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HIV Treatment Outcomes in Uganda: The Impact of Baseline Characteristics and Variability in Pharmacokinetics and Pharmacogenetics of Antiretroviral drugs



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ABSTRACT

Guidelines specify criteria for initiation and monitoring of antiretroviral treatment (ART), including options for first line regimens. Immunological progress remains a widely used form of HIV/ART monitoring and efavirenz, a preferred first line antiretroviral drug. CD4 cell values vary among HIV seronegative populations and among HIV patients starting ART. Variations in pharmacokinetics and pharmacogenetics of efavirenz are widely documented. This thesis explores the role of variability in relevant baseline immunological characteristics in HIV negative and positive populations and how variations in the pharmacokinetics and pharmacogenetics of efavirenz can affect HIV/ART response in Ugandan populations.

We conducted three sub-studies of HIV seronegative and seropositive Ugandans to address these issues. **Sub-study I; Paper 1:** 206 HIV seronegative Ugandans were recruited for a cross-sectional study of variations in CD4 reference ranges. We observed a CD4 reference range of 418-2105 cells/ μ L for this population, wider than ranges reported from other settings. Socio-economic status, altitude and prevalent tropical illnesses influenced CD4 reference values. **Sub-study II; Paper 2:** Using records for 426 HIV infected Ugandans treated between 2002 and 2007, we found that patients who started therapy at low baseline CD4 cell levels were less likely to achieve complete immunological recovery. **Sub-study III; Papers 3-6:** A total of 263 HIV positive, ART naïve Ugandans, of which 157 were TB co-infected, were recruited and followed up to 8 months after starting ART. Data from these 263 patients, and from 105 healthy volunteers, were used to describe efavirenz pharmacokinetics for the population, and to study the effect of HIV infection, pharmacogenetics and antituberculous treatment on the pharmacokinetics of efavirenz. Variations in efavirenz pharmacokinetics were observed, and 95% of the patients reached steady state maximum plasma concentrations (C_{max}) above the recommended range of 1-4 μ g/dl (3.2-12.6 μ mol/L). Efavirenz-related central nervous system (CNS) toxicity was observed in 40 (69%) of 58 patients who were evaluated, and 38 (95%) of the patients with CNS toxicity had efavirenz plasma concentrations that were above the recommended range. HIV patients displayed a 30% lower relative bioavailability of efavirenz compared to healthy volunteers while CYP2B6*6/*6 genotype had lower apparent oral clearance and higher plasma concentrations of efavirenz. Regardless of rifampicin co-treatment, efavirenz autoinduction was prominent in CYP2B6*1/*1 genotypes, and surprisingly, long-term efavirenz clearance was higher among patients receiving efavirenz-based HAART alone compared to those who were co-treated with rifampicin-based therapies. Population pharmacokinetic modelling of data from 99 of the HIV only patients showed that the efavirenz exposure was twice as high among patients homogenous for the CYP2B6*6/*6 mutation compared to those without the mutation. It was found that a daily dose of 450 mg efavirenz in the general Ugandan population and a dose of 300 mg in CYP2B6*6/*6 population gave adequate drug exposure.

Population characteristics, including immunological and genetic variations, affect the response to antiretroviral treatment, and population based CD4 cell values and pharmacogenetic-based dose modifications of antiretroviral therapies may improve HIV/ART outcomes.

Key words: ART Outcomes, Baseline Characteristics, CD4, Efavirenz, HIV, Pharmacogenetics,

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LIST OF ABBREVIATIONS

ABCB1	ATP-binding cassette, sub-family B
ACD4	Absolute CD4 Cells
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral treatment
AUC	Area Under the Plasma Concentration Time Curve?
CCR5	C-C chemokine receptor Type 5
CI	Confidence Interval
C _{max}	Maximum Concentration
C _{min}	Minimum Concentration
CXCR4	C-X-C chemokine receptor Type 4
CYP450	Cytochrome P450
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
GCP	Good Clinical Practice
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IQR	Interquartile Range
LMIC	Low and Middle Income Countries
mRNA	messenger RNA
MVEC	Microvascular Endothelial Cells
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogenase
NAT2	N-acetyltransferase 2
NCST	National Council of Science and Technology
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NONMEM	Non-linear mixed effects modelling
PCR	Polymerase Chain Reaction
PEPFAR	President's Emergency Fund for AIDS Relief
PLWH	People living with HIV
PMTCT	Prevention of Mother to Child Transmission
RNA	Ribonucleic acid
SD	Standard Deviation
SES	Social Economic Status
SNP	Single Nucleotide Polymorphism
t _{1/2}	Half-life
TB	Tuberculosis
UGT	Uridine 5'-diphospho-glucuronosyltransferase
V/F	Volume of Distribution after oral dose
VCT	Voluntary Counselling and Testing
VL	Viral Load
WHO	World Health Organization
UNAIDS	United Nations Programme on HIV/AIDS

1. INTRODUCTION

1.1. BACKGROUND ON HIV AND ANTIRETROVIRAL THERAPY

Although HIV (human immunodeficiency virus) still poses a major health threat worldwide with an estimated 34 million infected people [1], the epidemic has greatly been contained, resulting in reduction of HIV-related morbidity and mortality incidences. The positive shift largely followed introduction of antiretroviral therapy, and preventive measures that were instituted during the peak of the HIV epidemic.

1.1.1. Effect of antiretroviral treatment on the HIV burden

Antiretroviral therapy (ART) has been proved to improve lives of HIV infected persons [1, 2] by reducing HIV/AIDS-related mortality and morbidity. An estimated 2.5 million deaths were averted in low and medium income countries (LMIC) between 1995 and 2010 when antiretroviral therapy was introduced [1]. Of these averted deaths, 1.8 million were in sub-Saharan Africa, a region with over 69% of the estimated 34 million people living with HIV [1, 2]. The use of ART has also been shown to give protection to the general population by causing a reduction in the rate of new infections. There was an estimated 25% reduction in new infections in sub-Saharan Africa in 2011 (total 1.8 million new infections) compared to 2001 (2.4 million new infections) [2] and 27% fewer infections in 2010 compared to 1996 after introduction of ART [1]. Providing antiretroviral prophylaxis to pregnant women living with HIV prevented over 350 000 children from acquiring the infection between 1995 and 2010 [1], and there was a 20% reduction in the mother to child transmission between 2001 and 2011 [3]. At least 85% of the children who were protected from HIV live in sub-Saharan Africa, a region with the highest HIV prevalence among women of reproductive age [1, 3].

1.1.2. Antiretroviral treatment coverage

Antiretroviral treatment benefits spurred a progressive improvement in the access to antiretroviral drugs. The Global Fund and PEPFAR Fund among other donors ensured improved access of antiretroviral treatment to resource limited settings. In 2012, 9.7 million of the 34 million people living with HIV (PLWH) were estimated to be on ART in low and medium income countries [3], which is a six-fold increase from 2005 when only 1.3 million people were receiving ART [1]. The figures also represent an increase in ART coverage from 20% to 47% for low and mid income countries, Uganda inclusive. Figure 1 below shows a progressive increase in ART coverage between 2003 and 2010.

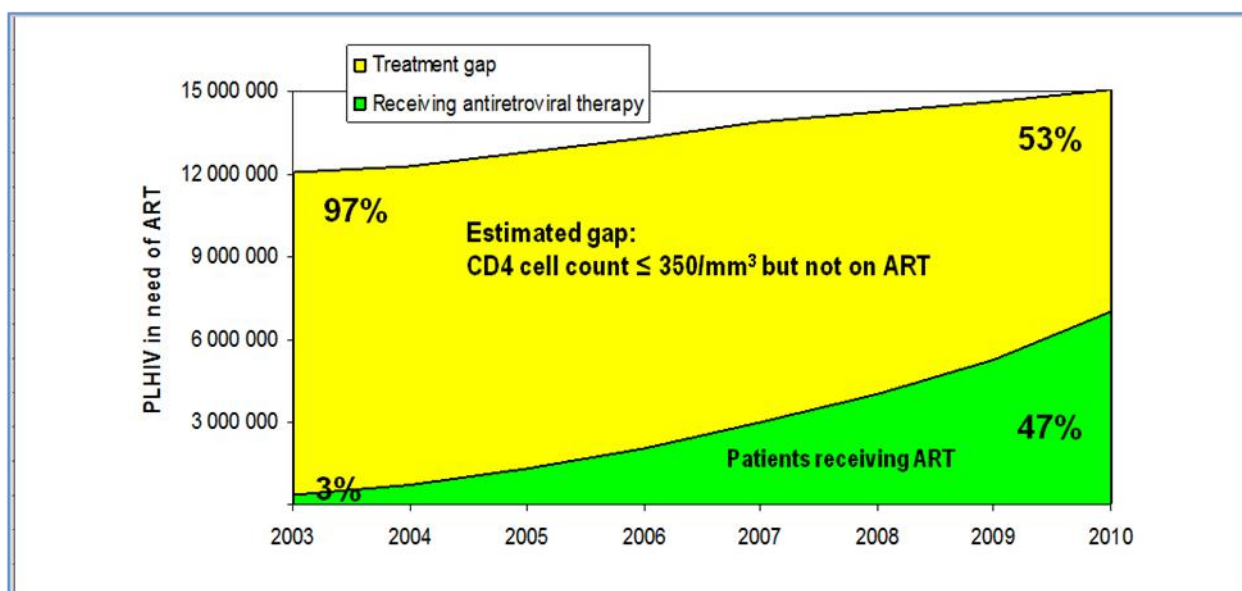


Figure 1: Progressive increase in antiretroviral treatment coverage in low and middle income countries between 2003 and 2010 [1, 4]

1.1.3. Treatment goals

The goals of initial HIV therapy as defined by the United States Department of Health and Human Services guidelines include restoration and preservation of immune function; control of HIV replication; prevention of HIV transmission; prevention of drug resistance; reduction of HIV-related morbidity and mortality; and improving quality of life [5, 6]. Nevertheless, regions and countries have not experienced the positive impact of ART in exactly the same way. Between 2005 and 2011, AIDS-related mortality increased by 21 percent in Eastern Europe and Central Asia, and by 17 percent in the Middle East and North Africa [2] while the annual number of newly infected people rose from 43 000 in 2001 to 59 000 in 2010 [1, 2]. However, in Western Europe and Southern Africa, the mortality and number of new infections decreased over the same time period [1, 2]. Uganda is one of the few African countries where the incidence of HIV has continued to increase, with the number of new infections rising by 11 %, between 2007 and 2010 [5, 6]. Timing of treatment initiation is one of the major factors implicated in determining antiretroviral treatment success.

1.2. WHEN TO START ANTIRETROVIRAL THERAPY

Not only the access of antiretroviral treatment, but also the timing of ART initiation has proved to play a key role in determining therapeutic outcomes, especially in resource-limited settings where the question of cost-effectiveness is paramount. The debate of when to start treatment lies

on balancing meeting the treatments goals and reducing unnecessary costs or unwanted effects related to long periods on ART.

Although other indicators of when to start treatment exist, CD4 T-lymphocyte cell counts are pivotal in defining HIV progress before and after starting treatment among adults and adolescents infected with HIV. T-lymphocytes are specialised white blood cells playing a central role both in antibody and cell-mediated immunity[7]. The human immunodeficiency virus attacks cells expressing the CD4 molecule, especially the T-Lymphocytes, causing abnormalities that include progressive depletion and qualitative dysfunction of the CD4-T-cell population [8-10]. This forms the basis of the progressive immunosuppression following HIV infection and the immunological recovery that follows viral suppression by antiretroviral therapy. During the initial stages of HIV infection, the bone marrow produces and replenishes the CD4-T-cell population but there is progressive injury and dysfunction of the endothelial cells of the bone marrow as HIV/AIDS advances [11, 12]. The pathological implications of HIV infection on the CD4 T-Lymphocytes hence form the focus on when to start antiretroviral therapy. Treatment should start in time to counteract the immunosuppressive effects of the virus, and perhaps before the bone marrow loses its capacity to replenish the invaded cell populations.

