PHARMACOGENETICS OF ANTIRETROVIRAL DRUGS USED FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV DURING PREGNANCY AND LACTATION

Thesis submitted in accordance with the requirements of the University of Liverpool for the

degree of Doctor in Philosophy by

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I declare that this thesis is the result of my own work and the material contained in it has not been presented, wholly or in part, for any other degree or qualification.

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LIST OF ABBREVIATIONS

μg	Microgram(s)
μL	Microlitre(s)
3TC	Lamivudine
95% CI	95% Confidence interval
ABC	Abacavir
ABCB	ATP-binding cassette transporters sub-family B
ABCC	ATP-binding cassette transporters sub-family C
ACTG	AIDS Clinical Trials Group
ADH	Alcohol dehydrogenase
ADME	Absorption, distribution, metabolism, and elimination
ADR	Adverse drug reaction
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
APV	Amprenavir
ART	Antiretroviral therapy
ARV	Antiretroviral
ATP	Adenosine triphosphate
ATV	Atazanavir
AUC	Area under the curve
AZT	Zidovudine
β	Regression coefficient
BCPA	Best Pharmaceuticals for Children Act
BCRP	Breast cancer resistance protein
BQL	Bellow quantification limit
CAR	Constitutive androstane receptor
CDC	Centers for Disease Control and Prevention
CL	Clearance
C_{max}	Maximum drug concentration
C_{min}	Minimum drug concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid

CV	Coefficient of variation
CYP	Cytochrome P450
DBMS	Dried breast milk spot
DBS	Dried blood spot
DLV	Delavirdine
DNA	Deoxyribonucleic acid
DPD	Dihydropyrimidine dehydrogenase
DRV	Darunavir
DTG	Dolutegravir
EFV	Efavirenz
EI	Entry inhibitor
ETR	Etravirine
ENT	Equilibrative nucleoside transporter
EVG	Elvitegravir
FDA	Food and Drug Administration
FI	Fusion inhibitor
FTC	Emtricitabine
FXR	Farnesoid X receptor
GST	Glutathione S-transferase
h	Hour(s)
HBD	Hydrogen bond donor
HCT	Haematocrit
HFE	Human hemochromatosis protein
HIV	Human Immunodeficiency virus
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
$IC_{50/90}$	Concentration required to produce 50/90% inhibition
ICRP	International Commission on Radiological Protection
IDV	Indinavir
IL	Interleukin
IQ	Inhibitory quotient
INSTI	Integrase strand transfer inhibitor
IQR	Interquartile range

Ka	Absorption rate constant
kg	Kilogram(s)
L	Litre(s)
LC-MS/MS	Liquid chromatography tandem mass spectrometry
Log_{10}	Logarithm to the base 10
LogP	Partition coefficient
LPV	Lopinavir
MEC	Minimum effective concentration
mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
MMPGL	Mass of microsomal protein per gram of liver
mRNA	Messenger RNA
MS	Mass spectroscopy
MTCT	Mother-to-child transmission
MVC	Maraviroc
NAT	N-Acetyltransferase
NFV	Nelfinavir
ng	Nanogram(s)
mM	Millimolar
mtDNA	Mitochondrial DNA
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NR1I2	Nuclear receptor subfamily 1 group I member 2
NR1I3	Nuclear receptor subfamily 1 group I member 3
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OCT	Organic cation transporter
PBPK	Physiologically based pharmacokinetic
PCR	Polymerase chain reaction
PEP	Post exposure prophylaxis

PMTCT	Prevention of mother-to-child transmission
popPK	Population pharmacokinetics
POR	Cytochrome P450 oxidoreductase
PREA	Pediatric Research Equity Act
PSA	Polar surface area
PXR	Pregnane X receptor
RAL	Raltegravir
RNA	Ribonucleic acid
RPV	Rilpivirine
RTV	Ritonavir
RXR	Retinoid X receptor
s	Second(s)
SD	Standard deviation
SLC	Solute carrier transporter
SNP	Single nucleotide polymorphism
SQV	Saquinavir
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TNF	Tumour necrosis factor
TPMT	Thiopurine-S-methyltransferase
TPV	Tipranavir
UGT	Uridine diphosphate glucuronosyltransferase
UK	United Kingdom
USA	United States of America
UTR	Untranslated region
VDR	Vitamin D receptor
WHO	World Health Organisation

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Siccardi M, **Olagunju A**, Simiele M, D'Avolio A, Calcagno A, Bonora S, Di Perri G, Owen A. Relative genetic contribution (rGC) for Cmin across a range of commonly prescribed antiretroviral drugs

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Submitted Manuscripts

Olagunju A, Bolaji O, Neary M, Back D, Khoo S, Owen A. Pregnancy affects nevirapine pharmacokinetics – evidence from a *CYP2B6* genotype-guided observational study.

Olagunju A, Khoo S, Owen A. Pharmacogenetics of nevirapine excretion into breast milk and infants' exposure through breast milk versus post-exposure prophylaxis.

Manuscripts in Preparation

Olagunju A, Rajoli RJR, Back D, Khoo S, Owen A. Prediction of infant exposure to maternal drugs through breast milk using physiologically-based pharmacokinetic modeling.

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GENERAL ABSTRACT

The use of antiretroviral therapy (ART) during pregnancy and lactation has significantly reduced the rate of mother-to-child transmission (MTCT) of HIV. However, pregnancy is known to affect the pharmacokinetics of many drugs, including key antiretroviral (ARV) drugs. In addition, ARV use during lactation raises questions about unintended exposure of breastfed infants to maternal drugs through breast milk. For drugs with significant genetic contribution to observed pharmacokinetic variability, we hypothesised that polymorphisms in drug disposition genes may accentuate or attenuate pregnancy-induced changes and/or breastfed infants' exposure. HIV positive pregnant women and nursing mothers taking efavirenz (EFV)- or nevirapine (NVP)-based ART were recruited from three hospitals in Benue State, Nigeria.

A novel strategy involving a preliminary pharmacogenetic association study was used to investigate the magnitude of pregnancy-induced changes in EFV and NVP pharmacokinetics in women stratified by single nucleotide polymorphisms (SNPs) in disposition genes. EFV apparent clearance (CL/F) was higher and AUC_{0-24}, C_{max} and C_{min} were significantly lower in pregnant compared with postpartum women. When stratified based on the SNP with the highest predictive power, pregnant women with *CYP2B6* 516GG genotype were especially at risk. In the NVP cohort, exposure was also significantly lower in pregnant compared with postpartum women. When stratified based on composite *CYP2B6* 516G>T and 983T>C genotypes, C_{min} was below target in most patients with combined *CYP2B6* 516GG and 983TT during pregnancy and postpartum. C_{min} was below target in at least 50% of pregnant women with one or two variant alleles, compared with 0% in postpartum women.

The intensive pharmacokinetics of EFV and NVP in breast milk and pharmacogenetic predictors were described for the first time. Breast milk pharmacokinetic parameters of EFV in breast milk differed significantly between patient groups stratified by *CYP2B6* 516G>T. The median time averaged milk-to-plasma concentration (M/P) ratio was 1.10 (range: 0.57-1.71) and the paediatric dose weight-adjusted exposure index was 4.05% (1.08-13.8). The resulting infant plasma concentration was influenced by *CYP2B6* 516G>T, highest up to 8 days of age at 1590 ng/mL (190-4631) and decreased by about 90% in the age stratum 9 days to 3 months. NVP AUC_{0-12}, C_{max} and C_{min} in breast milk were significantly lower in patients with composite *CYP2B6* 516GG/983TT than those with at least one variant allele. The M/P ratio was 0.88 (0.74-1.2) and paediatric dose weight-adjusted exposure index was 3.64% (1.99-9.88). Infant plasma concentration differed significantly based on *CYP2B6* 516G>T/983T>C and *CYP3A4* 20230G>A (*1G), highest in those exposed through both breast milk and post-exposure prophylaxis compared with either alone. A breastfeeding physiologically-based pharmacokinetic (PBPK) model to predict infant exposure to maternal drugs through breast milk was developed and validated, with over 90% of all individual observed data points within the predictive interval.

This thesis presents details about five different studies where these findings were observed. Their clinical implications in the context of current knowledge and practice were also explored.

CHAPTER 1

GENERAL INTRODUCTION

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1.1 HISTORY OF MOTHER-TO-CHILD TRANSMISSION OF HIV

Between October 1980 and May 1981, five young men were treated for *Pneumocystis carinii* pneumonia at three different hospitals in Los Angeles, California, United States. Those cases prompted the official recognition of an epidemic that is perhaps the defining public health issue of our time: HIV/AIDS.[1] By 1983, the risk of mother-to-child transmission (MTCT) of HIV was confirmed after reports of a number of paediatric cases of 'a new syndrome of acquired immunodeficiency' with clinical, histopathologic, and immunologic features similar to those described in adults (**Figure 1.1**).[2-5] Since there were no available treatment or prevention strategies, avoidance of pregnancy was the only known way to prevent MTCT in HIV positive women. The first Centers for Disease Control and Prevention (CDC) guidance released in 1985 also recommended advising HIV infected mothers to avoid breastfeeding for prevention of mother-to-child transmission (PMTCT) of HIV (**Figure 1.1**).[6] Since those early days, MTCT has remained the major route of paediatric HIV infection, with an estimated 260,000 new cases in 2012 alone,[7] about 80% of these occurring during pregnancy, labour and delivery, and 20% during breastfeeding.[8]

Therefore, the introduction of antiretroviral therapy (ART) consisting of a combination of three or more antiretroviral (ARV) drugs for PMTCT was arguably a major step in the efforts to prevent MTCT. This approach has been shown to reduce MTCT to less than 5% from 15-45% without intervention, even in breastfeeding populations.[9-12] The 2013 WHO consolidated guidelines recommend breastfeeding-limited (Option B) or lifelong (Option B+) maternal ART for PMTCT during pregnancy and breastfeeding. HIV-exposed, uninfected infants are given six weeks of daily nevirapine (NVP) if breastfed, or four to six weeks of daily NVP or twice daily zidovudine (AZT) if receiving replacement feeding for post-exposure prophylaxis (PEP).[13] Previous interventions involved monotherapy and/or short-course maternal and infant prophylaxis (**Figure 1.1**).
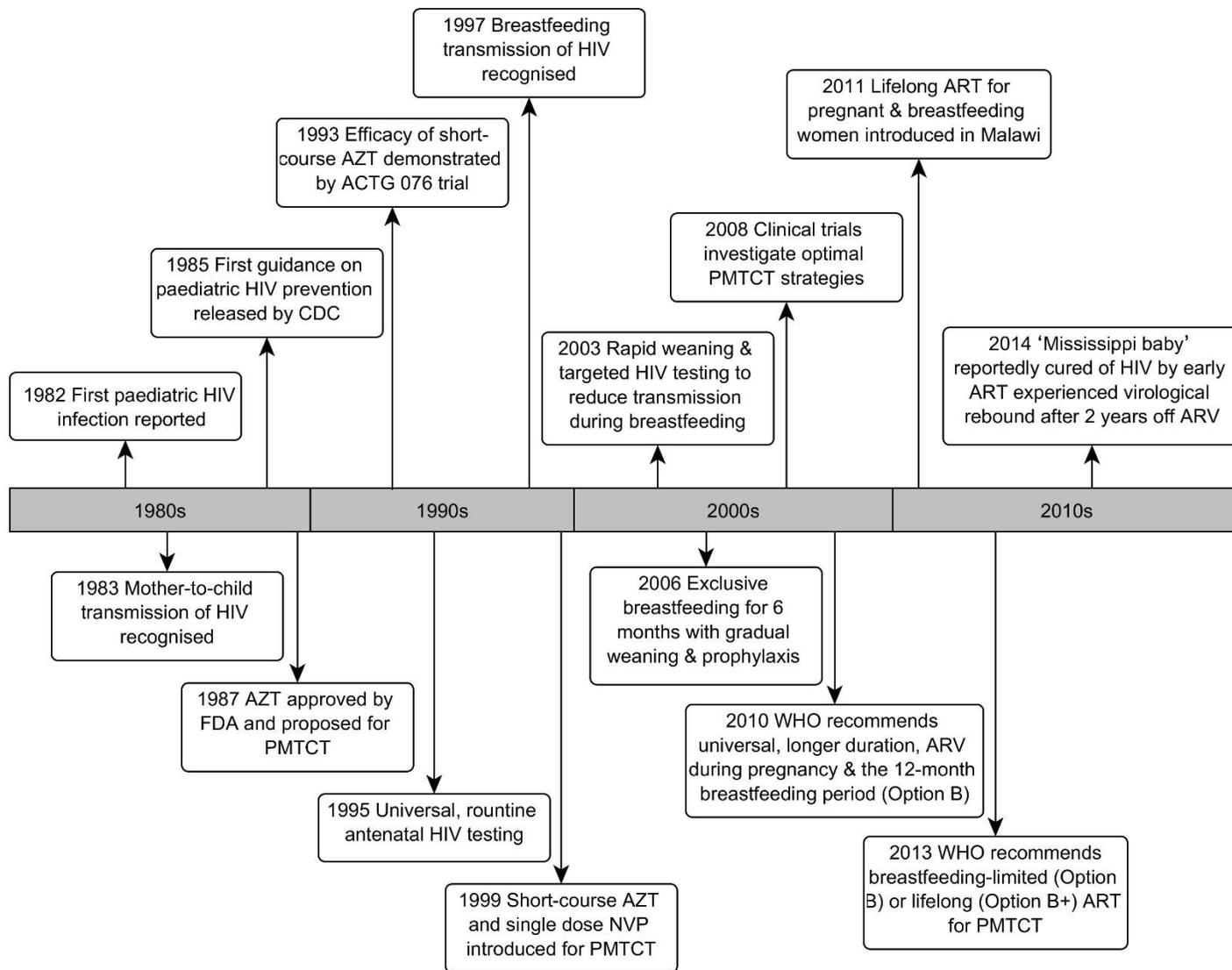
Figure 1.1 Significant developments in prevention of mother-to-child transmission of HIV since 1982, when vertical transmission of HIV was first reported (based on information from https://www.aids.gov/hiv-aids-basics/hiv-aids-101/aids-timeline/).

The AIDS Clinical Trials Group (ACTG) Study 076 was the first to demonstrate the efficacy of maternal and infant ARV prophylaxis for PMTCT.[14] Maternal AZT monotherapy from 14 weeks of pregnancy and during delivery, and a six-week infant AZT PEP reduced MTCT rates to 8% from 25% in non-breastfed infants. Initiating AZT monotherapy from 28 weeks of pregnancy achieved similar transmission rates in non-breastfed infants, suggesting that most of the remaining cases of transmission occurred during delivery.[15] Subsequently, a single maternal oral dose of 200 mg NVP at the onset of labour and 2 mg/kg to the newborn within 72 hours of life was shown to reduce MTCT in breastfeeding populations, though not as effectively as AZT monotherapy.[16] These observations prompted a trial of a combination of AZT monotherapy started during the third trimester and single-dose NVP given at the onset of labour. This further reduced MTCT to less than 2% in non-breastfed infants.[17] In addition, the probability of MTCT is significantly affected by the duration of AZT monotherapy and viral loads at baseline and at delivery (**Figure 1.2**).[18] These simple short courses of maternal and infant ARV prophylaxis for PMTCT were widely used in resource-limited settings.

In 2010, the WHO recommended ART for pregnant women with CD4 cell counts below 350 cells/mm^3. Women with higher CD4 counts were given AZT monotherapy plus intrapartum single-dose NVP, or triple ARV combination from as early as 14 weeks of pregnancy. In both cases, breastfed infants were given daily NVP PEP from birth until breastfeeding ends.[19] A total of 37 individual and combination ARVs have been approved by the FDA since 1987, with only 3 discontinued (**Figure 1.3**). ARVs are classified into six groups based on their mechanism of action: (1) nucleoside (or nucleotide) reverse transcriptase inhibitors (NRTIs); (2) non-nucleoside reverse transcriptase inhibitors (NNRTIs); (3) protease inhibitors (PIs); (4) integrase strand transfer inhibitors, (INSTIs); (5) entry inhibitors (EIs); and (6) fusion inhibitors (FIs). Current first-line ART
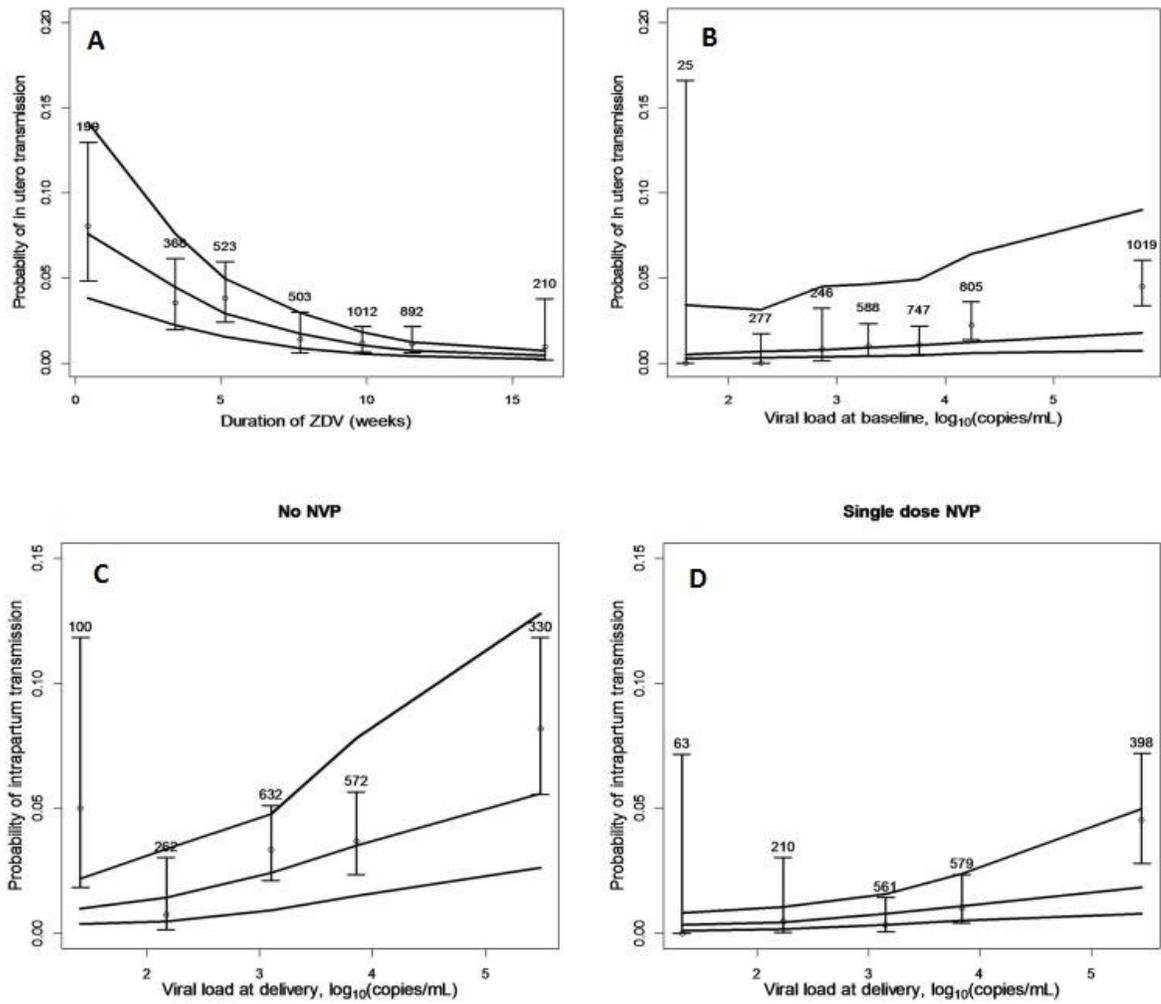
Figure 1.2 Mother-to-child transmission probabilities according to AZT duration, viral load at baseline/delivery, and NVP intake. A: Probability of in utero HIV transmission as a function of AZT duration; B: Probability of in utero HIV transmission as a function of viral load at baseline. The lines denote the median (5th and 95th percentiles) of model predictions. The open circles stand for the observed mean proportion of transmission, the solid vertical segments denote the corresponding 95% confidence intervals (numbers at top of each segment stand for the number of women in each time interval or VL interval); C: Probability of intra-partum HIV mother-to-child transmission as a function of viral load at delivery without single dose NVP; D: Probability of intra-partum transmission as a function of viral load at delivery with single dose NVP (Sripan et al. 2015).[18]
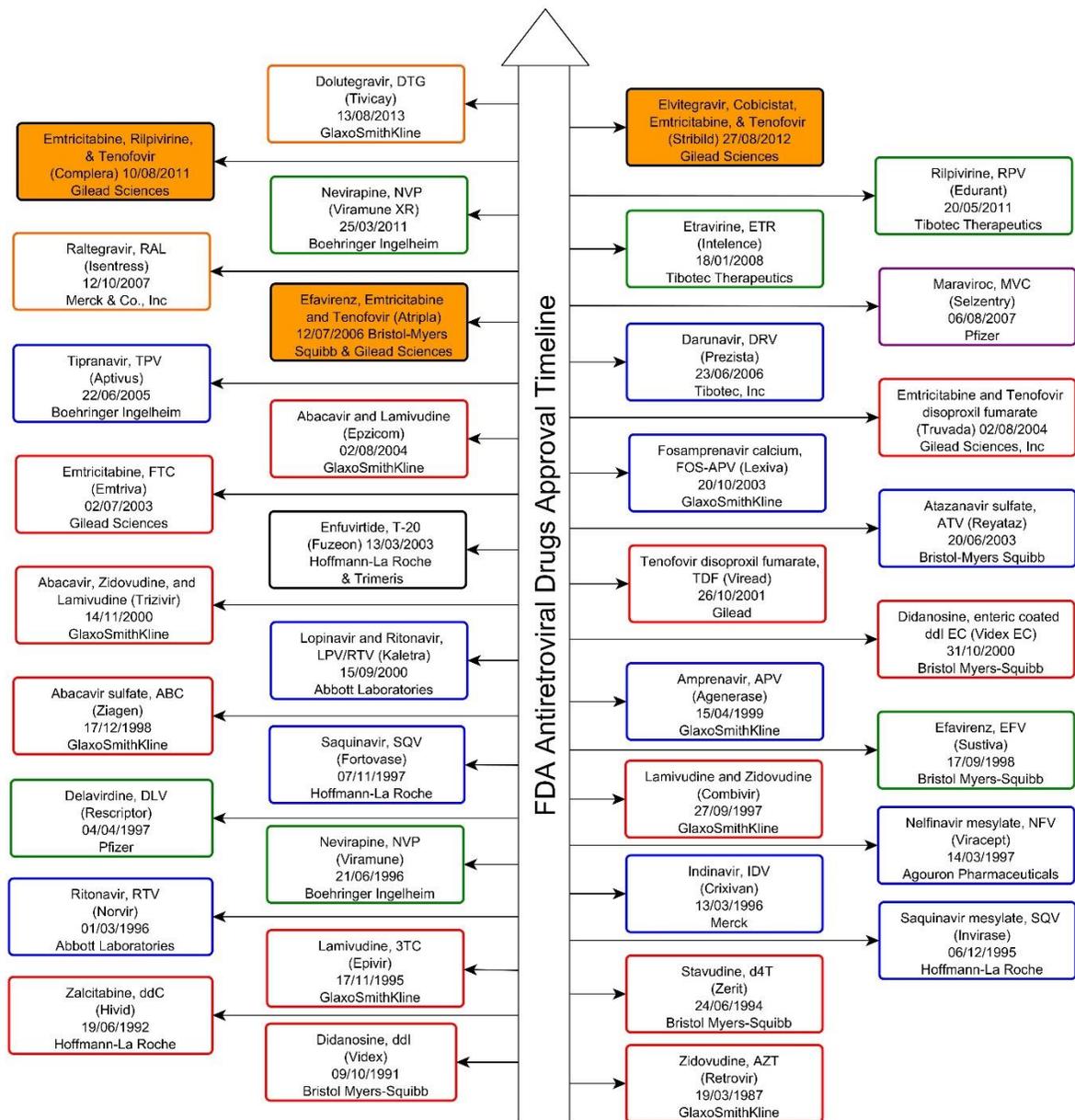
Figure 1.3 Antiretroviral drugs approval timeline by the FDA since 1987. Colour coding: red, nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs and NtRTIs); blue, non-nucleoside reverse transcriptase inhibitors (NNRTIs); blue, protease inhibitors (PIs); black, entry inhibitors (EIs); purple, fusion inhibitors (FIs); orange, integrase strand transfer inhibitors (INSTIs); orange filled, fixed dose combinations. Note: cobicistat is used as a pharmacokinetic enhancer in combination with elvitegravir, it has no antiviral activity.

regimens include a combination of three or more drugs from at least two classes (e.g. two NRTIs plus one NNRTI, PI or INSTI).[20] Although guidelines have since changed as new drugs became available, the goals of ART during pregnancy remain the same: to ensure rapid and durable virological suppression, restore immune system functions, and prevent MTCT. Achieving these requires optimal drug exposure during pregnancy, delivery and lactation.

However, considerable inter-individual variability in exposure to ARVs has been observed, often attributable to host genetic factors.[21] Additionally, many of the physiological changes associated with pregnancy affect the processes of drug absorption, distribution, metabolism, and excretion (ADME).[22] The use of ARVs during pregnancy and breastfeeding also raises questions about fetal and breastfed infants' exposure to maternal drugs in utero and through breast milk, respectively. This chapter reviews the effects of host genetics and pregnancy on the pharmacokinetics of ARVs as well as in utero fetal and breast milk infant exposure to maternal ARVs. A compelling argument is presented for possible accentuation or attenuation of pregnancy-associated pharmacokinetic changes in the presence of intrinsic genetically-induced variability.

1.2 GENETIC VARIATIONS AND ARV PHARMACOKINETICS/PHARMACODYNAMICS

About 20,000–25,000 protein-coding genes are estimated to be present in the human genome. Certain types of variations exist in individual genomes at frequencies > 5%: copy number variations (CNVs), insertion-deletion polymorphisms (indels), multiple-base nucleotide variations, single-nucleotide polymorphisms (SNPs), and short tandem repeats. Of these, SNPs (single-base differences between individuals) are the most common (> 90% of all sequence variations) with more than 22 million estimated to be in the human genome. SNPs occur within the coding, non-coding, or in the intergenic regions of genes. SNPs within the coding region (e.g., exon) that result in amino

acid substitution are described as nonsynonymous, while those that do not affect amino acid sequence are called synonymous SNPs. The two types of nonsynonymous SNPs, missense and nonsense, can alter protein activity and affect the disposition and/or efficacy of drugs that utilise the protein for metabolism, transport, or as target (**Figure 1.4**). Synonymous SNPs can affect gene expression and protein synthesis when present in the regulatory region of a gene (e.g., promoter region, intron), sometimes with functional consequences for drug disposition.
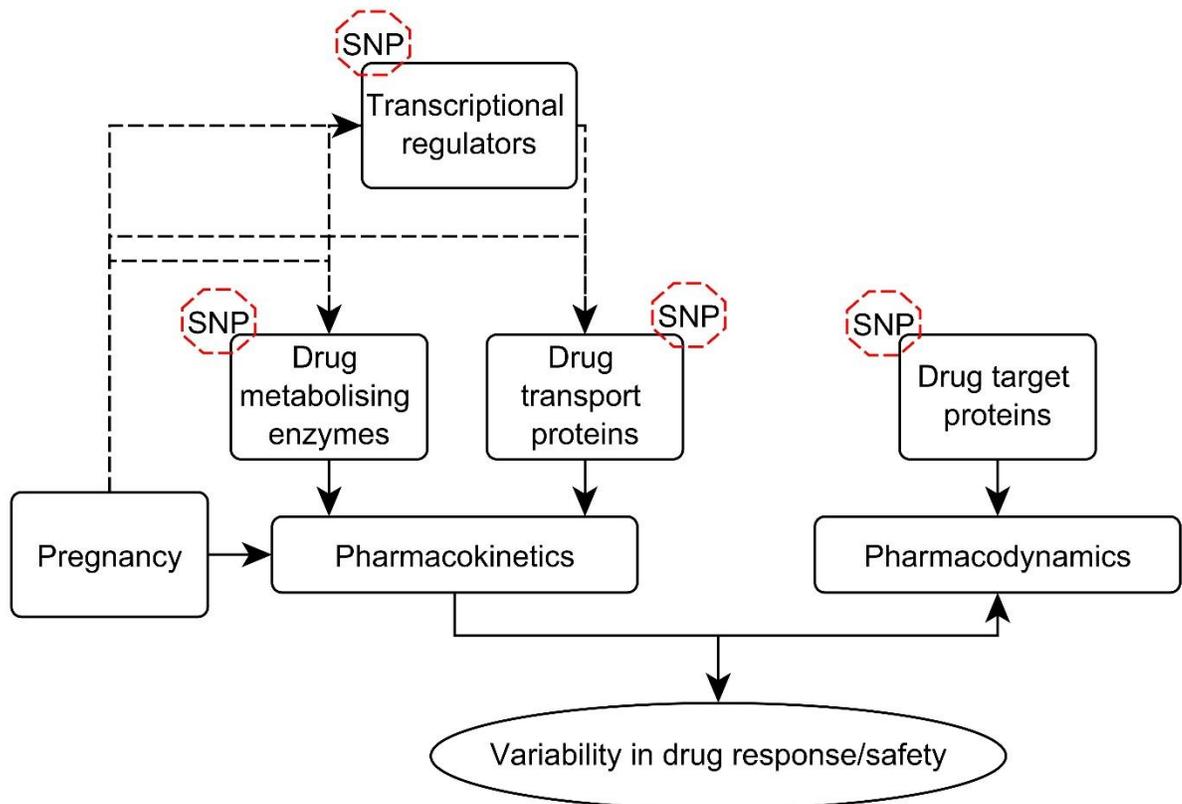
Figure 1.4 Sources of variability in drug response and safety. SNPs in genes that encode drug metabolising enzymes and transporter or their transcriptional regulator affect drug pharmacokinetics and those in genes encoding for drug target proteins alter pharmacodynamics. Pregnancy can affect pharmacokinetics by influencing the expression of metabolising enzymes, transporters, or their transcriptional regulators.

1.2.1 Drug Metabolising Enzymes

Most NRTIs undergo renal excretion as unchanged drugs while drugs in other classes undergo phase I metabolism by the cytochrome P450 (CYP) superfamily of isoenzymes and/or phase II metabolism by uridine 5'-diphospho-glucuronosyltransferase (UGT). The CYPs are involved in the metabolism of > 60% of all registered ARVs (**Table 1.1**), and thus constitute the most clinically relevant group of drug metabolising enzymes. About 60 different CYP genes and 58 pseudogenes have been identified and grouped into 18 families and 44 subfamilies based on similarities in their amino acid sequences. Of these genes and pseudogenes, 15 are known to encode different isoenzymes involved in drug metabolism in humans, including CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.[23] CYP genes exhibit considerable polymorphisms that cause gene deletions, premature stop codons or splicing defects, amino acid changes, and gene duplications. A number of non-CYP enzymes involved in drug metabolism also exhibit genetic polymorphisms that affect the metabolism and elimination of substrate drugs. Examples include dihydropyrimidine dehydrogenase (DPD), glutathione-S-transferase (GST), N-acetyltransferase (NAT), thiopurine-S-methyltransferase (TPMT), and UGT. A number of ARVs are substrates for UGT enzymes. UGT is involved in glucuronidation of endogenous compounds and xenobiotics, including the ARVs abacavir, raltegravir and dolutegravir (**Table 1.1**), contributing about 35% of all phase II drug metabolism.[24]

The resulting inter-individual differences in the number of functional variants or alleles of the affected gene encoding a specific enzyme is used to categorise patients into four general metabolic phenotypes. Poor metabolizers are individuals with defective or deleted alleles and abolished enzyme activity. Intermediate metabolizers carry either one functional and one defective allele, or two partially defective alleles, and in both cases have reduced activity of the enzyme. Extensive metabolizers have two functional alleles and normal enzyme activity. Individuals with a duplicated

or amplified gene variant, resulting in two or multiple copies of the functional allele and very high enzyme activity are classified as ultra-rapid metabolizers.[24] The clinical significance of a functional genetic polymorphism affecting a drug metabolising enzyme depends on the relative contribution of the isoenzyme to the overall metabolism of the drug and whether the parent compound and/or the metabolite is the pharmacologically active form (**Figure 1.4**). For instance, poor metabolisers of an active parent drug that is mostly dependent on a polymorphic enzyme for elimination are more likely to experience adverse drug reactions, whereas ultra-rapid metabolisers may experience lower efficacy with the same standard dosage regimen. The situation will be reversed for a prodrug. The ARV substrate specificity of known ARV metabolising enzymes are presented in **Table 1.1** and the functional consequences of reported SNPs on encoding genes are summarized in **Table 1.2**.

