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**OPTIMIZATION OF HIV AND
TUBERCULOSIS CO-TREATMENT
IN TANZANIAN PATIENTS:
EMPHASIS ON
PHARMACOGENETICS AND
DRUG INTERACTIONS**

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ABSTRACT

Treatment of HIV and its comorbidities particularly tuberculosis is complicated by wide inter-individual variations in drug exposure due to drug-interactions and variable expressions of drug metabolizing enzymes and transporters. Efavirenz in particular, is an antiretroviral characterized by wide inter-individual variation in exposure. Unfortunately, it also has narrow therapeutic range resulting into considerable proportions of patients with sub-therapeutic or supra-therapeutic concentrations. This thesis describes sources of variation in EFV plasma levels and evaluates its enzyme induction and hepatotoxicity; so as to inform appropriate dose optimization.

We recruited HIV infected patients with tuberculosis (arm2) and without (arm1) both in Tanzania and Ethiopia. Patients took EFV 600mg based antiretroviral therapy (HAART) and for arm2, rifampicin (RIF) based anti-TB was initiated 4 weeks before HAART. They were followed for 48 (arm1) and 52 (arm2) weeks to collect information on plasma efavirenz exposure (week 4 and 16), immunological and virological outcomes (weeks 0, 12, 24, 36 and 48), liver and renal functional tests and complete blood count (weeks 0, 2, 4, 6, 8, 12, and monthly thereafter) and genotyping for SNPs in EFV metabolizing enzymes CYP2B6*6 c.516G>T, CYP3A5 (*3, *6, *7), UGT2B7 -372G>A and drug transporters ABCB1 (c.3435C>T and c4036A>G) and SLCO1 (388A>G and 521T>C).

Among arm1, patients with CYP2B6 homozygous wild type (fast metabolizers) had significant decrease in mean EFV plasma level between week 4 and 16. Consequently, a significantly large proportion these patients had sub-therapeutic plasma level at week 16 compared to week 4, indicating prolonged auto-induction in these individuals.

Among arm2, only fast metabolizers had significantly lower efavirenz level, 4 weeks after HAART, compared to their counterpart in arm 1 implying that rifampicin induction occurred only in these individuals. Due to prolonged auto-induction in arm1 fast metabolizers, plasma levels between arm1 and arm2 were comparable at week 16 even when stratified by genotype implying that CYP2B6 genetic polymorphisms, but not rifampicin, influence efavirenz exposure after prolonged treatment.

ABCB1 4036A>G SNP was associated with higher EFV levels at week 4 while CYP3A5 (*3, *6, *7) alleles combined, were partially associated with variability in efavirenz metabolic ratio changes between week 4 and 16 among patients with CYP2B6*6/*6 genotype (poor metabolizers).

Ethiopians, even after controlling for genetic and other differences, had lower efavirenz exposure and lower immunological outcomes compared to Tanzanians.

Efavirenz induction of CYP3A4/5 among arm1 was highest (about 5 times) in poor and lowest (about 2 times) in fast metabolizers. After completion of TB therapy, induction dropped to 60% of its maximum, suggesting continued but lower induction of CYP3A4/5 by efavirenz.

EFV with or without rifampicin was associated with mild and transient elevation of liver enzymes. Only CYP2B6*6/*6 genotype and hepatitis C co-infection were associated with such elevations, suggesting that efavirenz is safe for both treatments but caution and monitoring of plasma levels among poor metabolizers and those co infected with hepatitis C should be exercised.

Time on therapy (with and without rifampicin co treatment), CYP2B6c516G>T, ABCB1c4036A>G, ethnicity and CYP3A5 (*3, *6, *7) alleles combined, can be used as priori for Bayesian estimation of individual pharmacokinetic parameters during dose adjustments.

The SNP, CYP2B6c516G>T, influences induction of CYP3A4/5 in gene dose dependent manner, therefore it should be considered during dose optimization of concomitant drugs taken with efavirenz.

It may be necessary to lower doses for concomitant CYP3A4/5 substrate drugs, whose doses were elevated during HIV/TB co treatment, after completion of TB therapy if such drugs have narrow therapeutic range.

LIST OF PUBLICATIONS

- I. Ngaimisi E, Mugusi S, Minzi OM, Sasi P, Riedel KD, Suda A, Ueda N, Janabi M, Mugusi F, Haefeli WE, Burhenne J, Aklillu E. Long-term efavirenz autoinduction and its effect on plasma exposure in HIV patients. *Clin Pharmacol Ther.* 2010 Nov; 88(5):676-84. Epub 2010 Sep 29
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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency Syndrome
ART	Anti-retroviral therapy
CAR	Constitutive Androstane receptors
CIOMS	Council for international organization of medical sciences
CYP	Cytochrome P450
DILI	Drug induced hepatotoxicity
FDA	United states of America Foods and Drugs Administration
HAART	Highly active antiretroviral therapy
HIV	Human Immune-deficiency virus
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
ICH	International Council for Harmonization
IRIS	Immune Reconstitution Inflammatory syndrome
LC-MS	Liquid Chromatography –Mass Spectrometry
MDCK	Madin-Darby Canine Kidney Epithelial Cells
MDR	Multi-Drug Resistant
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
PCR	Polymerase chain reaction
P-gp	P-glycoprotein
PIs	Protease Inhibitors
PLHIV	People living with HIV
PXR	Pregnane X –receptors
RFLP	Restriction Fragment length Polymorphism
SNP	Single Nucleotide Polymorphism
TB	Tuberculosis

XDR	Extended drug resistant
WHO	World Health Organization

1 Introduction

1.1 HIV BURDEN IN TANZANIA AND NEED FOR ITS TREATMENT

Tanzania is an east African country whose population size in 2010 was projected to about 43,188,000 people [1]. Of this population about 3% (1.3 million people) were estimated to be living with HIV by the same year,[2] a lower prevalence compared to 5.7% reported in 2007/2008.[3] The new estimates by UNAIDS indicate a stable prevalence HIV infection where about 1,600,000 people are now living with HIV in Tanzania.[4] One of the reasons for decline in prevalence of HIV/AIDS in Tanzania is the initiation of HIV care and treatment program in 2004 when prevalence was about 7%.[5] By extending lives and restoring productivity of HIV patients who were once severely ill, the program has significantly contributed towards increasing awareness on HIV/AIDS and reducing stigma on people diagnosed of AIDS; this has in turn boosted confidence in many to undergo HIV counseling and testing. Decrease in rate of new infections has also been attributed to reduction of HIV viral load among discordant couples and pregnant women undergoing treatment. Awareness of one's HIV-status has also reduced prevalence of risk behavioral practices among infected and no-infected individuals, underscoring the importance of HIV care and treatment programs in combating the HIV AIDS pandemic. Therefore, absence of HIV care and treatment programs not only could results into loss of lives; impacting social, micro and macro economies of countries but also would fuel the spread of the HIV. Understanding the importance of care and treatment programs, governments and partner health organizations including the WHO recommend expansion of the program to reach all people infected with HIV. However, by 2010 only 57% of people living with HIV (PLHIV) in Tanzania were enrolled into HIV/AIDS care and treatment program. This matched the low number of facilities offering HIV care and treatment services (only about 17% of total health facilities in the country). With the strategic objective by the Tanzania National AIDS Control Program (NACP) to strengthen and scale up HIV comprehensive care and treatment services to provide ART services to 90% of all PLHIV in need of ART by 2012, it is reasonable now to assume a wider coverage of the program in Tanzania.

1.2 TUBERCULOSIS BURDEN AND NEED FOR ITS TREATMENT

Tuberculosis (TB) is an infectious disease typically of the lungs (pulmonary tuberculosis), although other organs can be involved as well (extra-pulmonary tuberculosis). It is caused by different species of mycobacterium (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti* and *M. canetti*) and atypical mycobacterium (*M. Rhodococcus* sp, *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. intracellulare* and *Nocardia farcinica*); but usually it is *M. tuberculosis* that is associated with many cases of TB worldwide. *M. tuberculosis* also appears in different strains which differ in virulence, clinical presentation and hence their epidemiological profiles. The most prevalent spoligotype families of *Mycobacterium Tuberculosis* in Tanzania have been reported to include CAS, LAM, EAI and Beijing and were not been associated with multiple drug resistance in except for 2 cases which were found to have CAS strain.[6]

Incidence of TB (per 100,000 people) in Tanzania by 2012 has been estimated to about 177[7] compared to 297 of the year 2007,[8] a decline attributed to expanded TB and leprosy treatment program. Number of new TB infections has declined due to early detection and treatment of TB cases thanks to TB awareness campaign and strengthened collaboration between TB and HIV care and treatment programs. However, successes rate of TB treatment is still at about 87% [9]indicating late case presentation, inadequate treatment (poor adherence or sub-therapeutic drug concentrations) and drug resistance in some patients all of which could result into continued spread of TB, development and spread of MDR-TB and XDR-TB (extensively drug resistant TB). Indeed cases of MDR-TB in Tanzania range from cumulative 1% (cumulative cases from 1991 to 1999)[10] to 1.1% reported in 2010.[11] Therefore it is important to detect and treat all new cases of TB in order to save lives and prevent new TB infections. It is also important to achieve adequate treatment of TB cases in order to prevent emergence of MDR-TB or XDR TB

1.3 HIV/TB CO MORBIDITY: INCIDENCE, PREVALENCE AND NEED FOR CO-TREATMENT.

Infection with HIV causes a reduced immunity and thus reactivation of latent mycobacterium infections.[12] The HIV infection also increases expression of mycobacteria entry receptors on macrophages, impairs the macrophage bactericidal pathways, deregulate the macrophage chemo taxis, overturn the Th1/Th2 balance and impairs macrophage apoptotic response to M. tuberculosis and thus facilitating bacterial survival.[12] All these results into susceptibility of a HIV patients to new infections which progresses to active pulmonary tuberculosis and/ or extra pulmonary tuberculosis.[12] Similarly, mycobacterium infection in HIV patient accelerates the decline of immunity.[12] exposing the patient to attack by other opportunistic infection and death if not immediately treated after TB diagnosis. Therefore some patients with HIV infections also have active mycobacterium co infection (HIV/TB co infection) and most of these patients seek medical attention for tuberculosis symptoms without prior knowledge of their HIV status. Most often, they present with very low measures of immune function (CD4). In fact, TB diagnosis and treatment clinics have been important sites for HIV counseling and testing of patients diagnosed with TB. Unfortunately, HIV care and treatment clinics are not doing enough to identify HIV/TB co infected people; only about 20% (148,177) of patients with known HIV status have been screened for active TB status in Tanzania.[13] Data available is only on prevalence of HIV/TB co infection among TB patients. The 2012 global TB report indicate that, about 38% of tuberculosis patients were HIV infected in the year 2011 in Tanzania. It further reports that the number of reported cases of HIV/TB co infection increased drastically from below 5,000 in 2006 to around 20,000 in 2009 and has remained stable around this value up to 2011. It is probable that this increase was due to increase in case notification efforts and also due to spread of TB among immune-compromised HIV patients. This emphasizes the need to strengthen TB diagnosis efforts, HIV testing and screening HIV infected patients for TB.

HIV/TB co infection almost always represents advanced stages of both HIV and TB infection and patients are grouped into stage III of the HIV infection progression

according to WHO staging criteria. At this stage treatment of HIV is important and should be done immediately; treatment of TB is equally important at this stage as well, making co treatment of TB and HIV inevitable in these patients. Goals of co treatments are: to reduce the mycobacteria load and therefore make the patients noninfectious as soon as possible after TB diagnosis, to cure TB and prevent emergence of resistant TB strains, to reduce the HIV viral load and hence restore immunity as soon as possible for the patient to fight the TB infection, to achieve remission of the HIV viral load to undetectable levels within 6 month of treatment, to avoid development of HIV resistant strains, to restore the patient quality of life, to avoid immune reconstitution inflammatory syndrome (IRIS) and to avoid pharmacokinetic interactions and overlapping toxicities of anti-TB and anti-retroviral drugs (ARVs). However, achieving these goals has been a challenge due to limited information concerning *factors influencing* pharmacokinetic interactions and overlapping toxicities during co-treatment. It is still uncertain what dose adjustments for antiretrovirals is suitable during HIV/TB co treatment. The appropriate duration of TB treatment is also still uncertain as data indicate higher TB relapse rate among HIV infected compared to uninfected patients when undergoing a 6 month TB treatment while improved outcome was achieved for patients undergoing at least 8 month treatment. It is also still uncertain of incidence, severity, mortality, factors associated, and hazard/risk of overlapping toxicities during HIV/TB co treatment. It is felt that concomitant ARVs and anti-TB result into compounded toxicities; particularly drug induced liver injury (DILI) due to synergism between ARVs (protease inhibitors and non-nucleoside reverse transcriptase inhibitors) and anti-TBs (isoniazid, rifampin, and pyrazinamide). Incidences of DILI are reported to be higher during co treatment compared to HAART alone.[14] Similarly, it is felt that since HIV/TB co infected patients are most often severely immune-compromised, initiation of HAART in these individuals result into rapid immunological recovery (CD4 gain) and viral load decay leading to exacerbation of TB syndromes, particularly, pulmonary infiltrates and respiratory compromise, lymphadenopathy, and neurological deterioration culminating to severe morbidity and mortality (TB-IRIS). In fact, in trials to compare treatment outcomes of early versus late initiation of HAART relative to anti-TB, early initiations were associated with higher incidences of TB-IRIS compared to late initiations, although they were not fatal.[15-18]

Results from several cohort studies and randomized controlled trial indicated that early initiation of ART significantly reduces mortality of HIV/TB co infected patients. From SAPIT and CAMELIA trials, initiation of ARV at less than 8 weeks relative to initiation of anti-TB was a better compared to initiation after the intensive phase of anti-TB[19]. It is now certain that early initiation of antiretroviral improves survival of HIV/TB co infected patients and that when delayed (even for brief durations) mortality increases. Most guidelines now recommend initiation of HAART as soon as the patient is stable to tolerate it and preferably within 2 weeks of initiation of anti-TB.[20, 21]

1.4 HIV CO-MORBIDITIES: INCIDENCE, PREVALENCE AND NEED FOR CO-TREATMENT

As a result of immunity deterioration, HIV patients become susceptible to infectious diseases. The pattern of such diseases usually depends on immune status of the patients since susceptibility to different diseases is determined by stage of immune deterioration. Tuberculosis, malaria, bacterial pneumonia, candidiasis, herpes zoster, oral and genital herpes, staphylococcal skin infections and septicemia can occur in early HIV diseases as they could occur even in immune competent individuals; however, in HIV patients they are associated with greater morbidity, severity and longer recovery time. Malaria infection could also be a risk factor for HIV infection or transmission. It is most probable that immune activation during malaria infection elevates expression of CCR5 thus increasing susceptibility to HIV infection.[22, 23] Since HIV transmission is associated with higher viral load, the elevation of viral load that occur during malaria infection of HIV patients might contribute to enhanced HIV transmission in regions prevalent with malaria infections.[24, 25] These reciprocal interactions between malaria and HIV increase the incidences/prevalence of HIV/malaria co infection in malaria endemic regions.[25, 26] In similar fashion, sexually transmitted infections (STI) particularly genital herpes simplex, syphilis and gonorrhea increases susceptibility to HIV infection and for patients already infected they become more infectious.[27] Therefore HIV is also associated with higher incidences of HIV/STIs co infections.[27, 28] Helminthic infection has also been suggested to be an important risk factor for HIV infection and vice versa, [29, 30] such that, the incidences of HIV/helminthic co-infections in helminthes endemic areas are high.[31]

On the other hand, late stage of HIV infection is associated with diseases from pathogens/normal flora and malignancies that would not occur in an otherwise immune-competent individual (opportunistic infections). Most common of such opportunistic diseases include: cytomegalovirus (CMV) retinitis commonly associated with CD4 count less than 50, Mycobacterium avium complex (MAC) causing general sick feeling and weight loss, commonly associated with CD4 count less than 75, Pneumocystis pneumonia (PCP) commonly occurring at CD4 under 200, cryptococcal meningitis commonly occurring when CD4 is below 200, and Toxoplasmosis a protozoa infection occurring when CD4 count is under 100. The most common AIDS defining malignancies are also due to opportunistic viruses and they include Kaposi's sarcoma (human herpes virus 8), high-grade B-cell non- Hodgkin's lymphoma (Epstein-Barr virus) and invasive cervical cancer (human papilloma virus).