The first WHO guidelines set ART initiation when CD4 cell counts decline below 200 CD4 cells/mm³ for adults and adolescents in resource limited settings [13]. This was based on the observation that most life threatening opportunistic infections set in with severe immunosuppression when CD4 cell count falls below this threshold. Other criteria included advanced clinical staging of HIV/AIDS irrespective of CD4 cell counts. Following research findings of better treatment outcomes with earlier ART initiation, there have been progressive shifts in guidelines for ART initiation from <200CD4 cells/mm³ to <250 CD4 cells/mm³ followed by <350 CD4 cells/mm³[13-15]. The most recent WHO guidelines recommend HAART for all adults and adolescents infected with HIV with CD4 cell counts \leq 500 cells/mm³ [16]. Further, the guidelines recommend ART irrespective of CD4 count for HIV infected children under 5 years of age, pregnant and breastfeeding women with HIV, HIV-positive partners in serodiscordant relationships and HIV positive persons with active tuberculosis or hepatitis B co-infections [16]. It is evident that current thoughts for ART initiation are towards “test and treat”, i.e. starting all HIV positive persons on ART irrespective of their CD4 counts [13, 15-18].

1.3. MONITORING ANTIRETROVIRAL TREATMENT RESPONSE

Although the WHO and United States Department of Health and guidelines list several antiretroviral treatment goals, the financial implications related to the day-to-day monitoring of all these goals are beyond the possibilities of health systems in resource limited settings. The WHO recommends routine clinical and immunological monitoring of all HIV/ART patients in resource limited settings while other forms of monitoring, including virological and therapeutic drug monitoring, are reserved for cases of treatment failure and toxicity [13, 15]. This has led to immunological testing being the most available form of monitoring HIV/ART in resource limited settings, where the majority of PLWH reside.

Although immunological, virological and clinical monitoring is in place, the measures and values used in monitoring HIV/ART progress can be influenced by several factors, and this may affect the interpretation of antiretroviral treatment outcomes.

1.4. POPULATION VARIATIONS IN ABSOLUTE CD4 CELL COUNTS

Despite the usefulness of the CD4 T-Lymphocytes in monitoring HIV/ART progress, absolute CD4 cell counts may vary within and between populations of HIV seronegative individuals. Age, gender, altitude, nutrition and race are among several factors that have been shown to cause CD4 cell variations in a normal HIV seronegative population [19-32]. CD4 cell counts have been reported to be lower at higher altitudes and several studies have indicated higher values for women than men [28-30]. In addition, females have been shown to seroconvert and also reach AIDS at higher CD4 cell counts compared to men [33, 34], and there is some evidence that untreated persons with high CD4 cell counts or percentages may deteriorate faster than their counterparts with lower CD4 reference values [33-36]. This could have implications for treatment initiation. The variations caused by age seem to have the greatest challenge regarding the use of CD4 cells in HIV/ART management. The cell counts are highest during childhood life, declining slowly towards adulthood [21, 22, 26]. This has made it necessary to use CD4 cell percentages rather than counts in HIV managements for children below 5 years [14]. Other than HIV, other infections prevalent in sub-Saharan Africa can affect the immunity resulting in reduced CD4 cell [37-39]counts. Variations of CD4 cell counts can occur in one individual during the course of a day basing on level of stress, exercise or circadian cycle[40], and larger diurnal variations have been reported among HIV seronegative compared to seropositive individuals[41]. Despite these inter- and intrapopulation variations in CD4 cell reference values, similar CD4 cell reference

points are applied during ART initiation and monitoring. A better understanding of CD4 cell variations and their influence on treatment outcomes may improve HIV/ART response.

1.5. THE IMPACT OF BASELINE CHARACTERISTICS ON HIV/ART OUTCOMES

1.5.1. The effect of the virus characteristics on HIV/ART response

Characteristics of the Human Immunodeficiency Virus (HIV) may have direct effects on HIV/ART progress and therapeutic outcomes, or may indirectly affect the outcomes by interfering with drug disposition. While HIV type 2 is less virulent compared to HIV type 1 [42], and may hence cause limited immunosuppression over time, HIV-2 has been shown to have natural resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [43] and certain polymorphisms found in the HIV-2 protease gene confer resistance to protease inhibitors which target HIV-1 [43, 44]. Specific to HIV-1, certain subtypes were found to be more virulent in some populations causing faster disease progressions and death among untreated patients [45-49]. Data from Uganda and Kenya showed that untreated patients with HIV-1 sub-type D when compared to HIV-1 sub-type A, progressed faster to AIDS; had higher risk of mortality and faster rate of CD4 cell decline [45, 46, 48]. Unfortunately, the D-subtype was shown to be the predominant type in the Ugandan HIV infected population [48, 50]. Retrospective data from Senegal showed that although sub-type A was the most predominant subtype, patients infected with HIV-1 subtypes C, D and G had faster progression to AIDS compared to those with sub-type A [47]. While implications on treatment are still under investigations, studies have shown higher frequencies of both reverse transcriptase and protease mutations among treated patients with HIV1 subtype D compared to the other clades [51, 52]. The current antiretroviral agents target different viral proteins essential to the lifecycle of HIV, yet the different viral types or subtypes may possess distinct polymorphisms that alter their interaction with specific antiretroviral drugs (ARVs) [53]. For this matter, variability in HIV subtype can impact on the baseline susceptibility to ARVs, and may in the long run affect ART response and acquisition of ARV resistance. Since most drugs were developed with knowledge on subtype B, which is the most circulating clade in North America and Europe [52, 53], there might be a knowledge gap regarding susceptibility of some HIV clades to these drugs.

Baseline viral load (VL) been shown to affect HIV/ART outcomes. Patients with high baseline viral load (>100,000 copies) have poor clinical, virological and immunological success [54].

1.5.2. Effects of HIV/AIDS on drug disposition

HIV/AIDS is a disease that causes physiological and anatomical damage to structures which are relevant in the disposition of drugs and nutrients. Altered absorption of nutrients and medicines has been seen in patients with HIV and the functional impairment of the absorptive surface worsens with HIV progress [55]. Intra-luminal bacterial overgrowth, opportunistic infection, HIV enteropathy and immune dysregulation are among the possible causes of histological and functional impairment of the GIT in HIV infected persons [55]. A higher prevalence of HIV-infected patients with intestinal dysfunction was observed in Kampala, Uganda compared to London, UK [55]. Changes in acid production from parietal cells of the gastrointestinal tract have been observed in HIV [56] and the resultant changes in the pH can affect drug absorption. The P-glycoprotein is a drug transporter protein that has been shown to be overexpressed in cells of HIV-infected compared to un-infected individuals, and can lead to increased efflux of drugs. This could explain the reduced accumulation of zidovudine observed in CD4-T lymphocytes which overexpressed the P-glycoprotein[57].

Increased mean intestinal permeability was observed during HIV disease progress and patients with advanced HIV/AIDS had a three-fold increase of area under the curve (AUC) and a three-fold decrease in oral clearance of ganciclovir[58]. Erratic absorption and low bioavailability of zidovudine was observed in HIV patients with lower CD4 counts [59]while a group of French HIV patients showed enhanced saquinavir exposure in advanced disease [60] The effect of HIV/AIDS status on pharmacokinetics for most of the antiretroviral agents remains to be explored.

1.5.3. Effects of variations in CD4 cell characteristics

Variations in CD4 cell characteristics, including quality and quantity differences, can influence HIV infectivity, virulence and HIV/ART response. The 32–nucleotide deletion (Δ32) within the β–chemokine receptor 5 (CCR5), a co-receptor on the CD4-receptor bearing cells, has been shown to confer resistance to HIV infection [61]. In order to attach to the CD4-cell receptor, HIV requires CXCR4 and CCR5 chemokine co-receptors which are members of the G protein-coupled receptor [62]. The C-C chemokine receptor type 5 (CCR5) is the main co-receptor for the M-subtype (main HIV sub-type) during early stages of infection while the C-X-C chemokine Receptor type 4 (CXCR4) co-receptor is more virulent and comes into play in late stages of the disease [63]. There are however some cells, including the CD34(+) cells, which express both CD4-receptor and the CXCR4 co-receptor but remain non-permissive to infection by HIV following their ability to secrete high levels of CCR5-binding chemokines [64]. A recent study

showed that a high β -defensin genomic copy number observed in an African HIV/ART cohort was associated with high baseline VL and impaired immune reconstitution after 48 weeks of treatment with HAART [65]. β -defensins are a family of multifunctional peptides with a role in inflammation, and they have been shown to bind to HIV and inhibit its replication. These peptides show extensive variation in copy number of a given DNA sequence in a diploid genome causing alterations in their function [66]. The C868T single nucleotide polymorphism (SNP) in the CD4 receptor of infants born to HIV1-infected mothers were found to have a higher risk of acquiring HIV compared to infants without the mutation [67]. Chemokine receptor antagonists including those under development differ in their comparative capacities of blocking these receptors, and hence patients with mainly the CXCR4 co-receptors may not benefit from maraviroc which is one of the available CCR5-receptor antagonists [68-70]. Further differences in CD4 cells have been reported to be associated with differences in latency periods of HIV progress and those with longer latency period giving rise to slow progressors and elite controllers of HIV.

1.5.4. The effect of variations in baseline CD4 cell counts

The effect of baseline CD4 cell counts on antiretroviral treatment outcomes has previously been examined in ART cohorts in Europe, North America and parts of Africa [54, 71, 72]. These studies revealed that low baseline CD4 cell counts was independently associated with death, and immunological or virological failure [71, 72].

Patients with advanced HIV are thought to get T-cell activation and exhaustion, and the microvascular endothelial compartment (MVEC) of bone marrows of patients with lower baseline CD4 cell counts were shown to be heavily infected with HIV compared to the MVEC in patients with higher CD4 counts [11, 12, 73]. These patients had a reduced capacity to respond to cytokine signals that augment blood cell production indicating that the hematopoietic potential might be compromised in patients with severe immunosuppression. Low baseline CD4 counts has also been associated with increase toxicity of ARVs [74], and this effect on adverse drug reactions is not limited to antiretroviral therapy. Research has demonstrated an increasing risk of drug induced hepatotoxicity with reduced CD4 cell counts [75].

Recent research shows reduced HIV transmission among discordant couples whose HIV infected partners start treatment early [76-78]. The HPTN 052 trial that followed serodiscordant couples from several African countries and Asia (Botswana, Brazil, India, Malawi, South Africa,

Zimbabwe and Thailand) showed that providing antiretroviral therapy to the partner living with HIV immediately when CD4 cell count falls below 550 cells/mm³ was associated with a 96% reduction in the likelihood of HIV transmission compared to couples where ART was delayed until CD4 cell values fell below 250 cells/mm³ [78].

Moreover, there is additional evidence regarding the importance of baseline HIV characteristics in HIV/ART progress. A study that compared CD4 T cell restoration over 1 year between patients with primary and those with chronic HIV showed that immune reconstitution was faster and more complete among subjects who commenced antiretroviral therapy during primary HIV-1 infection [79]. A study that compared immunological and virological responses in ART naïve, HIV-1-infected adults at early stage of established infection (CD4 > 400) with HIV seronegative individuals showed that increments of CD4 T cell count in blood and percentage in lymph nodes were comparable to HIV-negative subjects by week 24 in blood and by week 48 in lymph nodes [80]. CD4 cell counts have been shown to predict survival of HIV treated children, while recent data indicate that HIV infected individuals starting treatment with CD4 cell counts ≥ 500 cells/mm³ would have a life expectancy comparable to that of the general population [81-84]. Functional cure of HIV was reported in an HIV infected child who was immediately started on ART instead of a conventional prevention of mother to child transmission (PMTCT) approach [85, 86] and in a group of 14 patients of the Visconti cohort whose treatment was started within 10 weeks of infection [87].

Theories of presence of the latent virus and/or provirus within resting CD4 cells throw some hope of future approaches towards HIV cure. It is believed that HIV can stay within resting CD4 cells as latent virus not undergoing replication, and/or as provirus which easily reverts to viral DNA production [88]. The average half-life of these resting CD4 cells is estimated at 44 months [88] and this theory provides prospective curative approaches including inducing latency of the virus and then eliminate the carrier CD4 cells or by controlling the provirus containing cells and hinder further viral DNA formation [89-91]. Suppression of productive infection has already been achieved in some CD4, CCR5 and CXCR4 expressing cells and in a human lymphoid cell line against the simian immunodeficiency virus (SIV) [91]. Other strategies include protecting the targeted cells (CD4 cells) and cause them to resist the infection by disrupting the CCR5 co-receptor [92, 93]. These reports show the necessity of preserving the CD4 cells against continued destruction by HIV. This can be achieved by early ART initiation, and the preserved CD4 cells would be used in better future treatment options, including inducing latency.