1.2.2 Drug Transporters

Membrane transport proteins facilitate the active movement of drugs across many endothelial and epithelial barriers in the brain, intestinal epithelial cells, hepatocytes, renal tubular cells, mammary glands, and placenta. By selectively facilitating the uptake of drugs into certain cells for access to target sites or for metabolism and elimination, while limiting it in others (e.g. blood brain barrier) to prevent toxicity, drug transporters play a key role in drug efficacy and safety, collectively termed pharmacodynamics. There are two classes of membrane transporters: the adenosine triphosphate binding cassette (ABC) family of efflux transporters, and the solute carrier (SLC) family of uptake transporters. The human ABC transporter family has 49 members further classified into 7 subfamilies based on similarities in amino acid sequences. Members include ABCB1 (also known as permeability glycoprotein, P-gp), ABCC1 (formerly known as multidrug resistance protein 1, MRP1), ABBC2 (formerly known as multidrug resistance protein 2, MRP2), and ABCG2 (formerly known as breast cancer resistance protein, BCRP). The 360 members of the SLC family are grouped into 46

Table 1.1 Antiretroviral drugs metabolism enzymes and transporters.

Drugs	Metabolism Enzymes	Transporters
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)		
Abacavir (ABC)	ADH, UGT	ABCB1, ABCG2
Didanosine (ddI)	Excreted unchanged	ABCC4, SLC28A2, SLC28A3, SLC29A1, SLC29A2, SLC29A3
Emtricitabine (FTC)	Excreted unchanged	ABCC1, ABCC4
Lamivudine (3TC)	Excreted unchanged	ABCG2, ABCC4, SLC22A1, SLC22A2, SLC22A3, SLC28A1, SLC28A3, SLC29A3
Stavudine (d4T)	Excreted unchanged	ABCC4, SLC28A1, SLC28A3, SLC29A3
Tenofovir (TDF)	Excreted unchanged	ABCB1, ABCC4, ABCC10, SLC22A6, SLC22A8
Zidovudine (AZT)	UGT2B7	ABCG2, ABCC4, SLC22A6, SLC22A7, SLC22A8, SLC22A11
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)		
Delavirdine (DLV)	CYP3A4	Unknown
Efavirenz (EFV)	CYP2B6, CYP2A6, UGT2B7, CYP3A4	Unknown
Etravirine (ETR)	CYP2C19, CYP3A4, CYP2C9	Unknown
Nevirapine (NVP)	CYP3A4, CYP2B6	ABCC10
Rilpivirine (RPV)	CYP3A4, CYP3A5	Unknown
Protease inhibitors (PIs)		
Amprenavir (APV)	CYP3A4	ABCB1
Atazanavir (ATV)	CYP3A4	ABCB1, ABCC1, ABCG2
Darunavir (DRV)	CYP3A4	ABCB1, SLCO1A2, SLCO1B1
Fosamprenavir (fAPV)	CYP 3A4	ABCB1
Indinavir (IDV)	CYP3A4	ABCB1, ABCC2
Lopinavir (LPV)	CYP3A4	ABCB1, ABCC1, ABCC2, SLCO1A2, SLCO1B1
Nelfinavir (NFV)	CYP3A4, CYP2C19	ABCB1
Ritonavir (RTV)[1]	CYP3A4, CYP2D6	ABCB1, ABCC2
Saquinavir (SQV)	CYP3A4, CYP3A5	ABCB1, ABCC2, SLCO1A2, SLCO1B1, SLCO1B3
Tipranavir (TPV)	CYP3A4	ABCB1

Table 1.1 continued

Drugs	Metabolism Enzymes	Transporters
Integrase Strand Transfer Inhibitors (INSTIs)		
Dolutegravir (DTG)	UGT1A1, UGT1A3, UGT1A9, CYP3A4	ABCB1, ABCG2
Elvitegravir (EVG)[2]	CYP3A4, UGT1A1, UGT1A3,	OATP1B1, OATP1B3
Raltegravir (RAL)	UGT1A1	ABCB1, SLC15A1, OAT1c,
Fusion Inhibitors (FIs)		
Maraviroc (MVC)	CYP3A4	ABCB1, SLCO1B1
Entry Inhibitors (EIs)		
Enfuvirtide (T-20)	Excreted unchanged	Unknown

[1]Ritonavir has a weak antiretroviral activity and is only used as a pharmacokinetic enhancer with other PIs; [2]Elvitegravir is used in combination with cobicistat, a pharmacokinetic enhancer with no antiviral activity. Abbreviations: ADH, alcohol dehydrogenase; UGT2B7, uridine diphosphate glucuronosyltransferase 2 family, polypeptide B7; *CYP2B6,* cytochrome P450, family 2, subfamily B, polypeptide 6; ABCB1, ATP-binding cassette, subfamily B, member 1; SLC28A2, solute carrier family 28, member 2; SLCO1A2, solute carrier organic anion transporter, family 1, subfamily A, member 2.

Table 1.2 Effects of SNPs in genes encoding metabolising enzymes, transporter, and nuclear receptors on antiretroviral drugs exposure.

Gene	SNPs	Observed effects
Metabolising enzymes		
CYP2A6[a][25,26]	1836G>T; 1093G>A	↑[EFV]
CYP2B6[a][24,25]	516G>T; 785A>G; 983T>C	↑[EFV], ↑[NVP]
CYP2C19[27]	rs4244285; rs12248560	↓[SQV], ↓[IDV], ↓[ATV], ↓[NVP]
CYP3A4[28]	*22	↑[LPV]
UGT1A1[29,30]	*28; 211G>A	↑ATV hyperbilirubinemia
UGT2B7[a][25]	735A>G; 802C>T	↔[EFV]; ↓[EFV]
Transporters (protein)		
ABCB1 (MDR1[a])[31-34]	3435C>T; 2677G>T; -129T>C	↓exp, ↓[NVP]_ht; ↓exp; ↓exp
ABCC2 (MRP2[a])[46,47]	1249G>A; -24C>T;	[SQV]↑; ↓[IDV], ↓TFV-ktd
ABCG2 (BCRP[a,b])[43,49]	C421A	↓exp/act
ABCC10 (MRP7[a,b])[35,36]	rs9349256 and rs2125739 (c.2759T>C)	↑TFV-ktd, ↓[NVP]
SLCO1A2 (OATP1A2[a])[37,38]	516A>C	↔[LPV]
SLCO1B1 (OATP1B1[a])[38,39]	521T>C	↑[MVC], ↑[LPV]
SLC22A6 (OAT1[b])	R50H	↑affinity for substrates
SLC22A1 (OCT1[a,b])[40-43]	Arg61Cys; P283L; -P341L	↑mRNA exp; ↓uptake
SLC22A2 (OCT2[a])[41,43,44]	T199I; -T201M and -A270S	↓uptake
SLC22A3 (OCT3[a,b])[40-42,45]	rs2292334; rs2048327; rs1810126; rs3088442	↑mRNA exp
Nuclear hormone receptors		
NR1I3 (CAR)[46]	540CC	↑EFV discontinuation
NR1I2 (PXR)[47]	63396 C>T	↓[ATV]

Footnotes: ↓, decreased; ↑, increased; ↔, no difference; a, expressed in the placental; b, expressed in the mammary glands. Abbreviations: act, activity; exp, protein expression; ktd, kidney tubular dysfunction; [X], drug exposure. Note: other transporters expressed in the placenta include ABCC1, ABCC5, SLCO1B3, SLC29A1, SLC29A2, SLC22A11, and SLC22A7.

subfamilies, including the organic anion transporting polypeptide (OATP), organic cation transporter (OCT), and organic anion transporter (OAT). Many of these transporters are involved in the disposition of ARVs (**Table 1.1**).

Inter-individual variations in the expression of drug transport proteins has been reported, resulting in variability in the disposition of substrate drugs. For instance, 20- to 55-fold variability in ABCB1 expression in human liver[48,49] and 3- to 6-fold variability in the small intestine have been observed.[50] Similar variability has been reported for other efflux transporters and many uptake transporters. This has partly been attributed to genetic variations in the genes encoding these drug transport proteins that affect their expression, substrate specificity, and/or intrinsic transport activity, and ultimately the disposition, efficacy, and safety of many substrate drugs[50,51] (**Figure 1.4**). The ARV substrate specificity of known ARV transporters are presented in **Table 1.1** and the functional consequences of reported SNPs on encoding genes are summarized in **Table 1.2**.

1.2.3 Nuclear Hormone Receptors (Transcriptional Regulators)

Nuclear hormone receptors are ligand-activated transcription factors (proteins) that regulate gene expression by interacting with specific DNA sequences and serve as on-off switches for transcription within the cell nucleus. They form part of the adaptive response to drug exposure and can also regulate gene expression in their non-ligand-bound form. Some of the well described receptors involved in the regulation of ARV disposition genes include nuclear receptor subfamily 1, group I, member 2 (NR1I2; also known as Pregnane X Receptor, PXR), NR1I3 (also known as Constitutive Androstane Receptor, CAR), Farnesoid X Receptor (FXR), Vitamin D Receptor (VDR), and arylhydrocarbon receptor (AhR).[52]

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Nuclear receptors are usually resident in cellular cytoplasm. Binding of ligands causes the activation of a common signalling pathway that results in translocation of the bound receptor to the nucleus where it heterodimerizes with retinoid X receptor (RXR) to form a complex which binds to hormone response elements (HREs) normally located in regulatory regions of target genes. The release of corepressor proteins and recruitment of coactivators stimulates the transcriptional machinery. Ligand binding to NR1I2, NR1I3, FXR, VDR, or AhR within the complex causes differential conformational changes that result in permissive, nonpermissive, or conditional transcriptional activation.[53] Importantly, post-translational modification of the receptor can also influence its ability to transactivate in the ligand-bound or non-ligand-bound form.

The importance of nuclear hormone receptors for ARV disposition lies in their regulation of drug metabolising enzymes and transporters genes. For instance, expression of the efflux transporter ABCB1 is regulated by NR1I2, NR1I3, and VDR.[54,55] CYP3A4 is known to share transcriptional regulation and induction pathways (mainly through NR1I2 and NR1I3) with other drug-metabolising enzymes, including CYP2B6, CYP2Cs and UGTs.[56-58] Inter-individual variability in regulation of gene expression by nuclear hormone receptors has been observed and reportedly associated with splice variants and genetic polymorphisms.[59] The nuclear hormone receptors involved in transcriptional regulation of different ARV disposition genes and reported SNPs are presented in **Table 1.2**.

1.3   ARV PHARMACOKINETICS DURING PREGNANCY AND POSTPARTUM

1.3.1   Pregnancy-Induced Physiological Changes Affecting Pharmacokinetics

The physiological changes associated with pregnancy are known to affect the pharmacokinetics of many drugs, including many ARVs.[22,60] Changes in drug absorption during pregnancy are caused by a number of factors, including up to 50% prolongation of gastric transit time as a result of increased progesterone levels, nausea and vomiting in early pregnancy, increase in gastric pH and the use of antacids to alleviate symptoms. Drug distribution is impacted by increase of up to 50% in cardiac output and blood volume, increase in water and sodium retention at the kidney and the resultant 6-8 L increase in body water. These lead to a significant increase in the volume of distribution of most drugs thereby reducing plasma drug concentrations. In addition, pregnancy results in 15% decline in plasma albumin and > 50% decline in alpha-1-acid glycoprotein concentrations, increasing the clearance of highly protein bound drugs because of increased percentage of unbound drug available for metabolism (influencing hepatic extraction ratio).

Alterations in the activities of several key hepatic drug metabolising enzymes during pregnancy have been reported, in most cases secondary to hormonal changes. For instance, a steady rise in plasma concentrations of estrogens and progesterone occurs during pregnancy, reaching up to 100-fold higher than pre-pregnancy levels at term.[61,62] In a study that characterised the effects of estrogen and progesterone on key CYPs, estradiol was shown to enhance CYP2A6, CYP2B6, and CYP3A4 expression, whereas progesterone induced CYP2A6, CYP2B6, CYP2C8, CYP3A4, and CYP3A5 expression. Estradiol was observed to increase CYP2C9 and CYP2E1 activities independent of mRNA expression levels by unknown mechanisms.[70] Pregnancy induces CYP2B6 expression through estradiol possibly through a mechanism involving its transcriptional

regulators NR1I2 and NR1I3.[63] Tracy et al. reported a 35-38% increase in CYP3A4 and CYP3A5

activities at all stages of pregnancy compared to postpartum.[64] UGT-mediated glucuronidation

of some drugs is also increased during pregnancy. For example, the clearance of

dihydroartemisinin (a UGT1A9 and UGT2B7 substrate), the active metabolite of the antimalarial

drug artesunate, increases by 42% during pregnancy.[65] The activity of alcohol dehydrogenase

(ADH) was reportedly higher during pregnancy in rodents.[66] Therefore, upregulation of enzyme

expression as a result of nuclear hormone receptor activation by rising female hormones is likely

one mechanism involved in observed pregnancy-induced changes in pharmacokinetics.[22] The

glomerular filtration rate in healthy women is at least 50% higher during pregnancy as a result

of 60-80% increase in renal blood flow. In addition, active renal tubular secretion increases

during pregnancy. These combine to increase the elimination of drugs that are excreted

unchanged by the kidneys.[67]

However, while pregnancy-induced changes in the pharmacokinetics of many drugs and the

inter-individual variability introduced by genetic polymorphisms in drug disposition genes are

individually well established, their combined effects have not been thoroughly investigated.

Available pharmacokinetic data of individual ARVs during pregnancy versus postpartum are

presented from Section 1.3.4. Where appropriate, logical assumptions are made about potential

accentuation or attenuation of any observed changes by host genetic variability.

1.3.2 Fetal Exposure to Maternal ARVs In Utero

Maternal drug use during pregnancy often leads to exposure of the fetus to the drug. In the case

of ARVs, the penetration of maternal drugs into fetal compartment may contribute to their

overall prophylactic effect.[68] Conversely, in utero fetal exposure to ARVs may have deleterious

effects as highlighted in reported cases of mitochondria toxicity, fetal growth restriction, heart

defects, and alterations in haematological parameters.[69-71] Some of the factors that determine the extent of drug penetration into the fetal compartment include maternal plasma concentration, physiochemical properties of the drug, placental blood flow, plasma pH, protein binding and placental drug metabolism. Perhaps more important in controlling in utero drug exposure are drug efflux and influx transporters: their placental expression, sub-cellular localisation, activity and substrate specificity.[72]

Fetal exposure to potentially harmful compounds from maternal circulation is prevented by a number of protective mechanisms. At least 18 transporters known to be relevant for ARV distribution are expressed on either the apical (maternal) or basolateral (fetal) membranes of the syncytiotrophoblast as well as the endothelium of fetal capillaries (**Figure 1.5**). The ABC efflux transporters limit fetal exposure to maternal ARVs,[73] while the SLC influx transporters, including the OATPs, OATs, OCTs and ENTs, facilitate the trans-placental passage of ARVs and distribution to the fetal compartment. In an extensive review of ARV penetration into the placenta and amniotic fluid, wide inter-individual variability and some class-specificity were observed with the following rank order of accumulation: raltegravir/NRTIs (tenofovir > AZT / lamivudine / emtricitabine / stavudine / abacavir) > NNRTIs (NVP > etravirine) > PIs > maraviroc / enfuvirtide.[68] Iqbal et al. reviewed the gestational changes in the expression and function of placental drug transporters as well as the associated genetic polymorphisms that can potentially affect fetal exposure.[74] The functional consequences of genetic polymorphisms in the encoding genes were highlighted by Ieiri et al.[75] The localisation of known ARV transporters on placental syncytiotrophoblast and reported functional SNPs are presented in **Table 1.2**. Other factors like drug-drug interactions, HIV infection and co-infections have been recognised to affect drug transport.[31,33,76-78] Drug-drug interactions at the human placenta can also affect fetal exposure

to maternal drugs.[79] Reported cord/maternal blood ratios for different ARVs are presented in sections 1.3.4 to 1.3.8.
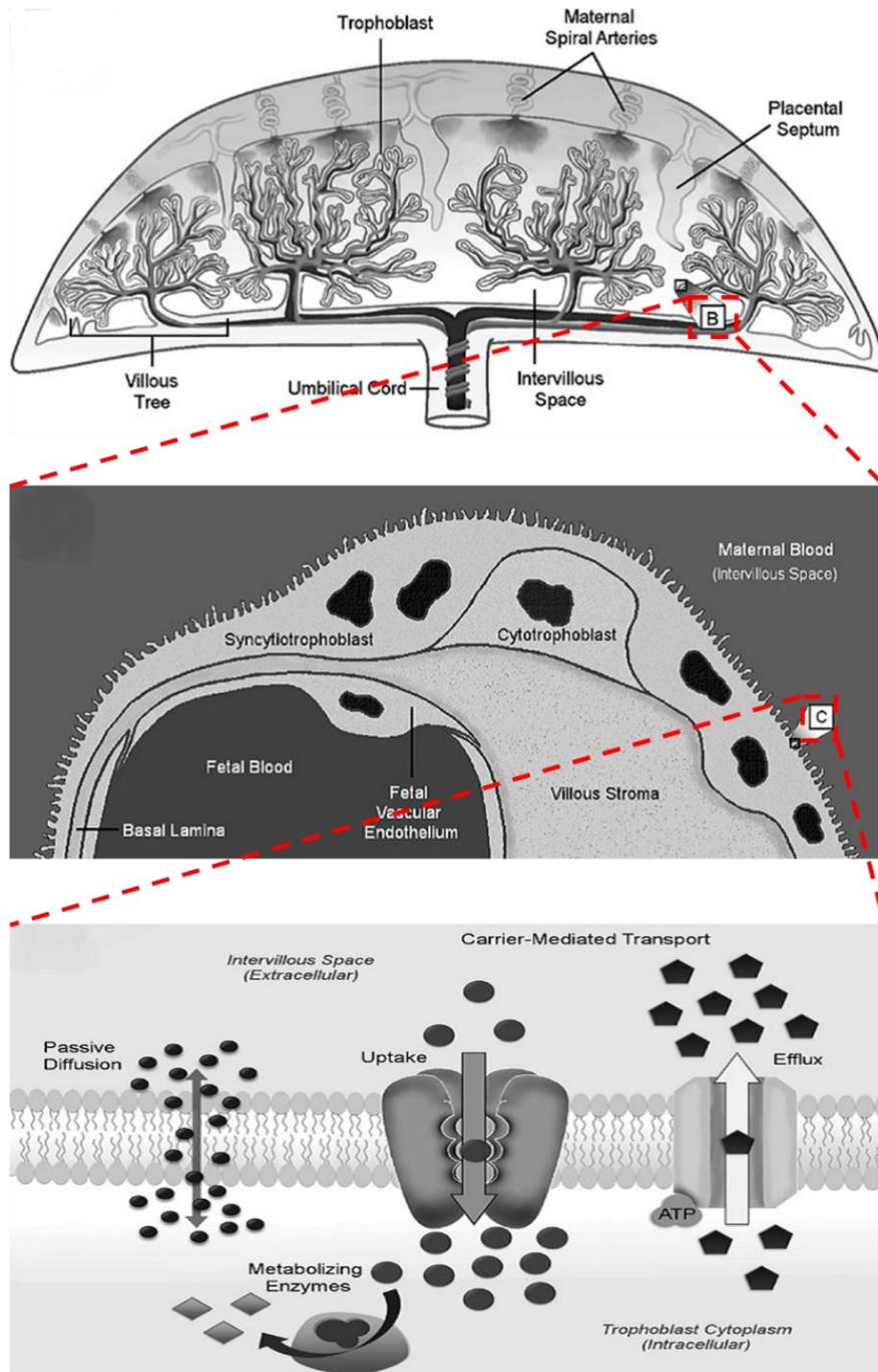
Figure 1.5 Mechanisms of maternal-fetal transfer. A: Overview of human placental morphology showing fetal vessels from the umbilical cord branching into villous trees, which are bathed by

Figure 1.5 continued

maternal blood entering the placenta via spiral arteries. Trophoblast cells on the surface of the villous structures separate the maternal blood in the intervillous space from the fetal circulation, as highlighted in B. B: Cellular components of a placental villus, wherein multinucleated syncytiotrophoblast cells are formed by fusion of the precursor cytotrophoblast cells. The trophoblast cells and the fetal vascular endothelial cells are separated by basal lamina. Several transport mechanisms within the trophoblast cell layer are highlighted in C. C: Transport mechanisms in trophoblast cells, with different molecules represented by different shapes. Passive diffusion is governed by the concentration gradient of any compound (xenobiotic or intermediary metabolite). Two types of carrier-mediated transport (uptake and efflux) involve transport proteins that span the phospholipid bilayer of the cell membrane. The biotransformation of molecules by metabolizing enzymes is also represented. Adapted from: Erik Rytting and Mahmoud S. Ahmed. Fetal drug therapy. In: Donald R. Mattison (eds), Clinical pharmacology during pregnancy. USA: Elsevier. First Ed. 2013. 55-72.

1.3.3 Exposure of Breastfed Infants to Maternal ARVs in Breast Milk

The WHO recommends exclusive, "on demand" breastfeeding starting within one hour of birth, up to 6 months of age, and continued with gradual introduction of appropriate complementary foods up to 2 years of age or beyond. In addition to its nutritional benefits, ready availability and affordability, the health benefits of breastfeeding for both infant and mother have long been recognised.[80-83] However, breastfeeding in the presence of maternal drug use is widespread despite lack of safety data for either proscriptions or permissive statements. In fact, over 90% of nursing mothers take at least one drug during the first week after delivery, 17% take at least one drug until 4 months after delivery, and 5% receive drugs for chronic conditions, giving rise to concerns over the presence of drugs in breast milk and their potential effects on the nursing infant.[84]

Making an informed decision requires accurate evaluation of the potential risks versus benefits based on knowledge of the extent of the drug's excretion in human breast milk.[85] For instance, HIV positive mothers breastfeed their babies while taking ARVs started before or during pregnancy for their own health and PMTCT. Determining the safety of maternal ARVs for the breastfed infant requires understanding their pharmacokinetics in breast milk.[86] Therapeutic ARV concentrations in breast milk can prevent ongoing localised replication of HIV and development of drug resistant virus,[87] which may otherwise be passed to infants who become infected if PMTCT fails.[88] On the other hand, exposure to subtherapeutic concentrations may lead viral resistance in infected infants who are diagnosed late while very high concentrations may lead to toxicity.

A recent systematic review of available studies on breast milk concentrations of 13 of the currently licensed ARVs indicated that while NRTIs and NNRTIs are excreted into breast milk in significant amounts and transferred to breastfeeding infants, the overall penetration of PIs into breast milk was low and tended to be undetectable in infant plasma.[89] The authors suggested that ARVs transfer to breastfed infants may explain the development of drug-resistant HIV in infants where maternal ARVs have failed to prevent transmission.[89] There are no data on infants' exposure to newer ARVs through breast milk. **Figure 1.6** illustrates five pathways of substance excretion into breast milk, including via transport proteins. The localisation of known ARV transporters in mammary gland epithelium and reported functional SNPs are presented in **Table 1.2**.

A major limitation hampering studies of breast milk pharmacokinetics is the inadequacy of available bioanalytical methods for the quantification of drugs in breast milk. The complexity of breast milk makes available methods either difficult to validate because of inadequate sample
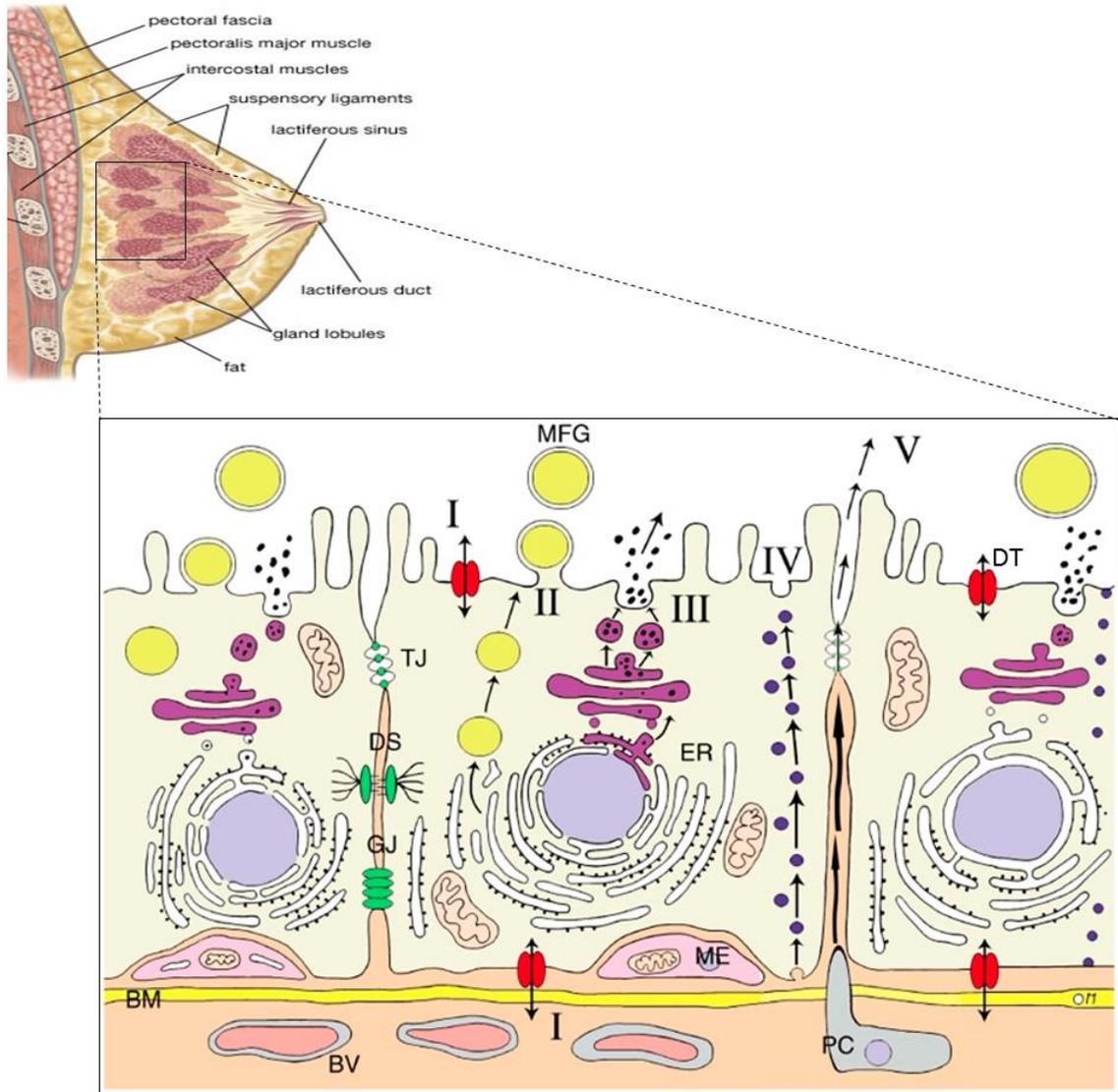
Figure 1.6 Mammary epithelium cell layer showing pathways of milk excretion. I: monovalent ions, water, glucose, lipids, drugs and xenobiotics move across apical and basal membranes via transporter proteins. II: milk fat and lipid secretion with formation of cytoplasmic lipid droplets moving to the apical membrane for release as milk fat globules (MFG). III, IV and V: exocytotic secretion, vesicular transcytosis and transport through the paracellular pathway for plasma components and leukocytes. V opens only during pregnancy, involution and in the event of inflammatory states such as mastitis. Abbreviations: BM, basement membrane; BV, blood vessel; DS, desmosome; DT, drug transporter; ER, endoplasmic reticulum; GJ, gap junction; ME, myoepithelial cell; PC, plasma cell; TJ, tight junction. Adapted from: www.ibmm.unibe.ch/content/groups/albrecht_group/.

clean-up or complicated due to multiple sample clean-up steps involving a combination of liquid-liquid and solid phase extraction in a single method.[90,91] In addition, lack of standardisation makes cross-study comparisons difficult, particularly given differential drug accumulation within specific fractions of milk. For instance, studies reporting the excretion of abacavir, efavirenz (EFV), etravirine, lamivudine, lopinavir, NVP, AZT, tenofovir and emtricitabine in human breast milk have used methods validated only in plasma.[92-97] A fully validated method was described for the quantification of lamivudine, lopinavir, nelfinavir, NVP, ritonavir, stavudine, and AZT (7 out of 25 ARVs in use) in breast milk.[98] However, the multiple extraction steps make the method complicated, time-consuming and expensive. An additional limitation is imposed by the ethical challenges associated with paediatric clinical research. Therefore, bridging the existing gaps in our understanding of breastfed infant exposure to maternal ARVs through breast milk will require adopting novel strategies that will overcome both the methodological and the ethical challenges presented by the currently available methods.

1.3.4 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

There are currently eight NRTIs approved for HIV treatment. All NRTIs are administered as prodrugs and must undergo intracellular metabolism by cellular kinases to their active triphosphate anabolites. Routine measurement of intracellular NRTI-triphosphate concentrations remains challenging. Comprehensive NRTI pharmacokinetic data available during pregnancy has subsequently been restricted to plasma NRTI concentrations but the relationship between NRTI plasma concentrations and drug efficacy/toxicity remains unclear.[99] Below is a summary of the effect of pregnancy on individual NRTI exposure and available pharmacogenetic data.

1.3.4.1 Zidovudine (AZT)

AZT is primarily eliminated through hepatic metabolism by UGT.[100] Several studies have reported that AZT pharmacokinetics during the third trimester of pregnancy is similar to non-pregnant adults.[101-103] However, lower AZT exposure during the third trimester versus the postpartum period has been reported[104] (**Table 1.3**; **Figure 1.7**). The standard dose of AZT is currently 300 mg, twice daily, and no adjustments are recommended during pregnancy. A lower dose of AZT, 200 mg twice daily, has been studied in non-pregnant adults with body weight < 60 kg due to concerns of higher rates of drug toxicity. Pharmacokinetic and efficacy data suggest that this lower dose may be preferable in this patient group[105,106] but has not been studied in pregnant women. Specific associations between in utero exposure to AZT and congenital heart defects and a long-lasting postnatal myocardial remodeling in girls have been reported.[71,107]

To date, no data on the effect of UGT host genetic polymorphisms on plasma AZT pharmacokinetics has been reported. Association between AZT-triphosphate concentrations and a SNP in *ABCC4* in HIV-infected non-pregnant adults has been reported.[108] Median AZT-triphosphate concentrations were 49% higher in *ABCC4* 3724G>A (rs11568695) carriers versus non-carriers ($P = 0.03$). ABCC4 has been shown to contribute to AZT uptake into rat conditionally immortalised syncytiotrophoblasts.[109] Therefore, the potential clinical implications of *ABCC4* polymorphisms during pregnancy, especially for fetal exposure, need to be explored. AZT readily crosses the placenta to the fetal compartment with cord-to-maternal blood concentration ratio of 1.13 to 1.27.[104] The M/P ratio varies widely with a pooled estimate of 0.80 (95% CI 0.76–0.85).[89]

Table 1.3 Summary of pharmacokinetic parameters of ARVs during pregnancy and post-partum from selected publications.