It has been determined however that, morbidity and mortality due to infectious diseases in HIV infected population is declining due to the current policy to initiate HAART at CD4 count above 350/mL and also due to prophylaxis against opportunistic infections.

Non infectious diseases however are becoming more important causes of co-morbidity and mortality in HIV infected patients. Such diseases are either related to age, HIV infection or are adverse outcomes of antiretroviral therapies. The risk for non-infectious diseases increases with age, therefore as HIV infected patients grows older the risk of non-communicable co-morbidities increases. Furthermore, HIV infection trigger a

cascade of immunological events sustained by HIV and non HIV related antigens that ultimately lead to immunological senescence and inflammatory aging thus accelerating the normal aging process. Specific antiretrovirals also interfere with specific physiologic and metabolic processes leading to adverse outcomes. Therefore despite adequate viral suppression, HIV infection and HAART have been associated with premature occurrence of age related diseases like diabetes mellitus, cardiovascular complications, renal diseases, musculoskeletal and neuropsychiatry disorders.

Several studies among HIV infected patients on HAART have determined that thymine analogs (stavudine and zidovudine) [32-35] and the protease inhibitors (indinavir, lopinavir/ritonavir)[35-37] are associated with of metabolic syndromes particularly lipodystrophy, insulin resistance, diabetes mellitus and lipohypertrophy. Risk for diabetes among patients with other underlying risk factors like genetic predisposition and unhealthy life style is therefore increased by ART use. Age specific relative risk for diabetes among HIV patients compared to non HIV patients was reported to range from about 8 in individuals age 18-24 to 2 in individuals above 65 years old.[38] Several studies have reported incidence of diabetes in HIV patients to range from 1 to 10 per 100 person years.[35, 37, 39] A cross section review of medical file of HIV/AIDS patients attending Muhimbili National Hospital in Tanzania determined about 25% prevalence of diabetes mellitus in this patient population.[40]

Although HAART reduces the risk for cardiovascular events, it has been observed that some risk persist even after adequate suppression of the viral load. This is attributed to persistent hyper-immunological events causing pro-atherogenic inflammations which lead to atheroma formation and hence cardiac diseases. Therefore risk for cardiac diseases increases with time on HAART. Certain antiretrovirals also increases this risk, for example, protease inhibitors have been associated with higher relative risk for myocardial infarction even after adjusting for lipid profiles. Tenofovir and abacavir have also been associated with increased risk for myocardial infarction while stavudine and zidovudine have not.

The incidences of non AIDS related cancer is also higher among HIV patients on HAART compared to general population, e.g. in a large HIV cohort incidence of basal cell carcinoma and melanoma were higher compared to the general population.[41] Similarly, incidences of cervical, anal, liver, and lung cancer, and Hodgkin lymphoma are higher in HIV patients despite being on HAART.[42-45] Accelerated aging of the immune system and therefore poor restoration of immunity despite HAART and aging of the HIV patient population itself account for reported higher prevalence of cancer among HIV patients.

Certain antiretrovirals have also been found to increase the age related risk for osteoporosis, bone fracture and frailty among HIV infected men and women. A meta-analysis determined that patients on HAART were more than 2 times likely to have osteoporosis compared to ART naïve HIV patients and among those on ARVs, patients on protease inhibitors were associated with 1.6 odds of having osteoporosis than others.[46] Tenofovir has also been associated with significant decline in bone mineral density and osteomalacia.[47, 48]

Other non-infectious complications that may be prevalent in HAART experienced HIV patients includes, Hypertension, Pulmonary hypertension, Cancer, Liver failure, Kidney failure, Peripheral neuropathy, depression, neuro-cognitive motor disorders and HIV associated dementia.[49]

1.5 CURRENT THERAPIES FOR HIV

Treatment of HIV/AIDS has evolved from mono-therapies to various combinations therapies. The first attempt to treat the disease with Zidovudine monotherapy encountered high rate of treatment failure and rapid emergence of resistant HIV strains. Discovery of other antiretrovirals targeting different stages of the HIV life cycle enabled treatment of HIV using drug cocktails. Such cocktails have also evolved from least effective or intolerable to highly effective and tolerable combinations. Combinations involving drugs from same class (mostly NRTIs) thus targeting same stage of HIV life cycle were clinically inferior to combination of drugs targeting different stages. Currently nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) form a back bone of most first line cocktails where by 2 NRTI are combined with either 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), Protease inhibitor (PI), fusion inhibitor (FI) or integrase inhibitor (II). Alternatively first line cocktails may be composed only of triple NRTIs when other drug classes are contraindicated or unavailable.

The choice of the 2 NRTIs to form the backbone has mostly depended on safety and resistance profile of the combination and possibility for salvage therapy if resistance emerges with first line therapy. For example zidovudine (AZT)/lamivudine (3TC) and stavudine (D4T)/lamivudine (3TC) are preferred combinations because they delay development of primer unblocking mutations (thymine analog mutations, TAM) which are responsible for multi-NRTI resistances. They also prevent development of M184V mutation responsible for resistance against 3TC and tenofovir (TDF). AZT/didanosine (DDI) or D4T/didanosine (DDI) combinations are not preferred because they lead to multi-NRTI resistance due to development of TAM and Q151M mutation.[50, 51] Although abacavir (ABC)/3TC combination would lead to M184V or M184 plus L74V mutations and salvage therapy with Zidovudine/3TC would restore susceptibility to 3TC, this combination is reserved for **second line HIV therapy** due to toxicity profile of abacavir (causing fulminant hepatic failure in individual with HLA-B5701 genotype). The TDF/3TC combination could lead to rapid emergence of M184V and K65R mutations responsible for resistance to lamivudine and tenofovir respectively[50]. Although salvage therapy with zidovudine containing regimen can restore susceptibility to 3TC, the use of TDF/FTC combination is associated with M184V mutations only and therefore preferred over TDF/3TC.[50] This backbone is also preferred for combination with efavirenz because it makes a cocktail of drugs with long half-lives and therefore suitable for once daily dosing.

In resource rich settings the choice of which drug class (among NNRTIs, PIs, FIs and II) to combine with NRTIs to make **first line therapies** has mostly depended on resistance testing to guide selection of suitable ARVs, but in resource limited settings it has depended on prevalence of primary or transmitted resistances, resistance barrier

of drug class, pill burden, side effects and toxicity profile of a drug class and costs of drugs.

Cost, limited resources and medication inconveniences associated with fusion and integrase inhibitors limits their use for treatment of HIV infection in developing countries. For example, while integrase inhibitor maraviroc requires HIV tropism test before it is prescribed, the fusion inhibitor enfurvitide requires subcutaneous injection twice daily. Furthermore these drugs are unaffordable to most developing countries. Therefore, it has been recommended by the WHO panel of experts to reserve these drugs to a **third line of HIV therapy**. [52]

Although resistance to NNRTIs develops relatively faster than to PIs (only one single nucleotide mutation in reverse transcriptase gene can cause cross resistance to all NNRTI while resistance to PIs requires accumulation of mutations in protease gene), they are preferred over PIs for first line therapies because of low pill burden and good tolerability hence better prospects of adherence of first line therapy. Most protease inhibitors are associated with gastrointestinal disturbances, hyperlipidemia, hyperinsulinemia, lipohypertrophy and fat redistribution. PIs including atazanavir have been associated with elevation of plasma cholesterol and triglycerides. They have been associated with increased risk for myocardial infarction and blood pressure elevation. Except for newer generation of protease inhibitors (darunavir and atazanavir) most PIs require twice or more daily drug dosing and others like ritonavir boosted lopinavir have variable dosing schedules in different age groups.

In treatment naïve patients with susceptible HIV, NNRTIs are very potent leading rapid suppression of viral load. In ACTG 5142 study efavirenz based first line therapy had superior virological outcome but lower CD4 rise compared to ritonavir boosted lopinavir first line. [53, 54] But was also associated with more drug resistances to 2 ARV classes. [53] In settings where genotyping for drug resistance cannot be done but prevalence of primary or transmitted resistance is less than 5%, NNRTIs are first line drugs of choice and **PIs form an important component of second line therapies** in patients who experience treatment failure from first line therapy. [52]

The choice of which of first generation NNRTIs to combine with 2NRTIs to make first line HAART has also depended on safety concerns, pill burden, drug-drug interactions and cost. Systematic reviews according to Cochrane guidelines have indicated equivalent efficacies of nevirapine compared to efavirenz based HAART. However, nevirapine use is limited by concerns over its association with higher incidence or prevalence of hepatotoxicity [55, 56] especially in HIV/hepatitis B or C co infected patients, women and men with absolute CD4 count above 250 and 400cell/mL respectively. [57, 58] Nevirapine was also associated with hepatic fibrosis in patients co infected with hepatitis B or C and therefore it has been advised to avoid this drug in such patients. Nevirapine use is also associated with cutaneous skin reactions manifested as rash and sometimes severe skin rash especially Steven-Johnson syndrome or toxic epidermal necrosis [55, 59] especially among patients with higher CD4 count as indicated above.

Nevirapine 200mg twice daily was associated with fewer toxic events compared to 400mg once daily and is therefore recommended for long term HAART.

Efavirenz is associated with lesser frequency of hepatotoxicity than nevirapine[55, 60] although some other analyses indicate no significant differences between the two drugs.[57] However, up to date, limited evidence exist as to which is associated with more hepatotoxic events compared to the other.

Although efavirenz is costly compared to nevirapine it is still a preferred drug due to tolerable side effects and simpler dosing schedule and therefore higher possibility for adherence. Efavirenz is associated with neuropsychiatric toxicity most commonly manifested as dizziness, hallucination, insomnia, vivid dreams or nightmares, inability to concentrate, anxiety and depression. Such condition are most often mild and resolve within 2-4 weeks, but for some individual particularly those with underlying neuropsychiatry problems they may be severe requiring switching from EFV to NVP.

Efavirenz was once contraindicated for use in pregnant women and children below 3 years old due to concerns over its teratogenicity effect observed in animal models. Recent evaluations of this toxicity in humans have indicated that it may be safe for use in these groups of patients.[61, 62]

In general therefore, nevirapine and efavirenz can be equally chosen for initiation of first line therapy but choice for nevirapine is affected by availability laboratory facilities to monitor liver functional test parameters. Preference for efavirenz over nevirapine and vice versa is also influenced by co-treatments where by clinically significant changes to pharmacokinetic parameters of relevant drugs should be avoided as discussed further below.

In situations where NNRTI or PIs are contraindicated or unavailable due to cost, triple NRTIs combinations can be alternative for first line treatment of HIV/AIDS. Although inferior to AZT/3TC/EFV[63], the triple NRTI combination of AZT/2TC/ABC is recommended as alternative in most guidelines. This is because subsequent analysis from observation cohorts demonstrated equivalent immune-virological outcomes of AZT/2TC/ABC compared to AZT/2TC +NNRTI.[64] A DART trial sub-study also reported that AZT/2TC/ABC combination achieved similar clinical outcomes to AZT/2TC/NVP combination although it had inferior virological and immunological outcomes. Furthermore compared to other triple NRTIs combinations[65, 66], AZT/3TC/ABC was associated low rate of mutations (mostly M184V and other NRTIs mutations but not TAMs)[63] that could be easily controlled with salvage second line therapies. However, AZT/3TC/ABC combination may be limited by hypersensitive to ABC in patients with HLA B5701 allele.

AZT/2TC/TDF combination is of interest particularly in resource limited setting where HLA 5701 allele cannot be genotyped to guide ABC use. Two studies have demonstrated that AZT/2TC/TDF combination achieves reasonable clinical, immunological and virological outcomes.[67] It is also observed that presence of AZT in this combination limits development of resistance against TDF (K65R mutation).[50] Therefore the combination has been proposed as an alternative to NNRTI+2NRTI combination when NNRTI is contraindicated or unavailable.[52]

1.6 CURRENT THERAPIES FOR HIV/TB CO TREATMENT

Tuberculosis treatment during HIV/TB co treatment is done in a similar way to treatment of tuberculosis alone. The WHO recommends that, for all new cases of all types of TB (except TB meningitis, bone and joint TB) patients should undergo directly observed daily intake of fixed dose combination (FDC) of 4 drugs namely rifampicin (R), isoniazid (I), pyrazinamide (Z) and ethambutol (E) for a period of two month (intensive phase treatment), followed by a four month directly observed daily treatment with rifampicin and isoniazid (continuation phase). For TB meningitis and bone and joint TB a 9-12 month course of treatment is recommended. Rifampicin and Isoniazid therefore form a back bone of fixed dose combinations for TB treatment throughout a course of therapy. Although rifampicin is a potent inducer of many drugs elimination pathways (enzymes and transporters) thus reducing plasma levels of concomitant medications for co-morbid conditions, its congener rifabutin (less potent inducer) is not affordable in resource poor countries. Therefore rifampicin is still a preferred anti-TB backbone in most countries. Evidences supporting current TB treatment guidelines are summarized in the 4th edition of WHO-Treatment of tuberculosis guidelines.[20]

There are limited choices of ARVs combinations for use during HIV/TB co treatment due to possibility of clinically significant drug-drug interaction and shared toxicity between certain anti-TB and antiretrovirals. Of particular importance is the pharmacokinetic interaction between rifampicin and most antiretrovirals through induction of drug metabolizing enzymes. Rifampicin induces cytochrome P450 enzymes particularly CYP3A4/5, CYP2B6, CYP2A6 and uridyl diphosphate glucuronyl transferase enzymes, particularly UGT2B7. These enzymes are responsible for metabolism of efavirenz (CYP3A4/5, CYP2B6, CYP2A6 and UGT2B7), nevirapine (CYP3A4/5, CYP2B6), protease inhibitors (CYP3A4/5) and zidovudine (UGT2B7). Therefore as indicated in table 1, rifampicin is associated with significant reduction in plasma exposure of protease inhibitors, delavirdine, nevirapine, efavirenz and zidovudine.