1.5.5. The effect of variation in other baseline characteristics

Other baseline factors including age, gender, HAART regimen, and drug adherence affect antiretroviral treatment outcomes. Young age has been associated with faster immunological response and lower mortality [54, 74]. Although data related to effect of gender is contradicting, some studies have shown lower mortality and better immunological/ virological responses among women compared to male treated patients [94, 95]. Additionally, thrombocytopenia and malnutrition were reported to be associated with increased mortality among HIV treated patients[95].

1.6. THE IMPACT OF HUMAN GENETICS ON ART OUTCOMES

Human genetics have been associated with antiretroviral therapeutic outcomes by either having an effect on drug disposition or by possibly having a direct effect on certain therapeutic outcomes [96-99]. Immunological recovery among HIV treated patients has been related to CYP2B6 and UGT2B7 genotypes [96]. Increase in the mean CD4 counts after ART initiation was shown to continue for a longer period among CYP2B6*6 and UGT2B7*2 carriers compared to CYP2B6*1/*1 and UGT2B7*1/*1 carriers [96]. CYP2B6 516/983, and CYP2B6 516/CAR (rs2307424) genotypes were associated with efavirenz plasma concentrations, efavirenz-related neuropsychiatric effects and early discontinuation of efavirenz [100-103]. ABCB1-3435 C>T has been associated with efavirenz related risk of virological failure [104] while CYP2B6 516 was associated with virological success among blacks [102]. An association was reported between drug induced liver injury (DILI) and CYP2B6*6 genotype, NAT2 slow acetylators and ABCB1 3435TT genotypes [99]. Although these effects could figuratively have followed high plasma efavirenz levels among patients of these genotypes, one of the studies did not show any association between efavirenz concentrations and DILI [99]. The HLA-B*5701 mutation is associated with increased risk of abacavir-related hypersensitivity reaction while CYP2C19 polymorphisms predict nelfinavir plasma levels and increased risk of virological failure to nelfinavir [105-107].

1.7. PHARMACOKINETICS AND PHARMACOGENETICS OF EFAVIRENZ

Efavirenz has a long half-life ($t_{1/2}$) of 52- 72 hours following a single dose, but the $t_{1/2}$ is reduced to 40-55 hours after repeated daily dosing owing to the drug inducing its own metabolism [108]. The steady state is reached within 6-10 days, and at steady state C_{max} concentration is $12.9 \pm 3.7 \mu\text{mol/l}$, trough concentration (C_{min}) $5.6 \pm 3.2 \mu\text{mol/l}$ and oral clearance of $0.18 \pm 0.072 \text{L/h/kg}$ [108, 109]. Efavirenz pharmacokinetics can be affected by several factors including diet, gender, genetic

polymorphisms and drug-drug interactions [109]. The isoenzyme Cytochrome P450, family2, sub-family B, polypeptide 6 (CYP2B6) plays the greatest role in efavirenz metabolism, with minor contribution from CYP3A4 and CYP3A5, CYP1A2, CYP2A6 and CYP2C9 isoenzymes [110-112]. The primary and major metabolite of efavirenz is 8-hydroxyefavirenz, and 7-hydroxyefavirenz is a minor metabolite [112]. However, 8-OH efavirenz may subsequently be hydroxylated to 8,14-dihydroxyefavirenz [112]. At normal efavirenz concentrations, CYP2B6 catalyses formation of both 8-hydroxyefavirenz and 8,14-dihydroxyefavirenz. CYP2C9 plays a minor role in formation of the 8,14-dihydroxyefavirenz, while other isoforms only catalyse the formation of 8-hydroxyefavirenz [112]. Among these enzymes, the catalytic potential is highest to lowest in CYP2B6, CYP3A5, CYP3A4, and CYP1A2, respectively. However, at very high efavirenz concentrations, CYP2A6, and CYP2C9 contribute to the formation of 8-hydroxyefavirenz in addition to CYP2B6, CYP3A5, CYP1A2, CYP3A4 [112]. CYP2A6 and CYP3A4 have shown prominence in efavirenz clearance when CYP2B6 is impaired [113]. UGT2B7 is also involved in efavirenz metabolism while ABCB1, a gene that encodes P-glycoprotein plays a role in efavirenz disposition.

CYP2B6 is primarily expressed in the liver where it contributes 1-10% of the total P450 enzymes, and with an inter-individual variability of up to 100 fold in the degree of protein expression and/or activity [114, 115]. The inter- and intraindividual variability in the protein expression and activity of the hepatic CYP2B is attributed to several factors including environment, age, gender, genetic polymorphisms, and induction or inhibition by other compounds. Regarding the genetic causes, single nucleotide polymorphisms (SNPs) cause variations in protein expression and enzymatic activities. The SNP c.516 G>T in the allele CYP2B6*6 has been associated with approximately 50–75% decreased protein level [116], and human liver microsome samples (HLM) of persons homozygous for the CYP2B6*6 polymorphism showed a 2-fold lower catalytic activity for efavirenz compared to the wild type [117]. Six polymorphic variants (CYP2B6.4 to CYP2B6.9) were shown to be alternatively expressed; CYP2B6*5 and *7 having increased metabolic activity compared to the wild type, CYP2B6* and *9 having reduced activity, while *8 had no activity for efavirenz [116]. Efavirenz pharmacokinetics is predicted by CYP2B6 polymorphisms: The (516G -> T and 983T --> C) polymorphisms being associated with low efavirenz clearance and high plasma concentrations [118-122], efavirenz toxicity, and early drug discontinuations [75, 102, 103, 123, 124]. Other low activity CYP2B6 alleles, including *11, *18, *27, *28, also predict high efavirenz plasma levels [102, 115, 119]. Five new variants CYP2B6*33, *34, *35, *36 and *37 were recently identified in a cohort of Rwandese HIV-1-infected patients and CYP2B6*33, *34,

*35 and *36 were shown to result in complete or almost complete loss of CYP2B6 metabolism of efavirenz [115, 125].

Ethnic differences exist in protein expression and activity of the CYP450 metabolising enzymes. CYP2B6*6 has been reported at frequencies of 15 to over 60% [115]; Asians at 10-21%, Caucasians at 14-27%, Africans at 33-50% and Papua New Guineans at 62%. Among the African population, CYP2B6*6 was found to be more frequent in Southern Africa (Zimbabwe and South Africa) compared to Eastern Africa (Uganda and Tanzania), and even within the Ugandan population, the frequency was higher among the persons of the Nilotics origin compared to Bantu group [126]. CYP2B6*18 (c.983C>T [I328T]) is currently thought to be the second most functionally deficient allele occurring at allele frequencies of 4–11% in African subjects [115, 127]. The polymorphisms are related to the racial differences in efavirenz pharmacokinetics and pharmacodynamics, and consequently, Caucasians have been shown to have higher clearance of efavirenz and nevirapine compared to persons of African and Asian origin [111, 128].

HIV/ART treated patients who are homozygous for CYP2B6*6 polymorphism were shown to have long term increase in C4 cell counts compared to the CYP2B6*1/*1[96] and Tanzanian treated patients had better increase in CD4 cell counts attributable to a higher prevalence of CYP2B6*6/*6 among Tanzanians compared to Ethiopians[129]. Sex differences in liver expression of the CYP450 activity have been observed with females' liver donors showing a higher expression and activity of CYP2B6 [115]. Women have also been shown to have higher CYP3A family isoform activity compared to men [57, 65]. However, these sex difference in CYP2B6 and 3A4 expression/activity does not match the elevated efavirenz plasma concentrations in females due to a large volume of distribution in females compared to males [122, 130].

There is a high variability in the metabolic activity of the CYP3A family with a 40-50 fold difference across populations [54]. Regarding CYP3A5 polymorphisms, CYP3A5*3 is most common in all ethnic groups at a prevalence of 90-93% among Caucasians, 65% in Hispanics, 60% in South Asia and 32% among African Americans [56, 57]. Although the CYP3A5*3 polymorphism is more prevalent in Caucasians compared to Africans, CYP3A family activity is lower in persons of African ancestry compared with Caucasians [53]. CYP3A5*6 and *7 are associated with reduced CYP3A5 activity, and were identified at frequencies of 10-22% among Africans but rare or nearly absent in the white population [131, 132]. Other identified CYP3A isoforms causing racial differences include CYP3A4*1B polymorphism occurring in Caucasians at a frequency of 9.3 - 11%, at 2 - 9.6% among Hispanic Americans, and 35 - 67% among African

Americans. Another candidate polymorphism that may explain differential CYP450 activity in different racial/ethnic groups is the recently identified CYP3A4*20 with no activity [53]. This recently identified SNP has a relatively high prevalence amongst differing ethnic groups: Caucasians (6%), African Americans (26%) and Chinese (22%). CYP3A4*22 is another newly identified SNP in intron 6 of CYP3A4 which showed lower expression in vitro [63]. Carriage of the CYP3A4*22 polymorphism has been associated with lower necessary statin doses and enhanced simvastatin-mediated cholesterol reduction in carriers (allele frequencies 4.3, 4.3 and 8.3% for African Americans, Chinese and Caucasians, respectively).

In the liver, Cytochrome P450 2B6 (CYP2B6) is involved in the metabolism of several drugs including antiretroviral agents efavirenz and nevirapine; antimalarial agents including artemisinin; but also other agents including bupropion, cyclophosphamide, ketamine and methadone. Other than the liver, CYP2B6 has been identified in several organs/systems including the lungs, bronchial tree and nasal mucosa; in the gastrointestinal tract, including colon and luminal lining of the intestinal cells; the skin; brain and the kidneys [133]. Functions in such organs include detoxification of toxins and pesticides at the skin and lung surface biotransformation of centrally acting agents. CYP3A4 is most abundant liver isoform, representing up to 50% of hepatic CYP450, while CYP3A5 only represents around 2% of the total liver CYP3A [134]. The CYP3A enzymes have also been identified in human gastrointestinal tract including the small intestines, oesophagus and colon where they are involved in the biotransformation of several carcinogenic compounds, and metabolism of some drugs[135].

1.8. PHARMACOGENETICS INFLUENCE ON EFAVIRENZ DRUG INTERACTIONS

Efavirenz interacts with other drugs causing changes in pharmacokinetic parameters of either efavirenz itself or the other drugs in the combination. Among the drugs that form important interactions with efavirenz are other antiretroviral agents, antituberculous drugs, antimalarial agents and drugs used against hepatitis. Efavirenz interacts with ritonavir darunavir, nelfinavir causing reduction in their AUC and/or plasma concentrations [136-138]. The fatality and proportion of HIV patients co-infected with tuberculosis in Sub-Saharan Africa, has called much attention to interactions between efavirenz and anti-tuberculosis drugs especially rifampicin. Efavirenz may cause reduction in plasma concentrations of rifampicin by inducing the CYP450 metabolising enzymes. Likewise through the induction of the CYP450 enzymes that metabolise efavirenz, rifampicin has been shown to increase the clearance of efavirenz concentrations hence lowering its concentrations [128]. For this reason, WHO proposed an adjustment in

efavirenz dosage from 600 to 800mg for patients who are above 50kg and are co-treated with rifampicin [15].

Most of the above drug-drug interactions act via CYP450 enzyme induction or inhibition. Rifampicin induces both CYP2B6 and CYP3A and even the CYP2A6 isoforms of the CYP450 enzymes resulting in increased metabolism of efavirenz. Rifampicin also induces ABCB1 which encodes P-glycoprotein, which is also involved in efavirenz disposition. However, recent findings have proposed a counter effect of other anti tuberculosis drugs. Rifampicin is usually given in combination with isoniazid, pyrazinamide and ethambutol, and with the exception of ethambutol, the other anti tuberculosis drugs are reported to have varying effects on the enzymes involved in efavirenz metabolism [139, 140]. The 7-hydroxylation of EFV among persons with CYP2B6*1/*1 genotype was reported to be inhibited by isoniazid, rifampicin and pyrazinamide. Isoniazid has the greatest inhibiting effect, including the inhibition of CYP2A6 and CYP3A4 enzymes whose activity increase at high efavirenz concentrations as those that occur in CYP2B6 slow metabolisers [139, 140] (Figure 2). Despite previous fears of reduced efavirenz exposure during rifampicin co-administration, several studies have failed to demonstrate this effect while others demonstrated a paradoxical increase in efavirenz levels during antituberculous treatment [115, 141-143].