Drug (FDA category)	Pregnancy AUC (µg.hr/mL)	C$_{max}$ (µg/mL)	C$_{min}$ (µg/mL)	Postpartum AUC (µg.hr/mL)	C$_{max}$ (µg/mL)	C$_{min}$ (µg/mL)
NRTIs						
3TC (C)[103]	4.9 (4.0 – 6.1)	1.3 (1.1 – 1.6)	-	5.1 (3.7 – 6.9)	1.2 (0.98 – 1.5)	-
ABC (C)[110]	5.9 (5.2 – 6.8)	1.9 (1.6 – 2.2)	BQL (BQL – 0.102)	5.4 (4.3 – 6.8)	2.1 (1.6 – 2.7	BQL (BQL – 0.203)
FTC (B)[111]	8.0 (7.1 – 8.9)	1.4 (1.2 – 1.6)	0.06 (0.04 – 0.06)	9.7 (8.6 – 10.9)	1.4 (1.3 – 1.7)	0.09 (0.07 – 0.01)
TDF (B)[112]	2.4 (2.1 – 2.6)	0.27 (0.22 – 0.33)	0.05 (0.04 – 0.06)	3.2 (2.7 – 3.7)	0.35 (0.29 – 0.42)	0.08 (0.06 – 0.09)
AZT (C)[104]	1.2 (±0.27)	1.04 (±0.45)	-	1.8 (±0.13)	1.2 (±0.01)	-
NNRTIs						
EFV (D)[113]	55.4 (13.5 – 220.3)	5.4 (1.9 – 12.2)	1.6 (0.23 – 8.13)	58.3 (22.7 – 214.4)	5.10 (1.96 – 11.42)	2.05 (0.31 – 8.43)
ETR (B)[114]	8.3 (2.7 – 31.0)	1.02 (0.26 – 3.47)	0.48 (0.08 – 1.94)	5.3 (2.1 – 16.4)	0.63 (0.30 – 1.60)	0.33 (0.07 – 1.14)
NVP (B)[115]	47.0 (41.5 – 58.9)	5.3 (4.7 – 6.3)	3.1 (2.7 – 4.1)	59.4 (53.6 – 69.6)	6.6 (5.9 – 7.7)	3.9 (3.5 – 4.9)
RPV (B)[116]	1.99 (0.56 – 4.31)	-	0.06 (BQL – 0.18)	2.58 (0.19 – 6.74)	-	0.10 (BQL-0.30)
PIs						
fAPV (C)[117]	32.4 (14.2 – 54.4)	5.93 (1.66 – 12.53)	1.70 (0.71 – 3.23)	50.7 (11.6 – 62.4)	5.7 (2.1 – 11.4)	2.43 (0.37 – 3.82)
ATV/r (B)[118]	41.9 (27.4 – 60.8)	3.6 (2.8 – 5.1)	0.7 (0.5 – 1.1)	57.9 (47.1 – 64.8)	4.1 (3.0 – 5.8)	1.2 (1.1 – 2.0)
ATV/r[a] (B)[119]	46.6 (11.0 – 88.3)	4.7 (0.88 – 7.5)	0.74 (0.14 – 2.1)	55.1 (9.9 – 99.5)	4.52 (0.93 – 9.45)	0.88 (BQL – 2.7)
DRV/r (B)[120]	63.5 (46.0 – 75.2)	5.78 (4.31 – 7.29)	1.17 (0.73 – 1.72)	103.9 (85.9 – 135.7)	8.11 (6.93 – 10.3)	2.78 (2.05 – 2.98)
DRV/r[a] (B)[120]	45.9 (29.3 – 52.5)	5.53 (4.44 – 7.10)	2.22 (1.68 – 3.26)	61.7 (49.7 – 80.9)	7.78 (6.11 – 9.54)	2.51 (2.04 – 3.27)

Table 1.3 continued

Drug (FDA category)	Pregnancy AUC (µg.hr/mL)	C$_{max}$ (µg/mL)	C$_{min}$ (µg/mL)	Postpartum AUC (µg.hr/mL)	C$_{max}$ (µg/mL)	C$_{min}$ (µg/mL)
IDV/r (C)[121]	16.1 (7.5 – 39.9)	3.5 (1.3 – 7.4)	0.13 (0.07 – 0.6)	27.1 (18.6 – 44.7)	5.5 (3.8 – 9.4)	0.28 (0.14 – 0.71)
LPV/r (C)[122]	58.0 (50.4 – 70.7)	7.5 (6.7 – 8.7)	2.5 (2.0 – 3.5)	89.4 (74.0 – 108)	10.0 (8.4 – 11.8)	4.7 (3.1 – 6.8)
LPV/r[a] (C) [123]	96 (43 – 198)	10.7 (5.8 – 19.1)	5.1 (1.5 – 12.2)	133 (66 – 237)	14.6 (9.8 – 22.8)	7.2 (2.8 – 21.0)
NFV (B)[124]	18.9 (3.6 – 53.7)	3.2 (0.9 – 6.5)	0.9 (<0.039 – 4.4)	30.8 (1.3 – 123.9)	4.6 (0.3 – 9.9)	1.1 (<0.039 – 4.8)
SQV/r (B)[125]	12.71 (8.96 – 26.93)	3.23 (2.68 – 6.03)	0.26 (0.23 – 1.01)	28.94 (14.55 – 58.49)	3.92 (2.96 – 5.66)	0.86 (0.25 – 2.21)
Others						
RAL (C)[126]	8.3 (3.0 – 17.5)	1.8 (0.69 – 6.3)	0.09 (0.014 – 0.47)	7.5 (2.0 – 25.5)	2.8 (0.35 – 8.9)	0.50 (<0.010 – 0.77)
MVC (B)[127]	2.72 (2.04 – 3.62)	0.45 (0.32 – 0.63)	0.11 (0.081 – 0.15)	3.65 (2.23 – 5.47)	0.65 (0.41 – 1.03)	0.13 (0.089 – 0.18)

Footnote: BQL, below quantification limit; a, a modified dose/dosage was used in this study. FDA categories: A, no evidence of risk; B, no evidence of risk in animal studies; C, adverse effect on the fetus demonstrated in animal studies but may be used if benefits outweigh risk; D, positive evidence of human fetal risk but may be used if benefits outweigh risk; X, risk of fetal abnormalities outweigh benefits.
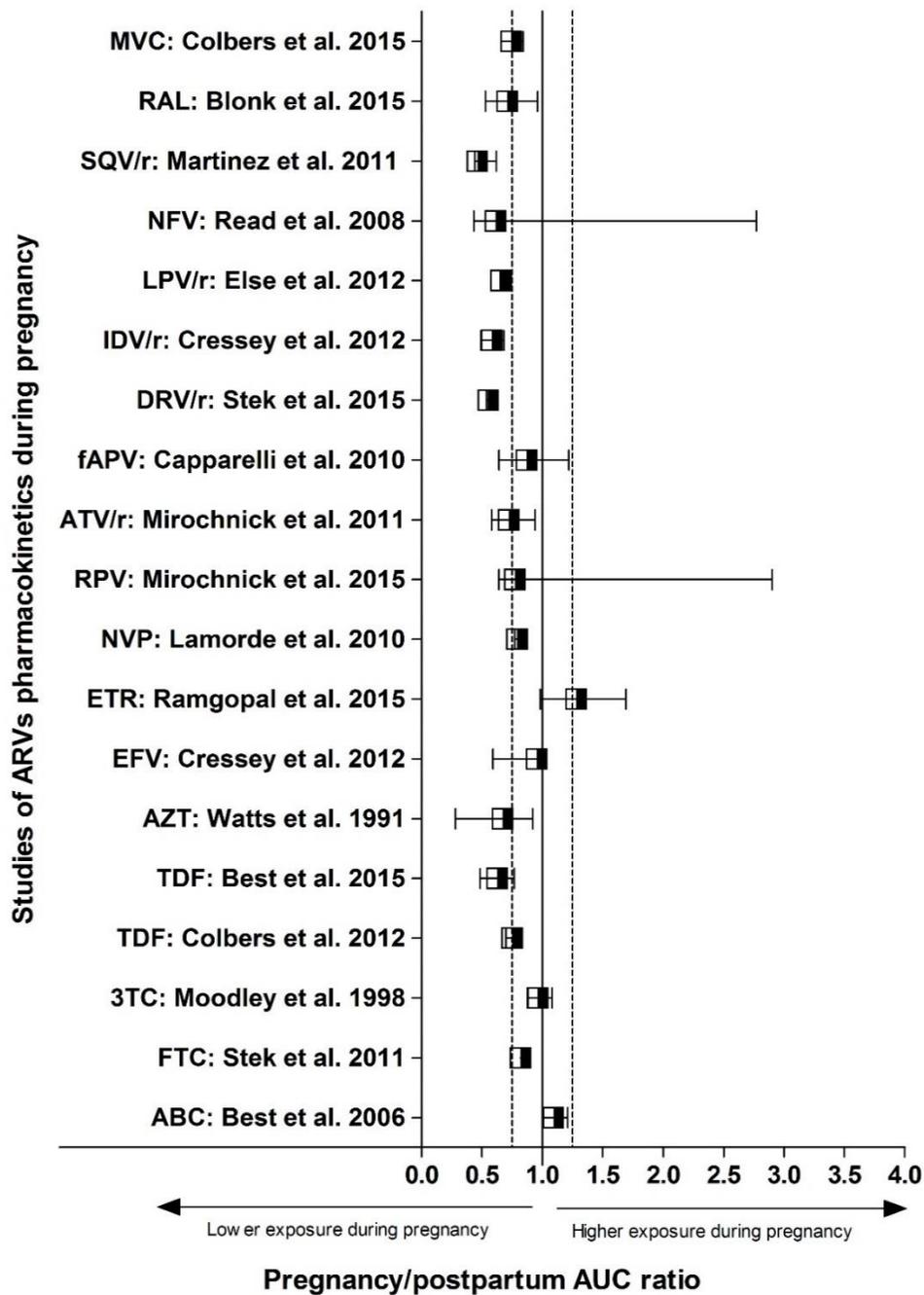
Figure 1.7 Forest plot showing studies of ARV pharmacokinetics in pregnancy vs postpartum. The solid line at 1.0 represents line of no difference and dashed lines represent ±25% difference. Abbreviations: 3TC, lamivudine; ABC, abacavir; APV, amprenavir; ATV, atazanavir; AZT, zidovudine; DRV, darunavir; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; IDV, indinavir; LPV, lopinavir; MVC, maraviroc; NFV, nelfinavir; NVP, nevirapine; RAL, raltegravir; RPV, rilpivirine; SQV, saquinavir; TDF, tenofovir disoproxil fumarate.

1.3.4.2   Stavudine (d4T)

The WHO ARV treatment guidelines recommend a 30 mg twice daily dose of d4T, a thymidine analogue, for adults.[67] The pharmacokinetics of d4T in pregnancy appears to be similar to those in HIV-infected men and no dose adjustment is recommended. A mean cord-to-maternal blood concentration ratio of 1.0 (95%CI 0.63-1.37) was reported.[128] The pooled estimate of M/P ratios from three different studies is 1.21 (1.07– 1.36).[89] It was widely used in resource-limited settings until recently when the WHO suggested that countries should phase it out and develop plans to move towards alternative first-line regimens, primarily due to d4T-associated lipodystrophy. No data are available on the role of transporter or metabolising enzymes genetic polymorphisms in d4T disposition but an association between human leukocyte antigen B*4001 (*HLA-B*4001*) and d4T-associated lipodystrophy was reported in a Thai cohort.[129]

1.3.4.3   Abacavir (ABC)

ABC is a guanosine analogue with recommended dosage of 300 mg twice daily or 600 mg once daily for adults. Best et al. reported that the pharmacokinetics of ABC was similar during the third trimester and postpartum (**Figure 1.7**; **Table 1.3**), and cord-to-maternal blood concentration ratio was 1.06.[110] In another study using a population pharmacokinetic (popPK) approach, ABC population clearance was similar between pregnant and postpartum women, including those on once daily 600 mg regimen.[130] A median M/P ratio of 0.85 has been reported.[93] ABC is metabolised by ADH and UGT but there are currently no reports on the influence of *ADH* or *UGT* polymorphisms on ABC pharmacokinetics. Potentially fatal ABC-induced hypersensitivity reactions occur in 5 to 8% of patients within 6 weeks of initiating treatment, necessitating immediate discontinuation and switching to an alternative regimen.[131] This has been associated with the presence of *HLA-B*5701* allele. Patients with this allele are at significantly higher risk of developing ABC-induced hypersensitivity reaction and should not receive abacavir.[132] This was later confirmed in the PREDICT-1 and SHAPE randomised controlled

trials,[133,134] establishing it as a routine pharmacogenetic testing used as standard of care for ART.[135]

1.3.4.4 Lamivudine (3TC)

3TC is a cytidine analogue excreted primarily unchanged via the kidneys. The recommended dosage for adults is 300 mg once daily or 150 mg twice daily. The pharmacokinetics of 3TC during the third trimester of pregnancy was reportedly similar to non-pregnant adults[103] (**Figure 1.7**; **Table 1.3**). Another study used a popPK approach and reported 22% higher oral clearance in pregnant women but a dose adjustment was not warranted.[136] 3TC crosses the human placenta with median cord-to-maternal blood concentration ratio of 1.06. But amniotic fluid concentrations were five times higher than in maternal plasma, suggesting passive diffusion across the amnion, fetal uptake, and fetal clearance.[137] Like AZT, 3TC is a substrate for ABCC4 and the *ABCC4* 4131G>T (rs3742106) carrier status has been associated with 20% higher 3TC-triphosphate intracellular concentrations (p = 0.004).[138] Breast milk concentrations were approximately 3-fold higher than maternal serum concentrations.[94] However, breast milk-ingested 3TC appears to be poorly absorbed in infants and present at low concentrations in the plasma, < 0.10 µg/mL.[95] 3TC is also a substrate for OCT1, OCT2 and OCT3,[41,139] all of which are expressed in the kidney, placenta and mammary gland (no OCT2 in the latter).[140] In a recent study Choi et al. observed reduced 3TC uptake in oocytes expressing OCT1-P283L and OCT1-P341L as well as those expressing OCT2-T199I, OCT2-T201M and OCT2-A270S variants compared to OCTs with the reference sequences.[43] The consequences of functional SNPs of OCTs on 3TC pharmacokinetics have not been studied in vivo. Such SNPs may also influence the distribution of 3TC to the fetal compartment as well as its excretion into breast milk.

Rare cases of lethal mitochondrial dysfunction in infants exposed in utero to AZT and 3TC were reported in the late 1990s.[141] These severe cases are clinically very similar to known genetically induced mitochondrial defects. Evidence of mitochondrial damage has been demonstrated in

HIV-uninfected infants exposed to AZT and 3TC for PMTCT.[142] Recent studies have associated mtDNA halogroup T/L1c and mtDNA 4917G (increased risk), *HFE* 845G>A (rs1800562) and *IL-12B* 3'-UTR*2 (rs3212227) (decreased risk), and *TNF*-α variants *1031*2* (rs1799964) and *308*2* (*rs*1800629) (increased risk) with NRTI-associated adult neuropathy.[143,144] Their roles in infant mitochondrial toxicity as a result of in utero exposure to maternal drug need further investigation.

1.3.4.5   Emtricitabine (FTC)

Like 3TC, FTC it is a cytidine analogue excreted primarily unchanged via the kidneys. The approved dosing in adults is 200 mg once daily. Within-subject comparisons of FTC pharmacokinetics during pregnancy and postpartum revealed significantly higher apparent clearance, and lower AUC and C_{min} during pregnancy[111] (**Figure 1.7**; **Table 1.3**). FTC is well distributed to the fetal compartment with mean cord-to-maternal blood concentration ratio of 1.2 (1.0-1.5). It is excreted into breast milk, achieving concentrations corresponding to approximately 2% of recommended oral neonatal dose.[97] It is a substrate for ABCC1,[145] which is expressed in both the placenta and mammary gland (**Table 1.2**).[140] However, no studies have assessed the influence of genetic polymorphisms on its disposition.

1.3.4.6   Tenofovir disoproxil fumarate (TDF)

TDF is a nucleotide analogue taken orally at a dose of 300 mg once daily for adults. It is a prodrug of tenofovir (TFV) and following oral administration and absorption, it undergoes esterase hydrolysis and is converted to TFV, a nucleotide (nucleoside monophosphate) analogue which is taken up by cells. Intracellularly TFV is rapidly phosphorylated by cellular nucleotide kinase to its active anabolite TFV diphosphate. TFV is primarily renally excreted unchanged through a combination of glomerular filtration and active tubular secretion.[146] Best et al. assessed the pharmacokinetics of TFV between 30 and 36 weeks gestation and 6 to 12 weeks postpartum.[147] Median (range) TFV AUC was significantly lower during the third trimester, 2.5 μg.hr/mL (1.1-

2.6) versus 2.96 μg.hr/mL (1.62-7.2) postpartum (p = 0.02). However, third trimester C_{min} were not different from postpartum values. A recent study reported a 25% reduction in TFV exposure during the third trimester compared to postpartum and the authors speculated that the decrease was due to reduced absorption and/or increased volume of distribution as no change in the terminal elimination half-life was observed[112] (**Figure 1.7**; **Table 1.3**). Benabound et al. studied the effects of pregnancy on TFV pharmacokinetics using a popPK analysis involving 46 pregnant and 156 non-pregnant HIV-infected women.[148] The apparent oral clearance was 39% higher in pregnant women and increased TDF dose for pregnant women from the second trimester until delivery was suggested.

The preferential transfer of TFV across the human placenta with cord-to-maternal blood concentration ratio of 1.04 (0.6 to 1.7)[147] compared to the blood-CSF barrier with CSF to plasma ratio of 0.057 (0.03-0.1)[149] may be indicative of differences in expression profiles of TFV transporters (**Table 1.2**). TFV is a substrate for ABCC4, ABCC10, OAT1, and OAT3.[35,150] OAT1, OAT3 and ABCC4 are expressed in choroid plexus epithelial cells of the brain but not in the placenta[151-153] while ABCC10 is expressed in both the placenta and CNS.[140] OATs are involved in the efflux of their substrates out of the CNS.[154,155] The mechanisms behind this observed difference have not been studied. However, combined with the fetal specific CSF-brain barrier,[156,157] it does suggest the protection of fetal CNS from exposure to maternal TFV. Carriers of *ABCC4* 3463A>G (rs1751034) were reported to have 35% higher intracellular TFV diphosphate concentrations than non-carriers (p = 0.04).[158] It is not known if *ABCC10* polymorphisms affect TFV uptake into the fetal compartment. TFV is excreted into breast milk but at low concentrations equivalent to 0.03% of neonatal dose.[97]

TFV is transported into proximal tubular cells by OAT1 and to a lesser extent by OAT3,[159] which are located on the basolateral membrane. It remains unknown if changes in OAT1 expression or activity play any role in increased TFV clearance during pregnancy, and if such changes vary in

genetically-defined sub-groups of pregnant women. The luminal efflux systems involved in the transport of TFV out of proximal tubular cells into the urine are not well described. Three transporters, ABCC2,[160] ABCC4[161] and ABCC10[35] have been reported to play a role in the active renal tubular efflux of TFV. A genetic variant of *ABCC2* (rs717620),[162] and two *ABCC10* SNPs (rs9349256 and rs2125739) were identified as predictors of TFV-induced nephrotoxicity.[35] Higher plasma TFV concentrations in patients with kidney tubular dysfunction have also been reported.[163] The implications of these SNPs on TFV pharmacokinetics during pregnancy and risk of tubular dysfunction is unknown.

1.3.5 Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Four NNRTIs are currently available for the treatment of HIV. NNRTIs are primarily metabolised by CYP enzymes and, with the exception of NVP, are highly protein bound. Therefore, drugs in this class are expected to be susceptible to temporal changes in hepatic enzyme activities and plasma protein fluctuations during pregnancy. In addition, inter-individual variability in the pharmacokinetics of NNRTIs has been associated with SNPs in genes encoding their metabolism enzymes, transporters, and associated nuclear hormone receptors.

1.3.5.1   Efavirenz (EFV)

EFV is the only ARV outside FDA category A-C (**Table 1.3**). Early reports of congenital neural tube defects resulting from first trimester exposure initially limited its use during pregnancy.[164] However, emerging data on its safety during pregnancy have provided some reassurance.[165-168] In 2010, the WHO updated PMTCT guidelines and recommended EFV use as part of ART after the first trimester.[67] Since 2013, EFV has been recommended as the preferred NNRTI component of ART across different populations, including at every stage of pregnancy and postpartum.[13] The first pharmacokinetic data of standard dose (600 mg, once daily) of EFV during the third trimester of pregnancy and postpartum were published in 2012 (**Figure 1.8**).[113] Despite

significantly higher oral clearance and lower C_{min} during the third trimester compared with postpartum, no significant changes in AUC and C_{max} were observed. Geometric mean ratios (90% CI) of pregnancy versus postpartum AUC and C_{max} were 0.97 (0.83-1.13) and 1.11 (0.99-1.24), respectively (**Figure 1.7**; **Table 1.3**). Cord-to-maternal blood concentration ratio was 0.49 (0.37–0.74). The authors concluded that no dose adjustment is necessary during pregnancy.
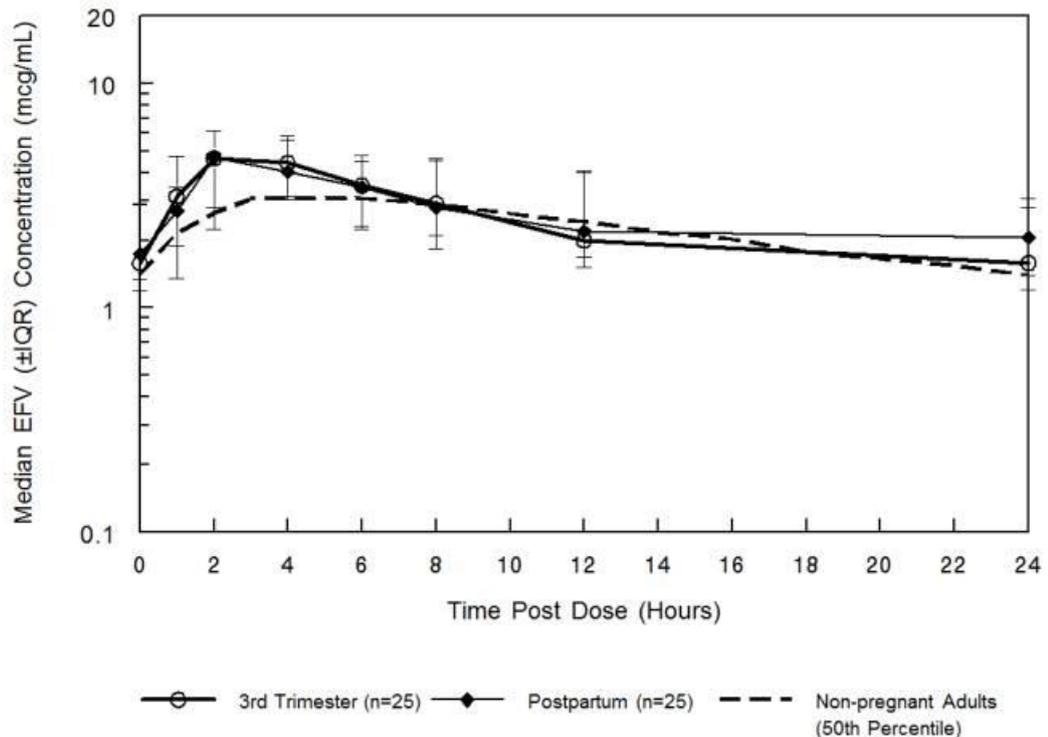
Figure 1.8 EFV median (IQR) concentration-time profiles in pregnant versus postpartum women and non-pregnant adults (Cressey et al. 2012).[113]

Considerable data are available on the effects of genetic polymorphisms in EFV disposition genes on its pharmacokinetics. EFV is primarily metabolised by CYP2B6[169] with a minor contribution from CYP2A6[170,171] and glucuronidation of the 8-hydroxy metabolite by UGT2B7.[172] Observed inter-individual variability has been attributed to polymorphisms in genes encoding these enzymes and NR1I3 (transcriptional regulator). The *CYP2B6* 516G>T (rs3745274), *CYP2B6* 983T>C (rs28399499), *CYP2A6* 1836G>T (rs8192726), *UGT2B7* 735A>G (rs28365062) and *UGT2B7* 802C>T (rs7439366), and *NR1I3* 540C>T (rs2307424) SNPs have been extensively investigated. In studies conducted in HIV positive Ghanaian patients, Kwara et al. identified

CYP2B6 516G>T, *CYP2A6* 1836G>T and/or *CYP2A6* 1093G>A (rs28399454) and *UGT2B7*1a*

carrier status as predictors of EFV plasma concentrations, accounting for 45.2%, 10.1% and 8.6%

of the total variance, respectively.[25,26] Rotger et al. also reported significantly higher plasma and

intracellular EFV concentrations and AUC in Caucasians with *CYP2B6* 516TT than in those with

CYP2B6 516GG genotype. *CYP2B6* 516TT was also associated with higher incidences of EFV

neuropsychiatric side effects.[173] The *CYP2B6* 983T>C polymorphism was associated with higher

EFV concentrations.[174] The *CYP2B6* 516TT and *NR1I3* 540CC genotypes were associated with

early treatment discontinuation, with CNS toxicity accounting for 94.7% of cases of

discontinuation.[46]

The effects of these SNPs that influence EFV pharmacokinetics in non-pregnant adults should be

studied during pregnancy. Induction of CYP2B6 expression is influenced by basal expression

levels which is affected by *CYP2B6* SNPs.[175] Therefore, it is possible that the magnitude of

pregnancy-induced changes will vary between genetically-defined subgroups of women.

Interestingly, consistent with inter-individual variability observed in non-pregnant patients due

to host genetic variations, C_{min} ranged from 0.23 to 8.14 µg/mL during pregnancy in the study by

Cressey et al, and 16% of the women had relatively high exposures with AUC between 146 and

220 µ.h/mL.[113]

EFV is excreted into breast milk with median (range) skim milk M/P ratio of 0.57 (0.35-1.03) and

infant plasma concentration of 0.87 µg/mL (0.31-1.51).[92] The recommended therapeutic range

is 1.0-4.0 µg/mL.[176] Maternal EFV concentration will be a major determinant of infant exposure.

However, EFV transport across the placenta and into breast milk is also likely to be important

but is, as yet, largely unstudied. Peroni et al. reported that EFV is a substrate of rat ABCG2 and

inhibits its own intestinal permeability by promoting over-expression of ABCG2 in the rat

gastrointestinal tract.[177] The polymorphic ABCG2 is highly expressed in the apical membrane of

human placenta syncytiotrophoblast[178] and human mammary gland during lactation.[179] It has

been suggested to play a role in protecting the fetus against exposure to potentially toxic compounds in maternal blood.[180] EFV is not a known substrate of any drug transporter in humans.[181]

1.3.5.2   Etravirine (ETR)

ETR is used as part of salvage therapy in treatment-experienced adult patients with drug resistant viral strains. A study that involved four HIV-infected pregnant women treated with ETR (in combination with darunavir and ritonavir) was the first to describe ETR pharmacokinetics during pregnancy and showed exposures similar to historical data in non-pregnant adults.[182] Two additional studies have since been reported with paired pregnancy and postpartum pharmacokinetic sampling, with evidence of reduced clearance and increase of 30-60% in C_{min}, C_{max}, and AUC observed during pregnancy[114,183] (**Figure 1.7; Table 1.3**). Virological suppression was maintained and there were no cases of MTCT. Reported cord-to-maternal blood concentration ratios varied significantly between the two studies, 0.32 (0.13-0.63)[183] and 0.76 (0.19-4.25).[114]

These results are consistent with ETR metabolic pathway. It is primarily metabolised by CYP2C19 (**Table 1.1**),[27] whose expression and activity declines during pregnancy.[184] CYP2C19 is highly polymorphic and the *CYP2C19**2 (rs4244285; high frequency in Asians) and *CYP2C19**17 (rs12248560; high frequency in Africans) alleles have been associated with poor and ultra-rapid metabolism of CYP2C19 substrates, respectively.[185,186] ETR clearance was 23% lower in *CYP2C19**2 (rs4244285) carriers than non-carriers and co-administration with darunavir/ritonavir or tenofovir reduces ETR clearance by 40% or more,[187] creating a potentially complex scenario of pharmacokinetic variability. However, these do not seem to have any unfavourable impact on ETR pharmacokinetics and efficacy during pregnancy.

1.3.5.3 Nevirapine (NVP)

NVP is initiated at a dose of 200 mg once daily for the first 2 weeks to allow for the auto-induction of metabolising enzymes that increases its clearance, followed by 200 mg twice daily if there are no clinical signs of rash. Initiating NVP in this step-wise fashion reduces the frequency of drug-associated rashes. The use of single dose NVP for PMTCT is no longer recommended; chronic NVP dosing is now used as part of first-line ART for treatment and PMTCT. The influence of pregnancy on NVP pharmacokinetics has been investigated in four different studies; three reported reduced exposure during pregnancy,[115,188,189] and one reported no difference.[190] In the study by Lamorde et al., C_{max} and AUC were 20% lower during pregnancy, and 67% had C_{min} below the 3000 ng/mL study target during the third trimester compared with 20% postpartum (**Figure 1.7**; **Figure 1.9**; **Table 1.3**).[115]
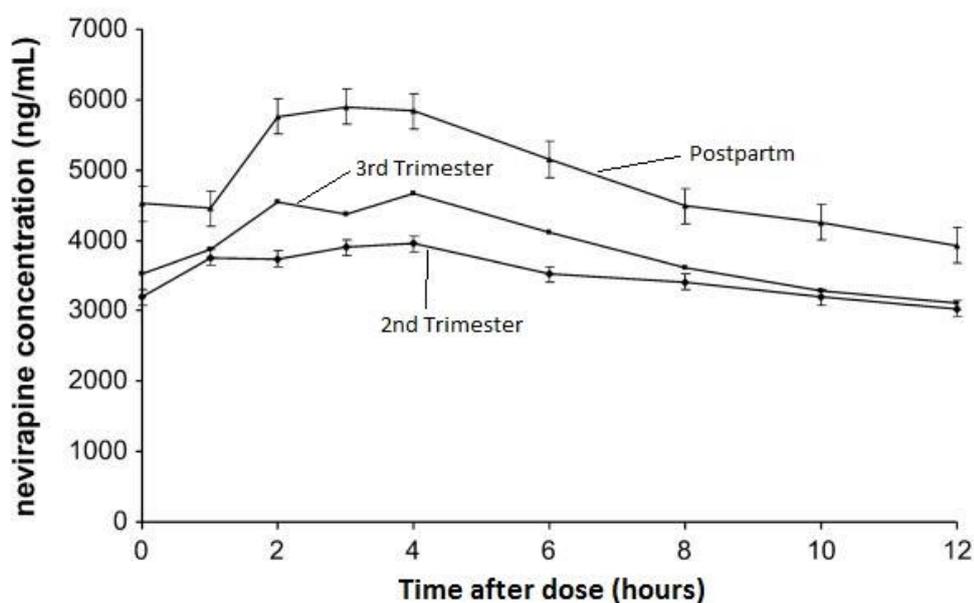
Figure 1.9 Nevirapine plasma concentration-time curve during pregnancy and postpartum (Lamorde et al. 2010)[115].