Table1: Effect of Rifampicin co treatment on ARV exposures

<i>Drug</i>	<i>Effect on AUC</i>	<i>Comments</i>
<i>Protease inhibitors</i>		
Saquinavir	40 % decrease[68]	Co-treatment not recommend
Ritonavir	42 % decrease[68]	Co-treatment not recommend
Indinavir	89 % decrease [69]	Co-treatment not recommend
Nelfinavir	82 % decrease[70]	Co-treatment not recommend
Amprenavir	81 % decrease[71]	Co-treatment not recommend
Lopinavir	75% decrease [72]	Co-treatment not recommend
Atazanavir	57 % decreases[73]	Co-treatment not recommended

<i>Nonnucleoside reverse transcriptase inhibitors (NNRTI)</i>		
Delavirdine	96 % decrease[74]	Co-treatment not recommended
Nevirapine	31- 40 % decrease[75-77]	Avoid lead in dosage, 200 twice daily, Some recommend 50% dose increment is [78]
Efavirenz	22-26% decrease [79, 80]	Mixed recommendations regarding efavirenz dose adjustments
<i>Nucleoside Reverse transcriptase inhibitors</i>		
Zidovudine	47% decrease[81]	No dose adjustment since intracellular levels may be adequate due to accumulation of AZT-triphosphate.
Others	None	

After initial studies indicated that rifampicin-efavirenz co administration decreased efavirenz exposure by 26% [80], initial recommendation were to increase efavirenz dose by up to 800mg.[82, 83] Studies involving few and predominantly Caucasian patients found that this increment during co treatment achieved similar exposure to standard dose and was effective and safe; [79, 84] supporting efavirenz dose increment during HIV/TB co treatment. However, a randomized controlled trial, comparing safety and efficacy of efavirenz 800mg versus 600mg during HIV/TB co treatment, found that 800mg was associated with more neuropsychiatric and hepatotoxic events.[85] A follow up FDA review of clinical cohort studies concluded, based on data available by 2007, that standard dose (600mg) was adequate during HIV/TB co treatment.[86] Following this and other expert opinions, it was recommended in most guidelines to avoid efavirenz dose increment during HIV/TB co treatment but use standard dosing during HIV and HIV/TB co-treatment. A second FDA review including more data available by 2011 has concluded that efavirenz dose should be increased to 800mg for patients weighting > 50kg [87]; supporting 2009 and 2011 British HIV association (BHIVA) recommendations to increase the dose among patients weighting > 50kg.[21] However, a caveat in most of these studies and recommendations is lack of patient stratification by CYP2B6 genotype. Functional polymorphism in genes coding for drug metabolizing enzymes results into phenotypes which differ in magnitudes of rifampicin induction. Therefore while there is no appreciable induction among poor metabolizers there may be clinically significant induction among intermediate and fast metabolizers. One dose fit all recommendation may not be applicable for efavirenz.[88]

In several cohort studies attempts to use nevirapine standard doses, including a 2 week lead in with 200mg once daily dosing, led to poor virological outcomes in the nevirapine arm.[89-91] This was attributed to sub-therapeutic levels in majority of patients during the lead in period. Attempts to increase nevirapine dosing from 200mg to 300mg twice daily, following a recommendation from a modeling study [78], was associated with more adverse events in the higher dose arm that led to premature termination of the trial.[89] Standard nevirapine dosing without the 2 week lead in during HIV/TB co treatment lead to comparable safety outcomes when compared to standard efavirenz HIV/TB co treatment.[92] Therefore in patients contraindicated to efavirenz, nevirapine 200mg twice daily without lead in is recommended.[92, 93]

Where both efavirenz and nevirapine are contraindicated or are unavailable, triple NRTIs is used for HIV/TB co treatment. However, it has been observed that this approach is inferior to efavirenz based HIV/TB co treatment.[63]

Attempts to co administer elevated doses of protease inhibitors with rifampicin to health volunteers resulted in hepatotoxic events especially when boosted PI followed rifampicin: [94-96] some plausible physiological explanations were offered.[94] Therefore, although implication of these events in health volunteers may be unclear to HIV/TB co treatment in patients, boosted PIs are not currently recommended for HIV treatment among patients already on rifampicin based anti-TB. However, patients already on boosted PI (second line therapies) can be treated with rifampicin based anti-TB.

Shared adverse events and toxicities between antiretrovirals and anti-TBs have also guided the choice of appropriate antiretroviral combination to be used during HIV/TB co treatment. The dideoxy-nucleotide derivatives, stavudine, didanosine and zalcitabine are avoided because they are commonly associated with toxic neuropathy, which is also a common isoniazid associated side effect of TB treatment. HIV/TB co treatment in which both isoniazid and stavudine were present was associated with increased risk for intolerable peripheral neuropathy leading to stavudine discontinuation.[97] Pyridoxine is usually prescribed to alleviate isoniazid associated polyneuropathy in patients undergoing TB treatment. However, the efficacy and appropriate pyridoxine dosage during HIV/TB co treatment are still uncertain [98], leaving only one option, to avoid or withdraw dideoxy-nucleotide derivative ARVs. Other anti-tubercular drugs with neurological toxicities include ethambutol (optic neuritis), ethionamide, cycloserine and streptomycin.

In addition to its pharmacokinetic interaction with rifampicin, nevirapine has been associated with higher frequencies of hepatotoxic events[91] compared to efavirenz and therefore relegated to second choice during HIV/TB co treatment due to shared hepatotoxicity from pyrazinamide, isoniazid and rifampicin. In fact, nevirapine was associated with more hepatotoxic events requiring its discontinuations during HIV/TB co treatment compared to efavirenz.[91, 99]

1.7 DRUG METABOLISM

Drug metabolism is enzyme catalyzed transformation of drug from one chemical entity to another so that they can be easily excreted from the body. Some drugs require two sequential stages of biotransformation (phase I and II) to achieve enough hydrophilicity for excretion while for some other drugs, either phase I or phase II only is required for excretion. Phase I reactions introduces or exposes hydroxyl, sulfhydryl or amine groups thus increasing drug solubility. Such groups are also functional site for phase II reactions which are conjugation reactions combining endogenous hydrophilic compounds (glucuronic acid, glycine, sulphate) with phase I products or parent drugs. Most phase I and II reactions occur in the liver as it expresses most of the xenobiotic biotransformation enzymes. Such enzymes includes cytochrome P450 (CYPs), monoamine oxidases (MAO), flavine monooxygenase (FMO) and uridine diphosphate-glucuronosyl transferase (UGT). The CYP enzymes are most important as they are involved in phase I metabolism of up to 80% of drugs in clinical use. Table 2 below summarizes some of CYP enzymes, some of their substrates, inducers and inhibitors.

Table 2: Examples of in vivo substrate, inhibitor, and inducer of specific CYP enzymes

CYP	Substrates	Inhibitors	Inducers
1A2	Amitriptyline, Clomipramine, Clozapine, Imipramine, Theophylline, R-Warfarin, Caffeine	Amiodarone, Cimetidine, Ciprofloxacin, Fluvoxamine	Omeprazole, Broccoli, Brussels sprouts, Cigarette Smoking
2A6	Aflatoxin B1, halothane, losigamone, methoxyflurane, cotinine, nicotine, valproic acid, efavirenz	Grapefruit juice, flavonoids, ketoconazole, methoxsalen, pilocarpine, tranlycypromine	rifampicin, phenobarbital
2B6	Bupropion, cyclophosphamide, efavirenz, ifosfamide, methadone, sorafenib	thiotepa, ticlopidine	Phenobarbital, phenytoin, rifampicin, efavirenz
2C19	Amitriptyline, Citalopram, Clopidogrel, Diazepam, Lansoprazole, Omeprazole, Pantoprazole, Proguanil, Propranolol, R-Warfarin	Chloramphenicol, Cimetidine, Ketoconazole, Lansoprazole, Omeprazole, Oxcarbazepine, Pantoprazole	Rifampicin, Carbamazepine
2C8	amodiaquine, cerivastatin,	Gemfibrozil,	rifampicin

	paclitaxel, repaglinide, sorafenib, torsemide	trimethoprim, glitazones, montelukast, quercetin	
2C9	Celecoxib, Diazepam, Diclofenac, Fluoxetine, Fluvastatin, Glibenclamide, Glimepiride, Glipizide, Ibuprofen, Irbesartan, Losartan, Meloxicam, Naproxen, Phenytoin, S-Warfarin	Amiodarone, Fluconazole, Isoniazid	Rifampicin, Secobarbital
2D6	Amitriptyline, Carvedilol, Chlorphenamine, Chlorpromazine, Clomipramine, Codeine, Dextromethorphan, Donepezil, Duloxetine, Fluoxetine, Haloperidol, Imipramine, Metoclopramide, Metoprolol, Ondansetron, Oxycodone, Paroxetine, Propranolol, Tamoxifen, Timolol, Tramadol, Venlafaxine	Amiodarone, Bupropion, Celecoxib, Cimetidine, Citalopram, Clomipramine, Duloxetine, Escitalopram, Fluoxetine, Levomepromazine, Paroxetine, Quinidine, Sertraline, Terbinafine	Rifampicin
2E	Enflurane, halothane, isoflurane, methoxyflurane, sevoflurane, acetaminophen, chlorzoxazone, ethanol, N, N-dimethylformamide, theophylline	diethyl-dithiocarbamate, disulfiram	Ethanol, Isoniazid
3A4/5	Amiodarone, Amlodipine, Aripiprazole, Atorvastatin, Buspirone, Ciclosporin, Clarithromycin, Dexamethasone, Diazepam, Diltiazem, Domperidone, Erythromycin, Estradiol, efavirenz, Felodipine, Fentanyl, Finasteride, Hydrocortisone, Indinavir, Lercanidipine, Methadone, Nelfinavir, Nifedipine, Progesterone, Ritonavir, Saquinavir, Sildenafil,	Cimetidine, Clarithromycin, Diltiazem, Erythromycin, Fluconazole, Indinavir, Itraconazole, Ketoconazole, Nelfinavir, Ritonavir, Saquinavir, Verapamil, Grapefruit Juice	Carbamazepine, Efavirenz, Nevirapine, Oxcarbazepine, Phenobarbital, Phenytoin, Pioglitazone, Rifampicin, St John's Wort

Simvastatin, Testosterone, Warfarin	Tacrolimus, Verapamil, R-		
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Sources: <http://medicine.iupui.edu/clinpharm/DDIs/table.aspx>., **P450 Drug Interaction Table**: Accessed on March 7, 2013 and <http://www.resourcepharm.com/pre-reg-pharmacist/substrates-inhibitors-and-inducers-of-the-major-CYP450-enzyme.html>., **Some Common Substrates, Inhibitors and Inducers of CYP450 Isoenzymes**: Accessed on March 7, 2013

1.8 PHARMACOGENETICS

In clinical practice, patients taking same medication at similar doses may respond differently to medications (some may experience treatment failure and others adverse reactions). Inherited and acquired traits have been attributed to this variability in drug response. The inherited traits influencing drug response may include: poor drug absorption, distribution, metabolism and renal excretion. Abnormal drug receptors also have been implicated to influence drug response. The inherited variability in drug response is most often attributed to small variations (changes) in DNA sequences for genes coding drug metabolizing enzymes, transporters and receptors. Such variations results from substitution of one nucleotide for another in a course of human evolution thus creating single nucleotide polymorphisms (SNPs) in a population. Knowledge of such SNPs can be used to predict drug response for an individual.

Pharmacogenetics therefore deals with examining inherited genetic variations that dictates drug response (exposure and clinical outcome) and explores ways by which such variations can be used to predict drug response. Most often pharmacogenetic studies involves DNA sequencing for genes encoding drug metabolizing enzymes and/or drug transporters in order to identify SNPs associated with variability in drug exposure and clinical response. Several SNPs exist in most CYP enzymes resulting into variable expression of the enzymes among individuals. A summary of SNPs for various CYPs can be found at <http://www.cypalleles.ki.se>. Frequencies of the SNPs differ among population and consequently frequencies of abnormal drug responses also differ. Therefore one dose may not fit all individuals and findings in one population may not always be extrapolated to others. Identification of SNPs influencing drug response and their relative frequencies among population may help in optimization of drug therapy.

1.9 ENZYME INDUCTION

One of the acquired traits that influence drug response is extensive drug metabolism due to induction by concomitant drugs or xenobiotics. As depicted the table 2, most of the CYP enzymes can be induced. Most often induction is due to increased gene

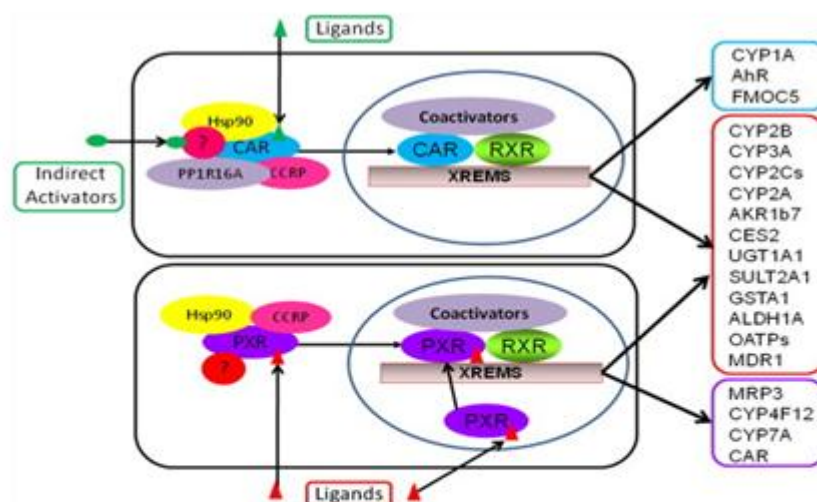


Figure 1. Schematic illustration of the activation mechanisms and target genes of CAR and PXR. CAR can be activated by either direct (ligand binding) or indirect mechanisms, while activation of PXR is purely ligand dependent. CAR and PXR shared target genes are grouped in a red box, CAR-specific targets in a blue box, and PXR-specific targets in a purple box (Adopted from Tolson, A.H and Wang, H., *Advanced Drug Delivery Reviews*, 2010. 62(13): p1238 – 1249,

expression at the protein level as a result of enhanced gene transcription. However, induction may also be through enzyme protein stabilization and increased mRNA translation.[100]

Induction through enhanced gene transcription follows several steps which include: inducer (ligand) binding to soluble nuclear receptors (NR) in the cytosol; translocation of the inducer-receptor complex to the nucleus; binding of the complex to xenobiotic response elements (near or in the gene promoter region); activation of the gene promoter to enhance gene transcription.

Several nuclear receptors exist, but constitutive androstane receptor (CAR) and pregnane X receptor are most important as they promiscuously bind several ligands and collaboratively activate transcription of a broad spectrum of distinct and overlapping genes encoding phase I, phase II drug-metabolizing enzymes and transporters. However, rate and magnitude of enzyme induction through these NRs differ among ligands suggesting differential activation of rate limiting step in the induction pathway. There exist agonist, partial agonist and antagonists for the nuclear receptors.[101] Figure 1, is a schematic representation of induction of CYP enzymes through the nuclear receptors CAR and PXR.