In addition to antituberculous drugs, efavirenz interacts with other drugs of importance to HIV burdened regions. Lower concentrations of artemether and lumefantrine have been detected among children treated with efavirenz [144] compared to those on lopinavir [144, 145], and subsequent poor treatment outcomes have been observed among efavirenz co-treated malaria patients compared to those co-treated with lopinavir [146]. These findings regarding antimalarial drugs presents efavirenz as a possible hindrance to malaria treatment while the six months treatment period with anti tuberculosis drugs is a challenge to both antiretroviral and the anti tuberculosis treatment outcomes. HIV-Hepatitis co-infection is also common, and efavirenz may lower the AUC of boceprevir and telaprevir which are forthcoming protease inhibitors against hepatitis C [147, 148].

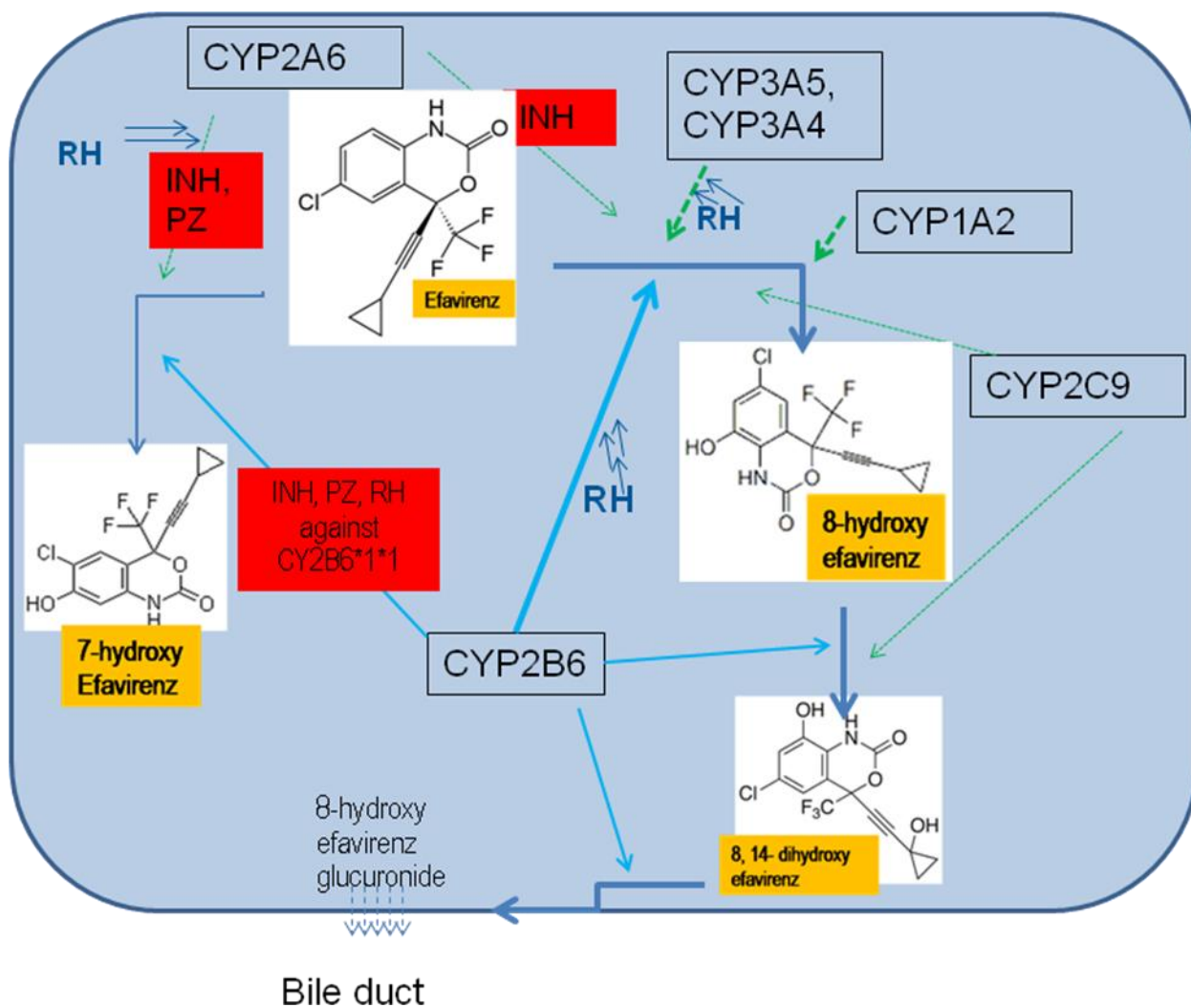


Figure 2: Diagrammatic representation efavirenz metabolism in the liver cells and the effect of anti tuberculosis drugs interactions [112, 139, 140]: CYP2B6 is the main enzyme that catalyses efavirenz metabolism with contributions from CYP3A4/5, CYP2A6, CYP2C9 and CYP1A2. Rifampicin (RH) enhances efavirenz metabolism while isoniazid (INH) and pyrazinamide(PZ) inhibits the metabolism.

2. RATIONALE

In resource limited settings, monitoring of HIV/ART usually relies on clinical and immunological staging. However, patients initiating treatment at the same disease staging and on the same HAART regimen may have varied therapeutic outcomes. Patients' baseline characteristics including disease staging, gender, age, and genetic differences may influence treatment outcomes including occurrence of adverse drug reactions. The influence by these additional factors may be through interference with the pharmacokinetics of the drug or through factors not yet well defined. Baseline CD4 cell characteristics including absolute numbers, are associated with HIV infectivity, ART response, and drug induced toxicity. On the other hand, pharmacogenetic variability is associated with efavirenz levels, toxicity, and treatment outcomes. The extent and role of variability of the genetics and baseline characteristics need much exploration in regions with high HIV burden.

This thesis describes variations in relevant baseline characteristics, and the effect these differences may have on the antiretroviral treatment outcomes. It also discusses pharmacogenetic variations that are relevant to HIV/ART therapy and their impact on treatment outcomes.

3. OBJECTIVES OF THIS WORK

3.1. GENERAL OBJECTIVE

The general objective of this thesis was to examine variations in baseline characteristics, pharmacokinetics and pharmacogenetics of antiretroviral drugs, and study their possible impact on HIV/ART outcomes.

3.2. SPECIFIC OBJECTIVES

- 3.2.1. To establish CD4 references as an HIV/AIDS baseline characteristic, and study their variations in the Ugandan population (Paper 1)

- 3.2.2. To examine the effect of baseline HIV/AIDS characteristics on selected ART outcome measures in Uganda (Paper 2)

- 3.2.3. To investigate the effect of HIV/AIDS on the pharmacokinetics of efavirenz in the Ugandan population (Paper 3)

- 3.2.4. To study variations in pharmacokinetics of efavirenz and their possible impact on therapeutic outcomes in the Ugandan population (Paper 4)

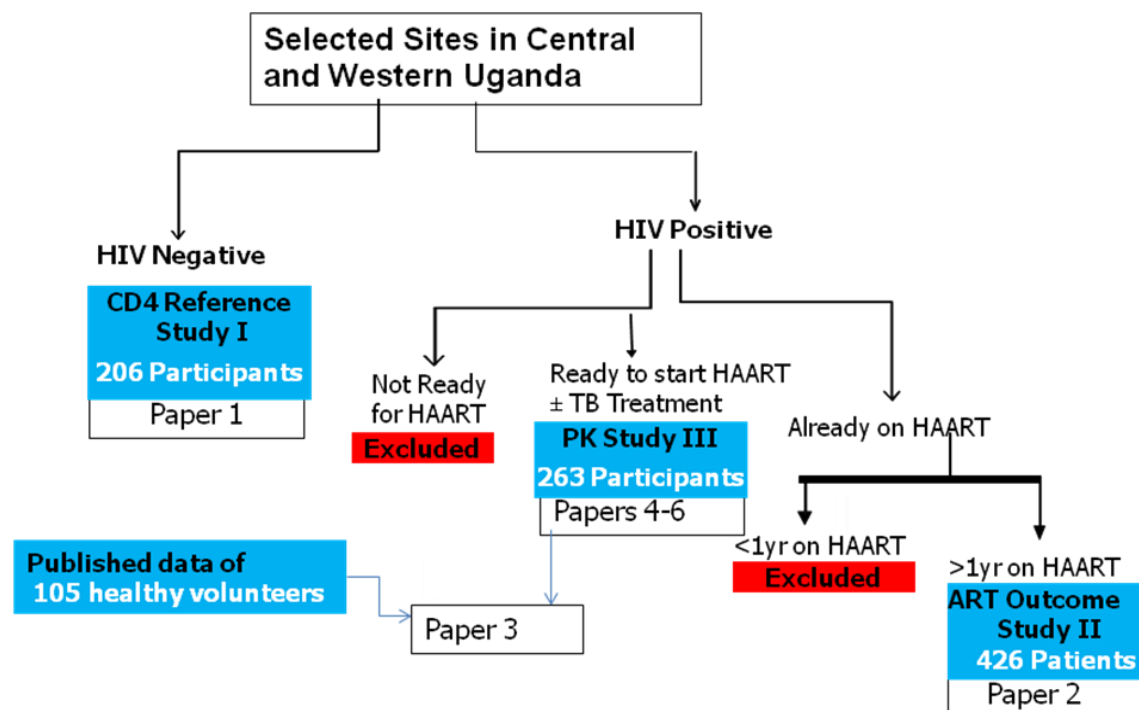
- 3.2.5. To study variations in pharmacogenetics of efavirenz and their possible impact on therapeutic outcomes in the Ugandan population (Papers 5 and 6)

4. MATERIALS AND METHODS

4.1. STUDY DESIGN

Three studies were conducted to address the objectives of this thesis. A cross-sectional study among healthy volunteers was carried out to investigate CD4 reference range variations (Sub-study I; **paper 1**), while retrospective data collected during routine HIV/ART care were used for the second objective (Sub-study II; **paper 2**). Objectives 3-5 were answered using a prospective study of patients with HIV infection, including those with and without TB co-infection (Sub-study III; **Papers 3-6**). These prospective patients' data were either analysed as data from HIV infected persons only (**papers 4 and 5**); or combined with published data on healthy volunteers (**papers 3**); or combined with TB co-infected patients' data (**paper 6**). The general methodology is displayed in figure 3.

Figure 3: General methodology of the design and participants used in investigating the variations in HIV baseline characteristics, pharmacokinetics and pharmacogenetics of antiretroviral drugs, and their impact on ART treatment outcomes



4.2. STUDY AREA AND POPULATION

The study was conducted at four sites selected in two regions of the country; two in the central and two in south-western regions of Uganda (fig.4). It was anticipated that the two regions vary in socio-demographics and perhaps genetically. The sites in central Uganda were Mulago and Butabika hospitals, both of which are in Kampala district, and the sites from western Uganda were Mbarara hospital and Bwera hospital in Mbarara District and Kasese Districts, respectively. Topographically, Kampala (central Ugandan region) lies at a relatively low altitude of about 914m ASL, compared to western Uganda region which includes the Rwenzori mountains reaching 5113 meters above sea level[149]. Kampala District is 238 square kilometres, more industrialised and populated compared to western Uganda[150]. It is estimated that 15 percent of the population in Kampala live below the poverty line while 30 percent of the population in western Uganda live below the poverty line[150]. Although both regions are inhabited by people of Bantu origin, slight differences occur in appearances, language, culture and practices, giving a possibility of slight genetic differences. The site selection was designed to capture possible in-country differences in socio-demographics and genetics. Sub-studies I (paper 1) and II (paper 2) were conducted in Kampala and Mbarara Districts while participants for sub-study III were recruited from Kampala and Kasese Districts. Figure 4 presents the study sites.

was done within communities around the study sites and volunteers who came to the study sites were screened for potential participation. On the other hand, HIV positive patients who were considered ready for ART initiation were screened for possible participation in the pharmacokinetic/pharmacogenetic studies. Healthy volunteers for the CD4 cell study were counselled and their physical status checked prior to having early morning blood drawn for both HIV testing and C4/CD8 cell establishment while HIV positive ART naïve patients used in the pharmacokinetic studies were admitted overnight for intensive sampling on days 1 and 14 of treatment, and then followed in the out-patient department for sparse sampling over 8 months. All HIV patients were started on 600mg efavirenz combined with 300 mg zidovudine and 150mg lamivudine in daily doses. The TB co-infected patients in Paper 6 were concurrently treated with rifampicin, isoniazid, pyrazinamide and ethambutol for two months followed by rifampicin and isoniazid for 4 months (2RHZE+4RH). Tuberculosis treatment for these patients was initiated 2 weeks prior to antiretroviral therapy. All patients were concurrently taking 960mg of cotrimoxazole daily as chemoprophylaxis for pneumocystis Jerovani pneumonia. Studying therapeutic outcomes needed a follow-up period that was longer than what was feasible for this work. Therefore, retrospective data was retrieved from files of HIV/ART patients who had been treated for 1-5 years (sub-study 2).