The *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) SNPs have been associated with higher NVP plasma concentrations. Penzak et al. reported NVP median plasma concentration of 7.61 µg/mL in individuals with *CYP2B6* 516TT genotype versus 4.18 and 5.56

µg/mL in those with 516GG and 516GT genotypes, respectively.[191] These findings were confirmed in a larger study where NVP plasma concentrations were 5.18 µg/mL (1.89-11.16), 6.13 µg/mL (0.15-11.69) and 6.69 µg/mL (4.16-21.03) in patients with *CYP2B6* 516 GG, GT and TT genotypes, respectively.[174] This latter study was also the first to demonstrate that carriers of *CYP2B6* 983 T>C SNP had significantly higher NVP plasma concentrations. Both *CYP2B6* 516G>T and *CYP3A5*3* (rs776746) were associated with NVP pharmacokinetics in a study conducted in Malawian population. The *CYP2B6* 516 T allele increased NVP AUC by 92% and *CYP3A5*3* decreased NVP AUC by 31% in a multivariable model.[192] Therefore, inclusion of host genetic polymorphisms in pharmacokinetic studies of NVP during pregnancy may help identify sub-populations of women who are at higher risk of subtherapeutic concentrations. To date, relatively little data have emerged on efflux or influx transporters capable of NVP transport. However, NVP has been shown to be a substrate for ABCC10 and SNPs within the *ABCC10* gene were associated with plasma concentrations of NVP.[36] This is an area where further mechanistic investigation is warranted since the identification of placental or mammary gland transporters may underpin studies to identify pharmacogenetic correlates of exposure of the fetus or infant, respectively.

Higher incidences of NVP related rash and hepatotoxicity have been reported in HIV-infected pregnant women with CD4 counts > 250 cells/mm^3 and NVP-containing ART is not recommended in these patients.[193,194] An association between *HLA-B*3505* and NVP-induced skin rash has been reported.[195] Furthermore, a genome-wide association study revealed that variations in the *CCHCR1* gene were strongly associated with NVP-induced skin rash.[196] Incorporating genetic and clinical risk factors may therefore have utility in reducing the incidence of NVP related-toxicities in women initiating NVP during pregnancy.

NVP excretion into human breast milk has been reported with M/P ratios of 0.67[94] and 0.75[95] resulting in 971 and 734-1032 ng/mL, respectively, in breastfed infants plasma. This is higher

than the > 100 ng/mL target on which current PEP dosing guideline is based[197] but less than the target > 3000 ng/mL in HIV-infected children.[198] However, breast milk ingestion of NVP has been associated with increased risk of cutaneous ADR in breastfed infants.[199] The new WHO guidelines that recommend NVP for PEP in breastfeeding populations means double exposure for the many of exposed infants in developing countries whose mothers also take NVP as part of ART. Therefore, double exposure to NVP from breast milk and PEP may predispose breastfed infants to more severe NVP-associated cutaneous and hepatic ADRs. There are no data on infant plasma concentration or ADR resulting from such double exposure and the potential influence of SNPs known to affect NVP pharmacokinetics.

1.3.5.4   Rilpivirine (RPV)

RPV is indicated for the treatment of HIV-1 at a dosage of 25 mg once daily in ART-naïve adults with HIV-1 RNA load of 100 000 copies/mL or less.[200] Very limited information is available on its pharmacokinetics during pregnancy. In two case reports, an average of 36, 21 and 49% reduction in AUC, C_{max} and C_{min}, respectively, were observed during pregnancy compared with postpartum.[201] However, virological suppression was sustained and there was no MTCT. Similarly, significant reductions in some pharmacokinetic parameters, including AUC and C_{min}, were observed in pregnant compared with postpartum women receiving the standard 25 mg daily dose in another study. However, the recommended AUC target was met in 94% (15/16), 93% (27/29), and 88% (23/26) at second trimester, third trimester, and postpartum, respectively (**Figure 1.7**; **Table 1.3**). Cord-to-maternal blood concentration ratio was 0.55 (0.38-0.83).[116]

RPV is metabolised by CYP3A enzymes and 99.7% bound to plasma proteins, mainly albumin. However, pregnancy-induced increase in CYP3A enzymes activity and decrease in albumin concentrations may partly explain the observed increase in RPV clearance. These cases highlight the need for caution in the use of 25 mg once daily RPV in pregnant women, especially in those

initiating therapy late in pregnancy. More studies are now warranted to further investigate these observations, including the implications of known *CYP3A4* and *CYP3A5* SNPs (**Table 1.2**).

1.3.6 Protease Inhibitors (PIs)

Ten PIs are currently available for the treatment of HIV. The PIs are primarily metabolised by the CYPs (**Table 1.1**) and are expected to be susceptible to pregnancy-induced changes in their pharmacokinetics like the NNRTIs.

1.3.6.1 Lopinavir/ritonavir (LPV/r)

LPV is one of the most widely studied ARVs during pregnancy. It is co-administered with low-dose ritonavir (RTV) to "boost" its pharmacokinetics. The standard dose of LPV/r in non-pregnant adults is 400/100 mg twice daily. LPV is primarily metabolised by CYP3A4 and CYP3A5. RTV is a potent CYP3A4 inhibitor and co-administration with LPV results in markedly higher LPV plasma concentrations. RTV is also a substrate for both CYP3A4 and CYP2D6.[202] Both LPV and RTV are ABCB1 and ABCC1 substrates[203,204] and highly bound (98%) to plasma proteins. LPV/r-containing first-line regimen is preferred in children younger than 3 years, and reserved as second-line regimen in older children and adults after failure of NNRTI-based first-line regimen, including in pregnant women.[13]

Stek et al. assessed standard LPV/r dosing during the third trimester and LPV exposure was approximately 50% lower compared to non-pregnant adults.[205] Mean (SD) LPV cord-to-maternal blood concentration ratio was 0.2 (0.13). Changes in RTV pharmacokinetic parameters were not statistically significant. The soft-gel capsule formulation used in this study has now been replaced with a tablet formulation, which has been shown to have improved bioavailability and reduces the impact of pregnancy (**Figure 1.7**; **Table 1.3**).[122] LPV exposure during the third trimester of pregnancy following standard dosing with the tablet formulation in Thai women was reduced by 22%.[206] Similar findings have been reported by Calza et al., Ramautarsing et al.

and Lambert et al.[207-209] A higher dose of 600/125 mg of the tablet formulation in American women provided comparable exposure to that observed in non-pregnant adults (**Table 1.3**).[123] There is ongoing debate regarding the optimal dose of LPV/r during pregnancy, which may differ between populations. Higher LPV plasma concentrations have been associated with *SLCO1B1* 521T>C (rs4149056) polymorphism,[38] and within a popPK model homozygosity for the C allele was associated with a 37% lower clearance.[39] The influence of *SLCO1B1* polymorphisms on LPV exposure during pregnancy should be investigated as the presence of this SNP may help compensate for the pregnancy-induced changes in some women. The possible influence of SNPs in *SLCO1B1*, *ABCB1* and *ABCC1* on transplacental transfer of LPV and RTV also warrants further study. LPV and RTV are mostly undetectable in breast milk.[98]

1.3.6.2 Atazanavir (ATV)

ATV is used in combination with low dose RTV (ATV/r) during pregnancy at 300/100 mg once daily. Conflicting data on ATV/r pharmacokinetics during pregnancy have been reported. Ripamonti et al. reported comparable AUC, C_{max} and C_{min} during pregnancy and postpartum.[210] Another study in which samples were collected at a single time-point similarly reported no difference in C_{min} between pregnancy and postpartum (n = 103).[211] In contrast, Mirochnick et al. observed significant differences in AUC, C_{max} and C_{min} with pregnancy versus postpartum ratios of 0.72, 0.47 and 0.58, respectively, without TDF, and 0.73, 0.61 and 0.67, respectively, with TDF (**Figure 1.7**; **Table 1.3**).[118] Two other studies have also reported significantly reduced ATV exposure with 300/100 mg ATV/r during pregnancy, with sustained virological suppression and no MTCT.[212,213] The 400/100 mg increased dose achieved exposure levels comparable to non-pregnant adults despite pregnancy-induced changes.[119] Median cord-to-maternal blood concentration ratio was between 0.14 and 0.23 but varied widely, 0.06-3.05 in one study.[212] Breast milk excretion of ATV has also been reported with a milk-to-plasma ratio of 0.13.[214]

ATV is metabolised by CYP3A enzymes. The *CYP3A5*1B* (rs28365095) allele has been associated with increased ATV clearance.[215] The *NR1I2* 63396C>T (rs2472677) has also been associated with unboosted ATV plasma concentrations[47] and a 17% increase in unboosted ATV clearance.[216] Furthermore, interaction between *CYP3A5* expressor status and *NR1I2* 63396C>T (rs2472677), were reported by Kile et al. ATV clearance was 37% lower in non-expressors of *CYP3A5* with *NR1I2* 63396CC genotype than those with CT or TT genotypes and 63% lower in expressors with *NR1I2* 63396CC genotype than those with CT or TT genotypes.[217] Based on available data, the effect of the *CYP3A5* expressor status on RTV boosted ATV during pregnancy may be limited. *ABCB1* 3435C>T (rs1045642) polymorphism may influence ATV plasma concentrations in the presence of RTV.[218] Investigating the effect of *ABCB1* 3435C>T on ATV exposure during pregnancy will be particularly interesting given that ABCB1 is expressed in the placenta.[219] ATV use is associated with hyperbilirubinemia and an association between the *UGT1A1*28* (rs8175347) and hyperbilirubinemia has been observed.[29] Neonatal *UGT1A1* 211G>A (rs4148323) and *SLCO1B1* 388G>A (rs2306283) polymorphisms, breastfeeding,[30] and ATV use during pregnancy[220] are risk factors for neonatal hyperbilirubinemia. The effects of these SNPs on ATV-associated neonatal hyperbilirubinemia during pregnancy and lactation warrant further investigation.

1.3.6.3   Darunavir (DRV)

DRV is used in combination with low dose RTV (DRV/r) at 800/100 mg once daily in treatment-naïve or treatment-experienced patients with no DRV-associated resistance mutations, or 600/100 mg twice daily in treatment-experienced patients with at least one DRV-associated resistance mutation. Findings from different studies have consistently indicated significantly reduced DRV exposure during pregnancy. Stek et al. assessed the pharmacokinetics of DRV during the third trimester and postpartum with once and twice daily dosing regimens. DRV clearance was significantly increased during pregnancy at both doses and the authors suggested using twice daily dosing during pregnancy to avoid subtherapeutic concentrations[120] (**Figure 1.7**;

Table 1.3). Other studies have reported similar findings.[221,222] However, virological suppression was maintained and there was no MTCT despite reduced exposure. Cord-to-maternal blood concentration ratio ranged from 0 to 0.82. *SLCO3A1* rs8027174 GT/TT genotypes were reportedly associated with 12% decrease in DRV clearance.[223] No other pharmacogenetic associations have been reported. Its excretion into breast milk has not been studied.

Other PIs, including amprenavir (APV/r), fosamprenavir (fAPV/r), nelfinavir (NFV/r), saquinavir (SQV/r) and indinavir (IDV/r), are rarely used during pregnancy. However, available pharmacokinetic data indicate a general trend towards significantly reduced exposure during pregnancy in this class of ARVs (**Figure 1.7**; **Table 1.3**).

1.3.7 Integrase Strand Transfer Inhibitors (INSTIs)

1.3.7.1 Raltegravir (RAL)

A number of case reports have documented rapid reduction in plasma HIV-1 RNA (> 2 log within 10 days) following treatment initiation or intensification with RAL in women presenting with high HIV-1 RNA late in pregnancy.[224-228] Comparisons of its pharmacokinetics during pregnancy versus postpartum showed significantly higher clearance and lower AUC, C_{max}, and C_{min} during pregnancy[126,229] (**Figure 1.7**; **Table 1.3**). Cord-to-maternal blood concentration ratio ranged from 0.09 to 2.3, suggesting significant but highly variable transfer into the fetal compartment.

RAL is metabolised by UGT1A1 which is highly polymorphic. Co-administration with ATV, a known UGT1A1 inhibitor, resulted in > 2-fold elevation of RAL plasma concentration, but the *UGT1A1**28/*28 (rs8175347) reduced function genotype was not associated in an Italian cohort.[230] In a comprehensive population pharmacogenetic-pharmacokinetic analysis, the rare *UGT1A9**3 (rs72551330) SNP was identified as possibly influencing RAL pharmacokinetics.[231] RAL is a substrate for ABCB1[31] and functional *ABCB1* SNPs may contribute to the observed inter-

individual variability in its trans-placental passage. Its excretion into breast milk and transfer to breastfed infants have not been studied.

Other INSTIs, elvitegravir (EVG) and dolutegravir (DTG), are relatively new and there are no data on their pharmacokinetics during pregnancy. Three clinical trials are planned or ongoing to investigate the safety and pharmacokinetics of DTG during pregnancy (ClinicalTrials.gov ID: NCT02245022; NCT02075593; NCT00042289). NCT00042289 will also investigate the pharmacokinetics of EVG (in combination with cobicistat) during pregnancy. Breast milk excretion of DTG and transfer to breastfed infants will also be investigated in NCT02245022. Early integration of host genetics into these trials will provide important information about any observed variability and potential treatment optimisation strategies.

1.3.8 Entry and Fusion Inhibitors

Very limited information is available on enfuvirtide (T-20) during pregnancy. Its use in late pregnancy for PMTCT in women presenting with virological failure and multi-class viral resistance mutations have been reported. T-20 does not cross the placenta[232-236] and has been associated with a case of MTCT despite adequate maternal virological suppression.[237] A case report was located on the use of maraviroc (MVC) during pregnancy with cord/maternal blood ratio of 0.30.[238] Colbers et al. evaluated MVC pharmacokinetics during pregnancy and postpartum. Its AUC, C_{max}, and C_{min} were 28, 30 and 15%, respectively, lower during pregnancy compared with postpartum.[127] Viral load was detectable in 24% of patients at delivery but a relationship with drug exposure could not be demonstrated and there was no MTCT.[127] MVC is a substrate for OATP1B1 and the *SLCO1B1* 521T>C (rs4149056) SNP has been associated with its plasma concentrations.[239]

1.4 THESIS OBJECTIVES

Highly efficacious regimens are now available to prevent MTCT and increasing numbers of women are using ARVs during pregnancy and postpartum. In 2014, UNAIDS proposed the 90-90-90 target, a new narrative on HIV treatment aimed at ensuring that 90% of people living with HIV know their status, 90% of HIV positive individual receive lifelong ART, and 90% of those receiving ART achieve virological suppression by the year 2020.[240] As highlighted above, questions remain about the potential accentuation or attenuation of pregnancy-induced changes in the pharmacokinetics of ARVs or breastfed infants' exposure by genetic polymorphisms in drug disposition genes. The overall aim of this thesis was to investigate the effects of host genetic variability on plasma and breast milk pharmacokinetics of two key ARVs (EFV and NVP) during pregnancy and lactation. Both drugs have significant genetic contribution to observed pharmacokinetic variability.[241] Genetic correlates of breastfed infants' exposure were also determined. In addition to highlighting novel strategies for conducting such investigations, it is expected that the data presented will fill important gaps in the medical literature and incentivise an evidence-based approach to pharmacogenetics-guided optimisation of ART in these special populations.

The magnitude of pregnancy-induced changes in the pharmacokinetics of EFV in genetically-defined subgroups of women was investigated in Chapter 2. Using a pharmacogenetic-guided approach, the effects of pregnancy on NVP pharmacokinetics was evaluated in Chapter 3. Chapters 4 and 5 detail the most comprehensive analysis to date of breast milk pharmacokinetics and breastfed infants' exposure to EFV and NVP. Conducting the studies described in Chapters 2 to 5 necessitated development and validation of novel methods for quantifying both drugs in dried breast milk spots and dried blood spots. These are reported elsewhere and cited in relevant sections of this thesis.[242,243] In a novel application of physiologically-based pharmacokinetic (PBPK) modeling, Chapter 6 describes the development

and application of a bespoke breastfeeding PBPK model to investigate breastfed infants' exposure to maternal drugs through breast milk.

CITATIONS

Olagunju A, Owen A and Cressey TR. Potential effect of pharmacogenetics on maternal, fetal and infant antiretroviral drug exposure during pregnancy and breastfeeding. *Pharmacogenomics*. 2012; 13(13): 1501-1522.

CHAPTER 2

PHARMACOGENETICS OF PREGNANCY-INDUCED CHANGES IN EFAVIRENZ PHARMACOKINETICS

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2.1 INTRODUCTION

The use of antiretroviral (ARV) drugs has reduced mother-to-child transmission (MTCT) of HIV from the baseline 20-45% without intervention to less than 5%.[9] The World Health Organisation (WHO) now recommends Option B or Option B+ for the treatment of HIV positive pregnant women and prevention of mother-to-child transmission (PMTCT) of HIV.[13] Under these options, all HIV positive pregnant women receive triple antiretroviral therapy (ART) started during pregnancy and continued until breastfeeding ends (Option B) or for life (Option B+) regardless of CD4 count and the HIV-exposed infants are given daily nevirapine post-exposure prophylaxis (PEP) from birth through age 4-6 weeks.[13] These simplified approaches are expected to significantly increase the number of pregnant women starting ARVs globally as seen in Malawi where Option B+ was first introduced.[244] If successfully implemented, these strategies could accelerate progress towards eradicating new paediatric HIV infections. Arguably, the single most important factor that determines the success of any PMTCT intervention is sustained suppression of viral replication by prescribed ARVs.

The physiological and anatomical changes associated with pregnancy are known to alter the pharmacokinetics of many drugs,[22] including efavirenz (EFV),[113] a popular component of first-line ARV regimens.[245] These changes include alterations in the expression and activity of hepatic drug-metabolizing enzymes, decreased plasma proteins, increased body water, increased gastric pH, and increased intestinal transit time.[22] The effects of these changes on the pharmacokinetics of specific ARVs have been reported and extensively reviewed.[60,246,247] Unfortunately, the historical exclusion of pregnant women from drug development trials has often led to lack of pregnancy-specific dosing guidelines.[248] In addition, significant inter-individual variability in the pharmacokinetics of many ARVs due to SNPs in drug disposition genes has been reported. For instance, the hepatic cytochrome P450 (CYP) isoenzyme, CYP2B6, accounts for over 90% of EFV metabolism.[249] Several SNPs in the *CYP2B6* gene have been associated with variability in EFV

pharmacokinetics across different populations: 516G>T,[250] 983T>C,[251] 785A>G,[252] and 485-18C>T.[253] SNPs in *CYP2A6*,[25] *NR1I3*,[254] *ABCB1*[255] and other genes which play some role in EFV disposition have also been associated with this variability.

Despite these observations, pregnancy-induced changes in the pharmacokinetics of EFV have not been well studied in the context of pharmacogenetic variability. We previously hypothesised that the magnitude of pregnancy-induced changes in pharmacokinetics may vary in genetically-defined subgroups of pregnant women.[247] In the present study, potential associations between EFV pharmacokinetics during pregnancy and postpartum and SNPs in *CYP2B6*, *NR1I3*, *CYP2A6*, and *ABCB1* were explored. The magnitude of pregnancy-induced changes in EFV pharmacokinetics in genetically-defined subgroups, stratified by the SNP with the highest predictive power, was then investigated.

2.2   METHODS

2.2.1 Patients

HIV positive pregnant women and nursing mothers were recruited from three hospitals in Benue State, Nigeria: Bishop Murray Medical Centre, Makurdi; St Monica's Hospital, Adikpo; and St Mary's Hospital, Okpoga. Potentially eligible subjects were identified using the current PMTCT record and invited to participate after an information session conducted in English and the local language. All participants gave prior written informed consent. Once subjects had signed a consent form, we ascertained their eligibility by examining their case notes and conducting a brief interview. The inclusion criteria included HIV positive and pregnant or breastfeeding, enrolled in the PMTCT programme and taking an EFV-containing regimen for at least the previous 4 weeks. Exclusion criteria included opportunistic infections (e.g. tuberculosis, pneumonia), severe illness, and concurrent treatment with other drugs or herbal medication with known or uncertain interaction with EFV. The protocol and the associated materials

transfer agreement were approved by the National Health Research Ethics Committee (NHREC), Abuja, Nigeria. Clinicaltrials.gov ID: NCT02269462.

2.2.2 Study Design

This was an observational study with an enrichment design[256,257] conducted in two phases. The primary objective of the preliminary phase was to identify SNPs associated with EFV concentrations and nine SNPs in an unselected cohort of HIV positive women during pregnancy and postpartum were investigated. In the second phase, the SNP independently associated with the highest predictive power during pregnancy and postpartum was used to stratify patients into three groups: non-carriers, heterozygotes, and homozygotes. Randomly selected patients from each group were re-recruited and invited for the intensive pharmacokinetic phase. Different groups of patients were evaluated during pregnancy and postpartum.

2.2.3 Sample Collection

In the preliminary phase, dried blood spots (DBS) samples were collected from all patients at a single, recorded time point post-dose. In the intensive pharmacokinetic phase, DBS samples were collected from patients preselected based on the SNP with the highest predictive power at 0.5, 1, 2, 4, 8, 12, 24 h after an observed evening dose of 600 mg EFV. DBS samples were collected after sterile skin cleaning and finger prick using a 2mm safety lancet (BD, Oxford, Oxfordshire, UK). The first drop of blood was discarded and subsequent blood drops were collected on Whatman 903® Protein Saver cards (GE Healthcare, Little Chalfont, Buckinghamshire, UK), dried at room temperature and stored with desiccant sachets in ziplock bags. Samples were shipped at room temperature to the Department of Molecular and Clinical Pharmacology, University of Liverpool, United Kingdom for analysis.

2.2.4 DNA Extraction and SNP Genotyping

Genomic DNA was extracted using E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) in accordance with the manufacture's protocol. The extracted DNA was quantified spectrophotometrically using NanoDrop® (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and stored at -20°C until analysis. Genotyping was performed by allelic discrimination real-time PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays for *CYP2B6* 516G>T (rs3745274; ID: C_7817765_60), *CYP2B6 983T>C* (rs28399499; ID: C_60732328_20), *CYP2B6* c.485-18C>T (rs4803419; ID: C_7817764_10), *NR1I3* c.540C>T (rs2307424; ID: C_25746794_20), *NR1I3* c.152-1089T>C (rs3003596; ID: C_16194070_10), *ABCB1* 4046A>G (rs1045642; ID: C_7586657_20), *ABCB1* 4036A>G (rs3842; ID: C_11711730_20), *ABCB1* 1236C>T (rs1128503; ID: C_7586662_10) and *CYP2A6* 48T>G (rs28399433; C_30634332_10) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to obtain allelic discrimination plots and make genotype calls.

2.2.5 EFV Quantification and Pharmacokinetic Analysis

EFV in DBS was quantified using a validated liquid chromatography-tandem mass spectrometry method described elsewhere.[258] Plasma concentrations were determined using $[DBS_{[EFV]}/(1-HCT)]*0.995$, where $DBS_{[EFV]}$ is EFV concentration in DBS, HCT is the patient-specific haematocrit and 0.995 is the fraction of EFV bound to plasma protein.[259] Minimum (C_{min}) and maximum (C_{max}) plasma concentrations were determined by direct inspection. The area under the concentration-time curve during the dosing interval (AUC_{0-24}) was calculated using the trapezoidal rule and the apparent clearance (CL/F) was calculated by dividing the dose by AUC_{0-24}.

2.2.6 Statistical Analysis

Compliance with Hardy-Weinberg Equilibrium was tested as described by Rodriguez et al.[260] Data were subjected to Kolmogorov-Smirnov normality test prior to statistical analysis. Relationships between continuous variables were tested by Pearson or Spearman correlation. Univariate linear regression analysis was conducted to identify variables associated with EFV plasma concentrations. Independent variables with $p \leq 0.05$ in the univariate analysis were included in a multivariate stepwise linear regression analysis. Bonferroni correction was used to adjust for multiple testing. Differences in EFV concentrations and pharmacokinetic parameters between patient groups were investigated using one-way analysis of variance (ANOVA) and Mann-Whitney U test. All statistical analyses were conducted using IBM ®SPSS® Statistics version 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism® 5 (GraphPad Software, Inc., La Jolla, CA, USA). All charts were plotted using GraphPad Prism® 5. The putative target for C_{min} was set at 1000 ng/mL, the suggested minimum effective concentration.[261,262]

2.3 RESULTS

2.3.1 Patients' Characteristics and Genotype Frequencies

Between December 2012 and October 2013 a total of 211 eligible HIV positive women enrolled in the PMTCT programme (77 pregnant and 134 postpartum) were recruited. Patient characteristics and genotype frequencies are summarised in **Table 2.1**. The mean (SD) duration on current ARV regimen was 23.9 months (16.3) in pregnant women and 17.6 months (14.1) in postpartum women, starting with baseline CD4 counts of 312 cells/mm^3 (195) and 380 cells/mm^3 (217), respectively. More than 70% of the pregnant women were in the third trimester. Most patients were taking regimens containing EFV, emtricitabine and tenofovir (> 60%) or EFV, lamivudine and zidovudine (> 20%). DBS samples were collected at 12.0 h (3.00-18.75) and 14.00 h (0.50-21.5) post-dose during pregnancy and postpartum, respectively, in the preliminary

phase. Comparison of observed and expected genotype frequencies showed that all nine SNPs were in Hardy-Weinberg equilibrium in both groups, $p > 0.05$.

Table 2.1 Characteristics[*] of patients during pregnancy and postpartum.

Characteristics	Pregnancy (n = 77)	Postpartum (n = 134)
Age (years)	28 (19-44)	29 (18-44)
Weight (kg)	58 (43-90)	57 (39-80)
Time since diagnosis (months)	28.4 (0.15-72)	21.1 (1.3-68)
Gestational/Postnatal age (weeks)	27.3 (1.1-37)	20.0 (0.29-75)
Trimester:		
1st	5%	NA
2nd	24%	NA
3rd	71%	NA
Drug regimen and CD4 count:		
TDF/FTC/EFV	63%	65%
3TC/AZT/EFV	23%	32%
3TC/TDF/EFV	11%	2%
3TC/ABC/EFV	3%	1%
Time post-dose (h)	12.0 (3.0-18.75)	14.0 (0.5-21.5)
Duration on regimen (months)	23.9 (16.3)	17.6 (14.1)
Baseline CD4 count (cells/mm^3)	312 (195)	380 (217)
CD4 change (cells/mm^3)	116 (202)	177 (217)
Genotype frequencies:		
CYP2B6 516G>T (rs3745274)	GG, 0.32; GT, 0.54; TT, 0.14	GG, 0.35; GT, 0.47; TT, 0.18
CYP2B6 983T>C (rs28399499)	TT, 0.75; CT, 0.25; CC, 0.00	TT, 0.80; CT, 0.20; CC, 0.00
CYP2B6 c.485-18C>T (rs4803419)	CC, 0.82; CT, 0.18; TT, 0.00	CC, 0.79; CT, 0.21; TT, 0.00
NR1I3 c.540C>T (rs2307424)	CC, 0.74; CT, 0.26; TT, 0.00	CC, 0.77; CT, 0.23; TT, 0.00
NR1I3 c.152-1089T>C (rs3003596)	TT, 0.20; CT, 0.61; CC, 0.20	TT, 0.19; CT, 0.54; CC, 0.27
ABCB1 4046A>G (rs1045642)	AA, 0.75; AG, 0.21; GG, 0.04	AA, 0.75; AG, 0.25; GG, 0.00
ABCB1 4036A>G (rs3842)	AA, 0.68; AG, 0.30; GG, 0.01	AA, 0.65; AG, 0.35; GG, 0.01
ABCB1 1236C>T (rs1128503)	CC, 0.69; CT, 0.30; TT, 0.01	CC, 0.76; CT, 0.23; TT, 0.02
CYP2A6 48T>G (rs28399433)	TT, 0.94; GT, 0.06; GG, 0.00	TT, 0.93; GT, 0.07; GG, 0.01

[*] Values expressed as mean (standard deviation) or median (range) or % of subjects.

2.3.2 Pharmacogenetics of EFV Exposure during Pregnancy and Postpartum

Of the nine SNPs investigated, only *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) were independently associated with EFV plasma concentrations during pregnancy with regression coefficients (95% Confidence Interval, CI) of 1530 ng/mL (771, 2290), $p = 1.4 \times 10^{-4}$ and 1660 ng/mL (454, 2860), $p = 8.0 \times 10^{-3}$, respectively (**Table 2.2**). In postpartum women, only *CYP2B6* 516G>T (rs3745274) was independently associated with EFV plasma concentrations and the regression coefficient was 1700 ng/ml (1250, 2150), $p = 1.7 \times 10^{-11}$ (**Table 2.2**). After Bonferroni correction for multiple testing only *CYP2B6* 516G>T (rs3745274) was associated during pregnancy and postpartum, with Boferroni p values of 1.8×10^{-3} and 2.0×10^{-10}, respectively. No other variable was significantly associated in either univariate or multivariate analysis (**Table 2.2**). Significant differences in EFV plasma concentrations between patients based on *CYP2B6* 516G>T (rs3745274) genotypes were observed during pregnancy and postpartum (**Figure 2.1**).

Therefore, patients were stratified based on their *CYP2B6* 516G>T (rs3745274) status for enrolment in the intensive pharmacokinetic phase. A total of 25 pregnant women (8 GG, 14 GT and 3 TT) and a different group of 19 postpartum women (6 GG, 7 GT and 6 TT) were successfully re-recruited.