1.10 DRUG-DRUG INTERACTION DURING HIV COMORBIDITIES TREATMENT

There is a concern with regard to safety and efficacies of current HIV/malaria co treatments. With regard to safety, amodiaquine has been associated with increased incidences of neutropenia and hepatotoxicity when concomitantly given with zidovudine and efavirenz based HAART respectively.[102, 103] With regard to efficacy some PIs and NNRTI significantly induce or inhibit drug metabolizing enzymes and transporters of which most antimalarials are substrates thus rising concern as to adequacy of exposure to such antimalarial.[104]

Hormonal contraception remains one of the preferred and practiced methods of pregnancy prevention among women.[105-107] However, clinically significant drug-drug interaction might exist between low dose contraceptives (pills) and NNRTIs and PIs. Anecdotal reports indicate that normal doses of oral contraceptives failed to prevent pregnancy in some women on HAART.[108] Pharmacokinetic studies have reported significant decrease in plasma exposure to estrogen and progesterone among women on NNRTI and some PI based HAART.[109, 110] Dose adjustments for oral contraceptives among women on ritonavir boosted atazanavir based HAART are recommended.[111]

The calcium channel blockers (diltiazem, verapamil and dihydropyridines derivatives) are metabolized by CYP3A which is induced by Efavirenz and nevirapine. The Liverpool HIV Pharmacology Group recommends dose adjustment of calcium channel blockers during co treatment.[112]

Co administration of sildenafil or doxazosin with efavirenz or nevirapine may lead to significant reduction in exposure to such anti-hypertensives while co administration with isosorbide dinitrate may lead to increased production of the active substance nitric oxide. Monitoring of clinical effect and dosage adjustments are therefore advised.[112]

Bonsetan is also an inducer of CYP3A such that co administration with efavirenz could potentially lead to significant decrease in plasma exposure to both. Monitoring of plasma exposure to efavirenz is therefore recommended.[112]

Losartan, an anti-hypertensive metabolized by CYP2C9 to an active metabolite; when co administered with efavirenz, plasma exposure to active metabolite could potentially be decreased since in-vitro studies have indicated that efavirenz inhibits CYP2C9.[112]

Clopidogrel is a pro-drug that is converted to its active metabolites via CYP3A4, 2B6, 2C19 and 1A2. Therefore efavirenz could increase exposure to active metabolite for clopidogrel. On the other hand clopidogrel inhibits CYP2B6 and could potentially increase efavirenz concentrations. It is recommended therefore to monitor both, clinical response to clopidogrel and efavirenz plasma levels during co administrations.[112]

Most lipid lowering agents (atorvastatin, lovastatin, pravastatin and simvastatin) are substrates for CYP3A, therefore their exposure significantly decreased when co

administered with efavirenz or nevirapine. It is recommended to monitor cholesterol levels periodically and adjust doses where applicable.[112]

Glibenclamide is metabolized mainly by CYP3A4 and therefore Efavirenz could potentially decrease its plasma exposure. It is recommended to monitor clinical effect and increase glibenclamide dosage when needed.[112]

Glipizide is metabolized mainly by CYP2C9 and therefore its plasma exposure could potentially be increased by efavirenz co co-administration. It is therefore recommended to monitor clinical effect and decrease glipizide dosage when necessary.[112]

Pioglitazone is metabolized mainly by CYP2C8 and to a lesser extent by 3A4, 1A2 and 2C9. In vitro data indicated strong inhibition of CYP2C8 by efavirenz such that co-administration could potentially increase pioglitazone concentrations. It is therefore recommended to monitor clinical effect and decrease pioglitazone dosage when necessary. [112]

1.11 DRUG INDUCED LIVER INJURY

Drug induced liver injury (DILI) is a hepatocellular chemical assaults which results into leaking of liver enzymes into plasma. Such assaults (whether it is direct stress on liver cells, impairment of mitochondria function or specific autoimmune reactions) cause changes in mitochondria permeability and hence triggers programmed cell death (apoptosis) or necrosis.

Generally there are rare incidences of DILI during normal clinical practice as most drugs pass through stringent scrutiny during pre-clinical and clinical drug development to identify their potential to cause DILI. Drugs showing potential to cause DILI are not approved for marketing. However, some drugs associated with very low true incidence of DILI can pass through the scrutiny. For such drugs cases of DILI are observed during clinical use when a multitude of patients have been exposed to the drug. Diagnosis of DILI is difficult due to several challenges and thus incidences of DILI in most settings are unknown. It is currently assumed that the worldwide incidence of DILI is about 14 cases per 100,000 person years

One of the challenges for diagnosis of DILI is that there are no pathognomic findings specific for DILI. Features of liver injury due to DILI are similar to those due to any other causes (infections, autoimmune, allergic disorders, cancer). To diagnose DILI therefore, one has to rule out all other causes first.

Another challenge is the low sensitivity or specificity of the currently used biomarkers for **liver injury**. Liver enzymes can also be found in other tissues and therefore injury to such tissue is associated with elevation of the enzymes as well. Currently, alanine amino transferase (ALT), which is abundant in the liver compared to other tissues and therefore more specific than aspartate amino transferase (AST), which is less abundant in the liver compared to myocardium, is a preferred biomarker. But it also has a limitation that mild elevations are not specific enough to indicate liver injury, they often are spurious and transient. Efforts are ongoing to identify and qualify other biomarkers of liver injury including: serum F protein,

arginase I, glutathione-S-transferase alpha (GST α), sorbitol dehydrogenase, glutamate dehydrogenase, paraxonase, malate dehydrogenase, and purine nucleoside phosphorylase.

Assessment of impairment of liver function is more specific at indicating liver injury than elevation serum enzymes levels. Impaired liver function is reflected by increase in prothrombin time (indicating failure to synthesize plasma protein), hepatic encephalopathy and elevations of plasma levels of indirect bilirubin (indicating a failure to conjugate bilirubin) and hence total bilirubin (TBL). Measurement of serum levels of direct and total bilirubin is commonly practiced to assess liver function. But usefulness of these biomarkers is limited by possibility that their serum elevation might be due to hemolysis, intrahepatic or extra hepatic cholestasis (non-inflammatory and inflammatory inhibition/repression of transporters for conjugated bilirubin and bile acids) and physical destruction of bile duct or obstruction of bile flow[113]. Since the liver secretes alkaline phosphatase (ALP) into bile, cholestasis is associated with elevation of serum ALP levels

Therefore for an individual patient, elevation of TBL, when associated with parallel elevation of serum ALT and AST with no elevation of serum ALP, is indicative of hepatocellular damage (failure to conjugate bilirubin). If other causes than drugs are ruled out then diagnosis of DILI is made. Elevations of serum TBL without corresponding increase in either ALT or ALP might indicate hemolysis or inhibition of liver bilirubin uptake[114] not associated with hepatocellular damage nor cholestasis.

When diagnosed early DILI is not fatal due to immediate withdraw of the culprit drug. But some drugs may have persistent hepatotoxicity even after withdraw, resulting into fatal hepatic failure if liver transplant is not done.

Most often jaundice is a hallmark of severe liver injury. Jaundice occurs after a period of bilirubin accumulation due to cumulative damage of hepatic cells. It is therefore important to monitor TBL and ALT levels rather than clinical symptoms of liver injury which appear late and may not be specific (e.g. fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever and rash).

Guidelines have been developed to harmonize criteria for early detection of DILI in clinical practice. In its guidelines for reporting adverse drug reactions, the Council for international Organization of medical sciences (CIOMS) defines DILI as an increase of over twice the upper limit of the normal range in ALT or conjugated bilirubin (CB), or a combined increase in AST, ALP and TBL, provided that one of these is present in excess of twice the normal level.[115] However, for some drugs these elevations are transient and no severe liver injury occur on continued therapy. But for some other drugs, progressive hepatocellular damage occur leading to serious liver injury. Patients diagnosed with DILI should then be closely monitored for time course of TBL, ALT and ALP and to rule out any other caused of such elevations.

In its guidance for industry, the FDA recommends that serum TBL elevations above 2 \times upper limit of normal (ULN) and parallel serum ALT elevation above 3 \times ULN (hy's law) are more predictive of severe liver injury and the culprit drug should be stopped immediately.[116] The FDA also recommend stopping the drug when serum

ALT or AST is $>8\times\text{ULN}$ at any time, ALT or AST $>5\times\text{ULN}$ for more than 2 weeks, ALT or AST $>3\times\text{ULN}$ plus fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia.

As indicated above drugs passing the scrutiny of preclinical and clinical drug development research may still cause severe DILI when marketed to wide and heterogeneous populations. This is because, for some drugs, susceptibility to DILI is both dose/plasma concentration and genetically determined. Therefore when drug concentration exceeds certain level in certain genotypes of individual severe DILI occur.

Post marketing cohorts and clinical trials are therefore useful at identifying drugs with potential to cause severe DILI during clinical practices. These exercises have in fact been useful at identifying drugs causing severe DILI and has enabled withdraw of the drugs from clinical use.

In FDA guidance for industry four signals are stipulated which can individually be useful indicators of a drug's potential to cause severe DILI during clinical use. Such indicators are: 1. observation of significant elevation of ALT ($>3\times\text{ULN}$) in trial subjects compared to a control group. Although a useful signal, it is not specific enough since several drugs may cause transient ALT elevation without severe DILI. 2. Finding hy's law cases among the trial subjects i.e. finding at least one or two patients having elevated levels of both serum ALT $>3\times\text{ULN}$ and TBL $>2\times\text{ULN}$ and no other explanation for such elevation can be found other than the drug under evaluation. This is more specific especially when it is accompanied with the first signal. 3. Marked elevations of ALT (to $5\times$ -, $10\times$ -, or $20\times\text{ULN}$) in modest numbers of subjects in the test drug group that is not seen (or is rare) in the control/placebo group. 4. Observation of one or more cases of newly elevated total serum bilirubin to $>2\times\text{ULN}$ in a setting of pure hepatocellular injury, with no other explanation, accompanied by an overall increased incidence of ALT elevations $>3\times\text{ULN}$ in the test drug group compared to placebo.

Application of such indicators in post marketing studies can also be useful at identifying potential for severe DILI of concomitant administration of drugs with shared hepatotoxicity.

1.12 THE NEED TO OPTIMIZE CO TREATMENT OF HIV AND ITS COMORBIDITIES

1.12.1 Factors influencing Efavirenz pharmacokinetics

1.12.1.1 Absorption: Influence of Fat meal and SNPs in drug transporter genes

Bioavailability of efavirenz from tablets, when taken without food, is 40-45%. [117] High fat meal increases exposure to efavirenz (area under the curve) by 22% and 28% for capsule and tablet formulations respectively. Although an in-vitro study indicated that efavirenz was not a substrate for MDR1, in-vivo studies have shown that SNPs in ABCB1 gene are associated with variation in plasma efavirenz exposures. [118-122] Efavirenz is substrate to a related transporter (ABCB5): [123] an in-vivo study has

indicated significant association between efavirenz plasma levels and SNPs in this transporter.[124]

Review of three population-pharmacokinetic analyses indicate that efavirenz absorption rate constant differ among populations (range from 0.15 h^{-1} to 1.39 h^{-1}).[118, 121, 125, 126] This implies that time to peak efavirenz concentration (T_{max}) and maximum concentration (C_{max}) varies between populations. It is observed that, time to peak efavirenz concentration remains relatively the same on multiple dosing. Measures of efavirenz exposure, (C_{max} , C_{min} , and AUC) increases proportionally with increase in efavirenz dose (from 200mg to 600mg). However, proportionality is lost at higher doses (1600mg) indicating saturation of absorption mechanisms.

1.12.1.2 Plasma, CNS and Intracellular Distribution: Influence of SNPs in drug transporter genes

Efavirenz is highly bound to plasma proteins, predominantly albumin. The extent of binding is comparable between patients on efavirenz based antiretroviral therapy (99.78% (99.74 to 99.80%))[127] and health volunteers (approximately 99.5 - 99.75)[117]. Total efavirenz plasma concentration is moderately correlated to unbound plasma concentration (Spearman's rank correlation coefficient =0.66).[128] Efavirenz distributes freely to central nervous system (CNS) reaching cerebral spinal fluid (CSF) protein-free concentration equivalent to unbound concentration in plasma (blood plasma to CSF ratio of 1.2 (0.97 to 2.12)).[127] CSF efavirenz concentration is controlled by passive diffusion from plasma and active efflux through drug transporters.[129] Total CSF efavirenz concentration is about 3 times higher than plasma unbound concentration[117] indicating that CNS accumulation is driven by protein binding. Efavirenz is 76.19% (74.47 to 77.15%) bound to CSF proteins.[127]

Efavirenz intracellular concentration is more correlated to unbound plasma concentration (Spearman's rank correlation coefficient =0.76) than total plasma concentration (Spearman's rank correlation coefficient =0.66)[128]. Due to its high lipophilicity, efavirenz has very high intracellular accumulation ratio (about 1190) compared to its unbound plasma concentration[128]. Intracellular protein free efavirenz concentration is still unknown. However, it might be highly correlated to both unbound and total plasma efavirenz concentration. None of the concomitant drugs have been reported to affect efavirenz protein binding or CNS and intracellular efavirenz distribution.

To date, only ABCB5 has been identified as efavirenz transporter.[123] It is expressed in many tissues including central nervous system, in testis, colon, stomach, mammary gland and retina. In MDKC cell lines the transporter is expressed on basal-lateral membrane indicating its importance in reabsorption or efflux from sanctuary sites.

1.12.1.3 Metabolism: Influence of Induction/inhibition, liver health status and SNPs in genes encoding efavirenz metabolizing enzymes

Efavirenz is metabolized to its main primary metabolite, 8-hydroxyefavirenz, by cytochrome P450 (CYP) enzymes 2B6, 3A4, 3A5, 2A6, 2C19, 2D6 and 1A2.[130] However, CYP2B6 is the main catalyst for efavirenz 8-hydroxylation.[131] It also undergoes 7-hydroxylation and N-glucuronidation by CYP2A6 [130] and UGT2B7 [132, 133] to minor primary metabolites 7-hydroxyefavirenz and efavirenz-N-glucuronide respectively. Formation of 8-hydroxyefavirenz and 7-hydroxyefavirenz account for 77.5% and 22.5% of efavirenz primary metabolism respectively.[130]

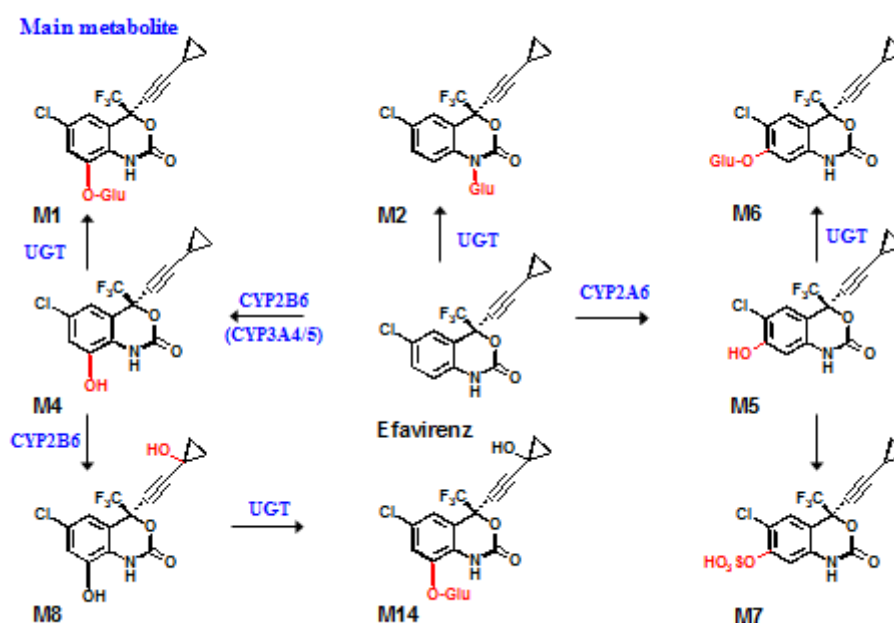


Figure 2: Efavirenz metabolic pathways

Assessment of N-glucuronidation has determined that it is a minimal pathway of efavirenz primary metabolism.[132]

Both 7 and 8-hydroxyefavirenz are further oxidised, primarily by CYP2B6, to dihydroxylated metabolites. 8, 14 dihydroxyefavirenz is a major secondary efavirenz metabolite in-vivo. The primary metabolites also undergo O-glucuronidation primarily by UGT2B7. This reaction is probably important in efavirenz excretion since it clears away 8-hydroxyefavirenz which is otherwise a mechanism based inhibitor of primary efavirenz oxidation. This is substantiated by the fact that, despite minor contribution of efavirenz N-glucuronidation pathway, functional polymorphisms in UGT2B7 are significantly associated with inter-individual variation in efavirenz plasma levels among patients on HAART.[134] Figure 1 display involvement of different enzymes in efavirenz metabolic pathways.

1.12.1.3.1 Single Nucleotide polymorphism in genes encoding efavirenz metabolizing enzymes

Efavirenz metabolizing enzymes are characterized by high variability in expression and catalytic activities among individuals leading to inter-individual variation in efavirenz exposure. The variability in expression and catalytic activities of the enzymes is primarily due to non-synonymous SNPs in genes encoding for the enzymes.