4.4. PROCEDURES FOR SAMPLE COLLECTION AND ANALYSIS

4.4.1. Blood sample collection and processing

Blood samples were collected to perform HIV serology testing, haematological values including CD4/CD8 cells, HIV1 RNA tests, efavirenz plasma concentrations and genotyping for CYP2B6 (*6 and *11), CYP3A5 (*3,*6 and*7) and ABCB1 (rs3842 and 3435C>T). For sub-study I; two blood samples, each 5 mL, were drawn from each participant through antecubital venous puncture using a Vacutainer (BD, Franklin Lakes, NJ) needle. One of the samples was collected in a 5 mL SST Gel and Clot Activator Vacutainer for HIV testing, and the second sample in a 5 mL EDTA-containing Vacutainer for determination of hematological indices. HIV tests were performed using a commercially available enzyme immunoassay kit (Welcozyme HIV-1 and -2 kit, Murex Diagnostics, Dartford, England), and discordant samples were confirmed using the Recombigen (*env* and *gag*) HIV-1 assay kit (Cambridge Biotech, Dartford, England). Samples for haematological tests were collected between 8 am to 11 am and analyzed within 4 hours using the PanLeucogating protocol.

Samples for determination of efavirenz plasma concentrations (sub-study3) were collected at 9 time-points over 24hour on days 1 and 14 for 66 ART naïve HIV patients, and then between 11-24hours over 8 months, for these and additional 197 HIV infected patients (157 HIV/TB co-infected). Four (4mL) of whole blood was drawn using an antecubital cannula and immediately centrifuged at 3000 rpm (1560 g) for 10 min. The plasma was stored at -70 1C until it was transported to Sweden for analysis using high-performance liquid chromatography (HPLC). Whole blood equivalent to 10mls were collected from each patient at enrolment and using this sample, genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Maxi Kit (QIAGEN GmbH, Hilden, Germany). Additionally, 10mls of blood for CD4 counts and HIV-RNA measures were collected at baseline and after every 3 months.

4.4.2. **CD4/CD8 estimation**

CD4/CD8 T lymphocytes and other haematological indices were performed on 206 HIV seronegative Ugandans. CD4/CD8 analysis was done using a double platform PanLeucogating method on an Epics XL-MCL Beckman Coulter flow cytometer (Beckman Coulter, Miami, FL). Using two MCL Epics XL tubes (Beckman Coulter), 10 µL of the PLG CD4 monoclonal antibodies (CD4 and CD45) was added to either of the tubes, and 10 µL of the PLG CD8 monoclonal antibody to the second tube. One hundred µL of well-mixed blood samples were pipetted into each of the tubes containing the monoclonal antibodies, gently vortexed, and incubated at 20°C-25°C in the dark for 10 minutes before 800 µL of the lyse was added. The mixture was further incubated at 20°C-25°C for 15 minutes, after which the tubes were loaded into the cytometer for analysis.

4.4.3. **Efavirenz plasma level quantification**

Blood samples drawn for the pharmacokinetic studies were immediately centrifuged at 3000 rpm (1560 g) for 10 min; plasma was stored at 70⁰C until the time of efavirenz quantification using High-performance liquid chromatography (HPLC) analysis. HPLC analysis was carried out at the Department of Laboratory Medicine, Karolinska University Hospital Huddinge (Karolinska Institute, Stockholm, Sweden), where reverse-phase HPLC with UV detection was used to determine the plasma efavirenz concentration. For HPLC, an Agilent Series 1100 (Agilent Technologies, Santa Clara, CA, USA), consisting of column compartment G1316A, degasser G132A, Quat pump G1311A, auto-sampler G1329A ALS, and diode array detector G1315B was used. The column used was Ace3C18, 3 mm, 50 _ 30mm (Advanced Chromatography Technologies, Aberdeen, UK) and the mobile phase consisted of 30% acetonitrile, 30% methanol, 4 mmol/L potassium hydroxide and 10 mmol/L acetic acid (pH 4.3).

Plasma proteins were precipitated with acetonitrile before centrifuging, after which 6 mL of the supernatant was injected and eluted at 0.80 mL/min for 3.5 min. The reference material was 99.9% efavirenz supplied by the WHO Collaborating Center for Chemical Reference Substances through Apoteket AB (Stockholm, Sweden), and the retention time was 2.42min as detected at UV-VIS 1, 210nm, UV-VIS 2, 220 nm. This method was linear, and the within-day coefficient of variation was 3.2, 3.3 and 5.1% at concentrations of 0.63 (n517), 2.53 (n517) and 6.31mg/L (n516), respectively, with a between-day coefficient of variation of 4.1% (n550) and a limit of quantification of 0.11mg/L.

4.4.4. Genotyping

Genomic DNA was isolated from peripheral blood leucocytes using a QIA amp DNA Maxi kit (Qiagen GmbH Hilden, German). Only SNPs that had been previously identified to be of clinical importance, including CYP2B6 (*6 and *11), CYP3A5 (*3,*6 and*7) and ABCB1 (rs3842 and 3435C>T) were determined on patient genomic DNA using the TaqMan method. Allelic discrimination reactions were performed using TaqMan (Applied Biosystems, CA, USA) genotyping assays: (C__7586657_20 for *ABCB1* 3435C>T, C__7817765_60, for *ABCB1* rs3842T>C, C__29560333_20, for *CYP2B6* 516G>T [*CYP2B6**6], for *CYP2B6* 136A>G [*CYP2B6**11], C__26201809_30 for *CYP3A5* 6986A>G [*CYP3A5**3], C__30203950_10 for *CYP3A5* 14690G>A [*CYP3A5**6]) and C__32287188_10 for *CYP3A5* g.27131_27132insT [*CYP3A5**7] on ABI 7500 FAST (Applied Biosystems, Foster City, CA). The final volume for each reaction was 10µl, consisting of 2x TaqMan Universal PCR Master Mix (Applied Biosystems), 20 X drug metabolising genotype assay mix and 10ng genomic DNA. The PCR profile consisted of an initial step at 50°C for 2 min and 50 cycles with 95°C for 10 minutes and 92°C for 15 minutes.

4.5. DATA MANAGEMENT, ANALYSIS AND MODELLING

For sub-study 1 (paper1), data were collected directly from patients using pre-tested data collection tools while clinical data for sub-studies II and III were collected from patient clinical files. All data were entered into excel files (sub-study 1), and Epi-info (sub-studies II and III). Also, electronic data, including pharmacokinetics, and pharmacogenetics analyses were extracted and entered into excel sheets. Double entries were made, followed by data cleaning prior to export into different softwares for analysis. Stata, Statistica and SPSS softwares were employed to calculate and compare means, medians and incidence rates that are reported in papers 1 and 2. WINOLIN software was employed for non-compartmental analysis (NCA) in paper 4, while NONMEM was used for population pharmacokinetics (PPK) analysis in papers 3, 5 and 6. The

effect of HIV/AIDS, pharmacogenetics, gender and other biological variables were tested by covariate analysis using a forward-selection ($\alpha=0.05$) followed by backward elimination ($\alpha=0.01$) method.

In paper 3; efavirenz pharmacogenetics and pharmacokinetic data for samples collected over 24 hours following the first dose of 600mg efavirenz based HAART were compared with published results from HIV seronegative Ugandans [130]. Population pharmacokinetic models were fitted to the data, using NONMEM VI software. The first-order conditional estimation method with interaction was used to describe the data. In paper 5; Pharmacokinetics data (556 steady-state plasma efavirenz concentrations collected over 5 different times per patient between 11-18hrs after dose) and pharmacogenetics data ((CYP2B6 (*6 & *11), CYP3A5 (*3,*6 & *7) and ABCB1 (c.4046A>G) for 99 (64 female) were simulated based on the AUC for the efavirenz 600 mg product label. A one-compartment population pharmacokinetic model with first-order absorption was used to estimate the genotype effects on EFV pharmacokinetics. Doses of 300, 450, and 600 mg were simulated for each of the six possible CYP2B6*6 and ABCB1 (c.4046A>G) combinations. In paper 6; sparse pharmacokinetics data collected over 8 months and pharmacogenetics data (CYP2B6 (*6 & *11), CYP3A5 (*3, *6 & *7) and ABCB1 (c4036A>G & c.3435C>T)) from ART naïve HIV patients (157 with and 106 without TB) were analysed. Efavirenz apparent oral clearance was determined on days 14, 56, 84 112, 140, 168, 196 and 224 and compared between the HIV only and HIV/TB co-infected group. The effect of covariates including genotypes, rifampicin, biochemistry data and demographic factors on apparent oral clearance of efavirenz at the different time points was assessed by Kruskal Wallis analysis of variance (ANOVA) using Statistica version 10 (StatSoft Inc., Tulsa, OK, USA). Graph Pad Prism version 5.0 for Windows (Graph Pad, La Jolla, CA, USA) was used for graphical presentation. $p < 0.05$ was considered as significant

5. RESULTS

5.1. VARIATION IN ABSOLUTE CD4 CELL REFERENCE VALUES FOR THE UGANDAN POPULATION; PAPER 1

The Ugandan HIV seronegative population displayed a great variability in absolute CD4 cell counts. A sample of 172 healthy volunteers had an absolute CD4 cell reference range of 418-2105 cells/ μ l, which is wider than the reference range 500-1500 cell/ μ l considered worldwide. During this study, absolute CD4 cell values were found to be affected by factors that included recent ailments, altitude and socio-economic status. Although 206 volunteers were enrolled as healthy participants at the time of the study, 34 (16.5%) of them had suffered recent infections that needed medical attention, and their mean CD4 cell value was lower than that of their healthy counterparts; 783 (CI 776-789) and 992 (CI 957-1026) cells/ μ l respectively (Fig. 5). For this reason, the results of the 34 volunteers were not included in the calculation of the country's reference ranges.

About 25% of the studied population had a median income of \$15/ month, indicating that they were living below the poverty line (less than 1 dollar/ day), another approximated 25% had a median monthly income of \$35 while the median monthly income of the 47.1% was \$100. Data on monthly income was not available for 10 of the volunteers. Participants living on <\$1/day had a statistically significantly lower mean CD4 cell count that their counterparts ($p=0.001$) (Table 1).

Fig. 5: Effect of recent acute illnesses on CD4 cell counts of HIV Seronegative Ugandans

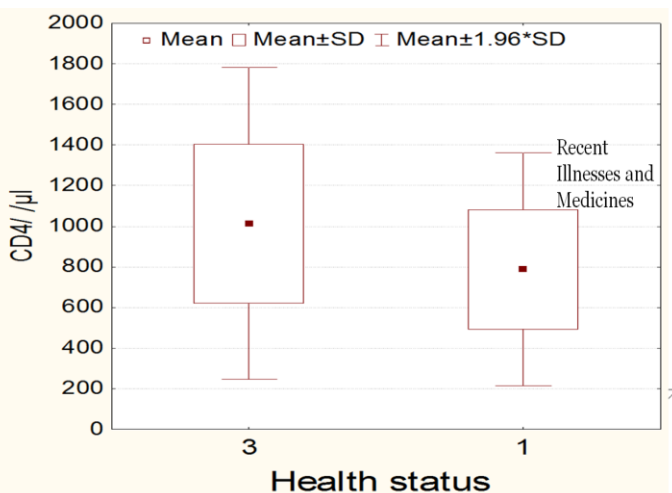


Fig. 3 shows a comparison in mean CD4 cell counts between healthy volunteers (category 3) and volunteers with recent ailments that needed medication (category 1). The mean CD4 cell count for health volunteers was higher for that for participants without recent ailments

Table 1: Effect of Socio-economic Status on CD4 Cell Counts of Seronegative Ugandans

Socioeconomic category	Mean CD4 (CI) (p value)
Lowest (23.3%) (\$15)	773 (675-872) (Ref)
Middle (23.5..%) (\$35)	1044 (926-1162) ($p=0.001$)
Highest (47.1%) (\$100)	1125 (1039-1211) ($p=0.001$)

About 38% of the participants were recruited from Mbarara district while the remaining 62% were from Kampala. The mean CD4 cell count for Mbarara participants; 742 (667-824) was lower than that of Kampala participants 1082 (1017-1151) (Fig. 6).