2.3.3 EFV Pharmacokinetics in Pregnancy versus Postpartum

A preliminary analysis was conducted to evaluate potential differences in EFV pharmacokinetic parameters between the different trimesters of pregnancy. There were no significant differences in CL/F and AUC_{0-24} ($p = 0.53$), C_{max} ($p = 0.98$) and C_{min} ($p = 0.57$) between the first two trimesters (n = 7) and the third trimester (n = 18). Therefore, all 25 pregnant women were included in subsequent analysis. A pooled comparison of EFV pharmacokinetic parameters showed a 42.6% increase in CL/F ($p = 0.023$), 29.8% decrease in AUC_{0-24} ($p = 0.023$) and 28.0% reduction in C_{min} ($p = 0.012$) during pregnancy compared with postpartum controls, although the

median (range) C_{min} was 1000 ng/mL (429-5190), a value within the suggested target.[176,261,262] There was no significant change in C_{max} (p = 0.072) (**Table 2.3; Figure 2.2A**). However, when stratified based on *CYP2B6* 516G>T (rs3745274), EFV CL/F increased by 100% during pregnancy
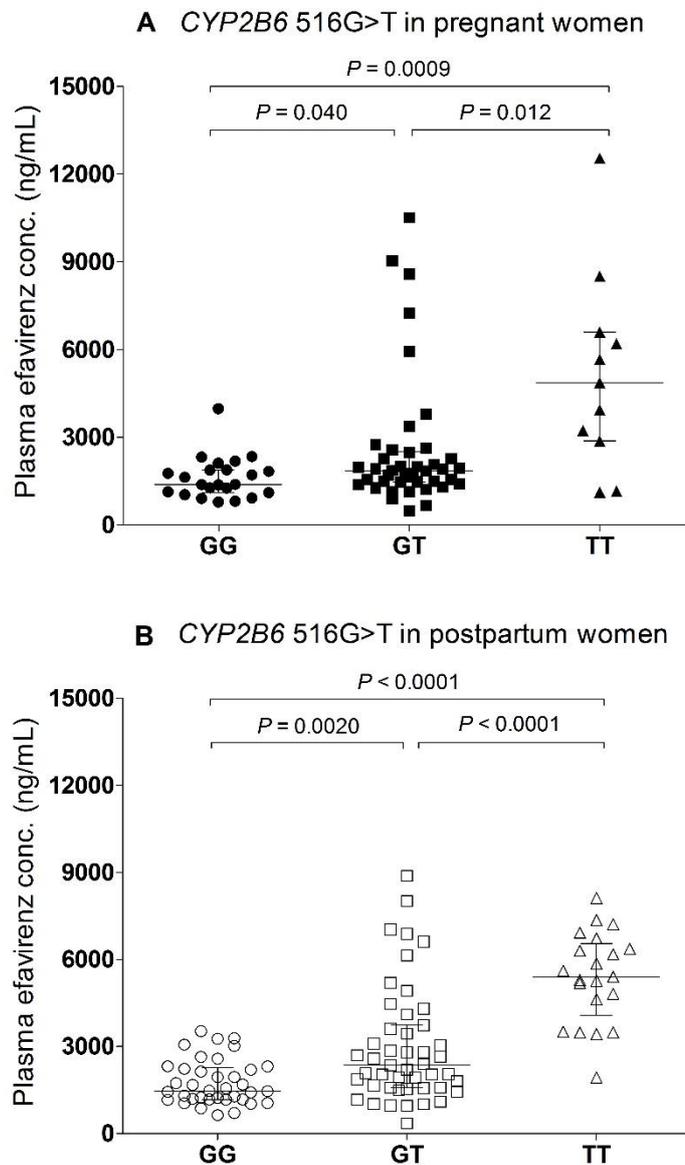
Figure 2.1 Association of *CYP2B6* 516G>T with EFV plasma concentrations during pregnancy and postpartum in the preliminary phase. *P* values on figures are for Mann-Whitney U test. EFV concentrations (median, IQR) during pregnancy were: GG (n = 23), 1390 ng/mL (1110, 1880); GT (n = 42), 1850 ng/mL (1450, 2510); TT (n = 11), 4860 ng/mL (2870, 6600); P_{ANOVA} < 0.0001 (**A**). Postpartum concentrations were: GG (n = 37), 1470 ng/mL (1170, 2270); GT ng/mL (n = 47), 2350 (1580, 3730); TT (n = 21), 5400 ng/mL (4070, 6550); P_{ANOVA} < 0.0001 (**B**).

Table 2.2 Association of patient characteristics and single nucleotide polymorphisms in *CYP2B6*, *NR1I3*, *CYP2A6*, and *ABCB1* with EFV plasma concentrations during pregnancy and postpartum.

	Univariate linear regression			Multivariate linear regression		
	β[a] (ng/mL; 95% CI)	*p* value	Bonferroni *p* value	β[a] (ng/mL; 95% CI)	*p* value	Bonferroni *p* value
Pregnant women (n = 77)						
Weight (kg)	-36.5 (-99.7, 26.9)	0.26				
Age (years)	-18.3 (-114, 77.2)	0.70				
Time post-dose (h)	-21.1 (-188, 146)	0.80				
Trimester	151 (-1290, 1590)	0.83				
CYP2B6 516G>T (rs3745274)	1530 (771, 2290)	1.4×10^{-4}	1.8×10^{-3}	1530 (771, 2290)	1.4×10^{-4}	1.8×10^{-3}
CYP2B6 983T>C (rs28399499)	1660 (454, 2860)	0.008	0.104			
CYP2B6 c.485-18C>T (rs4803419)	-321 (-1730, 1090)	0.65				
NR1I3 c.540C>T (rs2307424)	-493 (-1730, 746)	0.43				
NR1I3 c.152-1089T>C (rs3003596)	130 (-743, 1000)	0.77				
ABCB1 4046A>G (rs1045642)	531 (-488, 1550)	0.30				
ABCB1 4036A>G (rs3842)	19.3 (-1070, 1110)	0.97				
ABCB1 1236C>T (rs1128503)	87.9 (-1010, 1190)	0.87				
CYP2A6 48T>G (rs28399433)	158 (-2050, 2370)	0.89				
Postpartum (n = 134)						
Weight (kg)	-17.0 (-71.9, 38.0)	0.54				
Age (years)	4.20 (-67.3, 75.7)	0.91				
Time post-dose (h)	37.4 (-34.7, 110)	0.31				
CYP2B6 516G>T (rs3745274)	1700 (1250, 2150)	1.7×10^{-11}	2.0×10^{-10}	1700 (1250, 2150)	1.7×10^{-11}	2.0×10^{-10}
CYP2B6 983T>C (rs28399499)	209 (-718, 1140)	0.66				
CYP2B6 c.485-18C>T (rs4803419)	-470 (-1390, 453)	0.32				

Table 2.2 continued

	Univariate linear regression			Multivariate linear regression		
	β[a] (ng/mL) (95% CI)	*p* value	Bonferroni *p* value	β[a] (ng/mL) (95% CI)	*p* value	Bonferroni *p* value
Postpartum (n = 134) continued						
NR1I3 c.540C>T (rs2307424)	-792 (-1670, 87.9)	0.077				
NR1I3 c.152-1089T>C (rs3003596)	30.4 (-523, 584.0)	0.91				
ABCB1 4046A>G (rs1045642)	643 (-189, 1480)	0.13				
ABCB1 4036A>G (rs3842)	386 (-356, 1130)	0.31				
ABCB1 1236C>T (rs1128503)	404 (-368, 1180)	0.302				
CYP2A6 48T>G (rs28399433)	-31.7 (-1240, 1180)	0.959				

[*]β is the regression coefficient and represents incremental change in EFV plasma concentration per unit change in a patient characteristic (e.g. per kg body weight or per allele carried).

compared with postpartum controls (p = 0.0013) in patients with the *CYP2B6* 516GG genotype. The AUC_{0-24}, C_{max} and C_{min} were reduced by 50.6% (p = 0.0013), 17.2% (p = 0.14) and 61.6% (p = 0.0027) during pregnancy, with values of 25,900 ng.h/mL (21,700-32,600), 2640 ng/mL (1260-3490) and 592 ng/mL (429-917), respectively, compared with 52,400 ng.h/mL (32,600-64,000), 3190 ng/mL (2700-3800) and 1540 ng/mL (867-2310), respectively, postpartum. (**Table 2.3; Figure 2.2B**). No pregnancy-induced changes in pharmacokinetic parameters were apparent in patients with the *CYP2B6* 516GT genotype (**Table 2.3; Figure 2.2C**). In pregnant women with the *CYP2B6* 516TT genotype, EFV CL/F was 45.6% higher compared with postpartum controls (p = 0.095) and despite significant reduction (2890 ng/mL (2660-4030) versus 5130 ng/mL (3830-6740); p = 0.048), C_{min} was above 1000 ng/mL in all pregnant women with the *CYP2B6* 516TT genotype (**Table 2.3; Figure 2.2D**).

2.3.4 Effect of Pregnancy on Immunological Recovery

Baseline and antenatal or postpartum CD4 counts were available for 103 women (37 pregnant, 66 postpartum). In a pooled linear regression analysis, pregnancy was associated with lower most recent CD4 count, β = -112 cells/mm^3 (-193, -31.1), p = 0.0070. This association remained after adjusting for baseline CD4 count, but was lost after adding ART duration. Similarly, an association between most recent CD4 count and *ABCB1* 4036A>G (rs3842) in pregnant women observed in univariate analysis, β = -190 cells/mm^3 (-342, -39.0) and p = 0.015, was lost after adjusting for baseline CD4 count and ART duration. In postpartum women, *ABCB1* 4046A>G (rs1045642) was independently associated with CD4 change after adjusting for baseline CD4 count and ART duration, β = 154 cells/mm^3 (36.5, 271) and p = 0.011. No other associations were observed.

Table 2.3 EFV pharmacokinetic parameters (median, range) during pregnancy and postpartum based on *CYP2B6* 516G>T genotypes.

All (*CYP2B6* 516GG, GT and TT)	Clearance/F (L/h)	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)
Pregnancy (n = 25)	14.1 (2.96-27.7)	42,600 (21,700-203,000)	3490 (1260-14400)	1000 (429-5190)
Postpartum (n = 19)	9.89 (3.39-20.7)	60,700 (29,000-177,000)	4850 (2050-9760)	2030 (755-6740)
Pregnancy vs Postpartum: % change	42.6	-29.8	-28.0	-50.7
p value[a]	0.023	0.023	0.072	0.012
CYP2B6 516GG				
Pregnancy (n = 8)	23.2 (18.4-27.7)	25,900 (21,700-32,600)	2640 (1260-3490)	592 (429-917)
Postpartum (n = 6)	11.6 (9.37-18.4)	52,400 (32,600-64,000)	3190 (2700-3800)	1540 (867-2310)
Pregnancy vs Postpartum: % change	100	-50.6	-17.2	-61.6
p value	0.0013	0.0013	0.14	0.0027
CYP2B6 516GT				
Pregnancy (n = 14)	13.7 (2.96-23.3)	43,900 (25,700-203,000)	3660 (2490-14400)	1120 (571-5190)
Postpartum (n = 7)	11.9 (4.71-20.67)	50,700 (29,000-128,000)	4850 (2050-6780)	1520 (755-4860)
Pregnancy vs Postpartum: % change	15.1	-13.4	-24.5	-26.3
p value	0.85	0.85	0.43	0.63
CYP2B6 516TT				
Pregnancy (n = 3)	6.83 (5.22-8.15)	87,900 (73,700-115,000)	5770 (5320-5950)	2890 (2660-4030)
Postpartum (n = 6)	4.69 (3.39-5.35)	129,000 (112,000-177,000)	6940 (6370-9760)	5130 (3830-6740)
Pregnancy vs Postpartum: % change	45.6	-31.9	-16.9	-43.7
p value	0.095	0.095	0.024	0.048

[a]Mann-Whitney U test *p* value.
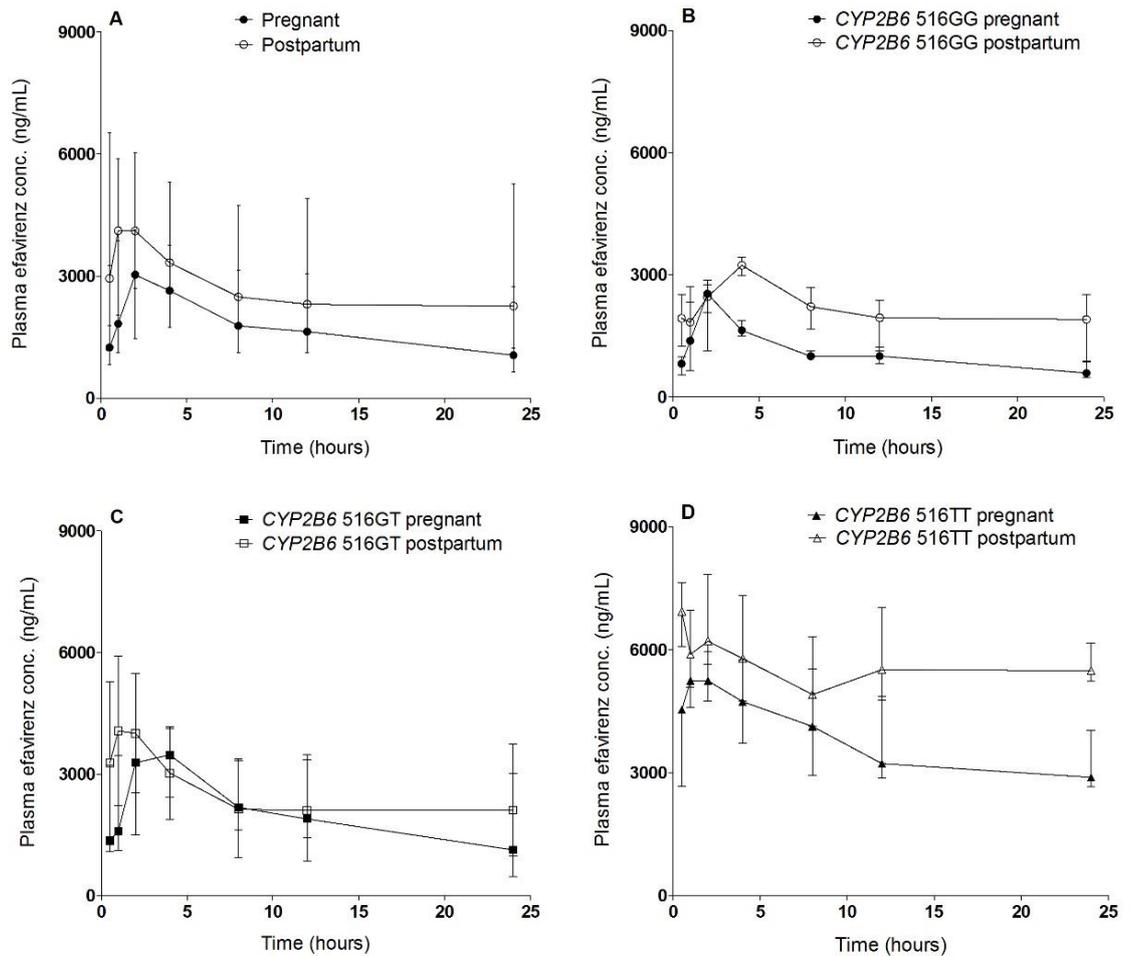
Figure 2.2 EFV concentration-time profiles (median, IQR) during pregnancy and postpartum. Global comparison shows decreased exposure during pregnancy (n = 25) compared with postpartum (n = 19), but the median C_{min} was still within target (**A**). Stratifying patients based on *CYP2B6* 516G>T status showed significantly reduced exposure during pregnancy (n = 8) compared with postpartum (n = 6) in patients with the GG genotype, with median (range) AUC_{0-24}, C_{max} and C_{min} of 25,900 ng.h/mL (21,700-32,600), 2640 ng/mL (1260-3490) and 592 ng/mL (429-917), respectively (**B**); no changes in patients with the GT genotype during pregnancy (n = 14) compared with postpartum (n = 7) (**C**); and despite significantly reduced exposure during pregnancy (n = 3) compared with postpartum (n = 6) in patients with the TT genotype, C_{min} was within suggested targets (**D**). Details are presented in **Table 2.3**.

2.4 DISCUSSION

The magnitude of pregnancy-induced changes in EFV pharmacokinetics in genetically-defined subgroups has been described for the first time. Our findings indicate that at the standard 600 mg dose, EFV exposure was significantly reduced during pregnancy, especially in patients with the *CYP2B6* 516GG genotype. Stratification of patients based on *CYP2B6* 516G>T (rs3745274) showed that EFV C_{min} was below 1000 ng/mL in 100% of pregnant women with the *CYP2B6* 516GG genotype, compared with 33% postpartum (**Table 2.3**). In fact, EFV plasma concentrations fell below 1000 ng/ml three or more times during the dosing interval in > 50% of pregnant women in this group (data not shown).

However, it should be noted that the 1000 ng/mL target[261,262] has been challenged on several grounds: (i) Firstly, the original evidence related virological failure to mid-dose plasma EFV concentrations, not C_{min},[176] (ii) secondly, earlier studies validating this target included treatment-experienced patients who might not have received sufficiently potent nucleoside backbone therapy,[263] (iii) thirdly, a randomised controlled trial observed no difference in virological failure rate at a reduced dose of EFV (400 mg), even though plasma exposures were significantly lower.[264,265] Nonetheless, our findings suggest that surveillance for MTCT in patients with the *CYP2B6* 516GG genotype taking EFV-based regimens during pregnancy may be justified. Whether particular scenarios (e.g. poor adherence, drug interactions, newly diagnosed pateints commencing EFV-based regimen late in pregnancy) may render this subgroup of patients at higher risk for treatment failure also needs to be investigated.

EFV metabolism to 8-hydroxy EFV by CYP2B6 has been reported to account for > 90% of its hepatic clearance when genotypes are not considered.[249] *CYP2B6* 516G>T (rs3745274) has been reported to decrease the rate of EFV 8-hydroxylation.[266] The magnitude of EFV autoinduction has been reported to be genotype-dependent, highest in patients with the *CYP2B6* 516GG genotype.[175] Interestingly, the basal expression of hepatic CYP2B6 is known to be highest in

CYP2B6 516GG homozygotes and lowest in *CYP2B6* 516TT homozygotes. Hence, the differences in inducible enzyme content in different genotypes may explain the observed differences in EFV autoinduction. A similar mechanism involving CYP2B6 induction by 17β-estradiol may explain the genotype-dependent differences in the magnitude of pregnancy-induced changes observed in the present study. The marked increase in 17β-estradiol plasma concentration during pregnancy has been shown to enhance CYP2B6 activity through activation of its transcriptional regulator, NR1I3 (also known as CAR, constitutive androstane receptor).[267] The lack of association between *CYP2B6* 983 T>C and EFV concentration[251,252] in the present study is most likely due to the relatively small sample size and absence of patients homozygote for this SNP.

To date, only one other study has reported EFV intensive pharmacokinetics during pregnancy.[113] In that study, the moderate increase in CL/F observed during pregnancy produced only a minimal reduction in C_{24} with the median within target. Similarly, a pooled comparison of EFV pharmacokinetics during pregnancy and postpartum in the intensive pharmacokinetic phase of the present study revealed significant change only in CL/F during pregnancy but the median C_{min} was within the suggested target. While plasma samples were used for drug quantification in the previous study, we used a fully validated DBS method which is associated with approximately 40% negative bias compared with plasma concentration.[258,259] However, we used patient-specific haematocrit correction which has been shown to give concentrations similar to plasma concentrations, with a negligible difference of 80 ng/mL (SD 310 ng/mL) between the two methods.[259] This approach was also used in the 'PROMOTE study' by Bartelink et al.[268]. In two other studies, sparse data were incorporated into population pharmacokinetic models to obtain estimates of CL/F and C_{min}. In one of the studies (the 'PROMOTE study'), the authors concluded that pregnancy did not affect EFV CL/F.[268] However, in the second study involving a mixed cohort of tuberculosis treated and non-tuberculosis treated pregnant women, a higher risk of C_{min} < 1000 ng/ml in fast CYP2B6 metabolisers compared with poor metabolisers was reported, though MTCT was rare.[269] The conflicting observations from the different studies may be a reflection of

differences in *CYP2B6* 516G>T (rs3745274) frequencies in the different study cohorts.[270] Suboptimal adherence was identified as a possible limitation in two of the studies.[113,269] The design of the present study involved an observed evening dose of EFV which ensured 100% adherence. The potential benefits of using the enrichment design in pharmacogenetic studies have been highlighted elsewhere.[257] In addition to matching patient characteristics in pregnant and postpartum groups, assigning patients to genotype-defined subgroups in an enrichment design is known to minimise potential variability.[256]

These observations should be interpreted in the context of a number of limitations. Maternal viral load and rate of mother-to-child transmission of HIV were not evaluated as part of the present study. The number of patients with available CD4 counts was limited and this precluded an adequate evaluation of potential differences in CD4 change between patient groups. In addition, the number of patients re-recruited for the intensive pharmacokinetic phase was limited (23 during pregnancy and 19 postpartum controls) particularly for genotype-based comparisons. We did not use pregnant women as their own postpartum controls.

In conclusion, these findings indicate significant reduction in EFV exposure during pregnancy, especially in patients with the *CYP2B6* 516GG genotype. Further studies to confirm these in a larger cohort as well as investigate the clinical implications are now warranted. Increasing numbers of HIV-positive pregnant women are receiving EFV during pregnancy.[13] Recent update about virological rebound in the 'Mississippi baby' thought to be functionally cured of HIV highlights the fact that the use of ARVs during pregnancy is currently the only proven way for PMTCT.[271] Due to uncertainty over the target minimum concentration for efficacy, these results do not preclude assessment of dose reduction strategies for EFV in special populations, but rather highlight the need for close pharmacokinetic and virological monitoring as well as integrated pharmacogenetics in any study design.[272,273]

CITATIONS

Olagunju A, Bolaji O, Amara A, Else L, Okafor O, Adejuyigbe E, Oyigboja J, Back D, Khoo S, Owen A. Pharmacogenetics of pregnancy-induced changes in efavirenz pharmacokinetics. *Clinical Pharmacology & Therapeutics*. 2015; 97(3):298-306.

Amara A, Else L, Tjia J, **Olagunju A**, Puls RL, Khoo S, Back DJ. A validated method for quantification of efavirenz in dried blood spots (DBS) using HPLC-MS/MS. *Therapeutic Drug Monitoring*. 2015; 37(2):220-8.

CHAPTER 3

PREGNANCY AFFECTS NEVIRAPINE PHARMACOKINETICS – EVIDENCE FROM A *CYP2B6* GENOTYPE-GUIDED OBSERVATIONAL STUDY

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3.1 INTRODUCTION

The 2013 WHO consolidated guidelines for treating and preventing HIV infection recommend efavirenz (EFV), tenofovir and emtricitabine (or lamivudine), available as a once daily fixed dose tablet, as the preferred first-line regimen in pregnant women.[13] This recommendation is partly based on potential clinical and programmatic advantages of a simplified regimen.[13] Like EFV, nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and both are known to be comparable in therapeutic efficacy. It is recommended that individuals who are clinically stable on a NVP-based regimen with no contraindications continue this regimen.[13] In addition, despite increasingly reassuring data on EFV safety throughout pregnancy,[274,275] its safety during pregnancy has not been definitively established. Some patients also experience treatment-limiting side effects after initiating EFV-based regimens. In either case, NVP is often the only available and recommended alternative, used in combination with two nucleoside reverse transcriptase inhibitors (NRTIs, e.g. zidovudine and lamivudine or tenofovir and emtricitabine) as the first-line regimen.[13]

As highlighted in Chapter 1, previous studies that investigated NVP pharmacokinetics in pregnant women have reported contradictory findings.[115,188-190] In addition, the relative genetic contribution to variability in NVP pharmacokinetics is >70% in both African and European populations.[241,276] NVP is metabolised by the cytochrome P450 enzymes CYP2B6 and CYP3A4[277] and is a substrate for the efflux transporter ABCC10.[36] Single nucleotide polymorphisms (SNPs) in *CYP2B6*, *CYP3A4*, and *ABCC10* genes have previously been associated with NVP pharmacokinetics. For instance, *CYP2B6* 516G>T and 983T>C have been associated with higher, and *ABCC10* 2843T>C (rs2125739) with lower plasma NVP concentrations.[36,174,278] The expression and activity of CYP2B6 and CYP3A4 are regulated by the nuclear receptors NR1I2 and NR1I3 and the cytochrome P450 oxidoreductase (POR), influenced by SNPs in the *NR1I2*, *NR1I3*

and *POR* genes.[279,280] The resulting inter-individual differences in exposure of the systemic circulation, target cells, and sanctuary sites to NVP may result in differential efficacy.[281,282]

The combined effects of these SNPs and pregnancy on NVP pharmacokinetics have not been studied. The potential effects of polymorphisms in drug disposition genes on pregnancy-induced changes in ARV pharmacokinetics was highlighted in Chapter 1[247] and this has now been confirmed in the case of EFV as highlighted in Chapter 2.[269,283] In fact, the contradictions about the effect of pregnancy on NVP pharmacokinetics could be a reflection of differences in the frequencies of genetic polymorphisms that affect its disposition in the different cohorts.[270]

In the preliminary phase of the present study, associations between nine SNPs in NVP disposition genes and its plasma concentrations in pregnant and postpartum women were investigated. The magnitude of pregnancy-induced changes was then investigated following genotype-guided stratification in an intensive pharmacokinetic phase.

3.2 METHODS

3.2.1 Patients

The study was conducted in three hospitals in Benue State, Nigeria: Bishop Murray Medical Centre, Makurdi; St Monica's Hospital, Adikpo; and St Mary's Hospital, Okpoga. Potentially eligible HIV-positive pregnant women and nursing mothers were identified using the current PMTCT records and invited to participate after an information session conducted in both English and the local Tiv language. Written informed consent was obtained and eligibility was ascertained by examining case notes and conducting a brief interview. The inclusion criteria included HIV-positive and pregnant or breastfeeding, enrolled in the PMTCT programme and taking a regimen containing 200 mg NVP twice daily for at least the previous 2 weeks. Exclusion criteria included opportunistic infections (e.g. tuberculosis, pneumonia), severe illness, and concurrent treatment with other drugs or herbal medication with known or uncertain

interaction with NVP. The protocol and the associated materials transfer agreement were approved by the National Health Research Ethics Committee (NHREC), Abuja, Nigeria. Clinicaltrials.gov ID: NCT02269462.

3.2.2 Study Design and Sample Collection

In the preliminary phase of the study, associations between nine SNPs in NVP disposition genes and its plasma concentrations in pregnant and postpartum women were investigated. For this, dried blood spots (DBS) samples were collected from all patients at a single, recorded time point post-dose. The magnitude of pregnancy-induced changes was then investigated following genotype-guided stratification in an intensive pharmacokinetic phase. DBS samples were collected from patients preselected based on their genotypes at 0.5, 1, 2, 4, 8, and 12 h after an observed dose of a regimen containing 200 mg NVP. DBS samples were collected after sterile skin cleaning and finger prick using a 2mm safety lancet (BD, Oxford, Oxfordshire, UK). The first drop of blood was discarded and subsequent blood drops were collected on Whatman 903® Protein Saver cards (GE Healthcare, Little Chalfont, Buckinghamshire, UK), dried at room temperature and stored with desiccant sachets in ziplock bags. Samples were shipped at room temperature to the Department of Molecular and Clinical Pharmacology, University of Liverpool, United Kingdom for analysis.

3.2.3 SNP Genotyping

Genomic DNA was extracted using E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA), quantified using NanoDrop® (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and stored at -20°C until analysis. Genotyping was performed by real-time PCR allelic discrimination assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays for *CYP3A4* -392A>G (*1B; rs2740574; ID: C_1837671_50), *CYP3A4* 20230G>A

(*1G; rs2242480; ID: C_26201900_30), *CYP2B6* 516G>T (rs3745274; ID: C_7817765_60), *CYP2B6* *983T>C* (rs28399499; ID: C_60732328_20), *NR1I3* c.540C>T (rs2307424; ID: C_25746794_20), *NR1I3* c.152-1089T>C (rs3003596; ID: C_16194070_10), *NR1I2* 63396C>T (rs2472677; ID: C_26079845_10), *POR* 1508C>T (*28; rs1057868; ID: C_8890131_30), and *ABCC10* 2843T>C (rs2125739; ID: C_16173668_10) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). Allelic discrimination plots and genotype assignments were made using Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

3.2.4 NVP Quantification and Pharmacokinetic Analysis

A previously described liquid chromatography-tandem mass spectrometry method was used to quantify NVP in DBS.[242] Plasma concentrations were determined using $[DBS_{[NVP]}/(1-HCT)]*0.6$, where $DBS_{[NVP]}$ is NVP concentration in DBS, HCT is the patient-specific haematocrit and 0.6 is the fraction of NVP bound to plasma protein.[259] The area under the concentration-time curve during the dosing interval (AUC_{0-12}) was calculated using the trapezoidal rule and the apparent clearance (CL/F) was calculated by dividing the dose by AUC_{0-12}. Minimum (C_{min}) and maximum (C_{max}) plasma concentrations were determined by direct inspection.

3.2.5 Statistical Analysis

Compliance with Hardy-Weinberg Equilibrium was tested as described by Rodriguez et al.[260] Data were subjected to Kolmogorov-Smirnov normality test prior to statistical analysis. Univariate linear regression analysis was conducted to identify variables associated with NVP plasma concentrations. Independent variables with $P \leq 0.2$ in the univariate analysis were included in a multivariate stepwise linear regression analysis. Bonferroni correction was used to adjust for multiple testing. Differences in pharmacokinetic parameters between patient groups were investigated using Mann-Whitney U test. Post hoc analysis of statistical power achieved was conducted using G*Power version 3.9.1.2 (Heinrich-Heine-University, Düsseldorf, Germany). All other statistical analyses were conducted using IBM ®SPSS® Statistics version 20.0

(IBM, Armonk, NY, USA) and GraphPad Prism® (GraphPad Software, Inc., La Jolla, CA, USA). All charts were plotted using GraphPad Prism® 5. The previously suggested minimum effective concentration of 3400 ng/mL[261] was set as the C_{min} target.

3.3 RESULTS

3.3.1 Patients

A total of 232 eligible patients (110 pregnant, 122 postpartum) were recruited between December 2012 and October 2013. Patient characteristics are summarised in **Table 3.1**. The median (range) duration on NVP-containing regimen was 37.5 months (0.97-75.7) in pregnant and 28.6 months (1.4-79.1) in postpartum women, starting at a baseline CD4 count of 241 c/mm^3 (31-864) and 241 c/mm^3 (31-1015), respectively. NVP was used in combination with emtricitabine and tenofovir (44% in pregnant versus 49% in postpartum women) or lamivudine and zidovudine (54% in pregnant versus 49% in postpartum women) or lamivudine and tenofovir. All nine SNPs were in Hardy-Weinberg equilibrium within the study population. Genotype frequencies are presented in **Table 3.1**.

3.3.2 NVP Pharmacogenetics in Pregnant and Postpartum Women

In the preliminary phase, samples were collected at 4.0 h (0.5-8.0) in pregnant and 4.0 h (1.25-8.25) post-dose in postpartum women. In pooled analysis, median (interquartile range; IQR) plasma concentration was 4130 ng/mL (3275, 5308) in pregnant compared with 5170 ng/mL (4120, 7380) in postpartum women (geometric mean ratio, 0.77; *p* value < 0.0001). Plasma concentrations below the recommended 3400 ng/mL were observed in 26% (29/110) of pregnant versus 14% (17/122) of postpartum women (**Figure 3.1A**).

As shown in **Table 3.2**, *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) were independently associated with higher NVP plasma concentration in pregnant and postpartum women. *CYP2B6* 983T>C (rs28399499) was included in postpartum multivariate analysis despite

Table 3.1 Characteristics of patients during pregnancy and postpartum.