Due to the large contribution of CYP2B6 primary and secondary efavirenz metabolism, such polymorphisms are of clinical significance. Several SNPs have been identified in the CYP2B6 gene including; CYP2B6 785A>G, which when it occurs alone it cause increased enzyme activity in-vivo. Other SNPs, CYP2B6 516G>T, 415A>G, 136A>G, 296G>A, 419G>A, 1172T>A, 983T>C, 1282C>A and 593T>C, cause decreased enzyme expression in-vitro. CYP2B6 516G>T mutation is also associated with decreased catalytic activity in-vitro. Furthermore, several such CYP2B6 SNPs are found in linkage disequilibrium resulting into functionally important haplotypes. A good example is a CYP2B6*6 allele, composed of 516G>T and 785A>G SNPs, which overall is associated with poor expression and reduced enzyme activity. Another example is CYP2B6*16 allele, composed of 785A>G and 983T>C SNMs, which result into poor enzyme expression. Therefore several CYP2B6 phenotypes exists which differ in efavirenz metabolic capacity and hence efavirenz exposure. Due to its relatively higher frequency among populations CYP2B6*6 allele is an important determinant of the variability in efavirenz exposure. Other alleles implicated to significantly influence efavirenz plasma level includes CYP2B6*16, CYP2B6*18, CYP2B6*27 and CYP2B6*28.

CYP2A6 is also a polymorphic enzyme with several of its alleles associated with decrease or loss of function [135]. A list of such allele can be found at www.cypallele.ki.se. When grouped together, the alleles were associated higher efavirenz exposure among CYP2B6 poor metabolizers [135]. Similar findings were observed by Kwara *et al.* [134]. These results corroborate its significant contribution towards efavirenz clearance (about 23%) and suggest that CYP2A6 is an alternative pathway to efavirenz elimination in CYP2B6 poor metabolizers.

Although N-glucuronidation of efavirenz forms a minor pathway of efavirenz disposition, in a study by Kwara *et al.*, carriers of UGT2B7*1a allele had higher efavirenz plasma levels compared to non-carrier. This effect was independent of CYP2B6*6 polymorphism implying that functional UGT2B7 polymorphism independently influences efavirenz disposition. As noted above this probably because UGT2B7 clears away a mechanism based inhibitor of CYP2B6, 8-hydroxyefavirenz.

1.12.1.3.2 Inducibility and inhibition of the enzymes: effect of concomitant drugs and Single Nucleotide Polymorphisms in genes encoding Nuclear Receptors.

In addition to genetic polymorphism, differential induction of enzyme expression causes variation in plasma exposure of its substrates. Induction of enzyme expression is through ligand binding to cytoplasmic nuclear receptors, an action which initiates a

cascade of processes which enhance gene transcription. Amount of exposure to xenobiotics and level of expression and ligand affinity of nuclear receptors might influence magnitude of enzyme induction. SNPs in genes encoding nuclear receptors and thus variability in expression and affinity of hCAR and PXR to ligands, has been implicated for variations in level of expressions of CYP2B6.[136, 137] SNPs in genes encoding drug metabolizing enzymes have also been implicated to determine extent of induction. For example, SNPs in CYP2B6 promoter and exon are shown to determine the rate and extent of CYP2B6 induction by hPXR ligands.[136, 137]

Due to differential exposure to xenobiotics, enzyme activities may differ by geographical regions. Enzyme activities may also differ depending on type of medication a patient is taking. Efavirenz auto induces its own metabolism causing wide inter-individual variation in exposure among patients (ranging from 58% to 120%).[121, 134, 138] Rifampicin, a potent inducer of efavirenz metabolizing enzymes (CYP2B6, 3A, 2A6 and UGT), has been associated with, on average, 22- 26 % decline in efavirenz exposure among patients undergoing co treatment[79, 80]. In some health volunteers, rifampicin caused up 100% decrease in one individual and 56% increase in another individual. Furthermore, in cohorts of HIV/TB patients, rifampicin is associated with wider inter-individual variation in efavirenz exposure compared to patients on HAART alone [84, 138]. This implies that rifampicin induction of efavirenz metabolism is highly variable possibly due to reasons stipulated above.

Other inducers causing variable exposure to efavirenz among patients include; carbamazepine, phenobarbital, phenytoin, and efavirenz itself. Identified inhibitors of efavirenz metabolisms include; orphenadrine, ticlopidine and thiotepa. Curcumin a constituent of turmeric is also a known inhibitor for efavirenz metabolism.

1.12.1.3.3 Liver health status:

Liver metabolism of efavirenz is a major route of efavirenz excretion. Therefore any comorbid condition or concomitant drugs, during HAART, impairing liver function might affect EFV metabolism consequently elevating its exposure. In a cross-sectional study, hepatitis B and C co morbidities were associated with significantly higher proportions of patients having efavirenz plasma level above therapeutic range. In the same study a higher proportion of patients with liver fibrosis had efavirenz over-exposure [139] indicating importance of liver function at determining efavirenz exposure. Although a case control study did not establish increased risk for efavirenz over-exposure among patients who are HIV/HCV or HIV/HBV co infected[140], prolonged survival and increased risk for chronic liver disease among such patients may cause efavirenz over-exposure after years of treatment. Efavirenz itself has been implicated to cause liver injury. Liver function is also impaired due to chronic alcoholism and other environmental intoxicants. Since HAART should be started in patients with chronic hepatitis C or cirrhosis and co-infection with hepatitis B, it is therefore important to monitor liver function to enable appropriate management of therapy.

Renal excretion is a minor route of efavirenz elimination and therefore minor impairment of renal function have no clinical relevance to efavirenz

pharmacokinetics. However, efavirenz is not recommended for patients with kidney failure.

1.12.2 Efavirenz pharmacokinetics-pharmacodynamics relationships

1.12.2.1 Efavirenz plasma level and virological and immunological outcomes

The hallmarks of HIV infection are elevations of plasma HIV viral load and immune compromise indicated by decline of blood absolute CD4 count and increased frequency of opportunistic infections. The HIV infection of various tissues and organs together with immune derangement causes various clinical features of the HIV disease, which resolve only after successful HAART.

Treatment outcomes of efavirenz based HAART, particularly virological suppression and central nervous system toxicity, have been associated with efavirenz plasma level. In studies by marzolin *et al.*, [121, 141] and Stahle *et al.*, [142] the suggested efavirenz plasma level cut offs of 1000ng/ml and 2200ng/ml had 70% and 80% probability for viral load suppression respectively. Furthermore, Marzolin *et al.*, indicated that levels above 4000ng/ml had increasing probability for central nervous system toxicity.

It has been indicated that the rate of immunological recovery after prolonged treatment (beyond 12 month of HAART) is dependent on sustained viral load suppression [143]. This emphasizes the importance of prolonged adequate efavirenz exposure at ensuring robust and sufficient immunological recovery. Since efavirenz has a narrow therapeutic range (1000ng/ml – 4000ng/ml) and wide inter-individual variability in its exposure (up to 120%), it is important to undertake monitoring of its plasma level and where information is available perform dose individualization.

1.12.2.2 Efavirenz plasma level and Liver injury

Several studies have linked efavirenz to liver injury [14, 144-149]. In some of the cases such injury led to acute liver failure [144, 145], while in others, although sometimes severe (grade 3 and 4), it resolved with discontinuation of efavirenz [14, 147-149]. It has been indicated by an in vitro study that, in clinically relevant efavirenz concentration ranges, hepatic mitochondria toxicity is concentration dependent [150]. This implies that high efavirenz concentrations are more hepatotoxic. Few studies have linked liver injury to efavirenz plasma level [14, 147]. In these studies higher efavirenz plasma level was associated with increased incidence of DILI in a cox regression analysis. Liver injury was also associated with reduced efavirenz metabolism due to a defective CYP2B6*6 allele [147].

1.12.2.3 Efavirenz plasma level and CNS toxicity

Efavirenz based HAART has been associated with increased rate of neuropsychiatry events in several studies [151-158]. Such events includes; dizziness, confusion, impaired concentration, insomnia, abnormal dreams, irritability, restlessness, anxiety, emotional instability, euphoria, post-traumatic stress disorder, obsessive-compulsive behavior, depression, hallucinations, agitation, sadness, and suicidal ideation.

Features associated with presentation and severities of the events have not been determined, but several studies have found higher rate of CNS toxicity in patients with higher efavirenz plasma level [141, 156, 159-161]. Marzolin *et al.*, found that patients with efavirenz mid dosing plasma levels (8-16 hours after drug intake) above 4000ng/ml were about 3 times more likely to develop neuropsychiatry events compared to patient in the range 1000ng/ml-4000ng/ml. In a study by Gutiérrez *et al.*, a lower cut off was recommended in order to avoid efavirenz CNS toxicity. In this study they found that patients with mid dosing efavirenz plasma level above 2740ng/ml were about 6 times more likely to develop neuropsychiatry events compared to lower levels.

These reports imply a tight efavirenz therapeutic range and thus dosage adjustments may be important rather than treatment discontinuation in order to avoid intolerable neuropsychiatry events.

1.12.2.4 Efavirenz plasma levels and Induction of drug metabolizing enzymes and transporters

Efavirenz display concentration dependent induction of several drug metabolizing enzymes and drug transporters. This property may have clinical implication particularly during treatment of HIV communicable and non-communicable comorbidities. Depending on the magnitude of induction, doses of concomitant medications used to manage such comorbidities may need to be adjusted in order to achieve the therapeutic goal of co-treatment.

Efavirenz, through activation of promiscuous nuclear receptors hPXR and h CAR, induces CYP3A enzymes. There is still a paucity of data regarding magnitude of this induction in clinical cohorts and clinical relevance of this induction. Recent studies have reported significant reduction in exposure to lumefantrine and artemether, two antimalarial which are substrate of CYP3A, among patients on HIV/malaria co treatment. Efavirenz has also been attributed to sub therapeutic progesterone levels (a CYP3A substrate) among HIV positive women taking oral contraceptive pills[162]. An invitro study indicated that CYP3A induction by efavirenz is concentration dependent in the clinically relevant concentration ranges. This implies that patients with higher efavirenz concentration are more induced than their counterparts. Indeed this has been shown in a pharmacogenetic study by Habtewolde *et al* [163].

Efavirenz also induces CYP2B6 [164] therefore interact with drug substrate for this enzyme system. Clinically significant interaction with methadone was observed among intravenous drug users. In such patients efavirenz significantly decreased methadone exposure by more than 60% causing withdraw syndromes that required 20% increase in methadone dose[165]. The complexity of efavirenz-methadone interaction reviewed by Rosario *et al.*, probably explains why despite 60% reduction in methadone exposure only 20% dose increase was enough[166]. In situations like these population pharmacokinetic-pharmacodynamic analysis are warranted for generation of appropriate algorithms of dose adjustments.

Efavirenz has also been implicated in induction of drug transporters including UGT1A1, bile efflux transporters, ABCB1, ABCG2, ABCC2, ABCC3, ABCC5, and

SLCO3A1 [167, 168]. This property most likely influences the pharmacokinetics of concomitant drugs substrates for these transporters.

1.13 STRATEGIES FOR OPTIMIZING THERAPIES

Therapies can be optimized through monitoring and ensuring adherence to medication, identifying and grading drug toxicities followed by halting or substituting a culprit drug, reviewing patient medication to identify inappropriate dosage, drug-drug and drug-co-morbidity interactions. Therapeutic drug monitoring (TDM) is an optimization strategy which involves adjusting drug dose so as to achieve the required therapeutic response in an individual patient. It is based on established functional drug exposure- response relationship. Therefore it requires appropriate measures of exposure and appropriate biomarkers/surrogate markers of response. Drug exposure in an individual is dependent on values for his pharmacokinetic parameters i.e. rate and extent of drug absorption (bioavailability), volume of distribution (plasma protein binding and tissue affinity) and drug clearance (metabolism and renal excretion).

Inherent patient characteristics (age, weight, body surface area (BSA), genotype, phenotype, co-morbid condition, co-medications) influencing pharmacokinetic parameters will determine drug exposure in this particular patient. Population pharmacokinetic studies are used to develop functional relationships between drug exposure and patient characteristics (covariates). When a well-established population pharmacokinetic model exists, patient characteristics can be used as surrogate measures of drug exposure. During TDM an individual's values for surrogate measures of drug exposure can be used for dose individualization. When surrogate measures for drug exposure are not available or are not established yet, but exposure-response relationship exists, direct measures of drug exposure (i.e. plasma drug concentrations or area under the curve of concentration time profile (AUC)) are used for dose adjustments.

Therefore, several strategies exist for dose individualization during TDM and, depending on which measures of drug exposure are used for dose adjustment, can be categorized into *priori* or *posterior* dose individualization strategy.

1.13.1 Priori dose individualizations

These don't involve determination of any biological fluid drug concentrations but assessment of patient surrogate measures of drug exposure (biometric or biological data) and then use of established graphs, charts, algorithms or tables to identify or calculate an appropriate dose for an individual. Such biometrical or biological data are those with established relationship to drug exposure or response. Age is a good example of surrogate marker for drug exposure. It is correlated in most cases to drug bioavailability, volume of distribution, hepatic and renal clearance. Most drugs therefore are dosed based on age. In general children require smaller doses compared to adult.

Weight and BSA based dosing are also common as they are related to some pharmacokinetic parameters for some drugs. Dosing based on renal function,

determined as creatinine clearance or glomerular filtration rate (GFR), is also a common *priori* dose individualization strategy for drugs whose renal clearance is a major component of their excretion.

However, no so common are genotype and phenotype based dosing. These approaches are used for drugs whose hepatic clearances vary widely in a population due to variability in catalytic capacity and expression of drug metabolizing enzymes (e.g. polymorphism in CYP2C9 determines hepatic clearance of warfarin). They are also used when variability in drug response is genetically determined (e.g. polymorphism in VKORC1 gene determines the anticoagulant activities for warfarin). Such variabilities may be due to genetic polymorphism or inter-individual variation in extent of exposure to inducers or inhibitors of drug metabolisms or receptors. Such inducers or inhibitors may be environmental chemicals in foods and beverages or medications. During genotype based dose individualization, a patient is genotyped and dose is identified or calculated from established genotype based dosing algorithms. During phenotype based dose individualization, a patient will be given a probe drug to assess the metabolic capacity for a particular enzyme. Based on metabolic ratio results (probe drug/probe drug metabolite), a patient is categorized as fast, intermediate or slow metabolizer. Then the patient is dosed according to established algorithm, charts or tables.

For some drugs several biometric or biological variables are surrogate markers for drug exposure. This happens when such variables, individually, explain a substantial proportion of the variability in pharmacokinetic parameters. In these situations, the variables are used to stratify the population into groups. Charts/tables for dosage for each subgroup are then prepared using group pharmacokinetic parameters. The charts are then used for identification of appropriate doses for an individual patient given his biometrical and biological data.

Biomarkers and surrogate markers of therapeutic response or toxicity also may useful criteria on which dose individualization are based. A good example is dose adjustments of warfarin are based on blood clotting time indicated by international normalized ratio (INR). In this algorithm, INR is determined at 3, 4, or 5 days of warfarin treatment and used to warfarin dose adjustment.

1.13.2 Posteriori dose individualizations

During this approach blood samples are taken for drug concentration measurement after initial dose(s) are given. These concentrations are then used to evaluate exposure in relation to target exposure, individual pharmacokinetic parameters and response. Posteriori dose individualization strategies include; test dose strategy, limited sampling protocol (LSP) and Bayesian methods.

The test dose strategy requires an established relationship between dose and target concentration at a specific time point after drug intake and treatment duration. When a test dose is given the measured concentration is compared to target concentration and then dose adjustment done. This strategy requires that blood sampling is done at the same time target concentration was determined.