**Makerere University;
Kampala, Central Uganda
Altitude=914 - 1,134 MASL**

Mean (95% CI)	Kampala (n=107)	Mbarara (n=65)
CD4 (cell/μl)	1082 (1017-1151)	742 (667-824)



**Mbarara District;
Western Uganda
Altitude=1,447- 5,113M ASL**

Figure 6: Effect of altitude on CD4 cell counts in HIV seronegative Ugandans

Kampala District, located in central Uganda is more industrialised and more populated while Mbarara District which is located in western Uganda is less industrialised, sparsely populated, and characterised by hills and mountains. Mean CD4 cell counts were higher for health volunteers from Kampala District.

5.2. THE EFFECT OF VARIATION IN BASELINE CHARACTERISTICS ON ART OUTCOMES IN UGANDA; PAPER 2

Despite the fact that WHO and national guidelines recommended commencement of ART at CD4 cell counts < 200 cell/μl, results from retrospective data collected over time from two HIV/ART centres in Ugandan showed a great variation in absolute CD4 cell counts both at baseline and during the course of treatment. The median baseline CD4 cell value was 109 cells/μl (IQR 41-184) with 27.5% of the population initiating treatment below 50 CD4 cells/μl. Owing to deterioration in WHO/CDC clinical stage to III or IV, 20.7% of the studied population were started on antiretroviral therapy before their absolute CD4 cell counts dropped below 200 cells/μl and above. During a follow-up period of 1061.1 personal years on HAART, the overall rate and extent of immunological recovery differed by time, baseline CD4 cell count, gender and site.

5.2.1. Effect of baseline CD4 cell count on immunological recovery

Using the reference range from sub study I, reported in paper 1 (418 – 2105 cells/ μ l) to define immunological recovery, we examined the effect of baseline absolute CD4 cell counts on immunological recovery for this population. Overall, 37.1% of the patients attained the target CD4 count of 418cells/ μ l; and the median baseline CD4 count for this group of patients was 142 cells/ μ l (IQR 72-212) while the median baseline CD4 count for the 62.9% non-recovering population was 86 cells/ μ L (IQR 34-155). Analysis based on baseline CD4 levels of 50, 100, 200 and 250 cells/ μ L showed a positive correlation between baseline CD4 counts and immunological recovery (Table 2). Patients with baseline CD4 counts of \geq 100 cells/ μ l reached the targeted 418 CD4 count 2.16 times faster than the patients who initiated treatment below 100 cells/ μ l while patients of baseline CD4 count \geq 200cells/ μ l reached the target 1.89 times faster than patients who initiated ART below 200 CD4 counts. Other than its effect on immunological recovery these data also demonstrated reduced incidence rates of new infections with increasing baseline CD4 count.

Table 2: Incidence rates of reaching 418 CD4 cells/ μ l among HIV infected Ugandans who initiated HAART at different CD4 counts

Baseline CD4 Category	n	Incidences of attaining 418 CD4 cells/ μ L	Incidence rate/ 1000 years	Rate ratio
\geq 250	30	15	284 (143-445)	1.49
0 - 249	396	143	191(152-230)	
\geq 200	88	43	329 (231-427)	1.89
0 - 199	338	115	174(134-214)	
\geq 100	231	107	274 (217-332)	2.16
0 - 99	195	51	127(80-174)	
\geq 50	309	122	227 (180-274)	1.60
0 - 49	117	36	142(79-205)	

5.2.2. Effect of other baseline factors

There were no statistically significant differences in gender or geographical areas with respect to baseline CD4 counts. Median baseline CD4 cell count for female (68.3%) was 118 (44-190) and

139 (33-152) for male patients; 107(39-177) and 109 (49-195) for Kampala (63.2%) and Mbarara, respectively. However, female patients demonstrated a faster CD4 increase and reached higher CD4 cell levels compared to male patients ($p=0.0005$). During the study we observed that absolute CD4 increase was faster among patients from Mbarara hospital (37.8%) compared to JCRC-Kampala ($p=0.0107$).

5.3. VARIATION IN EFAVIRENZ PHARMACOKINETICS AND ITS EFFECTS OF ART RESPONSE; PAPERS 3-4

5.3.1. Effect of HIV/AIDS on efavirenz pharmacokinetics; Paper 3

When pharmacokinetic data from 29 HIV infected, ART naive Ugandans were compared with data from 32 healthy Ugandans, no differences were observed in demographic characteristics between the HIV positive and negative participants. Slight differences were observed in the frequency of CYP2B6 and CYP3A5*1 genotypes between the HIV positive and negative study groups, but these differences did not reach statistical significance. Using covariate analysis, HIV/AIDS was identified as a statistically significant categorical predictor of efavirenz pharmacokinetics and HIV/AIDS patients displayed 30% (95% CI 18.7- 40.7) lower relative bioavailability than healthy subjects. Gender was a secondary factor that influenced efavirenz pharmacokinetics with females having a 2-fold (95% CI 1.53- 2.63) higher volume of distribution of the peripheral compartment after oral administration (V_2/F) than males. This increase in volume of distribution resulted in a 2-fold longer elimination half-life for females compared to males.

5.3.2. Variation in efavirenz pharmacokinetics among ART naive Ugandans; Paper 4

Data from 66 HIV positive, ART naive patients with complete pharmacokinetic profiles (9 data points over 24h) on days 1 and 14, showed great intra and intersubject variation in efavirenz concentrations and degree of autoinduction. The patients had normal biochemical results including liver and renal function tests results, mean weight was 51.7 kg (SD 9.2), average baseline CD4 cell count 249 cells/ μ l (SD 97) and baseline mean viral load of 107468 copies/mL. The day 14 efavirenz plasma concentrations varied from an average of 2.9 μ g/l (range 0.8–12.0) to 7.49 μ g/l (range 3.0–19.4) for minimum (C_{\min}) and maximum (C_{\max}) concentrations respectively. The interindividual variations were also observed in the apparent

oral clearance of the drug with ranges of 1.6–20.6 l/h, and 1.9–16.4 l/h for days 1 and 14 respectively. Although overall mean clearance did not change over the 2 weeks (mean 7.5l/h and 7.4l/h, for days 1 and 14 respectively), 41.9% of participants showed an average 95.8% increase in clearance while no increase was observed in the other patients. On day 14, efavirenz C_{max} was >4 mg/mL in 96.6% of participants, while 4.5% of the patients had $C_{min} <1$ mg/mL.

The tendency of efavirenz to induce its own metabolism impacted on day14 drug concentrations. The mean values for the efavirenz C_{max} and C_{mins} on day 14 were statistically lower in the group of patients who exhibited autoinduction ($p=0.0085$ and $p=0.01$, respectively). Among the 41.9% of the patients who exhibited autoinduction, there was a negative correlation between the degree of autoinduction and day 14 efavirenz C_{min} , and hence, patients with greater degrees of autoinduction had lower day 14 C_{min} compared to patients with a lower capacity of efavirenz autoinduction. No difference was observed in efavirenz pharmacokinetics among patients of different baseline CD4 cell counts. Surprisingly, we also observed a negative correlation between serum albumin level and efavirenz concentrations on day1, which could be another surrogate of HIV severity. This relation was however not maintained to day14 of treatment.

5.3.3. Effect of variability in efavirenz pharmacokinetics on ART response; Paper 4

Data on central nervous system (CNS) toxicity attributable to efavirenz treatment were available for 58 of the 66 patients, and among the reported symptoms were abnormal dreams/nightmares, insomnia, dizziness and headache. Of the 58 patients, 40(69%) reported symptoms related to CNS toxicity, and 38 (95%) of these had efavirenz plasma concentrations above the therapeutic range on day 14. The frequency of efavirenz-related CNS complaints that were graded as moderate to severe was higher among patients who maintained high efavirenz concentrations beyond 8 hour after the dose (Table 3).

Table 3: Day 14 efavirenz plasma concentrations and corresponding efavirenz-related central nervous system (CNS) adverse effects in 58 HIV-infected Ugandans during the first 2 weeks of treatment with efavirenz, lamivudine and zidovudine (Paper 4)

Efavirenz plasma concentration after observed dosing on day 14	Number of patients with moderate to severe (grade 2/3)* CNS symptoms	Number of patients with mild (grade 1)* CNS symptoms	Number of patients with no CNS symptoms	Total number per efavirenz plasma concentration category
> 4 µg/mL within 8h after dosing	3	16	8	27
> 4 µg/mL 8h after dosing	5	14	8	27
No plasma concentrations >4 µg/mL	0	2	2	4
Total number of patients per CNS toxicity category	8	32	18	58

*Severity grading of efavirenz-related adverse CNS symptoms was based on the WHO toxicity table, where ‘mild symptoms (grade 1)’ are transient (<48 h) with no medical intervention required, ‘moderate symptoms (grade 2)’ have mild-to-moderate limitations in activity and no or minimal medical intervention is required, ‘severe symptoms (grade 3)’ have marked limitations in activity and medical intervention or hospitalization is required, and ‘grade 4’ is life-threatening. No life-threatening efavirenz-related adverse reactions were observed during the 2 weeks of the study

5.4. VARIATION IN EFAVIRENZ PHARMACOGENETICS AND SUGGESTED DOSE MODIFICATIONS; PAPER 5

Modelling and simulation of the pharmacokinetics and pharmacogenetics data for 99 (64 females) HIV infected patients showed *CYP2B6**6 genotype to be a major predictor of exposure to efavirenz. Overall, the simulated mean AUC was 72523 Kg/L·h for the 600 mg dose for the Ugandan population, which is 1.25 fold times the mean AUC in the product label (58084 Kg/L·h). Subjects homozygous for *CYP2B6* *6 mutation exhibited a mean AUC of 12 x 10⁴ Kg/L·h, which is more than 2 times the mean AUC in the product label, and higher mean AUC was attained in *CYP2B6* *6/*6 genotype compared to *CYP2B6* *1/*1 ($p < 0.0001$). Mean AUC values for both wild type and heterozygous mutants were within the exposure range observed during clinical studies reported in the product label. These results are presented in figure 7. While plasma efavirenz exposure differed significantly between *CYP2B6**6 (*1/*1)/ homozygous wild type *ABCB1* (*c.4046A>G*) and *CYP2B6**6 (*6/*6) / mutant *ABCB1* (*c.4046A>G*) ($p < 0.0001$) no difference was observed between *CYP2B6**6 (*6/*6) / mutant *ABCB1* (*c.4046A>G*) and *CYP2B6**6 (*6/*6) /homozygous wild type *ABCB1* (*c.4046A>G*) $p < 0.53$. Simulated exposures for EFV daily doses of 300mg for *CYP2B6**6/*6 genotype, and 450mg for the Ugandan population; were found to be comparable to those for the product label (table 4). Efavirenz oral clearance was 2.2 and 1.7 fold higher in *CYP2B6**6 (*1/*1) and *CYP2B6**6 (*1/*6) compared *CYP2B6**6 (*6/*6) carriers, while a 22% increase in F1 was observed for the mutant *ABCB1* *c.4046A>G*.