Characteristics[a]	Pregnancy (n = 110)	Postpartum (n = 122)
Age (years)	29 (19-42)	30 (20-40)
Weight (kg)	61 (43-95)	57 (35-109)
Gestational/Postnatal age (weeks)	28.9 (6.0-38.6)	19.4 (1.4-73.9)
Trimester		
1st, 2nd, 3rd (%)	9, 32, 59	NA
Drug regimen and CD4 count		
FTC/TDF/NVP (%)	44	49
3TC/AZT/NVP (%)	54	41
3TC/TDF/NVP (%)	2	10
Time post-dose (h)	4.0 (0.5-8.0)	4.0 (1.25-8.25)
Duration on regimen (months)	37.5 (0.97-75.7)	28.6 (1.4-79.1)
Baseline CD4 count (cells/mm^3)	241 (31-864)	241 (31-1015)
CD4 change (cells/mm^3)	276 (5-1187)	351 (45-1108)
Genotype frequencies		
CYP3A4 -392A>G (*1B; rs2740574)	CC, 0.64; CT, 0.30; TT, 0.06	CC, 0.62; CT, 0.35; TT, 0.03
CYP3A4 20230G>A (*1G; rs2242480)	TT, 0.84; CT, 0.15; CC, 0.01	TT, 0.73; CT, 0.18; CC, 0.09
CYP2B6 516G>T (rs3745274)	GG, 0.35; GT, 0.44; TT, 0.21	GG, 0.36; GT, 0.43; TT, 0.21
CYP2B6 983T>C (rs28399499)	TT, 0.85; CT, 0.14, CC, 0.01	TT, 0.81; CT, 0.18, CC, 0.01
NR1I3 540C>T (rs2307424)	CC, 0.77; CT, 0.22; TT, 0.01	CC, 0.81; CT, 0.19; TT, 0.00
NR1I3 152-1089T>C (rs3003596)	TT, 0.30; CT, 0.52; CC, 0.18	TT, 0.20; CT, 0.54; CC, 0.26
NR1I2 63396C>T (rs2472677)	CC, 0.34; CT, 0.49; TT, 0.17	CC, 0.35; CT, 0.54; TT, 0.11
POR 1508C>T (*28; rs1057868)	CC, 0.68; CT, 0.28; TT, 0.04	CC, 0.72; CT, 0.27; TT, 0.01
ABCC10 2843T>C (rs2125739)	TT, 0.52; CT, 0.37; CC, 0.12	TT, 0.50; CT, 0.36; CC, 0.14

[a]Unless otherwise indicated, values are expressed as median (range). Abbreviations: FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; NVP, nevirapine; 3TC, lamivudine; AZT, zidovudine.

Table 3.2 Association of patient characteristics and single nucleotide polymorphisms in *CYP3A4*, *CYP2B6*, *NR1I3*, *NR1I2*, *POR*, and *ABCC10* with log_{10} plasma NVP concentrations during pregnancy and postpartum.

	Univariate linear regression		Multivariate linear regression	
	$β^a$ (log_{10} NVP conc., 95% CI)	*p* value	$β^a$ (log_{10} NVP conc., 95% CI)	*p* value
Pregnant women (n = 110)				
Weight (kg)	-0.001 (-0.005, 0.003)	0.69		
Age (years)	0.004 (-0.005, 0.012)	0.37		
Time post-dose (h)	-0.004 (-0.012, 0.004)	0.29		
Trimester	-0.004 (-0.086, 0.077)	0.92		
CYP3A4 -392A>G (*1B; rs2740574)	0.006 (-0.057, 0.068)	0.86		
CYP3A4 20230G>A (*1G; rs2242480)	0.075 (-0.020, 0.17)	0.12		
CYP2B6 516G>T (rs3745274)	0.041 (-0.008, 0.090)	0.098	0.061 (0.012, 0.11)	0.015[b]
CYP2B6 983T>C (rs28399499)	0.12 (0.026, 0.21)	0.012	0.15 (0.055, 0.24)	0.002
NR1I3 540C>T (rs2307424)	-0.091 (-0.17, -0.013)	0.022	-0.093 (-0.17, -0.018)	0.015[b]
NR1I3 152-1089T>C (rs3003596)	0.035 (-0.019, 0.089)	0.20		
NR1I2 63396C>T (rs2472677)	0.022 (-0.032, 0.075)	0.43		
POR 1508C>T (*28; rs1057868)	-0.005 (-0.071, 0.060)	0.87		
ABCC10 2843T>C (rs2125739)	-0.048 (-0.10, 0.005)	0.075		
Postpartum (n = 122)				
Weight (kg)	-0.001 (-0.005, 0.003)	0.58		
Age (years)	0.003 (-0.009, 0.016)	0.59		
Time post-dose (h)	0.010 (-0.027, 0.046)	0.60		
CYP3A4 -392A>G (*1B; rs2740574)	-0.005 (-0.070, 0.060)	0.88		
CYP3A4 20230G>A (*1G; rs2242480)	0.00 (-0.054, 0.054)	0.99		
CYP2B6 516G>T (rs3745274)	0.072 (0.024, 0.12)	*0.004*	0.10 (0.053, 0.16)	*1.1 x 10^{-4}*
CYP2B6 983T>C (rs28399499)	0.045 (-0.052, 0.14)	0.36[c]	0.11 (0.015, 0.21)	0.024[b]

Table 3.2 continued

	Univariate linear regression		Multivariate linear regression	
	β^a (log$_{10}$ NVP conc., 95% CI)	*p* value	β^a (log$_{10}$ NVP conc., 95% CI)	*p* value
Postpartum (n = 122) continued				
NR1I3 540C>T (rs2307424)	0.035 (-0.057, 0.13)	0.45		
NR1I3 152-1089T>C (rs3003596)	-0.036 (-0.090, 0.017)	0.18		
NR1I2 63396C>T (rs2472677)	-0.019 (-0.076, 0.038)	0.50		
POR 1508C>T (*28; rs1057868)	0.075 (-0.001, 0.15)	0.054	0.10 (0.026, 0.17)	0.008[b]
ABCC10 2843T>C (rs2125739)	0.005 (-0.048, 0.058)	0.84		

[a]β is the regression coefficient and represents incremental change in log$_{10}$ plasma NVP concentration (conc.) per unit change in a patient characteristic; [b]Bonferroni correction *p* value > 0.05; [c]*CYP2B6* 983T>C (rs28399499) was included in postpartum multivariate analysis despite *p* value > 0.2 in univariate analysis because of the observed association in the pregnant group.

p value > 0.2 in univariate analysis because of the observed association in the pregnant group. *NR1I3* 152-1089T>C (rs3003596) was associated with lower plasma concentration in pregnant women and *POR* 1508C>T (*28; rs1057868) with higher plasma concentration in postpartum women. No other associations were observed. Post hoc power analysis of the multivariate linear regression showed that the sample sizes of 110 and 122 achieved powers of 89.8% in pregnant and 99.8% in postpartum groups. To explore their combined effect, we stratified patients according to composite *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) genotypes: non-carriers, carriers of one allele of either SNP and carriers of two alleles of either SNP or one allele of both SNPs (designated 0, 1, and 2, respectively, in **Figure 3.1B**). Plasma NVP concentrations were significantly lower in pregnant (designated Preg in **Figure 3.1B**) versus postpartum (designated Postp in **Figure 3.1B**) women who were carriers of at least one allele of either SNP (**Figure 3.1B**). Plasma concentrations below the recommended 3400 ng/mL were observed in 46% versus 24% of pregnant versus postpartum women in non-carriers, 21% versus 13% in carriers of one allele of either SNP, and 13% versus 10% in carriers of two alleles of either SNP or one allele of both SNPs.

Patients were stratified according to composite *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) genotypes for the intensive pharmacokinetic phase. A total of 31 pregnant (6 non-carriers, *CYP2B6* 516GG and 983TT genotypes combined; 19 carriers of one allele of either SNP, *CYP2B6* 516GT or 983TC genotype; and 6 carriers of two alleles of either SNP or one allele of both SNPs, *CYP2B6* 516GT and 983TC combined, or 516TT, or 983CC genotype) and 29 postpartum (9 non- carriers, 10 carriers of one allele of either SNP, and 10 carriers of two alleles of either SNP or one allele of both SNPs) women were successfully re-recruited.
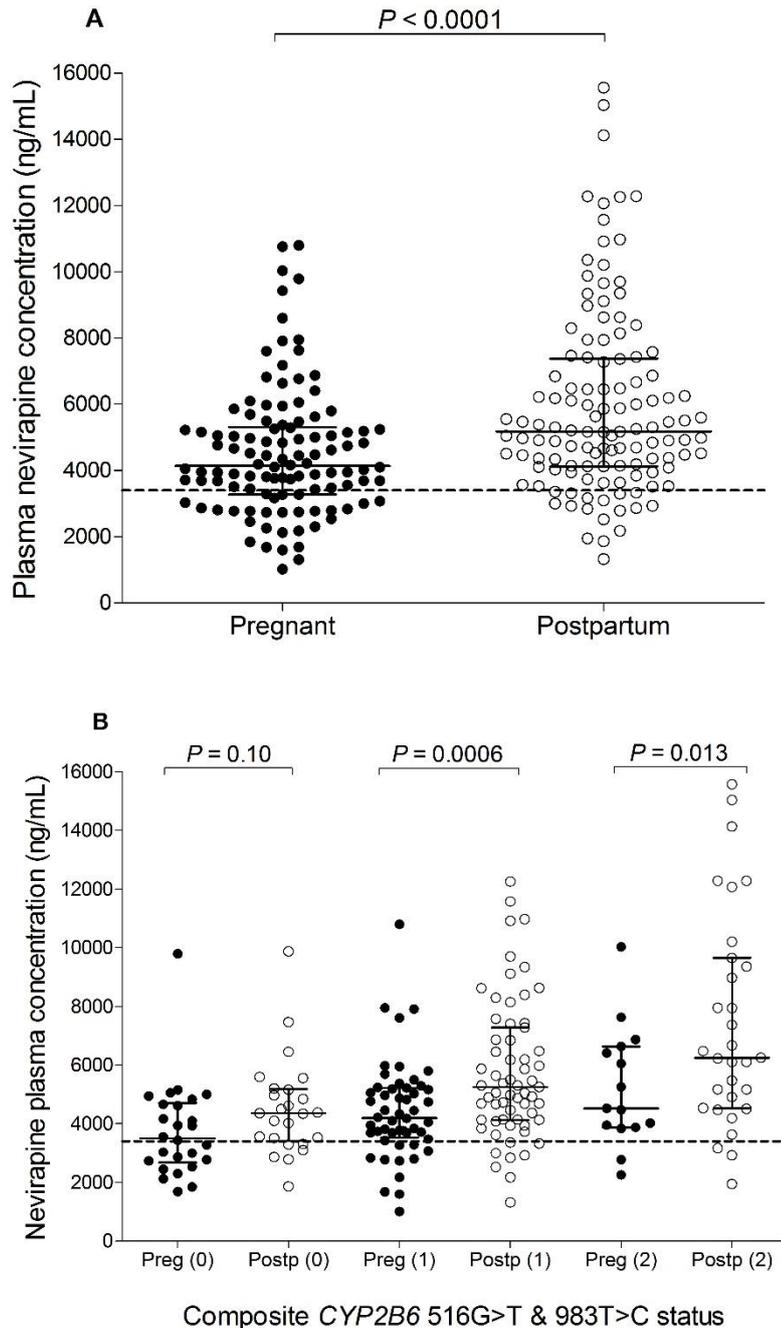
Figure 3.1 Plasma NVP concentrations in pregnant (closed circles) versus postpartum (open circles) women. In pooled analysis (A), median (IQR) plasma concentration was 4130 ng/mL (3280, 5310) in pregnant versus 5170 ng/mL (4120, 7390) in postpartum women (geometric mean ratio, 0.77; Mann-Whitney U test p value < 0.0001). When stratified based on composite *CYP2B6* 516G>T and 983T>C genotypes (B), NVP concentrations were significantly lower in pregnant (Preg) versus postpartum (Postp) women who were carriers of at least one allele of either SNP (0, non-carriers; 1, carriers of one allele of either SNP; 2, carriers of two alleles of either SNP or one allele of both SNPs). Dotted lines show target C_{min} of 3400 ng/mL.

3.3.3 Magnitude of Pregnancy-Induced Changes in NVP Pharmacokinetics

In pooled analysis, CL/F was 4.5 L/h (3.9, 5.1) in pregnant (n = 31) and 3.7 L/h (2.3, 4.6) in postpartum women (n = 28), representing 21.6% higher clearance during pregnancy (p = 0.022). The corresponding AUC_{0-12}, C_{max} and C_{min} were 19.2% (p = 0.022), 18.6% (p = 0.013), and 28.5% (p = 0.0021) lower, respectively. C_{min} was lower than target (3400 ng/mL) in 61% of pregnant compared with 25% of postpartum women (**Table 3.3; Figure 3.2**). When stratified based on composite *CYP2B6* 516G>T (rs3745274) and 983T>C (rs28399499) genotypes, CL/F was significantly higher during pregnancy compared with postpartum women, except in those with *CYP2B6* 516GG and 983TT genotypes combined. In this group of fast metabolisers, pregnancy was not associated with further increase in CL/F and the resulting C_{min} was 2470 ng/mL (2070, 5500) in pregnant and 2920 ng/mL (2440, 3300) in postpartum women, below target in 67% (4/6) and 88% (7/8), respectively (**Table 3.3; Figure 3.2**). In patients with *CYP2B6* 516GT or 983TC genotype, clearance was 40.6% higher in pregnant compared with postpartum women (p = 0.0009) and the corresponding AUC_{0-12}, C_{max}, and C_{min} were significantly lower. C_{min} was 3130 ng/mL (2990, 3540) in pregnant and 5590 ng/mL (4480, 7680) in postpartum women (p < 0.0001), and below target in 58% (11/19) and 0% (0/10), respectively (**Table 3.3; Figure 3.2**). Similarly, clearance was 51.7% higher in pregnant women with *CYP2B6* 516GT and 983TC combined, or 516TT, or 983CC genotype than in postpartum women (p = 0.008). The corresponding AUC_{0-12}, C_{max} and C_{min} were significantly lower, with C_{min} below target in 50% (3/6) of pregnant and 0% (0/10) of postpartum women (**Table 3.3; Figure 3.2**). Post hoc power analysis showed that 63.6% power was achieved in pooled analysis and 12.3, 98.6 and 59.0%, respectively, in the three genotype-guided comparisons.

Table 3.3 NVP pharmacokinetic parameters (median, IQR) in pregnant and postpartum women in pooled analysis and based on composite *CYP2B6* 516G>T and 983T>C genotypes.

Combined	CL/F (L/h)	$\text{AUC}_{0\text{-}24}$ (ng.h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	C_{min} < target
Pregnant (n = 31)	4.5 (3.9, 5.1)	44200 (39100, 51200)	4870 (4200, 5710)	3130 (2730, 3700)	61% (19/31)
Postpartum (n = 28)	3.7 (2.3, 4.6)	54700 (43900, 85300)	5980 (5090, 8630)	4380 (3380, 6030)	25% (7/28)
Pregnant versus postpartum (%)	21.6	-19.2	-18.6	-28.5	
p value[a]	0.022	0.022	0.013	0.0021	
CYP2B6 **516GG & 983TT**					
Pregnancy (n = 6)	5.7 (3.8, 7.0)	36500 (28800, 63200)	3900 (2860, 6600)	2470 (2070, 5500)	67% (4/6)
Postpartum (n = 8)	5.5 (4.7, 7.5)	36300 (26600, 42200)	4160 (3700, 5020)	2920 (2440, 3300)	88% (7/8)
Pregnant versus postpartum (%)	3.64	0.551	-6.25	-15.4	
p value	0.45	0.45	0.63	0.95	
CYP2B6 **516GT or 983TC**					
Pregnancy (n = 19)	4.5 (3.9, 4.8)	44600 (41800, 51200)	5260 (4220, 5640)	3130 (2990, 3540)	58% (11/19)
Postpartum (n = 10)	3.2 (2.0, 3.9)	63300 (51500, 103000)	7660 (5820, 10300)	5590 (4480, 7680)	0% (0/10)
Pregnant versus postpartum (%)	40.6	-29.5	-31.3	-44.0	
p value	0.0009	0.0009	0.0006	< 0.0001	
CYP2B6 **516GT & 983TC or 516TT or 983CC**					
Pregnancy (n = 6)	4.4 (3.7, 5.4)	45300 (37600, 54700)	4870 (4231, 6640)	3150 (2570, 3920)	50% (3/6)
Postpartum (n = 10)	2.9 (2.1, 3.8)	69800 (52800, 95900)	7280 (5680, 9310)	4910 (4150, 6100)	0% (0/10)
Pregnant versus postpartum (%)	51.7	-35.1	-33.1	-35.8	
p value	0.008	0.008	0.040	0.0027	

[a]Mann-Whitney U test *p* value.
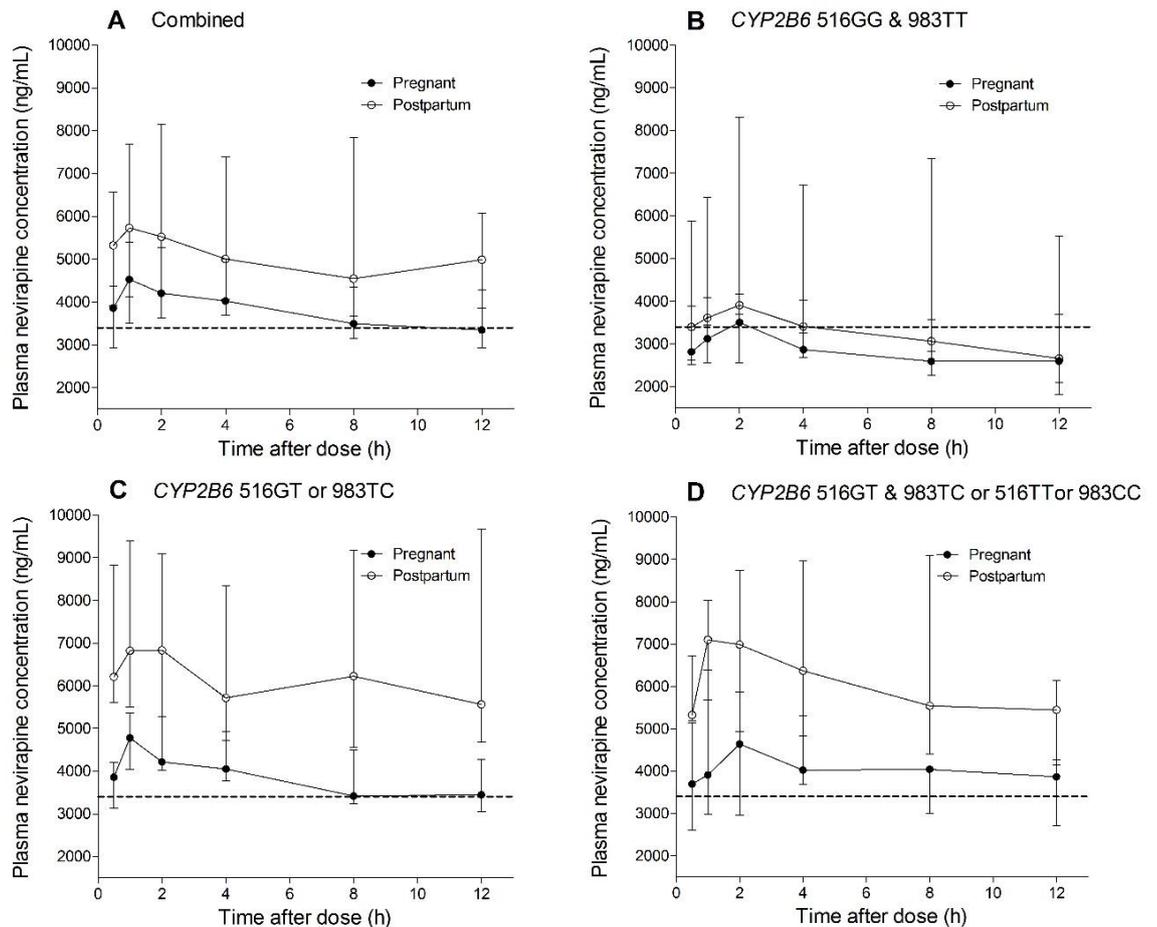
Figure 3.2 NVP concentration-time profiles in plasma of pregnant versus postpartum women. In pooled analysis, C_{min} was below target in 61% of pregnant compared with 25% of postpartum women (A). When stratified based on composite *CYP2B6* 516G>T and 983T>C genotypes, C_{min} was below target in: 67% (4/6) of pregnant and 88% (7/8) of postpartum women in *CYP2B6* 516GG and 983TT group (B); 58% (11/19) of pregnant and 0% (0/10) of postpartum women in the *CYP2B6* 516GT or 983TC group (C); 50% (3/6) of pregnant and 0% (0/10) of postpartum women in the *CYP2B6* 516GT and 983TC, or 516TT, or 983CC group (D). Solid lines and bars represent median and interquartile range while dotted lines show target C_{min} of 3400 ng/mL.

3.4 DISCUSSION

To our knowledge, the present study is the largest to date to investigate the effect of pregnancy on NVP pharmacokinetics and the first to do so in the context of host genetics. The findings showed significantly higher clearance in pregnant compared with postpartum women, except in

those with combined *CYP2B6* 516GG and 983TT genotypes. In pooled analysis and when stratified into three genotype groups, C_{min} was below the recommended therapeutic target (not PMTCT target) in 50-67% of pregnant women. These findings indicate that the effect of pregnancy on NVP pharmacokinetics is independent of patient genotype. In addition, C_{min} was below target in 88% of postpartum women with combined *CYP2B6* 516GG and 983TT genotypes, suggesting higher risk of suboptimal drug exposure both during pregnancy and postpartum in this group. A major limitation in the present study was the inadequate power achieved in the intensive pharmacokinetic phase. In addition, pregnant women were not used as their own postpartum control.

The effect of pregnancy on NVP pharmacokinetics have been investigated in four previous studies, three of which similarly reported significantly reduced exposure during pregnancy. NVP C_{min} below the target utilised by the investigators (3000 ng/mL) was reported in 67% (10/15) of pregnant women during the third trimester and in 26.7% (4/15) postpartum in one of the studies. Third trimester AUC_{0-12} and C_{max} were also significantly reduced.[115] This is similar to the 61 and 25% observed in the present study. A comparison of NVP pharmacokinetics between pregnant (n = 16) and non-pregnant women (n = 13) in an earlier study also revealed significant differences (>20%, $p < 0.02$) in CL/F, AUC_{0-12} and C_{max}.[188] Using sparse steady state NVP plasma concentrations available as part of routine care, Nellen et al. reported moderately lower exposure in pregnant (n = 45) versus postpartum (n = 152) women, 5200 ng/mL (IQR 3900-6800) versus 5800 ng/mL (4300-7700) ng/mL ($p = 0.08$).[189] This is in the region of the 4550 ng/mL (1950) versus 5960 ng/mL (2820) observed in the preliminary phase of the present study. Collectively with these findings, the current data contrast with those of a combined pharmacokinetic analysis of two Pediatric AIDS Clinical Trials Group studies (P1022 and P1026S, n = 26) in which NVP AUC and C_{min} during pregnancy and postpartum were reported to be comparable.[190] The physiological changes associated with pregnancy are understood to be

responsible for the reduced NVP exposure observed in the present and other studies. These have been well reviewed in Chapter 1.

The associations of *CYP2B6* 516G>T and *CYP2B6* 983T>C SNPs with plasma NVP concentrations observed in the present study are consistent with known NVP metabolic pathway and findings from other studies.[278] NVP is predominantly metabolised by CYP2B6 and CYP3A4 to 3- and 8-hydroxy and 2- and 12-hydroxy NVP, respectively.[277] The expression of both CYP2B6 and CYP3A4 in human hepatocytes are induced by NR1I2 (also known as PXR, pregnane X receptor) activation, while NR1I3 (also known as CAR, constitutive androstane receptor) activation predominantly induces the expression of CYP2B6.[56] Estradiol is upregulated during human pregnancy and has been shown to induce the expression of POR, NR1I2 and NR1I3.[267,284] The magnitude of pregnancy-induced changes in pharmacokinetics can be modulated by genetic polymorphisms in drug disposition genes.[247] For instance, pregnant women classified as extensive metabolisers based on their metabolic capacity (*CYP2B6* 516GG) were recently shown to have a higher risk of EFV concentrations below the recommended target compared with poor metabolisers (*CYP2B6* 516GT and TT).[269,283] In the present study, the impact of pregnancy on NVP pharmacokinetics was significant across all studied genotype groups, except in women with the *CYP2B6* 516GG and 983TT genotypes who were more likely to have C_{min} below the recommended target both during pregnancy and postpartum.

All CYP enzymes require POR for catalysis and SNPs in the *POR* gene affect CYP2B6 and CYP3A4 activities.[279] However, the associations observed between NVP plasma concentrations, and *NR1I3* 540C>T in pregnant and *POR* 1508C>T (not previously reported) in postpartum women were lost after Bonferroni correction. The observed differences in patterns of genetic associations between pregnant and postpartum women may reflect the complexity of the isoform-specific regulation of CYP enzymes by POR, NR1I2 and NR1I3 through estradiol and progesterone.[284,285]

These findings need to be interpreted with caution because the effects of the observed pregnancy-induced changes in NVP pharmacokinetics on efficacy and mother-to-child transmission risks are unknown. In fact, from two previous studies with data on infant HIV status postpartum, 0 out of 17 and 1 out of 25 infants became infected,[188,190] despite significantly lower exposure in one.[188] Though MTCT remained rare, most of the patients were treatment-experienced and virologically suppressed. Therefore, the findings cannot be extrapolated to those initiating therapy late in pregnancy.[286] However, these data might also challenge the appropriateness of the 3400 ng/mL therapeutic target for application in PMTCT, especially in women on suppressive therapy before pregnancy. Interestingly, the study by Lamorde et al. indicated that pregnant women who initiated therapy late were more likely to have suboptimal virological suppression (> 400 copies/mL), especially when C_{min} was below target.[115] Timely initiation of ART can reduce MTCT to less than 5% from the baseline 25-40% without intervention. However, about 250 000 infants are still infected every year (30 per hour).[7] Factors contributing to these residual transmissions are not known. Late interventions as a result of late diagnosis or acute maternal infection during pregnancy or during breastfeeding are common.[287-289] This can potentially result in detectable HIV RNA at third trimester and delivery, increasing MTCT risks.[290,291]

In conclusion, considering the pregnancy-induced changes in NVP pharmacokinetics observed in the present and previous studies, the potential effects on efficacy especially in women initiating therapy late in pregnancy now warrant further investigation. In addition to highlighting a possible source of residual MTCT in the ART era, this will also inform future studies on potential treatment intensification strategies.

CITATIONS

Olagunju A, Amara A, Waitt C, Else L, Penchala SD, Bolaji O, Soyinka J, Siccardi M, Back D, Owen A, Khoo S. Validation and clinical application of a method to quantify NVP in dried blood spots and dried breast milk spots. *Journal of Antimicrobial Chemotherapy*. 2015; 70(10):2816-22.

CHAPTER 4

BREAST MILK PHARMACOKINETICS OF EFAVIRENZ AND BREASTFED INFANTS' EXPOSURE IN GENETICALLY-DEFINED SUBGROUPS OF MOTHER-INFANT PAIRS: AN OBSERVATIONAL STUDY

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4.1 INTRODUCTION

Exclusive breastfeeding for the first six months of life, continued for up to two years with gradual introduction of safe and nutritionally adequate replacement feeding, is the recommended feeding option in the context of HIV/AIDS in resource-limited settings.[292-294] The potential benefits of avoiding or early cessation of breastfeeding in this population are largely offset by increased infant morbidity/mortality from causes other than HIV/AIDS, including malnutrition, diarrhoea and pneumonia.[295-298] Mother-to-child transmission (MTCT) during this period is prevented by maternal antiretroviral therapy (ART) started during pregnancy and continued until breastfeeding ends (Option B) or for life (Option B+).[244,299] The infant is given daily nevirapine (NVP) post-exposure prophylaxis (PEP) from birth until 4-6 weeks old,[299] which reduces MTCT to less than 5% in these settings from the baseline 20-45% without intervention.[9]

The current WHO guidelines recommend efavirenz (EFV) as the preferred non-nucleoside reverse transcriptase inhibitor component of first-line ART for adults across different patient populations, including nursing mothers.[13] However, EFV is not licensed for use in children < 3 months old or < 3.5 kg because optimal dosing and safety have not been evaluated.[300] However, it is increasingly used by nursing mothers and its presence in breast milk and breastfed infants' plasma have been reported, but in limited numbers of mother-infant pairs and at single time points after maternal dose.[92,301] Milk production, composition, and infant feeding patterns change during the dosing interval and may cause variations in the milk-to-plasma (M/P) concentration ratio, making single point estimates unreliable.[85,302] In addition, the only study that reported EFV concentration in breast milk used skimmed milk, which often yields lower drug concentrations than whole milk. A recently developed method for the quantification of EFV in dried breast milk spots[242] has now extended our ability to study the pharmacokinetics of EFV in whole milk during an entire dosing interval.

The influence of single nucleotide polymorphisms (SNPs) in drug metabolizing enzymes, transporter and nuclear receptor genes on plasma EFV concentration is well established.[303] The interindividual variability observed in EFV plasma pharmacokinetics in different populations has been associated with such SNPs.[304] We previously hypothesised that SNPs may affect EFV excretion into breast milk and transfer to breastfed infants.[247] Understanding the pharmacokinetics of EFV in human breast milk during an entire dosing interval and potential differences introduced by genetic polymorphisms are crucial for an accurate estimation of infant exposure.

In the present study, associations between EFV concentrations in plasma and breast milk of nursing mother-infant pairs and SNPs in *CYP2B6*, *NR1I3*, *CYP2A6*, *ABCB1*, *ABCB5* and *ABCG2* genes were explored. We then investigated EFV plasma and breast milk pharmacokinetics and breastfed infants' exposure in genetically-defined subgroups, stratified by the SNP with the highest predictive power.

4.2   METHODS

4.2.1   Patients

HIV positive nursing mothers and their breastfed infants were recruited from three hospitals in Benue State, Nigeria: Bishop Murray Medical Centre, Makurdi; St Mary's Hospital, Okpoga; and St Monica's Hospital, Adikpo. Potentially eligible subjects were identified using the current PMTCT delivery register and invited to participate after an information session conducted in English and the local Tiv language. All participants gave prior written informed consent. Once subjects had signed a consent form, we ascertained eligibility by examining case notes and conducting a brief interview. The inclusion criteria included HIV positive and breastfeeding, enrolled in the PMTCT programme and started EFV-containing regimen during pregnancy. Exclusion criteria (assessed at enrolment) included exclusive formula feeding, mixed feeding in

infants less than 6 months old, opportunistic infections (e.g. tuberculosis, pneumonia), severe maternal or infant illness, and maternal or infant treatment with drugs or herbal medication with known or uncertain interaction with EFV. The protocol and the materials transfer agreement were approved by the National Health Research Ethics Committee (NHREC), Abuja, Nigeria and Ethics and Research Committee, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. Clinicaltrials.gov ID: NCT02269462.

4.2.2 Study Design

This was an observational study conducted in two phases. In the preliminary phase, we explored associations between 12 SNPs in drug disposition genes and mid-dose plasma and breast milk EFV concentrations in an unselected cohort of HIV positive nursing mothers and their breastfed infants. In the intensive pharmacokinetic phase, the SNP independently associated with the highest predictive power was used to stratify mother-infant pairs into three groups: non-carriers, heterozygotes, and homozygotes. Randomly selected mother-infant pairs from each group were re-recruited and invited for the intensive pharmacokinetic phase.

4.2.3 Sample Collection

In the preliminary phase, paired dried blood spots (DBS) and dried breast milk spots (DBMS) were collected from mothers and DBS from infants at a single, recorded time point post-dose. In the intensive pharmacokinetic phase, maternal DBS and DBMS were collected at 0.5, 1, 2, 4, 8, 12 and 24 hours after an observed evening dose of 600 mg EFV and stored as previously described [242]. DBS samples were collected from infants at 2 h and 8 h after maternal EFV dose in the intensive pharmacokinetic phase. To reflect real-life situations, infant feeding times were not controlled; all infants were breastfed on demand. In addition, mothers took standard local meals about 30 min before drug administration. Samples were shipped at ambient temperature to the University of Liverpool, UK for analysis.