Limited sampling protocol involves finding a minimum number of plasma samples after drug intake (sparse samples) whose concentrations either individually or after integration (sparse AUC) are correlated to exposure, determined by intensive sampling (intensive AUC). When such correlation is established, target sparse concentration or sparse AUC are determined (based on exposure response relationship). To determine appropriate patient dose, sparse concentration or AUC after an initial dose are determined and compared to target sparse concentrations or AUC. Dose adjustments are done to an initial dose so that it achieves target sparse concentration or AUC.

Bayesian methods involve estimation of individual pharmacokinetic parameters using population pharmacokinetic parameters, individual values for biometric or biological variables and sparse plasma concentration data (measured at predetermined time points). They require computer software specially designed for population pharmacokinetic-pharmacodynamic modeling and Bayesian estimation of individual pharmacokinetic parameters. During Bayesian dose estimations, given a target concentration, determined from concentration response relationship, individual pharmacokinetic parameters are used for estimation of appropriate maintenance dose. The more variability in PK parameters is explained by biometric and biological variables, the better is the strategy at accurately estimating initiation and maintenance doses.

1.14 CURRENT PRACTICE OR RECOMMENDATIONS ON OPTIMIZATION OF EFAVIRENZ DOSAGE

A manufacturer's recommended efavirenz dose for adult patients weighting above 40kg is 600mg taken on empty stomach preferably at bedtime. In children, the manufacturer recommends efavirenz dosage to be based on weight and provides a table for efavirenz doses in different weight categories[80].

However, several studies have recommended efavirenz dose adjustment when given in different situations. Furthermore different methods have been used for dose optimization in these different situations. In these studies, Bayesian estimation of individual pharmacokinetic parameters was used as for dose optimization.

1.14.1 Recommended efavirenz dose adjustment during treatment of HIV alone

In absence of CYP2B6 genotype data Cabrera et al., recommended assessing efavirenz plasma levels to establish whether a patient is poor metabolizer or not (CYP2B6 phenotyping). For poor metabolizers they recommended stepwise dose reduction (while maintaining trough concentration within 1000-4000ng/ml) from 600mg, to 400 mg, and finally to 200mg.

Genotype based efavirenz dosing was recommended and used by Gatanaga *et al.* In their study, they established that efavirenz dose could be empirically reduced to 400mg in patient with high mid dosing plasma level and with CYP2B6*1/*26 heterozygote, CYP2B6*6/*6 homozygotes, and CYP2B6*6/*26 heterozygote. They

later genotyped efavirenz naïve patients and initiated those with CYP2B6*6/*6 and CYP2B6*6/*26 genotypes on 400mg dose. In all these patients efavirenz plasma levels were above therapeutic range.

Nyakutira *et al.*, used data from a sample of Zimbabwean HIV patients to estimate values for efavirenz population pharmacokinetic parameters (mean and variances) stratified by CYP2B6 genotype and sex. These parameters were used to simulate virtual patients which were then used to test which priori doses (500, 400, 300 mg) could achieve the pre-defined therapeutic range (1000-4000ng/ml). They concluded that efavirenz dose could be decreased to 500mg, 400mg and 300mg for female with CYP2B6*1/*6 genotype, male with CYP2B6*6/*6 genotype and female with CYP2B6*6/*6 genotype respectively.

Cabrera *et al.*, used their previously developed efavirenz population pharmacokinetic model to perform Bayesian estimation of patient pharmacokinetic parameters. In this model CYP2B6*6 genotype was the only covariate on oral clearance and was used together with plasma concentrations measured at 2 different time points to estimate PK parameters for patients. Targeting concentrations within therapeutic range for their patient with CYP2B6*6/*6 genotype, efavirenz dose was decreased to 400mg and finally was maintained at 200mg.

1.14.2 Recommended efavirenz dose adjustment during HIV/TB co-treatment

Lopez et al empirically chose efavirenz 800mg dose to compare with 600mg in a randomized trial among 16 HIV/TB patients. They found that efavirenz 800mg among HIV/TB patients had comparable exposure to 600mg among patients on HAART alone. Subsequently, it was recommended by some experts to increase efavirenz dose by 33% in all patients undergoing HIV/TB co treatment.[82]

In some cohort studies and a randomized clinical trial the 800mg dose was associated with increased incidence of neuropsychiatry events and hepatotoxicity particularly among patients with native African descents. In a study among Caucasian patient, 800mg dose was not associated with supra-therapeutic efavirenz concentration nor toxicity. Due to lack of superiority of 800mg dose on virological responses, some other experts suggest that the standard dose (600mg) is adequate during HIV/TB co treatment. However, in pediatric patients, the standard dose was associated with sub therapeutic plasma levels in majority of patients both during and after rifampicin co treatment [169], indicating under dosing in this patient population.

In a therapeutic drug monitoring activity for two patients, by Cabrera *et al.*, efavirenz dose was increased in steps of 200mg empirically. The dose increments were guided by efavirenz plasma level measurements, a goal being to achieve therapeutic concentrations. In one patient, the final maintenance dose was 1000mg while in the other it was 1600mg [170]. These doses were well tolerated. After completion of TB treatment efavirenz exposure rose and the doses had to be decreased in steps up to 33% reduction to return the levels into the therapeutic range.

Genotype based dosing of efavirenz during HIV/TB co treatment has been recommended after findings that CYP2B6*6 allele is associated with higher efavirenz exposure and CNS toxicity even among patients undergoing rifampicin based TB treatment [171, 172]. Standard doses of efavirenz may be adequate only in patients with CYP2B6*6/*6 genotype.

1.15 CURRENT PRACTICE ON OPTIMIZATION OF CO-CONCOMITANT DRUGS AMONG PATIENTS ON EFAVIRENZ

Efavirenz induces several drug metabolizing enzymes. This may result in sub-therapeutic plasma level of concomitant drugs which are substrates of the enzymes. Such induction may be variable among patients causing variation in exposure to concomitant drugs and thereby treatment outcomes of co-morbidities. Variable outcome was noted among patients taking methadone for treatment of drug-withdraw syndromes, whereby when efavirenz was initiated, about 80% of the patients experienced withdraw syndromes after 8-10 days of co administration. In these patients dose individualization was done based on clinical response. Methadone dose was increased empirically in steps of 10 mg until withdraw syndromes disappeared. Finally, methadone dose increase ranged from 15-30 mg [165] implying variation in efavirenz-methadone pharmacokinetic interaction.

Despite significantly big reduction in exposure (AUC) to artemether (-51% to -79%), Dihydroartemisinin (-46% to -75%) and Lumefantrine (-21% to -56%) in patients and health volunteers co treated with efavirenz [173, 174], no study has indicated dose increase for Artemether-Lumefantrine (ALu) when administered co infected patients. This is because no study has evaluated the clinical significance of this interaction among HIV/malaria co infected patients. Studies are needed to characterize population pharmacokinetics of artemether and lumefantrine and evaluate its relationship to parasite clearance among patients undergoing HIV/malaria co treatment.

To date no dose adjustments for oral contraceptive pills when co administered with efavirenz, rather use of alternative or additional methods of contraception have been recommended.[175] Characterizations of population pharmacokinetics of oral contraceptive agents (ethinyl estradiol, levonorgestrel, norethindrone and norgestimate) and evaluation of clinical significance of such reduced exposure are required for better dose adjustments of oral contraceptive pills.

In general no dose adjustments for anti-hypertensive, antidiabetics, statins and many other drugs which when co administered with efavirenz results into significant reduction of their exposure.

2 RATIONALE OF THIS PHD THESIS

Some studies have indicated variability in treatment outcomes among patients on efavirenz based HAART. Such variability has been observed even in adherent patients and has therefore been attributed to inherent wider inter-individual variability of efavirenz oral clearance and plasma levels. Assessments of factors contributing such wide variability in efavirenz exposure have identified polymorphisms in CYP2B6, CYP2A6 and UGT2B7 gene to account for some of such variability. Co-treatment with rifampicin increases such variability and this has been attributed to variable induction of efavirenz metabolizing enzymes among patients. However, a large component for such variability remains unexplained.[134]

To optimize efavirenz dosage it is important to investigate more sources/factors for such wide variability in exposure and treatment outcomes of efavirenz based HAART both in HIV and HIV/TB co-infected patients. Such factors would therefore form rich *priori* for precise Bayesian estimation of individual pharmacokinetic parameters, and hence appropriate dosage optimization.

On the other hand, efavirenz induces enzymes involved in metabolism of most drugs used for treatment of HIV co morbidities. However, insufficient evaluation of pharmacokinetic and clinical significance of such induction has been done; partly because of lack of appreciation of the extent efavirenz can induce such enzymes. This is further reflected in absence of adequate studies on dose optimization of concomitant drugs taken during efavirenz based HAART. Characterization of efavirenz induction of various enzymes among patients would form a step stone towards evaluation of clinical significance of such induction and ultimately dose recommendations for concomitant drugs.

Several studies have established preponderance to cause liver injury by efavirenz. However, incidences and severity of such toxicity have varied between studies indicating variable inter-individual susceptibility to efavirenz induced liver injury. To explain such variability, genetic, co-morbidities, concomitant medications and other environmental exposures have been hypothesized to contribute. There is still insufficient studies on susceptibility to DILI during efavirenz based HAART. It is important therefore to be vigilant of DILI in the antiretroviral therapy era when overlapping toxicities from drugs taken concomitantly have been shown to increase incidence and severity of other adverse events.

In this thesis we highlight on long term efavirenz auto induction, expounding on its pharmacogenetic and environmental aspects. We further elucidate the pharmacogenetic aspects of efavirenz - rifampicin interaction. We also emphasize on the importance for therapeutic drug monitoring and efavirenz dose adjustments. We also establish covariates which should be considered when preparing algorithm for efavirenz dose optimization including; time after initiation of HAART, geographical region or environmental aspects, genetic polymorphism and gene-gene interaction, hepatic diseases and rifampicin co treatment.

3 OBJECTIVES

3.1 GENERAL OBJECTIVE

This work had the general aim of exploring sources of variation in efavirenz pharmacokinetics and its enzyme inductive and toxic effects. These are necessary requirements for optimization of HIV and Tuberculosis co treatment and treatment of other comorbidities.

3.2 SPECIFIC OBJECTIVES

The specific objectives were:

1. To investigate the effect of gender, CYP2B6, CYP3A5 and ABCB1 gene polymorphisms on long-term efavirenz auto induction among Tanzanian HIV patients on HAART alone
2. To comparatively examine long-term efavirenz auto induction in presence and absence of rifampicin co treatment among Tanzanian HIV and HIV/TB co infected patients stratified by CYP2B6 gene polymorphisms
3. To evaluate the importance of ethnicity in determining efavirenz pharmacokinetics, auto-induction and immunological outcomes
4. To assess the effect of CYP2B6, CYP3A5, UGT2B7 and ABCB1 gene polymorphisms on magnitudes of CYP3A4/5 induction during efavirenz alone and with rifampicin co-treatment in HIV patients.
5. To investigate the timing, incidence, clinical presentation, pharmacokinetic and pharmacogenetic predictors of drug induced liver injury (DILI) during efavirenz alone and with rifampicin co-treatment in HIV patients.

4 MATERIAL AND METHODS

4.1 STUDY DESIGN

This thesis represents a subset of work and secondary analyses of the main project, which is a multicenter clinical trial project entitled “Optimization of TB-HIV treatment in Africa”. The main project was designed as a treatment, non-randomized, open label, active control, parallel assignment and population steady state pharmacokinetic and pharmacogenetic study. Therefore data generated were, as secondary analyses, analyzed as either parallel-group prospective cohorts studies (Paper II), (Paper III), (Paper IV) and paper V or prospective cohort study (Paper I).

4.2 STUDY AREA AND STUDY POPULATION

Patients were recruited between September 2007 and June 2010 at Muhimbili National Hospital (MNH), Infectious Disease Centre (IDC) and Mwananyamala Municipal Hospital all within Dar es Salaam city, Tanzania and also at Black-lion hospital in Addis-Ababa-Ethiopia. Patients were recruited into two arms: Arm-1 were HIV infected, without active TB, antiretroviral naïve patients while arm2 were HIV and tuberculosis co infected patients naïve to antiretroviral and who had not been on anti-tuberculosis for previous 5 years from day of recruitment. Further inclusion criteria for both arms were: newly diagnosed HIV patients with CD4 count < 200 cells/mm³, adult males & females age > 18 years and able to give consent to participate in the study. Exclusion criteria for both arms were: severely ill patients with Karnofsky score < 40%, patient receiving medications that are contraindicated or not recommended for use with EFV, serum Aminotransferases (ALT) raised above 3 folds, hemoglobin < 8 gms/dL, pregnancy or breast feeding, previously exposed to ART in PMTCT/ PEP, prisoners, presence of persistent diarrhea or malabsorption that would interfere with the subject's ability to absorb drugs, drug or alcohol abuse that may impair safety or adherence or interfere with the study results. A subset of arm1 Tanzanian patients (n=128) with complete set of 4th and 16th plasma efavirenz and metabolite concentrations were used for evaluation of efavirenz long term auto induction (paper I), this subset together with another similar subset of arm2 patient (n=54) were used for comparison of long term auto-induction between the two arms (paper II). Subsets of arm 1 patients recruited both in Tanzania (n=209) and Ethiopia (n=285) were used to compare efavirenz pharmacokinetics and pharmacogenetics of long-term auto induction in the two geographically separated and culturally different ethnic groups (paper III). A subset of arm1 Tanzanian patients (n=41) and another subset of arm2 Tanzanian patients (n=23) with complete set of 4 beta hydroxycholesterol and cholesterol plasma concentrations were used for assessment of CYP3A4/5 induction (paper IV). Subsets of arm1 and arm2 Tanzanian patients were used to assess drug induced liver injury (paper III)

4.3 DRUG TREATMENTS AND DATA COLLECTION

All patients received efavirenz 600 mg based highly active antiretroviral therapy (HAART) together with two nucleoside reverse transcriptase inhibitors (NRTIs). The Nucleoside back bones were zidovudine 300mg + lamivudine 150mg or stavudine

30mg +lamivudine 150mg twice daily or tenofovir 300mg +emtricitabine 200mg once daily.

HIV/TB co infected patients also received anti-tuberculosis (anti-TB) drugs which were initiated 4 weeks prior to initiation HAART. The anti-TB drugs included rifampicin, isoniazid, ethambutol and pyrazinamide and were given in 2 phases: intensive and continuation phase as recommended in Tanzanian TB treatment guidelines. The drugs were taken under directly observation of the nurse or treatment assistant to ensure adherence.

In case of any other co morbidities, patients were treated according to existing treatment guidelines: However, the following drugs were contraindicated: voriconazole, astemizole, ergot derivatives, midazolam, triazolam, bepridil, cisapride, pimozide and St John 's wort.

Before recruitment all patients were screened and for those who met inclusion criteria, baseline clinical and laboratory characteristics were assessed and recorded into case report forms. The characteristics included: demographic information, body weight, physical signs and symptoms, CD4 cell count, viral load, liver function tests (LFT), kidney function tests, smear status, hematology tests. Blood samples for genotyping of human CYP2B6, CYP3A5, UGT2B7 and ABCB1 genes were collected at baseline and at any other visit if missed at baseline.

Patients prognosis were monitored by monthly assessment of body weight, physical signs and symptoms, body systems integrity, liver function tests (LFT), kidney function tests, hematology tests. After every 2 months patients were assessed for sputum smear status. In both arms, plasma HIV-1 viral load and CD4 count were further assessed at 12th, 24th and 48th week after initiation of HAART.

