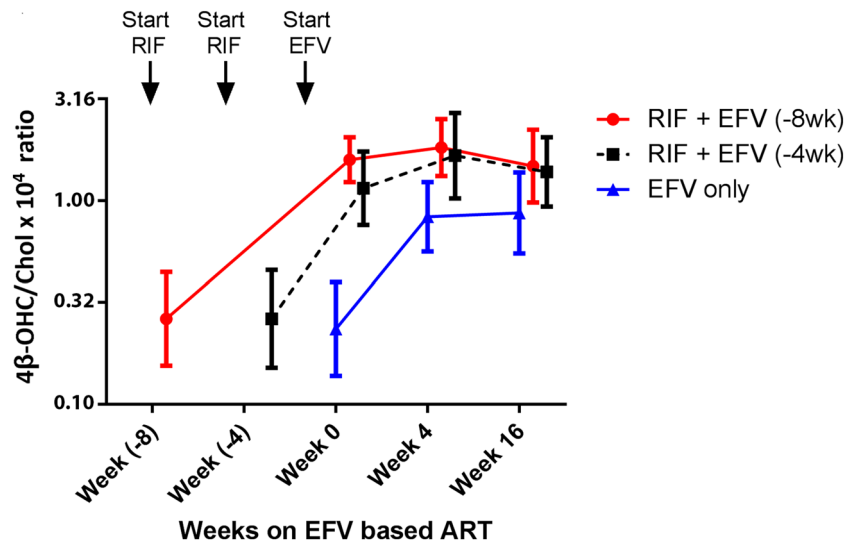
### 3.4 | Effect of sex, CYP2B6, CYP3A5, ABCB1, SLCO1B1, and UGT2B7 genotype

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 4 $\beta$ -



**FIGURE 4** Change in plasma 4 $\beta$ -hydroxycholesterol/cholesterol (4 $\beta$ -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 4 $\beta$ -OHC/Chol ratio was used to plot the graph. Central points denote the geometric mean and the vertical bars show SD



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 4 $\beta$ -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 4 $\beta$ -OHC/Chol ratio at week 16 of ART (Table 3).

### 3.5 | Correlation between plasma efavirenz and 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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

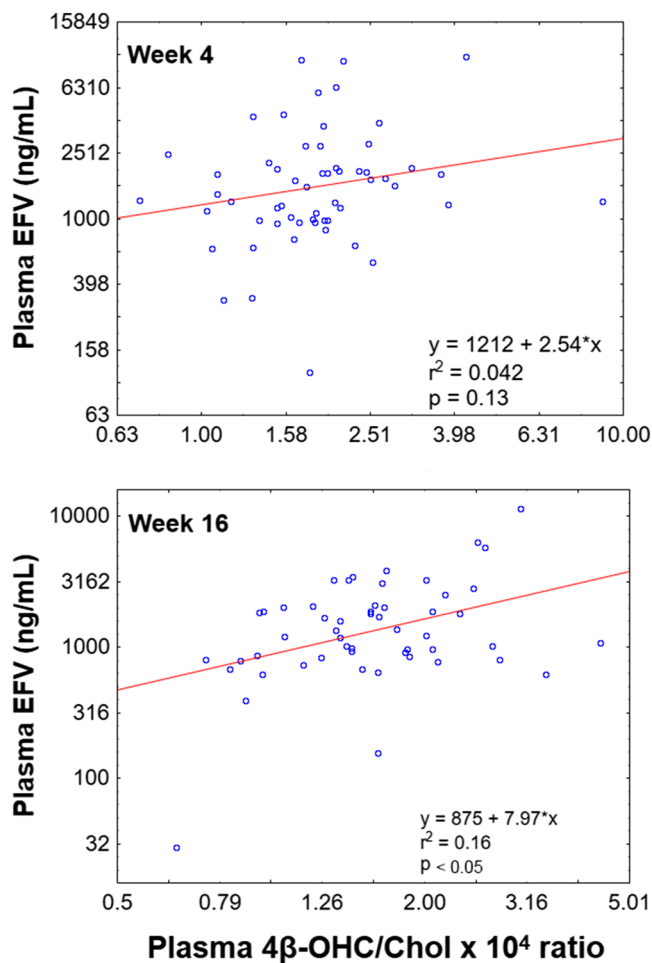
following treatment with an inducer drug is indicative of CYP enzyme induction and metabolism-based drug-drug interactions (DDI) (Gu et al., 2018; Tang, Lin, & Lu, 2005). The plasma 4 $\beta$ -OHC/Chol ratio is the preferred non-invasive endogenous biomarker for CYP3A-mediated clinical DDIs, where CYP3A 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 4 $\beta$ -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 TB co-infection, no significant differences in pre-treatment plasma 4 $\beta$ -OHC concentrations and 4 $\beta$ -OHC/Chol ratios before starting treatment was observed between the different treatment groups (Table 2). The change in plasma, cholesterol, 4 $\beta$ -OHC concentrations and 4 $\beta$ -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

**TABLE 3** Predictors of 4 $\beta$ -hydroxycholesterol/cholesterol (4 $\beta$ -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

Variable	Log 4 $\beta$ -OHC/Chol ratio at week 4 of ART				Log 4 $\beta$ -OHC/Chol ratio at week 16 of ART			
	Univariate		Multivariate		Univariate		Multivariate	
	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 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·ml <sup>-1</sup> )	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·ml <sup>-1</sup> )					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- $\beta$  hydroxycholesterol/cholesterol ratio (4 $\beta$ -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-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 anti-retroviral 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 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$  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 *ABCB1*c.3435C>T is associated with high plasma 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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 4 $\beta$ -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).

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## 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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