4.2.4 DNA Extraction and Genotyping

Genomic DNA was extracted using E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) in accordance with the manufacturer's protocol. DNA was quantified spectrophotometrically using NanoDrop® (Thermo Fisher Scientific Inc., Wilmington, DE, USA) before storage at -20°C. Genotyping was performed by real-time PCR on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays (**Table 4.1**) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to obtain allelic discrimination plots and make genotype calls.

Table 4.1 TaqMan® assays

Gene and SNP	Reference SNP ID	Product ID
CYP2B6 516G>T	rs3745274	C_7817765_60
CYP2B6 983T>C	rs28399499	C_60732328_20
CYP2B6 c.485-18C>T	rs4803419	C_7817764_10
NR1I3 c.540C>T	rs2307424	C_25746794_20
NR1I3 c.152-1089T>C	rs3003596	C_16194070_10
ABCB1 4046A>G	rs1045642	C_7586657_20
ABCB1 4036A>G	rs3842	C_11711730_20
ABCB1 1236C>T	rs1128503	C_7586662_10
CYP2A6 48T>G	rs28399433	C_30634332_10
ABCB5 c.2908G>A	rs6461515	C_25621077_20
ABCG2 c.1728-46G>A	rs2231164	C_15922479_10
ABCG2 78551A>G	rs2622604	C_9510352_10

4.2.5 EFV Quantification and Pharmacokinetic Analysis

EFV in DBS and DBMS was quantified using validated liquid chromatography-tandem mass spectrometry methods described elsewhere.[242,258] Plasma concentrations were determined using [DBS$_{[EFV]}$/(1-HCT)]*0.995, where DBS$_{[EFV]}$ is EFV concentration in DBS, HCT is the patient-specific haematocrit and 0.995 is the fraction of EFV bound to plasma protein.[259] Minimum (C$_{min}$)

and maximum (C_{max}) plasma concentrations were determined by direct inspection. The area under the concentration-time curve during the dosing interval (AUC_{0-24}) was calculated using the trapezoidal rule and the apparent clearance (CL/F) was calculated by dividing dose by AUC_{0-24}. Maximum (InfantDose$_{max}$) and average (InfantDose$_{avg}$) infant EFV doses from breast milk were calculated using equations 4.1 and 4.2, respectively. Paediatric dose (for children aged ≥ 3 months and weighing ≥ 3.5 kg)[300] weight-adjusted exposure index (EI$_{paediatric}$) and maternal dose weight-adjusted exposure index (EI$_{maternal}$) were calculated using equations 4.3 and 4.4, respectively.

$$\text{InfantDose}_{max} \, (\mu g/kg/day) \; = \; 150 * MilkC_{max} \tag{4.1}$$

$$\text{InfantDose}_{avg} \, (\mu g/kg/day) \; = \; 150 * MilkAUC_{0-24}/24 \tag{4.2}$$

$$\text{EI}_{paediatric} \, (\%) \; = \; 100 * InfantDose_{max}/Dose_{paediatric} \tag{4.3}$$

$$\text{EI}_{maternal} \, (\%) \; = \; 100 * InfantDose_{max}/Dose_{maternal} \tag{4.4}$$

where 150 is the average volume of infant daily milk intake in mL/kg/day; MilkC$_{max}$ is the maximum EFV concentration in breast milk (μg/mL); MilkAUC$_{0-24}$ is EFV breast milk AUC$_{0-24}$ (μg.h/mL); 24 is the dosing interval in h; Dose$_{paediatric}$ is the weight-adjusted licensed paediatric dose of EFV (μg/kg/day); Dose$_{maternal}$ is the weight-adjusted licensed adult dose of EFV (μg/kg/day).

4.2.6 Statistical Analysis

Compliance with Hardy-Weinberg Equilibrium was tested as previously described.[260] Data were subjected to Kolmogorov-Smirnov normality test prior to statistical analysis. Relationships between continuous variables were tested by Pearson or Spearman correlation. Univariate linear regression analysis was conducted to identify variables associated with EFV concentrations in maternal plasma, infant plasma and breast milk. Bonferroni correction was used to adjust for multiple testing. Independent variables with Bonferroni p value ≤ 0.1 in the

univariate analysis were included in a multivariate stepwise linear regression analysis. Differences in EFV concentrations and pharmacokinetic parameters between patient groups were investigated using one-way analysis of variance (ANOVA) and Kruskal-Wallis or Mann-Whitney U test. Trend across groups was investigated using Cuzick's test on StatDirect (StatsDirect Ltd, Altrincham, Cheshire, UK). Post hoc analysis of statistical power achieved was conducted using G*Power version 3.9.1.2 (Heinrich-Heine-University, Düsseldorf, Germany). All other analyses were conducted using IBM ®SPSS® Statistics version 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism® (GraphPad Software, Inc., La Jolla, CA, USA).

4.3   RESULTS

4.3.1 Patients' Characteristics

Between December 2012 and October 2013, 134 eligible HIV positive nursing mothers and their breastfed infants were recruited. Maternal and infant characteristics are summarised in **Table 4.1**. The mean (standard deviation, SD) duration on current ART regimen was 17.6 months (14.1), starting with baseline CD4 count of 380 c/mm^3 (217). Most patients were taking EFV, emtricitabine and tenofovir (65%; 87/134) or EFV, lamivudine and zidovudine (32%; 43/134). Infants less than 6 weeks of age (22%; 29/134) were taking NVP PEP. Genotype frequencies are summarised in **Table 4.1**. All twelve SNPs were in Hardy-Weinberg Equilibrium.

4.3.2 Factors Associated with Plasma and Breast Milk EFV Concentrations

In the preliminary phase, samples were collected at 14 h (0.50-21.5) post maternal EFV dose and median (range) maternal plasma, breast milk and infant plasma EFV concentrations were 2310 ng/mL (632-8880), 2280 ng/mL (475-10800) and 173 ng/mL (46.0-4630), respectively (**Table 4.3**). There were strong to moderate positive correlations in EFV concentrations between maternal plasma and breast milk, Pearson's r = 0.73 (0.62, 0.81), R^2 = 0.53, p < 0.0001; maternal

plasma and infant plasma, Pearson's r = 0.58 (0.45, 0.69), R^2 = 0.33, p < 0.0001; and breast milk

and infant plasma, Pearson's r = 0.46 (0.28, 0.61), R^2 = 0.21, p < 0.0001 (**Figure 4.1**).

Table 4.1 Characteristics of nursing mother-infant pairs.

Mothers (n = 134)[a]	
Age (years)	29 (18-44)
Weight (kg)	57 (39-80)
Time since diagnosis (months)	21.1 (1.3-68)
Infants (n = 134)[a]	
Age (weeks)	20.0 (0.29-75)
Weight (kg)	5.8 (2.2-10)
Gender (Female)	52% (70/134)
Maternal drug regimen and CD4 count[a]	
TDF/FTC/EFV	65% (87/134)
3TC/AZT/EFV	32% (43/134)
3TC/TDF/EFV	2% (3/134)
3TC/ABC/EFV	1% (1/134)
Time post-dose (h)[b]	11.8 (5.5)
Duration on regimen (months)[b]	17.6 (14.1)
Baseline CD4 count (c/mm^3)[b]	380 (217)
CD4 change (c/mm^3)[b]	177 (217)
Maternal genotype frequency	
CYP2B6 516G>T (rs3745274)	GG, 0.36; GT, 0.45; TT, 0.19
CYP2B6 983T>C (rs28399499)	TT, 0.79; CT, 0.21; CC, 0.00
CYP2B6 c.485-18C>T (rs4803419)	CC, 0.79; CT, 0.21; TT, 0.00
NR1I3 c.540C>T (rs2307424)	CC, 0.77; CT, 0.23; TT, 0.00
NR1I3 c.152-1089T>C (rs3003596)	TT, 0.19; CT, 0.54; CC, 0.27
ABCB1 4046A>G (rs1045642)	AA, 0.75; AG, 0.25; GG, 0.00
ABCB1 4036A>G (rs3842)	AA, 0.65; AG, 0.35; GG, 0.01
ABCB1 1236C>T (rs1128503)	CC, 0.76; CT, 0.23; TT, 0.02
CYP2A6 48T>G (rs28399433)	TT, 0.93; GT, 0.07; GG, 0.01
ABCB5 c.2908G>A (rs6461515)	GG, 0.45; GA, 0.45; AA, 0.10
ABCG2 c.1728-46G>A (rs2231164)	GG, 0.68; GA, 0.30; AA, 0.02
ABCG2 78551A>G (rs2622604)	AA, 0.84; AG, 15; GG, 0.01
Infants' genotype frequency	
CYP2B6 516G>T (rs3745274)	GG, 0.45; GT, 0.67; TT, 0.18

[a]Unless otherwise indicated, values are expressed as median (range) or
% (number) of subjects; [b]mean (SD).
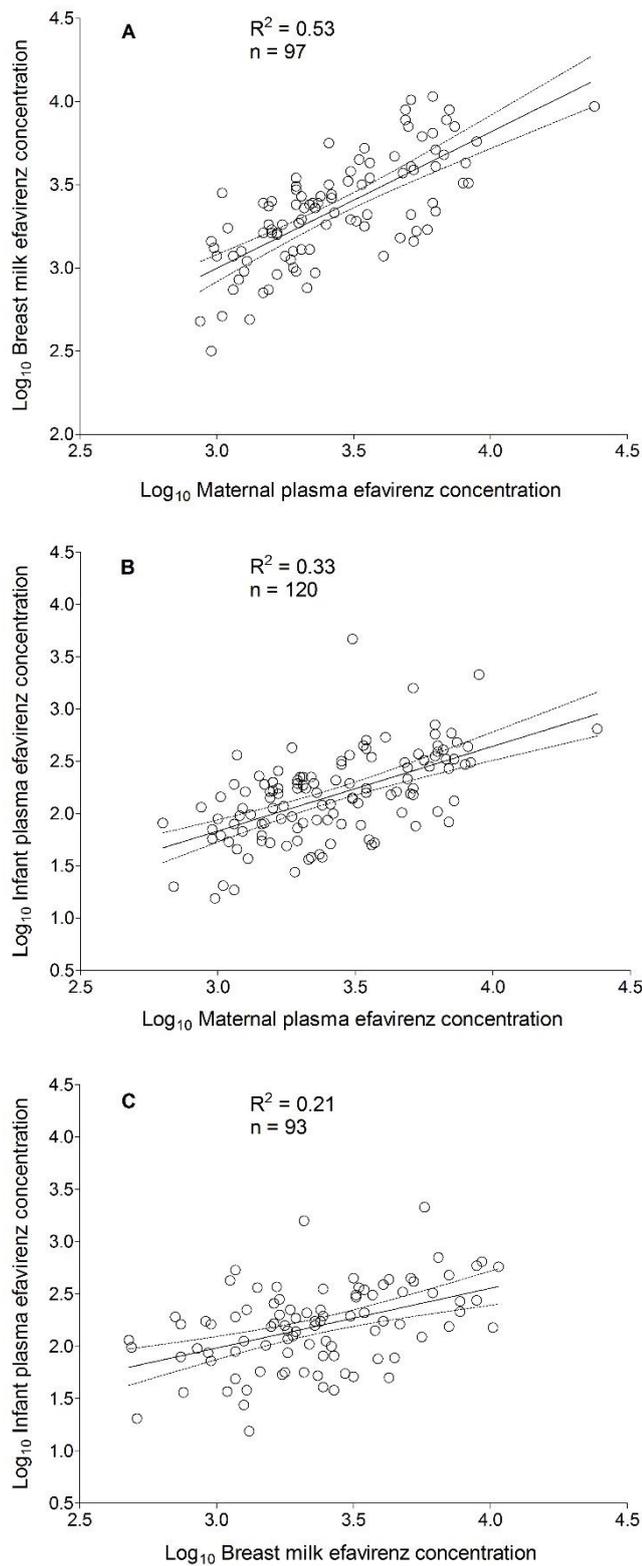
Figure 4.1. Correlations between EFV concentrations in maternal plasma and breast milk (A), maternal plasma and infant plasma (B), and breast milk and infant plasma (C). Solid lines represent mean values and broken lines represent 95% confidence intervals.

Of the 12 SNPs investigated, only maternal *CYP2B6* 516G>T (rs3745274) was independently associated with EFV concentrations in maternal plasma, breast milk and infant plasma. In addition, infant age and time post maternal EFV dose were independently associated with infant plasma EFV concentration (**Table 4.2**). The regression coefficients (β), which represents incremental change in $\log_{10}$ EFV concentration per unit change in patient characteristics, are presented in **Table 4.2**. In a separate analysis, both infant *CYP2B6* 516G>T (p = 0.019) and composite infant/maternal *CYP2B6* 516G>T genotypes (p = 0.006) were independently associated with infant $\log_{10}$ EFV concentration after adjusting for breast milk concentration and infant age with β values of 0.105 (0.018, 0.193) and 0.033 (0.010, 0.057), respectively. More than 99% statistical power was achieved in the multiple linear regression for plasma (maternal and infant) and breast milk concentrations.

Significant differences were observed in maternal plasma EFV concentrations based on *CYP2B6* 516G>T genotypes (p < 0.0001): GG (n = 42), 1660 ng/mL (632-3610); GT (n = 52), 2390 ng/mL (951-8880); and TT (n = 23), 5400 ng/mL (1920-8110). Breast milk concentrations also varied based on maternal *CYP2B6* 516G>T genotypes (p = 0.0002): GG, 1610 ng/mL (475-5580); GT, 2370 ng/mL (713-10300); and TT, 4070 ng/mL (995-10800). A similar trend was observed for infant plasma concentrations based on both maternal and infant *CYP2B6* 516G>T genotypes (**Table 4.3** and **Figure 4.2**). After excluding infants less than 10 days old with residual intrauterine exposure, plasma EFV concentrations in infants based on maternal *CYP2B6* 516G>T genotypes were: GG: 120 ng/mL (46.0-429); GT: 157 ng/mL (48.5-590); and TT: 329 ng/mL (75.5-705). There was a significant trend towards higher infant plasma EFV concentration from GG/GG to TT/TT composite maternal/infant *CYP2B6* 516G>T genotype, Cuzick's test for trend p value was < 0.0001 (**Figure 4.3**). Therefore, maternal *CYP2B6* 516G>T (rs3745274) was used to stratify mother-infant pairs and a total of 29 (GG, 10; GT, 11; TT, 8) were invited for the intensive pharmacokinetic phase.

Table 4.2 Linear regression analysis showing associations of patient characteristics and maternal single nucleotide polymorphisms in drug disposition genes with $\log_{10}$ EFV plasma concentrations in maternal plasma, breast milk and infant plasma.

	Univariate linear regression			Multivariate linear regression		
Patient characteristic	β^a ($\log_{10}$ EFV conc., 95% CI)	*p* value	Bonferroni *p* value	β^a ($\log_{10}$ EFV conc., 95% CI)	*p* value	Bonferroni *p* value
Mother (n = 134)						
Weight (kg)						
Maternal plasma	-0.002 (-0.011, 0.008)	0.73				
Breast milk	-0.005 (-0.013, 0.004)	0.30				
Infant plasma	-0.004 (-0.013, 0.004)	0.32				
Maternal age (y)						
Maternal plasma	-0.0003 (-0.012, 0.012)	0.96				
Breast milk	0.006 (-0.007, 0.019)	0.089				
Infant plasma	-0.002 (-0.014, 0.01)	0.71				
Infant age (months)						
Maternal plasma	0.002 (-0.016, 0.02)	0.80				
Breast milk	0.003 (-0.021, 0.027)	0.80				
Infant plasma	-0.026 (-0.043, -0.009)	0.004	0.064	-0.032 (-0.047, -0.016)	9.9×10^{-5}	0.0016
Time post-dose (h)						
Maternal plasma	0.002 (-0.01, 0.014)	0.78				
Breast milk	0.011 (-0.006, 0.027)	0.20				
Infant plasma	0.016 (-0.005, 0.028)	0.006	0.096	0.017 (0.007, 0.027)	0.001	0.016
***CYP2B6* 516G>T (rs3745274)**						
Maternal plasma	0.24 (0.17, 0.31)	1.9×10^{-9}	3.0×10^{-8}	0.24 (0.17, 0.31)	1.9×10^{-9}	3.0×10^{-8}
Breast milk	0.18 (0.098, 0.27)	4.7×10^{-5}	7.5×10^{-4}	0.18 (0.098, 0.27)	4.7×10^{-5}	7.5×10^{-4}
Infant plasma	0.18 (0.10, 0.26)	2.3×10^{-5}	3.7×10^{-4}	0.19 (0.11, 0.27)	6.0×10^{-6}	3.7×10^{-4}

Table 4.2 continued

Patient characteristic	Univariate linear regression			Multivariate linear regression		
	β^a (log$_{10}$ EFV conc., 95% CI)	p value	Bonferroni p value	β^a (log$_{10}$ EFV conc., 95% CI)	p value	Bonferroni p value
CYP2B6 983T>C (rs28399499)						
Maternal plasma	0.061 (-0.098, 0.22)	0.45				
Breast milk	0.043 (-0.13, 0.22)	0.63				
Infant plasma	-0.051 (-0.21, 0.11)	0.53				
CYP2B6 c.485-18C>T (rs4803419)						
Maternal plasma	-1.3E-5 (-0.17, 0.17)	1.00				
Breast milk	0.008 (-0.18, 0.2)	0.93				
Infant plasma	-0.038 (-0.21, 0.14)	0.67				
NR1I3 c.540C>T (rs2307424)						
Maternal plasma	-0.091 (-0.24, -0.061)	0.24				
Breast milk	0.025 (-0.14, 0.19)	0.76				
Infant plasma	-0.044 (-0.20, 0.11)	0.57				
NR1I3 c.152-1089T>C (rs3003596)						
Maternal plasma	-0.009 (-0.11, 0.087)	0.85				
Breast milk	-0.020 (-0.12, 0.08)	0.69				
Infant plasma	0.042 (-0.055, 0.14)	0.39				
ABCB1 4046A>G (rs1045642)						
Maternal plasma	0.12 (-0.026, 0.27)	0.11				
Breast milk	-0.011 (-0.17, 0.15)	0.89				
Infant plasma	-0.095 (-0.24, 0.053)	0.21				
ABCB1 4036A>G (rs3842)						
Maternal plasma	0.037 (-0.1, 0.18)	0.59				

Table 4.2 continued

Patient characteristic	Univariate linear regression			Multivariate linear regression		
	β^a (log$_{10}$ EFV conc., 95% CI)	*p* value	Bonferroni *p* value	β^a (log$_{10}$ EFV conc., 95% CI)	*p* value	Bonferroni *p* value
Breast milk	0.071 (-0.075, 0.22)	0.34				
Infant plasma	0.084 (-0.054, 0.22)	0.23				
ABCB1 **1236C>T (rs1128503)**						
Maternal plasma	0.067 (-0.066, 0.2)	0.32				
Breast milk	-0.030 (-0.18, 0.12)	0.69				
Infant plasma	-0.052 (-0.19, 0.082)	0.44				
CYP2A6 **48T>G (rs28399433)**						
Maternal plasma	0.058 (-0.22, 0.34)	0.68				
Breast milk	0.11 (-0.40, 0.18)	0.44				
Infant plasma	0.019 (-0.26, 0.30)	0.89				
ABCB5 **c.2908G>A (rs6461515)**						
Maternal plasma	0.043 (-0.053, 0.14)	0.38				
Breast milk	0.070 (-0.034, 0.18)	0.18				
Infant plasma	-0.039 (-0.14, 0.057)	0.42				
ABCG2 **c.1728-46G>A (rs2231164)**						
Maternal plasma	-0.020 (-0.15, 0.11)	0.76				
Breast milk	0.11 (-0.050, 0.27)	0.18				
Infant plasma	0.010 (-0.12, 0.14)	0.88				
ABCG2 **78551A>G (rs2622604)**						
Maternal plasma	-0.029 (-0.19, 0.13)	0.72				
Breast milk	-0.065 (-0.24, 0.11)	0.47				
Infant plasma	-0.11 (-0.27, 0.047)	0.16				

$^a\beta$ is regression coefficient, which represents incremental change in log$_{10}$ EFV concentration per unit change in a patient characteristic.
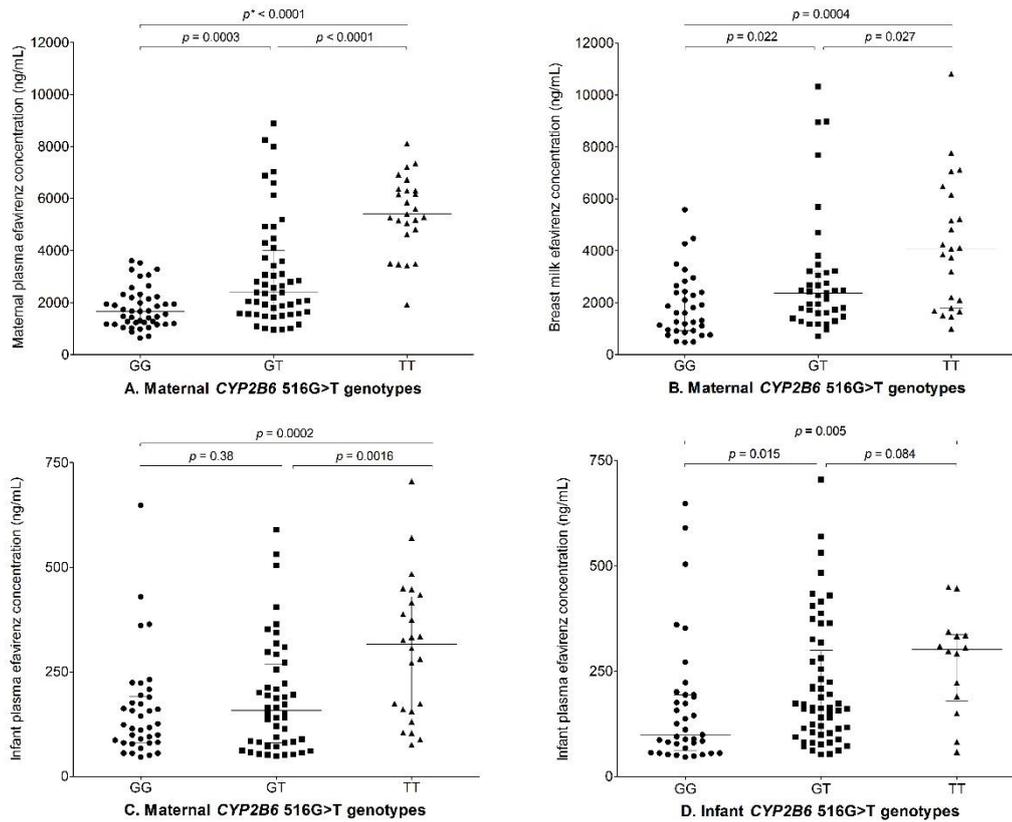
Figure 4.2. Associations between maternal *CYP2B6* 516G>T genotype (GG, 42; GT, 52, and TT, 23) and EFV concentrations in maternal plasma (A), breast milk (B), infant plasma (C), and infant *CYP2B6* 516G>T genotype (GG, 45; GT, 67; and TT, 18) and EFV concentrations in infant plasma (D). Bars represent median (IQR) and *p* values are for Mann-Whitney U test.

4.3.3 Full Pharmacokinetic Profiles of EFV in Plasma and Breast Milk

EFV concentration-time profiles in maternal plasma and breast milk in the entire population and in *CYP2B6* 516GG, *CYP2B6* 516GT, and *CYP2B6* 516TT groups are presented in **Figure 4.4**. The corresponding pharmacokinetic parameters are presented in **Table 4.3**. In pooled analysis, median (range) pharmacokinetic parameters in maternal plasma versus breast milk were: AUC_{0-24}, 60700 ng.h/mL (26800-177000) versus 68500 ng.h/mL (26300-257000); C_{max}, 4630 ng/mL (2050-9760) versus 5390 ng/mL (1430-18400); and C_{min}, 2030 ng/mL (755-6740) versus 1680 ng/mL (316-9570). Wide intra- and inter-individual variability in the M/P ratio during the dosing interval were observed. The time averaged M/P concentration ratio was
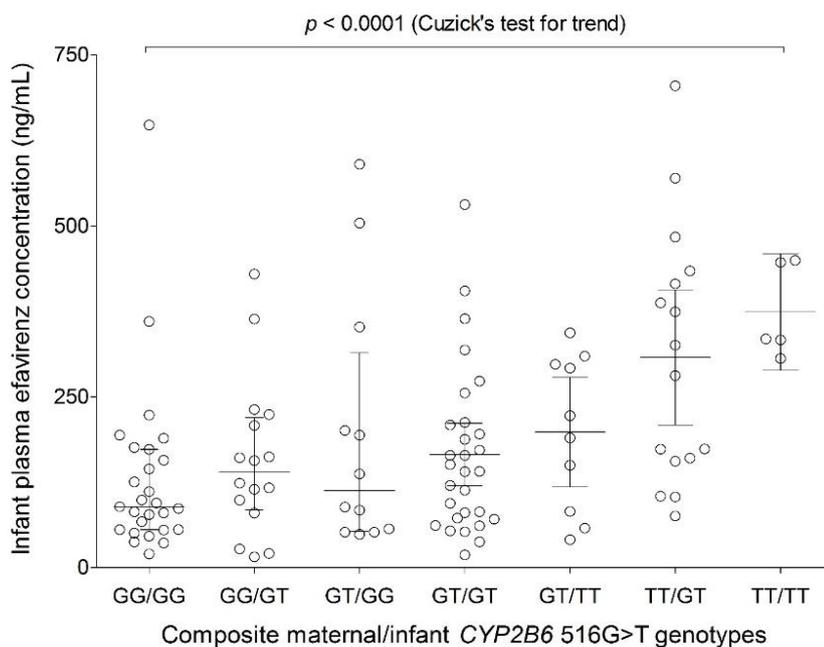
Figure 4.3. Effect of composite maternal/infant *CYP2B6* 516G>T genotype on infant plasma EFV concentration (median, IQR). A significant trend towards increasing infant plasma EFV concentration from GG/GG to TT/TT composite maternal/infant *CYP2B6* 516G>T genotype was observed.

1.10 (0.57-1.71) and M/P AUC_{0-24} ratio was 1.13 (0.50-1.93) (**Table 4.3; Figure 4.4**). EFV pharmacokinetic parameters in plasma and breast milk differed significantly between patients stratified by *CYP2B6* 516G>T genotypes (p <0.05). As with plasma, EFV breast milk AUC_{0-24}, C_{max} and C_{min} were significantly lower in nursing mothers with *CYP2B6* 516GG compared with *CYP2B6* 516GT or *CYP2B6* 516TT genotypes (**Table 4.3; Figure 4.4**). The study was sufficiently powered to detect differences between GG vs TT (100% power) and GT vs TT (99.9% power), but not between GG vs GT groups (26% power).

4.3.4 Breastfed Infants' Exposure to EFV from Breast Milk

The average infant EFV dose from breast milk, calculated using AUC_{0-24}-derived average milk concentration during the dosing interval and 150 mL/kg/day as average milk intake, was 428 µg/kg/day (164-1610). Using EFV breast milk C_{max} as the maximum breast milk concentration,

Table 4.3 EFV pharmacokinetic parameters (median, range) in plasma and breast milk and breastfed infants' exposure.