For assessment of efavirenz long-term auto-induction (paper I, paper II and paper III), 8 ml of blood sample in duplicate were collected 16 hours post dose on the 4th and 16th weeks of HAART initiation; for quantification of plasma and intracellular of EFV and its metabolite concentrations. Efavirenz metabolic ratio was used for CYP2B6 phenotyping (paper I). Efavirenz plasma levels at 4th and 16th were evaluated as risk factors for liver injury (paper V). Cell preparation tubes were used for blood collection and peripheral mononuclear cells (PBMC) isolations.

For assessment of CYP3A induction (paper IV), 5ml of blood samples were collected in EDTA tubes at baseline, 4th and 16th weeks among arm1 patients: for arm2, blood samples were collected at day 0, 1, 3, 7, 14, 21, 42, 56 after completion of TB treatment. From these samples plasma was isolated for quantification of cholesterol and 4 beta hydroxyl cholesterol levels.

4.4 QUANTIFICATION OF PLASMA AND INTRACELLULAR EFAVIRENZ AND 8-HYDROXY EFAVIRENZ CONCENTRATIONS

Four and sixteen weeks after initiation of EFV based HAART, 16h post-dose duplicate blood samples were collected in vacutainer CPT tubes (Becton Dickinson, Heidelberg, Germany). Blood samples were centrifuged (1700 g for 20 min) and

plasma and peripheral blood mononuclear cells (PBMC) were prepared as described by Burhenne *et al.*, [176] and stored at -80°C. Samples were sent on dry ice to the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Germany where plasma and intracellular efavirenz and 8-hydroxyefavirenz concentrations were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS). The determination of efavirenz and 8-hydroxyefavirenz concentrations was performed as described previously, [177] with some modifications. In brief, protein precipitation with ice-cold acetonitrile containing the deuterated and ¹³C-labeled internal standards was used for sample preparation and extraction. Extracts were chromatographed on a Phenomenex Synergi Fusion RP column with an eluent consisting of acidified 5 mmol/l ammonium acetate buffer, acetonitrile, and methanol. Efavirenz and 8-hydroxyefavirenz concentrations were quantified using ¹³C₆-efavirenz and 2H₄-8-hydroxyefavirenz as internal standards and electrospray tandem mass spectrometry in the selected reaction-monitoring mode. The lower limits of quantification in plasma were 10.0 ng/ml for efavirenz and 0.4 ng/ml for 8-hydroxyefavirenz. The efavirenz (8-hydroxyefavirenz) calibration range was 10–10,000 ng/ml (0.4–400 ng/ml). Linear regression with 1/× weighing resulted in correlation coefficients of $r^2 > 0.99$. Accuracy and precision (within-batch and batch-to-batch) of the assay fulfilled all recommendations of US Food and Drug Administration guidelines.

4.5 QUANTIFICATION OF PLASMA CHOLESTEROL AND 4B-HYDROXYCHOLESTEROL CONCENTRATION

Cholesterol was determined by a commercial enzymatic 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). Plasma 4β-hydroxycholesterol was quantified by gas chromatography mass spectrometry with instrument settings and sample preparation procedure reported by Bodin *et al.* and Diczfalussy *et al.*, respectively. [178, 179]

4.6 GENOTYPING FOR CYP2B6, CYP3A5, UGT2B7, SLCO1B1 AND ABCB1

Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Maxi Kit (QIAGEN GmbH, Hilden, Germany). Genotyping for the common functional variant alleles in five relevant genes for efavirenz disposition were carried out at the division of clinical pharmacology, Department of laboratory medicine, Karolinska Institutet Stockholm, Sweden. Genotyping were done by real time PCR using pre-developed Taqman assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) according to the manufacturer's instructions. Allelic discrimination reactions were performed using TaqMan® (Applied Biosystems, CA, USA) genotyping assays with the following ID number for each SNP: (C__7586657_20 for ABCB1 c.3435C>T rs1045642, C__11711730_20 for CYP2B6*6 c.516G>T rs3745274, C__30720663_20 for UGT2B7 -372G>A rs7662029 (UGT2B7*2b,*2c,*2d,*2f), C__26201809_30 for CYP3A5*3 6986A>G rs776746, C__30203950_10 for CYP3A5*6 14690G>A g.14690G>A,

C__32287188_10 for CYP3A5*7 g.27131_27132insT rs241303343, C__1901697_20 for SLCO1B1 388A>G rs2306283 (*1b) and C__30633906_10 for SLCO1B1 521T>C rs4149056 (*5) on ABI 7500 FAST (Applied Biosystems, Foster City, CA). The final volume for each reaction was 10µl, consisting of 2x TaqMan Universal PCR Master Mix (Applied Biosystems), 20 X 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 with 95°C for 10 min and 92°C for 15 sec. Genotyping for SLCO1B1 388A>G (rs2306283) and 521T>C (rs4149056) in Tanzanian subjects was done using LightCycler® based method [22]. Haplotype analysis was done using Haploview v.4.1 software.

4.7 DATA MANAGEMENT AND ANALYSIS

All data recorded in case report forms (hard copies) were transcribed into electronic copies by double entry into a database tailored to store data emanating from this project. Data from electronic data sources (LCMS quantifications, real time PCR, light cycler, Roche/Hitachi Modular instrument) were also transferred into the data base. For a particular analysis necessary data was extracted from the database formatted and analyzed using appropriate software. Microsoft excel, R statistical software, and SPSS were using for appropriate data management. Statistical analyses were performed using STATA version 9, STATISTICA version 10, R statistical software version 2.10 and 2.15, and SPSS version 16.

4.7.1 Statistical analysis

Mean (SD), median (interquartile range), and proportions were used to describe patients' baseline characteristics (paper I). Student's t-test and z-test were used to compare the continuous and categorical baseline characteristics between arms 1 and 2 (paper II and paper III). Descriptive statistics for plasma efavirenz steady-state levels and efavirenz/8-hydroxyefavirenz ratio were summarized by median (interquartile range) (paper I).

The Shapiro–Wilk W-test (paper I) and Kolmogorov–Smirnov test (paper II) were used to investigate normality of distribution. Normality was assured by conversion of the data to log 10 values before statistical analysis (paper I, II, III, V) but for CD4 count, square root values had normal distribution but not log 10 values (paper III) .

Plasma efavirenz concentration and metabolic ratio (MR) at the two sampling points (weeks 4 and 16) were compared using the Wilcoxon matched-pair test (paper II). One-way ANOVA (paper II) was used to test for any significant difference in mean log-transformed plasma EFV and 8-hydroxyefavirenz concentrations between the different genotype groups in arms 1 and 2 and between the values at week 4 and week 16 of EFV treatment.

Repeated-measures ANOVA (paper I) and two way repeated measure ANOVA (paper II and III) was used to investigate the influence of gender and genotype on the change in mean log-transformed values of efavirenz and efavirenz/8-hydroxyefavirenz ratio: It was also used to compare mean CD4 counts between

treatment weeks and to assess interaction between patient country, CYP2B6 genotypes and duration of therapy to influence CD4 gains.

A χ^2 -test was used to compare the numbers of patients with plasma EFV levels <1 µg/ml, 1–4 µg/ml, and >4 µg/ml (paper I and II). Hardy–Weinberg equilibrium was assessed using the χ^2 -test.

Univariate and multivariate logistic regression methods were used to assess risk factors associated with plasma levels <1 µg/ml or >4 µg/ml (**See results section in this thesis**)

Hierarchical multivariate linear regression model building in SPSS (paper III) was used to assess factors influencing CD4 gain and Efavirenz plasma and intracellular levels.

Univariate and multivariate Cox proportional hazards regressions (paper V) was used to determine factors associated with higher incidence for DILI.

4.7.2 Nonlinear mixed effect modeling

This technique was used to simultaneously estimate model parameters (typical population values), population variability of model parameters, predictors (covariates) for model parameters and measurement errors (residuals) for CD4 count–time profile (paper III) and cholesterol and 4 beta hydroxycholesterol kinetics (paper IV).

NONMEM version 7.2 was used and analyses were executed using Pearl-speaks-NONMEM (PsN) version 3.5.3. Pirana (version 2.4.0) was used for NONMEM analyses documentation. Xpose package version 4.3.2 and ggplot2 package version 0.9.2.1 as implemented in R version 2.15 were used for the generation of diagnostic plots.

4.8 ETHICAL CONSIDERATIONS

The studies conformed to the Helsinki declaration and were conducted according to the recommendation of the International conference on Harmonization (ICH). In brief, only patients consenting on written consent forms were recruited. Patients were reimbursed for their time and cost to attend clinics but no incentives were given. Participants were informed that their participation is voluntary and had right to withdraw from the study without giving reasons. Participants were kept anonymous in all publications except to the primary study teams and attending clinic staff. Ethical clearance was sought from Institutional Review Board of the Muhimbili University of Health and allied Sciences, IRB of Faculty of Medicine, Addis Ababa University and Karolinska Institutet. Blood sample transfers between countries were done following signing of material transfer agreement between the respective universities.

5 RESULTS

5.1 EFFECT OF GENDER, CYP2B6, CYP3A5 AND ABCB1 GENE POLYMORPHISMS ON LONG-TERM EFAVIRENZ AUTO INDUCTION (PAPER I)

Overall there was 19% decrease in efavirenz concentration between week 4 and 16 which was in parallel with increase in 8-hydroxyefavirenz (primary metabolite) concentration and hence 32% decrease in metabolic ratio (efavirenz/8-hydroxyefavirenz). Only CYP2B6 gene polymorphism was associated with variation in extent of change in log transformed efavirenz, 8-hydroxyefavirenz and metabolic ratio between week 4 and 16. Patients with CYP2B6*1/*1 genotype had higher and significant decrease in efavirenz concentrations between week 4 and 16 compared to carriers of CYP2B6*6 allele. ($p=0.0001$)

CYP2B6 gene polymorphism was also associated with variation in efavirenz plasma levels at week 4 ($CV^1=76.5\%$). The concentration varied in gene dose dependent manner: Patients with CYP2B6*1/*1 (homozygous wild type) had lowest levels while those with CYP2B6*6/*6 had the highest levels ($P < 0.0001$). Since significant decrease was only observed in homozygous wild type, higher variability in efavirenz plasma level was observed at week 16 ($CV=90.2\%$) compared to week 4: significantly associated with CYP2B6 polymorphism ($p<0.0001$).

Gender only influenced the extent of increase in log transformed 8-hydroxyefavirenz concentration but not decrease of efavirenz or metabolic ratio between week 4 and 16. Levels of log transformed 8-hydroxyefavirenz were significantly higher in women at week 16 compared to men at week 16 and women and men at week 4. ($p=0.004$)

CYP3A5 gene polymorphism only influenced metabolic ratio (a phenotypic measure of efavirenz metabolism) but not efavirenz plasma level: association with metabolic ratio (MR) was observed at week 4 ($P = 0.037$) but not at week 16 ($p=0.13$) and there was a tendency to association with the extent of change in MR between week 4 and 16. Gene interaction between CYP2B6 and CYP3A5 to influence MR was also observed. Patients with CYP2B6*6/*6 genotype had highest MR values (poor metabolizers) compared to carriers of CYP2B6*1 allele, but among them, mean values for log transformed MR were lowest for those with two CYP3A5 functional alleles (CYP3A5*1/*1) and highest for those without any functional allele (CYP3A5*0/*0). However, these differences were not statistically significant.

ABCB1, 3435C>T polymorphism had no influence of efavirenz, its metabolite nor its metabolic ratio.

The influence of CYP2B6 gene polymorphism on magnitude of change of efavirenz levels between week 4 and 16 was reflected on its influence on parallel change in proportions of patients in sub therapeutic, therapeutic and supra-therapeutic efavirenz

¹ CV=coefficient of variation

levels between the two weeks. As reported in paper I *“Among subjects with CYP2B6*1/*1 and *1/*6 genotypes, the proportion of subjects with <1 µg/ml at week 16 rose by 67 and 25%, respectively, whereas it remained virtually unaltered at only 5% among slow metabolizers”*.

5.2 COMPARISON OF LONG-TERM EFAVIRENZ AUTO INDUCTION AND KINETICS IN PRESENCE AND ABSENCE OF RIFAMPICIN CO TREATMENT STRATIFIED BY CYP2B6*6 GENOTYPES (PAPER II)

5.2.1 Comparison of long term auto induction

Unlike in patients on efavirenz based HAART alone (Paper I), where significant changes ($p < 0.05$) in efavirenz, 8-hydroxy efavirenz and metabolic ratio between week 4 and 16 were observed, no such significant changes were observed ($p > 0.05$) in patients initiated on rifampicin based anti-TB therapy followed by efavirenz based HAART (after 4 weeks) regardless of CYP2B6 genotype.

5.2.2 Comparison of efavirenz kinetics

After 4 weeks of HAART (8 weeks on rifampicin and 4 weeks on rifampicin + efavirenz co administration in arm2), patients on co administration had 32% lower efavirenz concentration compared to patients taking efavirenz alone (arm1) ($p = 0.005$). This difference decreased to 4% after 16 weeks of HAART ($p = 0.12$). When the two arms were stratified by CYP2B6 genotype, patients in arm2 with CYP2B6*1/*1 genotype had significantly lower efavirenz concentration after 4 weeks of HAART compared to their arm 1 counterpart ($P = 0.03$). The differences were not significant when arm2 with CYP2B6*1/*6 and CYP2B6*6/*6 genotype were compared to their arm 1 counterparts. By contrast, within the same genotype, no significant difference in EFV plasma concentration was observed between arms 1 and 2 by week 16 of EFV therapy.

The magnitude of variability in efavirenz plasma concentration among arm 2 patients at week 4 ($CV = 81\%$) was higher than that observed in arm1 (76.5%). It was still higher in arm 2 at week 16 ($CV = 111\%$) compared to arm1 ($CV = 90\%$).

There was a significant interaction between absence or presence of rifampicin co-therapy and duration of efavirenz therapy in determining efavirenz concentration ($p = 0.023$).

Plasma concentration of 8-hydroxyefavirenz were also lower among arm2 compared to arm1 patients both at week4 ($p = 0.06$) and week 16 ($p = 0.02$). When the two arms were stratified by genotype, arm2 patients with CYP2B6*1/*1 genotype had significantly lower levels compared to their arm 1 counterparts both after 4 ($p = 0.04$) and 16 ($p = 0.001$) weeks of HAART. No significant difference were observed among arm2 with CYP2B6*1/*6 and CYP2B6*6/*6 genotypes compared to their arm 1 counterparts at both time points.

5.2.3 Comparison of proportion of patients in sub-therapeutic, therapeutic and supra-therapeutic ranges

Rifampicin co administration influenced proportions of patients with efavirenz levels below 1 µg/ml (sub-therapeutic), within 1–4 µg/ml (therapeutic range), and above 4 µg/ml (supra-therapeutic) at week 4. This influence was significant only among patients with CYP2B6*1/*1 genotype: a greater proportion of arm2 patients with CYP2B6*1/*1 genotype had sub-therapeutic levels as compared to their arm1 counterpart ($\chi^2 = 6.26$, $P = 0.04$). No such difference was observed among CYP2B6*1/*6 and CYP2B6*6/*6 genotypes

Similar to arm1, CYP2B6 genotype had significant influence on proportion of arm2 patients in different efavirenz therapeutic ranges: A higher proportion of arm2 patients with CYP2B6*1/*1 genotype had sub-therapeutic levels both at week 4 and at week 16 ($\chi^2 = 18.80$, $P = 0.00086$; and $\chi^2 = 17.78$, $P = 0.001$, respectively). However, there was no significant change in proportion of patients in different therapeutic ranges between week4 and 16.

A multivariate logistic regression analysis revealed that, the likelihood of sub-therapeutic levels at week 4 was 3 times higher in arm-2 compared to arm-1 ($p=0.0008$) and it was 5 times higher in patients with CYP2B6*1/*1 compared to CYP2B6*1/*6 genotype ($p=0.003$). Similarly, the likelihood of sub-therapeutic levels at week 16 was 3 times higher in arm-2 compared to arm-1 patients ($p=0.026$) and was 7 and 20 times higher in patients with CYP2B6*1/*1 compared to patients with CYP2B6*1/*6 ($P=0.0001$) and CYP2B6*6/*6 ($p=0.008$) genotypes respectively.