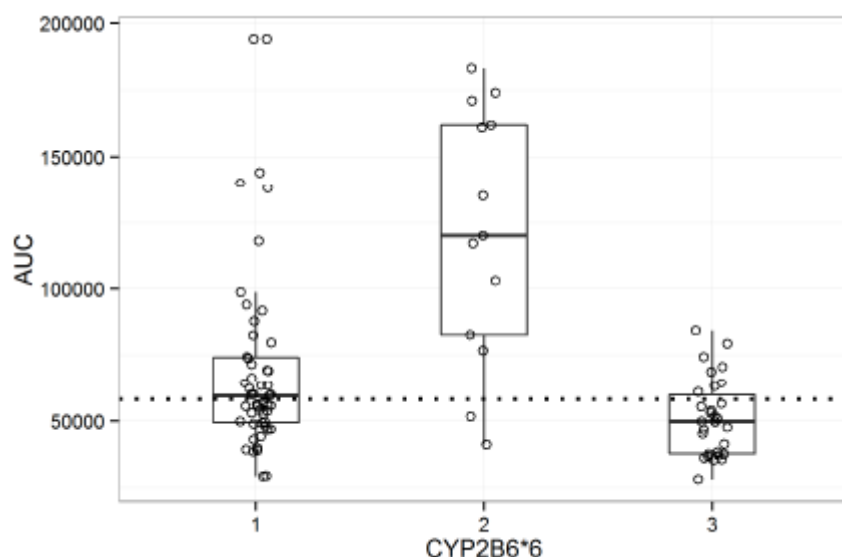


Figure 7: Distribution of estimated patients AUC values, stratified by CYP2B6 genotype. Groups 1, 2 and 3 represent patients with CYP2B6*1/*6, CYP2B6*6/*6; and CYP2B6*1/*1 genotypes, respectively, while the transverse dotted line represents mean AUC for the product label. The estimated AUC of patients homozygous for the CYP2B6*6 mutation were higher than the mean for the product label

Table 4: Simulation based AUC for 300, 450, and 600 mg doses in various genotype groups

CYP2B6*6	Number of observation ²	Dose= 300mg daily		Dose= 450mg daily		Dose= 600mg daily		
		Mean	95% CI	Mean	95% CI	Mean	95% CI	
All participants	All	47000	3.63	1.57-7.58	5.44	2.35-11.06	7.25	3.14-14.75
ABCB1 (c4046A>G) wt	*1/*1	17500	2.5	1.28-5.41	3.75	1.92-3.50	5.0	2.56-8.49
	*1/*6	2000	3.16	1.63-5.34	4.74	2.45-8.01	6.32	3.27-10.68
	*6/*6	10500	5.57	2.85-9.53	8.36	4.27-14.29	11.14	5.7-19.05
ABCB1 (c4046A>G) mut	*1/*1	4000	3.20	1.65-5.41	4.80	2.48-8.11	6.41	3.31-10.81
	*1/*6	9000	4.05	2.10-7.05	6.07	3.14-10.58	8.10	4.19-14.10
	*6/*6	4000	7.12	3.63-12.20	10.67	5.45-18.30	14.23	7.26-24.40

² The frequency of each group in these simulations reflects the population frequency of the groups in the observed patients' data

The simulation based mean AUC for the 300mg dose in the CYP2B6*6/*6 genotype group and that for the 450mg dose in the entire Ugandan population were comparable to the mean AUC of 58084 Kg/L·h in the product label.

Viral load fell precipitously on treatment with only six (6.1%) patients having HIV RNA > 40 copies/mL after 84 days of treatment. No trend with exposure was noted for these six patients, and their AUC were found to be either within range or above the product label AUC, implying that baseline viral resistance might have played a role

5.5. THE EFFECT OF VARIATION IN PHARMACOGENETICS ON EFAVIRENZ DRUG-DRUG INTERACTIONS; PAPER 6

This dataset consisted of 263 HIV ART naïve patients, 60% (n =157) co-infected with TB, with efavirenz concentrations collected over 252 days. The HIV, TB co-infected patients had lower average body weight than the HIV only group, 50.3kg versus 54.9 kg respectively (p<0.0001); lower mean baseline CD4 cell counts 88 versus 150 cells/μl (p<0.001); and lower mean albumin levels, 2.8 versus 3.9 g/dl' p<0.001. CYP2B6*6 homozygous genotype was observed at 10% for the entire study population but 13.5% among the HIV/TB patients and at 7.3% among the HIV only group.

5.5.1. Autoinduction during efavirenz-rifampicin co-treatment

Overall, maximal enzyme induction was observed at day 168 in both treatment groups although slight differences were observed between the rifampicin and non-rifampicin co-treatment groups. HIV patients co-infected with TB received at least 2 weeks of rifampicin therapy before efavirenz treatment was initiated, and as result, these patients had significantly higher apparent oral efavirenz clearance compared to patients without rifampicin during early efavirenz therapy on day 14 (p=0.03) but no effect was observed on day 56 or thereafter. Increasing efavirenz apparent oral clearance trends from day 14, peaking on day 168 and dropping back by day 224 were observed for all study participants (day 14: 12.65, day 168: 14.10 and day 224: 12.19).

5.5.2. Effect of genotype

Long-term induction was predicted by CYP2B6*6. Comparison of enzyme induction by CYP2B6*6 genotypes during efavirenz treatment alone and during efavirenz plus rifampicin treatment showed CYP2B6*1 carriers to have exhibited enzyme induction in a gene dependant manner. Maximum difference over time was observed among CYP2B6*1/*1 followed by *1/*6 while *6/*6 individuals exhibited no difference in clearance. Notably among CYP2B6*1/*1 genotype, the effect of efavirenz auto-induction on its own long-term clearance was much higher when given alone than during rifampicin co-administration in (Fig. 8).

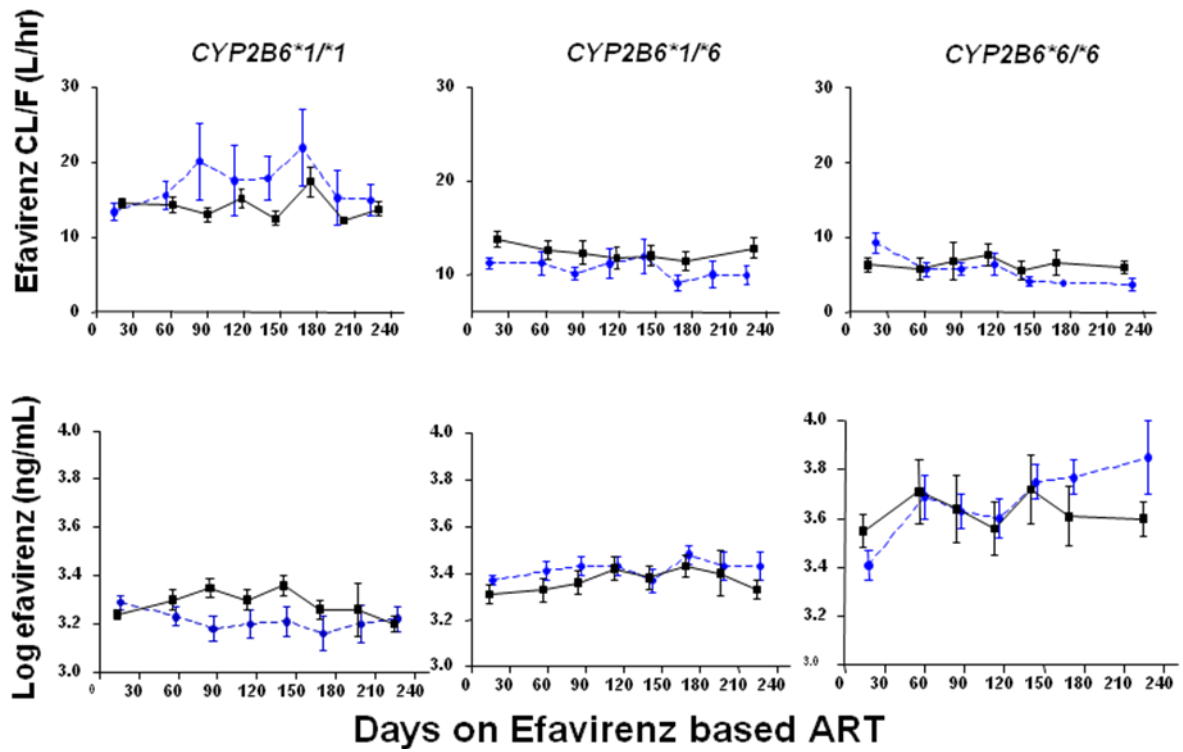


Figure 8: Comparison of efavirenz apparent oral clearance and plasma concentrations between "efavirenz only" and "efavirenz-rifampicin" treated patients, stratified by CYP2B6 genotype
 Full lines represent "Rifampicin co-treated" patients while dotted blue lines represent the "Efavirenz only" group. A paradoxical effect was observed with efavirenz apparent oral clearance being higher in "Efavirenz only" group with CYP2B6*1/*1 genotype compared to the Rifampicin co-treated patients (upper graphs). An increase in efavirenz plasma concentrations over time was observed among the CYP2B6*6/*6 genotype (lower graphs)

6. GENERAL DISCUSSION

The thesis highlights population variations in CD4 cell reference values, which is the main immunological marker of HIV/ART progress in adults and adolescents. The magnitude and importance of variations in pharmacokinetics and pharmacogenetics of efavirenz, a key first line antiretroviral drug, are also highlighted.

The first study (**paper 1**) demonstrated a wide reference range of 418-2105 CD4 cells/ μ L. The range presents a 5-fold variation in normal absolute CD4 cell values for the country. The reference range is also wider than what has been reported from most countries and regions [28, 29]. However, it is consistent with results from one of the previous studies in Uganda[32]. There are reports of faster CD4 cell deterioration among populations with higher CD4 cell counts [35, 36] and wide reference ranges such as the one reported in this thesis may place persons with high CD4 cell values at risk of developing advanced HIV while waiting for treatment initiation at specific CD4 cell counts.

Further still, sub study demonstrated variations in absolute CD4 cell counts with relation to socio-demographics (paper 1). High altitude and the low socio-economic status of Mbarara participants were possible causes of their low CD4 cell reference values. However, low socio-economic status would have been associated with a low mean haemoglobin value but on the contrary, participants from Mbarara had significantly higher mean values for neutrophils and haemoglobin level (p values 0.001 and 0.003 for neutrophils and haemoglobin, respectively) compared to Kampala participants. It was therefore concluded that the observed regional difference in mean CD4 cell values was due to altitude. This observation is consistent with findings from other studies that reported haematological changes at high altitude including reduction in CD4/CD3 subsets; and increment in neutrophils and red blood cell component [24, 25]. Although the effect of socio-economic status has previously only been investigated among HIV seropositive individuals, this thesis reports low CD4 cell counts among HIV seronegative individuals with lower income. Some of the factors that are frequent among persons at low socio-economic status, including malnutrition and frequent ailments, could partly be responsible for the low CD4 cell counts at low income [151, 152]. Research among HIV positive patients has demonstrated a higher relative risk of death or developing AIDS among HIV/ART patients with low income even when their baseline CD4 cell counts were comparable to the patients of higher income [153-155]. Findings from this study may indicate that effect of low income on immunity may be prevailing even before people

acquire HIV, and hence putting them at risk of poor HIV/ART outcomes that is reported in those studies [153-155]. The effect on parasitic and other viral infections in lowering CD4 cell counts among both HIV negative and positive patients has been reported [37, 39, 156-158]. The decision on when to initiate ART among HIV patients co-infected with acute illnesses gets complicated by the additional influence of the illnesses on CD4 cell values.

In substudy II (**paper 2**), we observed a low median CD4 cell count for patients who started ART during the selected study period. We further observed a low proportion (37.1%) of patients who had complete immunological recovery compared to proportions reported from Europe, North America, and particularly from the Swiss cohort where only 1/3 of the patients had immunological failure[159]. Evidence from these studies indicates that the reduced capacity of immunological recovery observed in my study patients was caused by delayed initiation of ART [71, 72, 159, 160]. This was further confirmed by the fact that the median CD4 cell counts for the immunological responders was higher than that of immunological failures, 142 (IQR 72-212) and 86 (IQR 34-155), respectively. The bone marrow has been shown to have reduced haemopoetic potential during advanced HIV [11, 12] and there is evidence that HAART started as early as during primary HIV leads to faster and more complete immune reconstitution [79, 80, 161, 162]. In the light of these findings, early ART would preserve the haemopoetic potential of the bone marrow and hence lead to complete immunological recovery.

Even though females have been reported to have higher CD4 cell reference values, and that there are reports of faster HIV progression among patients with higher CD4 cell reference values [32-34, 36], it is interesting to note that we and other researchers [94, 95] observed better clinical and immunological response among female patients. There could be some inherent factors causing the female seronegative population to have higher CD4 counts and HIV/ART female patients to have faster immunological recovery. Regarding ART response by study region, we had expected Kampala participants to have a faster immunological recovery given a longer HIV/ART experience at JCRC (HIV/ART centre in Kampala) compared to Mbarara. It is difficult to understand why Mbarara participants showed a faster immunological recovery than Kampala patients in sub-study II yet lower CD4 reference values were observed for Mbarara healthy volunteers in sub-study I. This finding could have been due to a random effect or to reasons not well understood. However, given all these factors that influence CD4 reference values and CD4 cell recovery, it is possible that waiting for all HIV positive patients to deteriorate to specified CD4 cut off values for treatment, may subject some patient populations to a risk of losing lose their immune-haemopoetic potential [11, 12]. Much earlier ART initiation during primary HIV,

or an approach of “test and treat” could lead to better HIV/ART outcomes including functional cure or preservation of the immune system until an opportunity of better approaches including inducing latency of the virus within the CD4 cells [79, 85-87, 90].