A multivariate logistic regression analysis also revealed that, the likelihood of supra-therapeutic efavirenz levels at week 4 was 3 and 38 times higher among patients with CYP2B6*1/*6 ($p=0.026$) and CYP2B6*6/*6 genotypes ($p<0.00001$) respectively, compared to patients with CYP2B6*1/*6. The likelihood of supra-therapeutic levels was still 3 and 32 times higher for patients with CYP2B6*1/*6 ($p=0.004$) and CYP2B6*6/*6 genotypes ($p=0.00001$) respectively at week 16 compared to patients with CYP2B6*1/*1 genotype.

5.3 IMPORTANCE OF ETHNICITY IN DETERMINING EFAVIRENZ PHARMACOKINETICS, AUTO-INDUCTION AND IMMUNOLOGICAL OUTCOME (PAPER III)

After pooling efavirenz plasma level data from Ethiopian and Tanzanian patients variability was higher at week 4 (CV=90.2%) compared to week 16 (CV=84.4%).

In hierarchical multivariate linear regression analysis, controlling for genetic differences between the two ethnic groups, patient ethnicity was a significant predictor of efavirenz plasma level at week 4 ($p=0.035$) but not at week 16 ($p=0.08$).

Similar to findings in paper I and paper II, CYP2B6*6 gene polymorphism was a significant predictor of the pooled efavirenz plasma level both at week 4 ($p<0.0001$) and 16 ($p<0.0001$).

Single nucleotide polymorphism in the gene for the drug transporter P-glycoprotein, ABCB1 4036A>G, was significantly associated with variability in efavirenz plasma level at week 4 ($p=0.002$) but not at week 16 (not a predictor even at univariate analysis, $p=0.98$)

Although CYP3A5 gene polymorphism explained some variability in efavirenz plasma level at week 4 following a univariate linear regression analysis, after controlling for other variables (patient country, hepatitis B co infection and other genetic polymorphisms), it was no longer a significant predictor of efavirenz plasma level at a multivariate analysis. Neither was it associated with efavirenz levels at week 16.

Patient ethnic group was also a significant predictor for week 4 ($p=0.009$) and 16 ($p=0.006$) intracellular efavirenz levels, after controlling for plasma efavirenz concentration and other variables (genetic polymorphism in CYP2B6, CYP3A5, and SLCO1B1 and body mass index, hepatitis B co infection and baseline alanine aminotransferase levels). However, plasma efavirenz concentration accounted for much of the variability in intracellular concentrations both at week 4 (27%, $p<0.0001$) and 16 (18%, $p<0.0001$).

Patient ethnic group also determined CD4 cell count both at week 24 ($p=0.004$) and 48 ($p=0.002$) being higher in Tanzanian compared to Ethiopian. Intracellular efavirenz level at week 4 ($p=0.013$) and a trend by ethnic group ($p=0.059$) were predictors of gain in CD4 count by week 12. However, maximum gain (population typical value) in CD4 cells depended on baseline CD4 count (higher for those with CD4 cell count >98) and patient ethnic group (higher in Tanzanians).

5.4 EFFECT OF CYP2B6, CYP3A5, UGT2B7 AND ABCB1 GENE POLYMORPHISMS ON MAGNITUDES OF CYP3A4/5 INDUCTION DURING EFAVIRENZ ALONE AND WITH RIFAMPICIN CO-TREATMENT IN HIV PATIENTS (PAPER IV)

The kinetics of 4 β hydroxycholesterol during induction and fall of induction was adequately described by a one compartment enzyme turn over model: its production depended on cholesterol and enzyme (CYP3A4/5) amount at a given time point, while its elimination depended on amount of 4 β hydroxycholesterol pool (first order kinetics). Enzyme amount depended on rate of induction or decay of induction and its rate of degradation (product of enzyme amount and degradation rate constant at a given time point). The enzyme degradation half-life (turn over value) was fixed to previously reported value of about 3 days, but we estimated very high population variability for this parameter (about 379%)

CYP2B6*6 genetic polymorphism influenced magnitude of induction of CYP3A4/5: arm 1 patients with CYP2B6*1/*1 genotype had lowest population typical value for maximum folds of induction (1.8) compared to CYP2B6*1/*6 (3.4) and CYP2B6*6/*6 (4.7). The population unexplained variability in the maximum folds of induction was 56%.

CYP3A5, UGT2B7 and ABCB1 genetic polymorphisms did not influence the magnitude of CYP3A4/5 induction.

Rate of 4 β hydroxycholesterol formation in arm1 patients at steady state of efavirenz based HAART (mean value = 2.3×10^{-7} /h) was significantly smaller than rate of formation in arm2 just before completion of rifampicin based anti-TB (typical value = 3.6×10^{-7} /h), p-value <0.0001, but **not** significantly higher than rate of formation in arm2 after maximum decay in enzyme amount following completion of TB therapy (mean value = 1.9×10^{-7} /h), p-value = 0.06. No gene polymorphism was identified to influence the rate constants of 4 β hydroxycholesterol formation neither in arm1 nor arm2 patients.

In arm2 patients, enzyme amount dropped to about 60% of its initial value after completion of anti-TB therapy and continuation of efavirenz based HAART. No gene polymorphism was identified to influence the magnitude of enzyme decay.

4 β -hydroxycholesterol elimination followed first order kinetics with long half-life of about 11 days.

5.5 TIMING, INCIDENCE, CLINICAL PRESENTATION, PHARMACOKINETIC AND PHARMACOGENETIC PREDICTORS OF DRUG INDUCED LIVER INJURY (DILI) DURING EFAVIRENZ WITH OR WITHOUT RIFAMPICIN CO-TREATMENT IN HIV PATIENTS (PAPER V)

In general there was transient, mild to moderate elevation of liver enzymes in both arms. Some of such elevations met the CIOMS criteria for diagnosis of drug induced liver injury (DILI). Median time to DILI differed between arm1 (2 weeks) and arm2 (5 weeks) patients. No DILI occurred after 12 weeks of HAART. The incidence of DILI in arm2 patients (10.0%, 10.7 per 1000 person-week) was almost two folds compared arm1 patients (5.9%, 6.3 per 1000 person-week), though not statistically significant (p=0.07). The only significant predictors of DILI were hepatitis C co-infection and CYP2B6*6 allele. Patients co infected with hepatitis C (anti-HCV antibody positive) or carriers of CYP2B6*6 allele had 5.32 and 2.83 times hazard of developing DILI than non-infected or non-carriers respectively.

6 DISCUSSION

One of the major findings of this work in the context of optimization of efavirenz dosage is that: efavirenz exhibit prolonged auto induction (up to 16th week of therapy) among Tanzanian patients (paper I). This might be due to slower induction rate by efavirenz as a result of some unknown rate limiting steps in the molecular pathways of induction. However, this property is not observed among patients already on rifampicin therapy probably because rifampicin activates all mediators of induction (paper II): rifampicin was found to have relatively high induction rate constant for CYP2B6 (0.6 /day) and CYP3A4 (0.65 /day).[180] This prolonged efavirenz auto induction was reflected in high proportion of patients below therapeutic range after 16 weeks of therapy; indeed, several cohort studies have indicated treatment failure after a median time of 4 months on HAART despite initial clinical recovery.[181-185] It was also reflected in comparable efavirenz concentrations between patients on efavirenz alone and those on efavirenz + rifampicin co treatment at week 16 despite their significant difference at week 4. This implies that erroneous conclusion can be made, about rifampicin induction, that it does not affect efavirenz plasma level; if the comparisons are made after maximum induction by efavirenz is attained.[171, 186, 187]

Depending on baseline induction of efavirenz metabolizing enzymes, which may vary between ethnic groups or geographical locations due to differences in cultural or environmental exposure, prolonged auto induction may differ between populations. This was the case when efavirenz levels were compared across duration of therapy between Ethiopian and Tanzanian patients (paper III). Therefore, for some populations particularly Tanzanians, optimization of efavirenz dosage for patients who are on efavirenz based HAART should consider duration since initiation of HAART.

Another major finding, in the context of efavirenz dose optimization, is that CYP2B6*6 allele influenced magnitude of induction of CYP2B6 gene expression. Among patients on efavirenz based HAART alone, prolonged induction was only significant in non-carriers of this mutation (paper I). Therefore, in addition to time on therapy, optimization of efavirenz dose should also consider CYP2B6 genotype. Only individual with CYP2B6*1/*1 genotype may need dose increment after prolonged therapy (16 weeks in this case).

Another finding from this work is the influence of ethnicity (geographical, cultural or environmental influence) on variability in efavirenz exposure (paper III). Similar finding has been reported previously; controlling for CYP2B6 and CYP3A5 genetic polymorphism, Haas *et al.*, found that, Hispanics had significantly lower clearance compared to African American [188]. In the current study, this influence was further reflected in the significant differences in immunological outcomes between the 2 ethnic groups. To our knowledge no study has been designed to compare outcomes of anti-retroviral therapies (ART) across populations. Only a subgroup analysis of a clinical trial determined that, Asian population (90% recruited in Bangkok) had fewer individuals with treatment and virological failures compared to other regions.[55] This finding therefore is a rationale for studies designed to compare ART outcomes

across geographic regions. It also implies that efavirenz dose individualization should, in addition to other factors, consider patient ethnicity.

The finding that ABCB1 c4036A>G mutation predicted efavirenz plasma level at week 4 was not unexpected as it has been reported to influence oral bioavailability of this compound after single dose administration;[118] and plasma levels at steady state.[122] It has also been reported to influence intracellular efavirenz plasma level [189], something which we also found (paper III). Others have also found that this polymorphism influences HIV suppression after 6 months of therapy [122]. Therefore, our finding compliment other findings and implies that, this gene polymorphism should also be considered when individualizing efavirenz dose.

Replicating other's findings, we also found that CYP2B6*6 allele influenced efavirenz plasma level among patients on HAART with or without rifampicin co administration (paper I, II and III). In fact, it was a major predictor of pooled (Ethiopian and Tanzanian) efavirenz plasma level both at week 4 and week 16 compared to other variables (paper III). This is consistent with the effect of this mutation on the catalytic activity of the CYP2B6 enzyme. It was observed that CYP2B6 c.516C>T mutation (CYP2B6*allele) resulted into splice variants of messenger RNA (mRNA) and enzymes with reduced catalytic activities.[190, 191] Thus homozygous carriers of this mutation are poor metabolizers, heterozygous carriers are intermediate and homozygous non carriers are relatively extensive metabolizers of efavirenz. This variability in metabolic capacity among individuals results into variability in exposure. Therefore, CYP2B6*6 genetic polymorphism is an important covariate in optimization of efavirenz dosage regardless of duration of therapy, rifampicin co administration (concomitant medications) or ethnicity (geographical location). Other researchers have recommended *priori* efavirenz dose individualization based on this gene polymorphism (genotype based dosing). Our finding of other factors responsible for the variation of efavirenz plasma level will serve to fine tune dose optimization to reduce variability in exposure even further; this will ensure consistent treatment outcomes across individuals and populations.

The influence of rifampicin on efavirenz exposure was significant but influenced by CYP2B6 polymorphism in gene dose dependent manner at week4. This influence was not seen at week 16 possibly due to attainment of comparable induction by efavirenz among arm1extensive metabolizers. Efavirenz might achieve comparable magnitude of induction as rifampicin after long term treatment in CYP2B6 extensive metabolizers. In fact a larger proportion of these patients had sub-therapeutic plasma levels with or without rifampicin co administration at week 16. This emphasizes that, with or without rifampicin co administration, patients with CYP2B6*1/*1 are prone to treatment failure and may need efavirenz dose increment.

The importance of CYP3A5 gene polymorphism to inform dose individualization seems to be minor at the moment. However, its importance in determining efavirenz metabolism in poor CYP2B6 metabolizers implies that it may be important in dose individualization in these individuals. Considering the role of CYP3A in efavirenz metabolism,[192] its significance should be investigated further by studies involving many patients with this CYP2B6*6/*6 genotype.

With regard to optimization of dosage for concomitant medications during efavirenz based HAART, we found that CYP2B6 gene polymorphism influences the variability in CYP3A4/5 induction in gene-dose dependent manner (paper IV). This is through its influence on efavirenz plasma exposure; therefore poor metabolizers for efavirenz have higher exposure and hence higher induction of CYP3A4/5. The approximately 5 folds increase in CYP3A4/5 activity in these individuals implies that, they are susceptible to treatment failure of co-morbidities if CYP3A4/5 substrate drugs are taken without some dose increment. Therefore, genotyping for CYP2B6*6 gene polymorphism to guide efavirenz dosage and reduce inter-individual variability in efavirenz exposure, will reduce variation in treatment outcomes of both HIV infection and co-morbidities.

The finding that efavirenz sustains only about 60% of CYP3A4/5 induction after completion of rifampicin based anti-TB therapy implies that, dose adjustments made for concomitant CYP3A4/5 drug substrates during HIV/TB co treatment may need to be changed. If doses were increased for drugs with very narrow therapeutic margin, dose reduction of up to about 1.6 folds may need to be done at about 15 days after completion of TB therapy.

In our assessment of liver injury biomarkers (ALT, AST and direct bilirubin) in both arm1 and 2 patients, we did not find any of the FDA suggested indicators for drug's potential to cause severe DILI during clinical use (a necessary requirement for drug withdraw from clinical use). Therefore although from elsewhere, efavirenz has been associated with fulminant hepatic failure, we did not find its potential to cause severe DILI in Tanzanian patients. We therefore support its continued clinical use with or without rifampicin in Tanzania. However, a finding of mild and transient elevation of liver injury biomarkers associated with CYP2B6*6 allele (a determinant of efavirenz exposure) implies that, supra-therapeutic exposures to efavirenz should be avoided. Since efavirenz concentration versus probability of hepatotoxicity relationship has not been established (dose-response curve). Maintaining efavirenz therapeutic range may be a good strategy to avoid hepatotoxicity. This can only be achieving by efavirenz dose individualization, either through stratification (genotype and ethnicity) based dosing (priori dose individualization) or target concentration intervention (posteriori dose individualization). Furthermore, patients with liver diseases particularly hepatitis C should be careful monitored when undergoing efavirenz based HAART to avoid drug-disease interaction to cause severe hepatic injury.

7 CONCLUSIONS AND RECOMMENDATIONS

Our studies have assessed factors which should be considered when setting algorithms for efavirenz dose optimization, these should include: duration of therapy at least among Tanzanians, CYP2B6*6, ABCB1 c4036A>G and CYP3A5 genetic polymorphisms, rifampicin co treatment and patient ethnicity (geographical location). These factors can be used for priori and posteriori dose individualization. We also recommend further studies evaluating the influence of these factors on treatment outcomes particularly: virological suppression, immunological improvements and CNS toxicity.

This thesis also concludes that, it is necessary to carry out dose optimization for concomitant CYP3A4/5 drug substrate taken during efavirenz with or without rifampicin co-treatment. Factors influencing efavirenz exposure as stipulated above should be considered when optimizing dosage for these concomitant medications. However, we also recommend further studies to assess clinical relevance of efavirenz induction of CYP3A4/5.

The thesis also supports continued clinical use of efavirenz with or without rifampicin on grounds that it might be safe to the liver; at least among Tanzanian patients when supra-therapeutic efavirenz concentration are avoided. We therefore recommend CYP2B6 genotype based dose individualization or target concentration intervention to reduce efavirenz dose in order to achieve therapeutic range in patients with over-exposure.

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