In sub-study III (papers 3-6), we observed variability in efavirenz pharmacokinetics with HIV/AIDS, CYP2B6 genotype and gender being the predictors of efavirenz pharmacokinetics. Exploration of the effect of HIV/AIDS on efavirenz pharmacokinetics showed a 30% lower relative bioavailability of efavirenz among HIV infected individuals compared to healthy volunteers, after a single dose (**paper 3**). Similar finding of altered pharmacokinetics of drugs during HIV/AIDS have been reported, including findings of lower plasma concentrations of atazanavir among HIV/AIDS patients compared to healthy volunteers [163] and reduced bioavailability of zidovudine in advanced HIV/AIDS [59]. The reduced bioavailability of efavirenz, and other drugs may be related to HIV/AIDS inflammatory processes leading to functional impairment of the absorptive surface [55]. However, effects of other factors including changes in expression of P-glycoprotein and ABCB1 may be implicated. The reduced bioavailability of zidovudine have a link to a report of overexpression of the p-glycoprotein on CD4 cells of HIV infected persons, which caused reduced accumulation of zidovudine within the cells [57]. The p-glycoprotein expression was found to increase as HIV/AIDS advances [57] and this protein acts as an efflux pump for a number of antiretroviral drugs. Changes in P-glycoprotein expression is a possible explanation for the low bioavailability of efavirenz in my HIV/AIDS participants. This study was the first step to demonstrate the effect of HIV disease on pharmacokinetics of antiretroviral drugs.

Further attempts to explore the effect of HIV/AIDS on pharmacokinetics of efavirenz at steady state by using 66 HIV infected patients with different baseline CD4 counts (**paper 4**) showed no associations between CD4 counts and efavirenz exposure. With reports of increasing expression of the p-glycoprotein in advancing HIV[57], we had expected to see a difference in the bioavailability of efavirenz between severely immunosuppressed and the less immunocompromised patients. However, patients in this study had a mean baseline CD4 count of 249 cells/ μ l; SD 97, and this narrow spectrum of baseline CD4 cell counts cannot be used to make conclusions. The contradicting findings from these two papers (3 and 4) could also imply that the direct effect of HIV/AIDS on efavirenz bioavailability may be short lived. If the observed effect was indeed due to functional impairment of the absorptive surface which occurs in HIV/AIDS, the inflammation is likely to clear following treatment initiation resulting into early stabilisation of the absorptive surface. The quick restoration of the function of the absorptive

surface that follows treatment initiation could also account for the loss of the albumin influence on day 14 efavirenz concentrations compared to what was observed on day 1 of this study. Paper 4, however, reports great variability in efavirenz concentrations and auto-induction capacity of the patients, and the vast majority (95%) of patients who reported central-nervous efavirenz toxicity had concentrations above therapeutic ranges. Studies have previously reported CNS toxicity among patients with high plasma concentrations [57, 97, 115, 164], but the proportion of patients with the CNS side effects observed in my study was relatively high, and this could be due to the high prevalence of CYP2B6*6 polymorphism in the African population [122, 126, 130].

The differences in efavirenz concentrations and clearance related to the CYP2B6 genetic variability reported in papers 3-6 of this thesis have previously been documented [102, 113, 115, 129]. Individuals who are homozygous for the CYP2B6*6 wild type have been reported to metabolise efavirenz much faster, and show greater tendencies of efavirenz to induce its own metabolism compared to persons with the mutation [96, 102, 115, 118, 119, 122, 128-130]. On the contrary, individuals homozygous for the mutation show less auto-induction, are slow metabolisers of efavirenz and tend to have high efavirenz concentrations compared to the persons without the mutation [115, 118, 119, 122, 130]. The high efavirenz plasma concentrations among the CYP2B6*6 group of patients has previously been correlated with high rates of efavirenz toxicity and discontinuation [103, 115]. Dosage modifications proposed earlier [165] and in this thesis may reduce the frequency of CNS toxicity in the African population. Findings reported in paper 4 are complimented with our findings in paper 6 where we observed a gene dependent increase in efavirenz clearance over time; greatest in CYP2B6*1/*1, followed by CYP2B6*1/*6, while the CYP2B6*6/*6 did not demonstrate any tendency to autoinduction. The patients who showed minimal tendencies of autoinduction and had high efavirenz concentrations in paper 4, are likely to have CYP2B6*6/*6 genotype. Pharmacogenetic- directed dosing of patients or patient populations has been suggested to reduce efavirenz-related toxicity [113].

Our findings in **paper 5** showed that using the current 600mg efavirenz dose, efavirenz exposure at steady state is anticipated to be twice as high for the CYP2B6*6/*6 genotype, predisposing these patients to adverse effects. Further modelling of the data predicted efavirenz dosage adjustment of 300mg to achieve adequate exposure and pharmacodynamic effects for the CYP2B6*6/*6, while 450mg would achieve similar pharmacodynamic effects in the Ugandan population. These predictions are in line with previous findings: Nyakutira et al. predicted 35% dosage reduction for the Zimbabwean population to achieve adequate efavirenz plasma

concentrations [122], while Gatanaga et al. observed adequate viral suppression at 400mg among the CYP2B6*6/*6 carriers [165]. Consideration of these dosage reductions could reduce the efavirenz-related CNS toxicity in the African population, and especially the CYP2B6*6/*6 carriers [100-103]. However, additional effects of prolonged CD4 cell increase has been documented among CYP2B6*6/*6 carriers following administration of 600mg of efavirenz, and it is not clear if this advantage would be maintained after the suggested dosage adjustments [96, 102, 129].

Our investigation of the implications of rifampicin co-treatment on the efavirenz plasma exposure in the Ugandan HIV patients showed that CYP2B6 genotype rather than rifampicin co-treatment explained the long-term effect of efavirenz autoinduction and plasma concentrations (**paper 6**). With the documented effect of rifampicin on inducing a number of enzymes that metabolise efavirenz, including CYP2B6, CYP2A6, CYP3A4, and UGT7B [141], we expected a reduction of efavirenz plasma concentrations over time, following rifampicin administration. We however noted that irrespective of rifampicin administration, patients with CYP2B6*1/*1 genotype had the highest increase in clearance resulting in reduced efavirenz concentrations over time, and that the clearance was higher when efavirenz was given alone compared to when it was co-administered with rifampicin. There are similar reports from studies where the expected effect of rifampicin reducing efavirenz concentrations was not demonstrated when efavirenz was co-administered with rifampicin based TB treatment [128, 141, 142]. There are even studies where a paradoxical increase in efavirenz concentrations was observed during rifampicin based TB-treatments [143]. Unlike when pharmacokinetics of efavirenz are studied with rifampicin given in isolation, antituberculous treatment often contain isoniazid and pyrazinamide, which have been shown to inhibit enzymes involved in efavirenz metabolism [139]. Isoniazid inhibits the CYP2A6 and CYP3A4 enzymes which play a significant metabolizing role at high efavirenz concentrations [113, 139, 140]. Also isoniazid, pyrazinamide and probably rifampicin were shown to inhibit the alternative pathways of 7-hydroxylation of efavirenz among the CYP2B6*1/*1 fast metabolisers, in addition to inhibition of CYP2A6 by both isoniazid and pyrazinamide [139]. High isoniazid concentrations such as those that occur in NAT2 slow acetylators were seen to greatly inhibit the 7-hydroxylation in CYP2B6*6/*6, and this can cause paradoxical increase in efavirenz concentrations when co-administered with rifampicin-based TB therapy [139]. The impact of genetics on isoniazid metabolism is not well documented in most African populations, Uganda inclusive, but 68.7% of Ethiopian TB/HIV patients were reported as NAT2 slow acetylators [99]. The paradoxical increase in efavirenz concentrations during HAART-TB treatment that is

secondary to slow isoniazid metabolism may be wide spread in the African population, and regardless of rifampicin administration, genetic based efavirenz dose modification need to be considered.

6.1. METHODOLOGICAL CONSIDERATIONS

The study employed several methodological approaches and this was considered important in answering the different study objectives requiring different study populations and outcome measures. The study investigated the effect of a single exposure (HIV/AIDS) on two aspects/outcomes (pharmacokinetics and pharmacodynamics) of ART, with the specific measurements approached using different approaches. While a cross-sectional study approach adequately addressed the question of CD4 reference ranges, the rest of the research questions needed data that was collected over time.

Investigating the effect of HIV/AIDS on efavirenz pharmacokinetics using different study populations revealed results that may have been missed if a single study population and design was employed. A single dose study showed HIV/AIDS patients to have a lower relative bioavailability compared to healthy volunteers and this finding would have been missed with a single prospective cohort of patients. Moreover the study in patients revealed no difference in pharmacokinetics of efavirenz at different disease severities, and this would have created an overall impression that HIV/AIDS has no effect on pharmacokinetics of efavirenz. Although still, we think that a sample comprising a wide range of baseline CD4 counts would have been more reliable to study the effect of HIV severity on efavirenz pharmacokinetics. This was not feasible with the current study since the investigator was not providing antiretroviral therapy but rather relied on patients deemed ready for ART under the national programme and hence had no control on patients' baseline characteristics.

A longitudinal study design with a prospective cohort would have been the best approach for sub-study II investigating the effect of HIV severity on ART outcomes. However, it would have been difficult to obtain a sufficient sample size within the available time and related resources. For that matter, a retrospective data review was considered despite the challenges of documentation and data storage in low resource settings. Moreover, the data not routinely collected in low resource settings including viral load assays, could have affected interpretation of results. Findings from this study however, provide valid evidences comparable to operational research findings using

data collected from HIV/ART files used in routine practice. This provides good evidence of the outcomes from a day to day operation of HIV/ART clinical practice.

The short prospective pharmacokinetic study yielded clinical data over a period of 8 months. Although this clinical data was utilised in the pharmacokinetics-pharmacodynamics data reported in paper 5, the period may not be sufficient to make strong clinical judgements.

In the pharmacogenetic studies it would have been interesting to include participants from additional regions, especially the Nilotic group in northern Uganda, for purposes of capturing the different genetic representation of the country. However, prevailing circumstances including financial constraints, accessibility and previous security issues caused exclusion of the region. The populations included in the study may not give good genetic representation of the country, but does give a picture of the importance of pharmacogenetic variations within the country.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. GENERAL CONCLUSION

Just as the virus and host-cell characteristics vary and affect HIV/ART outcomes, variations in relevant baseline characteristics including CD4 cell values and pharmacogenetics, influence HIV/ART outcomes. My study population varied greatly in CD4 cell reference values, and pre-treatment CD4 cell counts affected immunological recovery. CYP2B6*6 genotype influenced efavirenz pharmacokinetics, and a high proportion of HIV infected Ugandans exhibited high efavirenz plasma levels and central nervous adverse effects. Given the great diversity observed among Africans, the impact of these un-addressed factors could be implicative in HIV/ART outcomes in sub Saharan Africa.

7.2. RECOMMENDATIONS

7.2.1. Recommendations for findings in sub-studies I and II

My study shows the value of early ART initiation. Approaches including the “test and treat”, or “ART initiation during primary HIV infection” should be considered. The approaches would address the current challenge of optimal timing for antiretroviral treatment, and could have added advantages including functional cure for some HIV infected patients. Setting CD4 cell limits for treatment initiation may not be addressing all the challenges related to population variations in CD4 cell values, thus putting some HIV infected patients at risk of delayed treatment.

7.2.2. Recommendations for findings in sub-study III

Efavirenz is a widely used antiretroviral drug, including among patients co-infected with TB, malaria and hepatitis, due to its relatively low cost and high efficacy. Population and pharmacogenetics based dosing is recommended, including the suggested 450 mg dose for an African population, and 300mg dose for the CYP2B6*6/*6 genotype, that are suggested in this thesis. The dosing should be in the light of reducing efavirenz related adverse effects and at the same time maintaining the efficacy advantages previously observed among the CYP2B6*6/*6 genotype.

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