Preliminary phase pharmacokinetic data	Pooled (n = 117)	*CYP2B6* 516GG (n = 42)	*CYP2B6* 516GT (n = 52)	*CYP2B6* 516TT (n = 23)
Maternal plasma EFV conc. (ng/mL)	2310 (632-8880)	1660 (632-3610)	2390 (951-8880)	5400 (1920-8110)
Breast milk EFV conc. (ng/mL)	2280 (475-10800)	1610 (475-5580)	2370 (713-10300)	4070 (995-10800)
Infant plasma EFV conc. (ng/mL)	173 (46.0-4630)	124 (46.0-4630)	164 (48.5-2150)	333 (75.5-1590)
Intensive pharmacokinetic parameters	**n = 29**	**n = 10**	**n = 11**	**n = 8**
Plasma				
Cl/F (L/h)[a]	9.89 (3.39-22.4)	12.2 (9.37-22.4)	10.0 (4.71-20.7)	4.64 (3.39-5.35)
AUC_{0-24} (ng.h/mL)[a]	60700 (26800-177000)	49400 (26800-64000)	59700 (29000-128000)	130000 (112000-177000)
C_{max} (ng/mL)[a]	4630 (2050-9760)	3220 (2310-4630)	4750 (2050-6780)	6940 (5560-9760)
C_{min} (ng/mL)[a]	2030 (755-6740)	1640 (861-2310)	1580 (755-4860)	5150 (3830-6740)
Breast milk				
AUC_{0-24} (ng.h/mL)[b]	68500 (26300-257000)	55000 (29200-105000)	60600 (26300-206000)	105000 (68100-257000)
C_{max} (ng/mL)[c]	5390 (1430-18400)	4020 (2400-8450)	4540 (1430-9220)	8920 (5810-18400)
C_{min} (ng/mL)[d]	1680 (316-9570)	1120 (534-2430)	1500 (316-6070)	2480 (1500-9570)
Time averaged M/P conc. ratio	1.10 (0.57-1.71)	1.22 (0.61-1.71)	1.08 (0.57-1.57)	0.98 (0.59-1.66)
M/P AUC_{0-24} ratio	1.13 (0.50-1.93)	1.23 (0.71-1.93)	1.18 (0.73-1.73)	0.95 (0.57-1.66)
Infant exposure				
Average infant EFV dose (µg/kg/day)	428 (164-1610)	344 (182-656)	379 (164-1290)	656 (426-1610)
Maximum infant EFV dose (µg/kg/day)	809 (215-2760)	603 (360-1270)	681 (215-1380)	1340 (872-2760)
Average $EI_{paediatric}$[e] (%)	2.14 (0.82-8.05)	1.72 (0.91-3.28)	1.90 (0.82-6.45)	3.28 (2.13-8.05)
Maximum $EI_{paediatric}$[e] (%)	4.05 (1.08-13.8)	3.02 (1.80-6.35)	3.41 (1.08-6.90)	6.70 (4.36-13.8)
$EI_{maternal}$[e] (%)	7.69 (2.04-26.2)	5.73 (3.42-12.1)	6.47 (2.04-13.1)	12.7 (8.28-26.2)
Infant plasma EFV conc. 1[f] (ng/mL)	173 (27.2-1590)	166.10 (27.3-208)	88.53 (37.7-273)	293 (103-1590)
Infant plasma EFV conc. 2[f] (ng/mL)	146 (29.9-1130)	133.54 (30.0-223)	85.83 (45.2-313)	342 (113-1130)

Table 4.3 continued

^{a}, ^{b} and ^{c}: Significant differences between genotype groups (Kruskal-Wallis test) at 0.001, 0.05, 0.01 and levels, respectively; ^{d}: no significant difference between genotype groups (Kruskal-Wallis test); ^{e}: EI$_{paediatric}$ and EI$_{maternal}$ represent paediatric and maternal dose weight-adjusted exposure indices, respectively (average EI$_{paediatric}$ was calculated by replacing InfantDose$_{max}$ with InfantDose$_{avg}$ in equation 4.3); ^{f}: Infant plasma EFV conc. 1 and 2 represent infant plasma concentrations 2 h and 8 h after maternal dose, respectively.
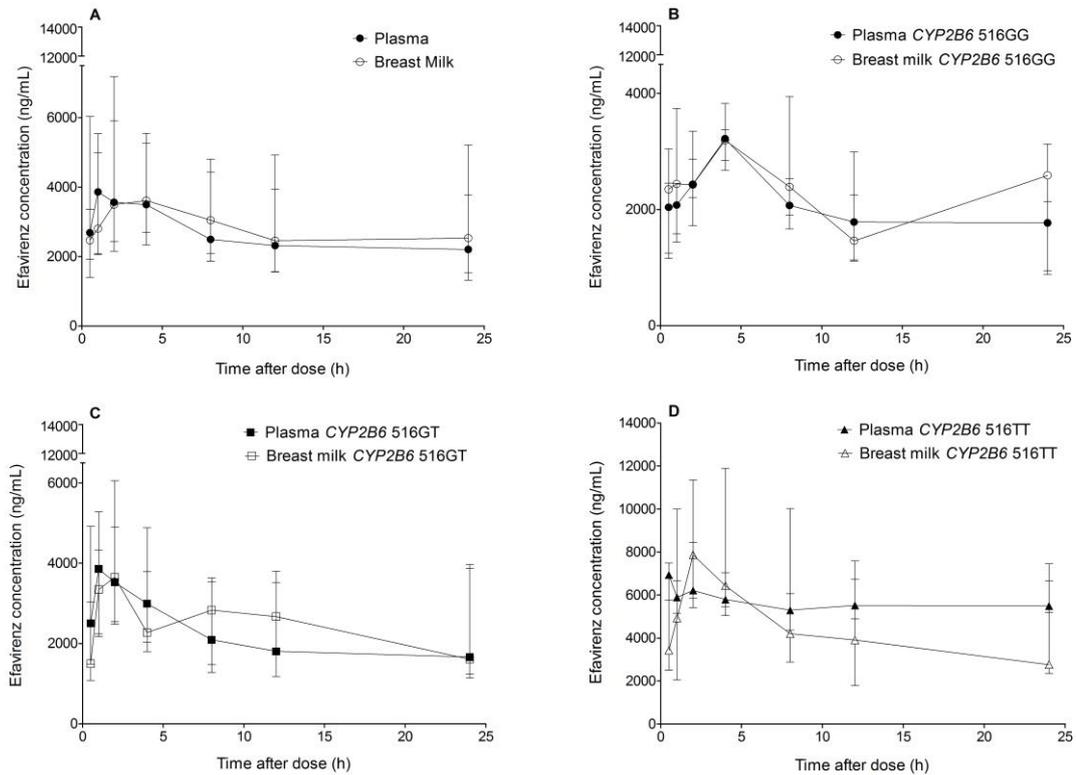
Figure 4.4. EFV concentration-time profiles in plasma and breast milk of nursing mothers: in pooled analysis (n = 29) (A); with *CYP2B6* GG genotype (n = 10) (B); with *CYP2B6* 516GT genotype (n = 11) (C); and with *CYP2B6* 516TT genotype (n = 8) (D). Values are plotted as median (IQR). Details are presented in **Table 4.3**.

the maximum EFV dose from breast milk was 809 µg/kg/day (215-2760). In pooled analysis, paediatric dose (for children aged ≥ 3 months and weighing ≥ 3.5 kg)[300] weight-adjusted exposure index was 4.05% (1.08-13.8) and maternal dose weight-adjusted exposure index was 7.69% (2.04-26.2). When stratified by maternal *CYP2B6* 516G>T, paediatric and maternal dose weight-adjusted exposure indices were highest in *CYP2B6* 516TT group with values of 6.7 (4.36-13.8) and 12.7 (8.28-26.2) respectively. Infant plasma concentrations did not change significantly during the dosing interval, with no significant differences (*p* > 0.05) between concentrations at 2 h and 8 h after maternal EFV dose, with an average of 157 ng/mL (28.6-1360), highest in infants of *CYP2B6* 516TT mothers with a value of 315 ng/mL (108-1360) (**Table 4.3; Figure 4.5**). Infant EFV concentration decreased from 1590 ng/mL (190-4631) in 2-8 days
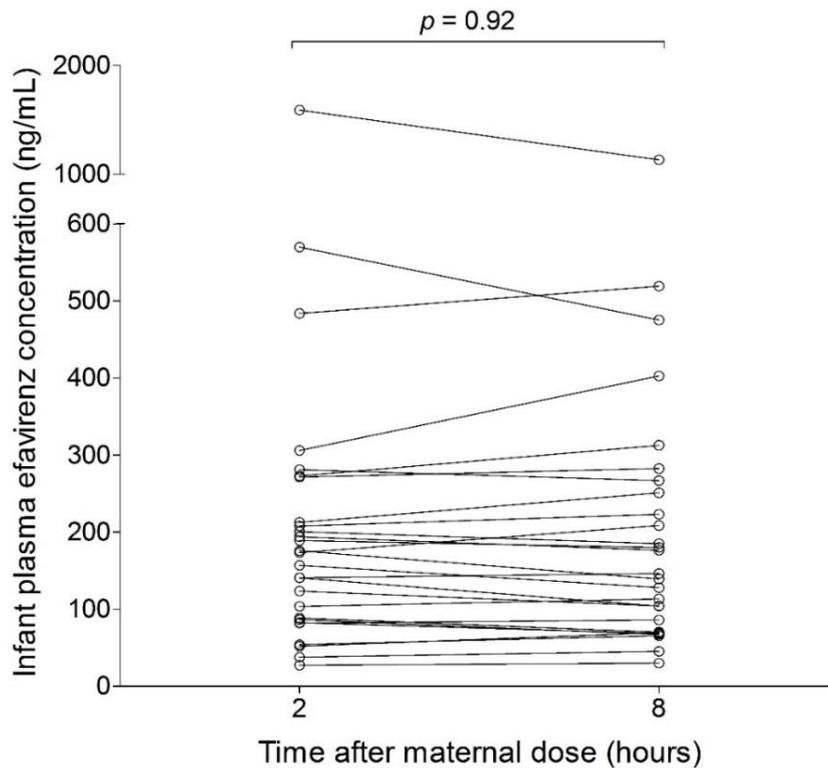
Figure 4.5. Infants' plasma EFV concentrations during dosing interval. In pooled analysis and when stratified based on maternal genotype, there were no significant differences in infant plasma EFV concentrations at 2 h and 8 h after maternal dose.

old infants, to 194 ng/mL (51.9-705) in 9 days-3 months old, 149 ng/mL (51.8-325) in > 3-6 months old, and 102 ng/mL (40.8-590) in > 6 months old. All infants in this cohort achieved EFV concentrations above the IC_{50} of 0.51ng/mL for wild-type HIV-1 in protein-free medium, and more than 75% achieved greater than 100 x IC_{50}.[305] About 96% (129/134) achieved the protein binding-corrected IC_{50} of 36 ng/mL reported by Acosta et al.,[306] but only 57% (76/134) achieved the corresponding IC_{95} of 126 ng/mL.

4.4 DISCUSSION

To our knowledge, this is the first study describing EFV pharmacokinetics in human breast milk during the entire dosing interval in genetically-defined subgroups of nursing mothers. This approach highlights possible worst case scenarios of infant exposure to drugs from breast milk

in terms of maternal drug metabolism capacity and infant feeding time. It is also the largest study of EFV excretion into human breast milk and transfer to breastfed infants. Findings indicated that maternal *CYP2B6* 516G>T was associated with EFV concentrations in maternal plasma, breast milk and infant plasma. With a paediatric dose weight-adjusted exposure index of 4.05% (1.08-13.8), the overall infant exposure to maternal EFV from breast milk was relatively low, highest in infants of mothers with the *CYP2B6* 516TT genotype at 6.7% (4.36-13.8). The infant plasma EFV concentrations also varied depending on maternal and infant *CYP2B6* 516G>T genotypes, suggesting a role for *CYP2B6* SNPs in EFV disposition in neonates and supporting a recent FDA recommendation for *CYP2B6* 516G>T-guided dosing of EFV in children between 3 months and 3 years old.[300] CYP2B6 is known to be expressed in infants and to contribute to EFV pharmacokinetic variability in children.[307,308] The relatively high infant plasma EFV concentrations in the early neonatal period which rapidly declined after day 8 is consistent with observations of in utero exposure to EFV and persistence in plasma up to day 7 after delivery.[113,269] Significant increases in the expression of CYP2B6 after the neonatal period may also play a role.[307]

The EFV dose below which there is no clinically significant effect in infants is unknown. An exposure index of no more than 10% weight-adjusted therapeutic paediatric dose has been proposed as a safety threshold for infant exposure to maternal drugs from breast milk.[85] There was no report of drug-related adverse events in any infants in the present study, suggesting EFV ingestion through breast milk is unlikely to result in toxicity. However, CNS disturbance cannot be ruled out as it is impossible to diagnose in infants. Additionally, exposure to subtherapeutic EFV concentrations through breast milk raises concerns about potential development of resistance in the rare event of PMTCT failure as reported for other drugs.[309,310] About 96% and 57% of infants achieved plasma EFV concentrations above the published protein binding-corrected IC_{50} and IC_{95}, respectively, for wild-type HIV-1.[306]

Studies in chronically infected patients have demonstrated limited compartmentalisation and clonal amplification of functional HIV-1 in human breast milk, suggesting ongoing blood-to-breast milk seeding of virus, followed by transient local replication in breast milk.[311,312] Therefore, therapeutic concentrations of EFV in breast milk may play an important role in preventing ongoing replication of HIV in mammary glands and development of resistance,[87] which may otherwise be passed to infants if PMTCT fails.[88,313] These findings support recent updates in treatment guidelines recommending the use of EFV-based ART started during pregnancy and continued until breastfeeding ends (Option B) or for life (Option B+) for PMTCT.[13]

These findings should be interpreted in the context of certain limitations. The study was not adequately powered to detect differences in pharmacokinetic parameters between the GG (n = 10) vs GT (n = 11) groups; only 26% statistical power was achieved. Although we observed higher EFV concentrations in 22% of the infants receiving NVP PEP (data not shown), we are unable to make firm conclusions about the interaction since PEP is given to younger infants who also have residual EFV from intrauterine exposure. As a result of practical challenges associated with plasma collection in resource-limited settings, samples were collected as DBS. However, the negative bias associated with EFV quantification in DBS was adequately compensated using a validated method correcting for patient haematocrit and protein binding.[259] Dried breast milk spot allowed for EFV quantification in whole milk,[242] a major difficulty associated with liquid milk.[91]

In conclusion, most breastfed infants are exposed to less than 10% of weight-adjusted licensed paediatric dose of EFV through breast milk. Further studies to monitor the long-term safety, including possible emergence of resistance in infants who may become infected if PMTCT fails, are now warranted.

CITATIONS

Olagunju A, Bolaji O, Amara A, Waitt C, Else L, Adejuyigbe E, Siccardi M, Back D, Khoo S, Owen A. Breast milk pharmacokinetics of efavirenz and breastfed infants' exposure in genetically-defined subgroups of mother-infant pairs: an observational study. *Clinical Infectious Diseases*. 2015; 61(3):453-63.

Olagunju A, Bolaji O, Amara A, Waitt C, Else L, Soyinka J, Adeagbo B, Adejuyigbe E, Siccardi M, Back D, Owen A, Khoo S. Development, validation and clinical application of a novel method for the quantification of efavirenz in dried breast milk spots using LC-MS/MS. *Journal of Antimicrobial Chemotherapy*. 2015; 70(2):555-61.

CHAPTER 5

PHARMACOGENETICS OF NEVIRAPINE EXCRETION INTO BREAST MILK AND INFANTS' EXPOSURE THROUGH BREAST MILK VERSUS PROPHYLAXIS

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5.1 INTRODUCTION

The risk of HIV transmission through breast milk was first recognised following case reports of HIV diagnosis in breastfed infants of women who were infected postnatally through blood transfusion or heterosexual exposure.[314-319] In additional to intrauterine and intrapartum transmissions, breastfeeding is estimated to account for 15% (95% CI: 7-22) additional risk of transmission in women with established infection.[320] Postpartum maternal HIV infection is associated with higher risk of breast milk transmission, estimated at 29% (16-42). HIV is present in breast milk[321] and findings from several animal studies have suggested potential transmission mechanisms, including intestinal mucosal cell infection by cell-free HIV,[322] transepithelial transport by intestinal M cells,[323] and transcytosis across the intact epithelial barrier.[324] Although avoidance of breastfeeding is an effective strategy to prevent postpartum MTCT, it is precluded by lack of access to safe and nutritionally adequate alternatives in resource-limited countries with the highest burden of the disease. Evidence from numerous clinical trials has shown that exclusive breastfeeding for the first six months of life along with maternal and infant antiretroviral therapy reduces MTCT to less than 2%.

One of the early PMTCT interventions involved intrapartum single dose of 200 mg NVP in combination with zidovudine monotherapy and a single 2 mg/kg infant dose within 72 hours of birth. Although this strategy significantly reduced MTCT rates,[17] about 35% of women and 53% of infants developed slow-clearing resistant virus, compromising future NVP-based treatment options in infants who may become infected.[313,325] Therefore, current guidelines recommend longer term or lifelong maternal ART (Option B or B+) started before or during pregnancy, or as soon as possible after postpartum diagnosis in combination with daily infant NVP post-exposure prophylaxis (PEP) for up to 6 weeks of age.[13] As previously highlighted, NVP is an important component of first-line ART regimens (as an alternative to efavirenz) and it is widely used in women with established infection or those initiating treatment postpartum.[13] In addition to further reducing MTCT rates, ART prevents the development and clonal amplification of NNRTI-

resistant HIV variants in systemic and mammary gland compartments observed with the use of intrapartum single dose NVP.[326,327]

Maternal drug use inevitably results in breastfed infant drug exposure. However, the level of exposure is within the recommended (albeit arbitrary) limit of 10% paediatric or maternal therapeutic dose for most drugs. Occasionally, serious adverse drug reactions and fatality have resulted from infant exposure to maternal drugs through breast milk. For instance, the use of codeine by nursing mothers has been associated with potentially fatal CNS depression in breastfed infants.[328,329] The mechanism involves the accumulation of its active metabolite, morphine, in mothers with the *CYP2D6* ultra-rapid genotype. In addition to the potential for adverse drug reaction (ADR) if infants are exposed to sufficiently high levels of ARVs through breast milk, exposure to subtherapeutic drug concentrations creates a selective pressure for the emergence of drug resistant virus. The concentration of NVP in breast milk and plasma of breastfed infants have been reported in several studies.[89] However, infant exposure to maternal NVP through breast milk were confounded with additional exposure from infant PEP.[89] In addition, samples were collected only at a single time-point during the dosing interval, limiting their utility in assessing potential risks.[85] A recently described method for the quantification of NVP in dried blood spots (DBS) and dried breast milk spots (DBMS) has opened up more opportunities for conducting clinical pharmacokinetic studies in nursing mother-infant pairs.[242]

For drugs with significant genetic contribution to observed inter-individual variability in plasma pharmacokinetics, potential variations in breast milk pharmacokinetics and infant exposure attributable to host genetics warrant investigation.[247] NVP is predominantly metabolised by CYP2B6 and CYP3A4, and is a substrate for the efflux transporter ABCC10. Genetic variations in NVP disposition genes are known to affect its pharmacokinetics, including *CYP2B6* 516G>T and 983T>C, and *ABCC10* rs2125739.[36,174,330] However, their effects on NVP breast milk pharmacokinetics and breastfed infant exposure have not been investigated.

The present study assessed breast milk pharmacokinetics of NVP during an entire dosing interval as well as genetically-driven differences between mother-infant groups. Infant plasma NVP concentrations were compared between infants exposed through breast milk alone, PEP alone, and combined breast milk and PEP.

5.2 METHODS

5.2.1 Patients

This was part of an observational study to evaluate the effects of genetic factors on the pharmacokinetics of antiretroviral drugs during pregnancy and lactation (Clinicaltrials.gov ID: NCT02269462). As previously described,[283,331] HIV positive nursing mothers and their breastfed infants were recruited from three hospitals in Benue State, Nigeria: Bishop Murray Medical Centre, Makurdi; St Mary's Hospital, Okpoga; and St Monica's Hospital, Adikpo. Written informed consent was obtained and eligibility was ascertained by examining case notes and conducting a brief interview in English and the local Tiv language. In this sub-study, HIV positive nursing mothers who started NVP-containing regimen before or during pregnancy and continued during breastfeeding were included. Exclusion criteria were assessed at study enrolment and included exclusive formula feeding, mixed feeding in infants less than 6 months old, opportunistic infections (e.g. tuberculosis, pneumonia), severe maternal or infant illness, and maternal or infant treatment with drugs or herbal medication with known or uncertain interaction with NVP. Ethical approval was obtained from the National Health Research Ethics Committee (NHREC), Abuja, Nigeria.

5.2.2 Study Design and Samples Collection

The purpose of the preliminary phase was to explore associations between SNPs in NVP disposition genes and its breast milk and plasma concentrations in an unselected cohort of nursing mother-infant pairs. For this, paired DBS and DBMS samples were collected from

mothers and infants at a single, recorded time point after maternal dose of regimen containing 200 mg NVP. In the intensive pharmacokinetic phase, differences in plasma and breast milk pharmacokinetic parameters and measures of breastfed infants' exposure was investigated in mother-infant pairs following genotype-guided stratification. For this, maternal DBS and DBMS were collected at 0.5, 1, 2, 4, 8, and 12 hours after an observed dose of regimen containing 200 mg NVP and stored as previously described.[242] DBS samples were collected from infants at 2 h and 8 h after maternal dose. To reflect real-life situations, infant feeding times were not controlled and all infants were breastfed on demand. In addition, mothers took standard local meals about 30 min before drug administration. Samples were shipped at ambient temperature to the University of Liverpool, UK, for analysis.

5.2.3 SNP Genotyping

E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) was used to extract genomic DNA from DBS. Extracted DNA was quantified using NanoDrop® (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and stored at -20°C until analysis. Genotyping was conducted as previously described.[283] TaqMan® Genotyping Master Mix and assays for *CYP3A4* - 392A>G (*1B; rs2740574; ID: C_1837671_50), *CYP3A4* 20230G>A (*1G; rs2242480, ID: C_26201900_30), *CYP2B6* 516G>T (rs3745274; ID: C_7817765_60), *CYP2B6 983T>C* (rs28399499; ID: C_60732328_20), *NR1I3* c.540C>T (rs2307424; ID: C_25746794_20), *NR1I3* c.152-1089T>C (rs3003596; ID: C_16194070_10), *NR1I2* 63396C>T (rs2472677; ID: C_26079845_10), *POR* 1508C>T (*28; rs1057868; ID: C_8890131_30), and *ABCC10* 2843T>C (rs2125739; ID: C_16173668_10) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). Allelic discrimination plots and genotype assignments were obtained using Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Compliance with Hardy Weinberg Equilibrium was evaluated as previously described.[260]

5.2.4 NVP Quantification and Pharmacokinetic Analysis

NVP in DBS and DBMS was quantified as previously described using a validated LC-MS/MS method.[242] Plasma concentrations were determined using $[DBS_{[NVP]}/(1-HCT)]*0.6$, where $DBS_{[NVP]}$ is NVP concentration in DBS, HCT is the patient-specific haematocrit and 0.6 is the fraction of NVP bound to plasma protein.[259] Minimum (C_{min}) and maximum (C_{max}) plasma concentrations were determined by direct inspection. The area under the concentration-time curve during the dosing interval (AUC_{0-12}) was calculated using the trapezoidal rule and the apparent clearance (CL/F) was calculated by dividing the dose by AUC_{0-12}. Infant NVP dose from breast milk and exposure indices (infant PEP, infant treatment, and maternal doses) were calculated using equations described in Chapter 4.[331]

5.2.5 Statistical Analysis

Pearson correlation was used to test relationships between continuous variables and univariate linear regression analysis was conducted to identify variables associated with breast milk and plasma NVP concentrations. Independent variables with $P \leq 0.2$ in the univariate analysis were included in a multivariate stepwise linear regression analysis. Bonferroni correction was used to adjust for multiple testing. Differences in pharmacokinetic parameters between patient groups were investigated using Mann-Whitney U test. All other statistical analyses were conducted using IBM ®SPSS® Statistics version 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism® (GraphPad Software, Inc., La Jolla, CA, USA). All charts were plotted using GraphPad Prism® 5. The previously suggested minimum effective concentration of 3400 ng/mL[261] was set as the C_{min} target.

5.3 RESULTS

5.3.1 Patients' Characteristics

A total of 122 eligible HIV positive nursing mothers and their breastfed infants were recruited between December 2012 and October 2013 (**Table 5.1**). The median (range) duration on current NVP-containing regimen was 28.6 months (1.4-79.1), starting at a baseline CD4 count of 241 c/mm^3 (31-1015). NVP was taken in combination with emtricitabine and tenofovir disoproxil fumarate (49%), lamivudine and zidovudine (41%), or lamivudine and tenofovir disoproxil fumarate (10%). Genotype frequencies are presented in **Table 5.1**; all nine SNPs were in Hardy Weinberg equilibrium. Infants were 19.4 weeks (1.4-73.9) old and 50% (61/122) were receiving NVP PEP at doses determined by their age and weight in line with current guidelines (**Table 5.1**).

5.3.2 Pharmacogenetics of NVP in Plasma and Breast Milk

NVP concentrations (median, range) in maternal plasma (n = 122), breast milk (n = 65), and infant plasma (n = 93; excluding those receiving NVP PEP) were 5170 ng/mL (1320-15600), 4830 ng/mL (1360-16300), and 660 ng/mL (104-3090), respectively. Very strong to moderate positive correlations in were observed between maternal plasma and breast milk (n = 65), maternal and infant plasma (n = 93), and breast milk and infant plasma (n = 53; **Figure 5.1**).

Of the nine SNPs investigated, only *CYP2B6* 516 G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) were independently associated with higher NVP concentrations in maternal plasma and breast milk (**Table 5.2**). *CYP2B6* 983T>C which was included in the multivariate analysis for plasma concentration despite *P* > 0.2 in univariate analysis because of numerous literature supporting its role. Therefore, mother-infant pairs were stratified based on composite maternal *CYP2B6* 516 G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) genotypes for the intensive pharmacokinetic phase. *POR* 1508C>T (*28; rs1057868) and *NR1I3* 152-1089T>C (rs3003596) were associated with higher plasma and lower breast milk NVP concentrations, respectively; the former was lost after Bonferroni correction (**Table 5.2**).

Table 5.1 Characteristics of nursing mother-infant pairs.

Mothers (n = 122)	
Age (years)	30 (20-40)
Weight (kg)	57 (35-109)
Infants (n = 122)	
Age (weeks)	19.4 (1.4-73.9)
Weight (kg)	6.0 (2.4-10.0)
Gender (% Female)	54 (66/122)
Receiving NVP PEP (%)	23.8 (29/122)
Maternal drug regimen and CD4 count[a]	
FTC/TDF/NVP (%)	49
3TC/AZT/NVP (%)	41
3TC/TDF/NVP (%)	10
Time post-dose (h)	4.0 (1.25-8.25)
Duration on regimen (months)	28.6 (1.4-79.1)
Baseline CD4 count (c/mm^3)	241 (31-1015)
CD4 change (c/mm^3)	351 (45-1108)
Maternal genotype frequency	
CYP3A4 -392A>G (*1B; rs2740574)	CC, 0.62; CT, 0.35; TT, 0.03
CYP3A4 20230G>A (*1G; rs2242480)	TT, 0.73; CT, 0.18; CC, 0.09
CYP2B6 516G>T (rs3745274)	GG, 0.36; GT, 0.43; TT, 0.21
CYP2B6 983T>C (rs28399499)	TT, 0.81; CT, 0.18, CC, 0.01
NR1I3 540C>T (rs2307424)	CC, 0.81; CT, 0.19; TT, 0.00
NR1I3 152-1089T>C (rs3003596)	TT, 0.20; CT, 0.54; CC, 0.26
NR1I2 63396C>T (rs2472677)	CC, 0.35; CT, 0.54; TT, 0.11
POR 1508C>T (*28; rs1057868)	CC, 0.72; CT, 0.27; TT, 0.01
ABCC10 2843T>C (rs2125739)	TT, 0.50; CT, 0.36; CC, 0.14

[a]Unless otherwise indicated, values are expressed as median (range). Abbreviations: 3TC, lamivudine; AZT, zidovudine; FTC, emtricitabine; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

Significant variations in breast milk NVP concentrations were observed between women in the three groups: 3670 ng/mL (2330-5240) in *CYP2B6* 516GG & 983TT (n = 12); 4830 ng/mL (1360-10500) in *CYP2B6* 516GT or 983TC (n = 33); and 6270 ng/mL (3350-16300) in *CYP2B6* 516GT & 983TC or 516TT or 983CC (n = 16). The corresponding maternal plasma concentrations were 4360 ng/mL (1870-9880) (n = 25), 5250 ng/mL (1320-12300) (n = 61), and 6250 ng/mL (1950-15600) (n = 31), respectively (**Figure 5.2**).
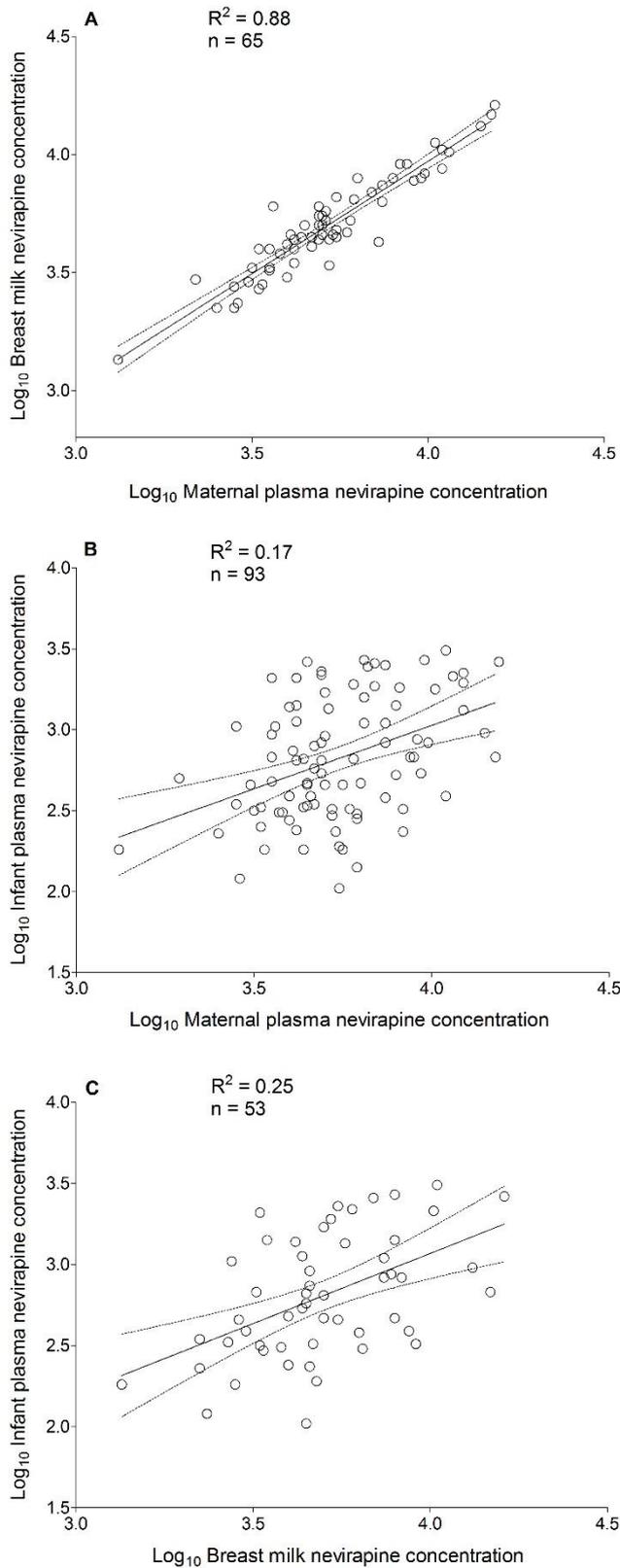
Figure 5.1 Correlations between NVP concentrations in maternal plasma and breast milk (A); maternal plasma and infant plasma (B); and breast milk and infant plasma (C). Solid lines represent mean values and broken lines represent 95% confidence intervals.

Table 5.2 Linear regression analysis showing associations of patient characteristics with $\log_{10}$ NVP concentrations in breast milk and plasma.

Patient characteristics	Univariate linear regression β^a ($\log_{10}$ NVP conc., 95% CI)	p value	Multivariate linear regression β^a ($\log_{10}$ NVP conc., 95% CI)	p value
Maternal plasma (n = 122)				
Maternal weight (kg)	-0.001 (-0.005, 0.003)	0.58		
Maternal age (y)	0.003 (-0.009, 0.016)	0.59		
Infant age (months)	-0.002 (-0.019, 0.014)	0.77		
Time after maternal dose (h)	0.010 (-0.027, 0.046)	0.60		
CYP3A4 -392A>G (*1B; rs2740574)	-0.005 (-0.070, 0.060)	0.88		
CYP3A4 20230G>A (*1G; rs2242480)	0.00 (-0.054, 0.054)	0.99		
CYP2B6 516G>T (rs3745274)	0.072 (0.024, 0.12)	*0.004*	0.10 (0.053, 0.16)	*1.1 x 10^{-4}*
CYP2B6 983T>C (rs28399499)	0.045 (-0.052, 0.14)	0.36	0.11 (0.015, 0.21)	0.024^c
NR1I3 540C>T (rs2307424)	0.035 (-0.057, 0.13)	0.45		
NR1I3 152-1089T>C (rs3003596)	-0.036 (-0.090, 0.017)	0.18		
NR1I2 63396C>T (rs2472677)	-0.019 (-0.076, 0.038)	0.50		
POR 1508C>T (*28; rs1057868)	0.075 (-0.001, 0.15)	0.054	0.10 (0.026, 0.17)	0.008^c
ABCC10 2843T>C (rs2125739)	0.005 (-0.048, 0.058)	0.84		
Breast milk (n = 65)^b				
Maternal weight (kg)	0.001 (-0.003, 0.004)	0.77		
Maternal age (y)	0.005 (-0.008, 0.018)	0.43		
Infant age (months)	-0.011 (-0.028, 0.005)	0.17	-0.015 (-0.029, 0.00)	0.049^c
Time after maternal dose (h)	0.027 (-0.009, 0.064)	0.14		
CYP3A4 -392A>G (*1B; rs2740574)	-0.010 (-0.11, 0.094)	0.85		
CYP3A4 20230G>A (*1G; rs2242480)	0.02 (-0.050, 0.089)	0.57		
CYP2B6 516G>T (rs3745274)	0.089 (0.020, 0.16)	0.013	0.16 (0.084, 0.23)	*6.0 x 10^{-5}*
CYP2B6 983T>C (rs28399499)	0.11 (-0.030, 0.25)	0.12	0.18 (0.042, 0.31)	0.011^c

Table 5.2 continued

Patient characteristics	Univariate linear regression		Multivariate linear regression	
	β^a (log$_{10}$ NVP conc., 95% CI)	*p* value	β^a (log$_{10}$ NVP conc., 95% CI)	*p* value
Breast milk (n = 65)^b continued				
NR1I3 540C>T (rs2307424)	0.11 (-0.020, 0.24)	0.095		
NR1I3 152-1089T>C (rs3003596)	-0.077 (-0.15, -0.004)	0.039	-0.11 (-0.18, -0.052)	0.001
NR1I2 63396C>T (rs2472677)	-0.006 (-0.084, 0.073)	0.89		
POR 1508C>T (*28; rs1057868)	0.044 (-0.065, 0.15)	0.42		
ABCC10 2843T>C (rs2125739)	0.020 (-0.059, 0.10)	0.61		
Infant plasma (n = 93)^b				
Infant weight (kg)	-0.054 (-0.11, 0.00)	0.05		
Maternal age (y)	-0.024 (-0.049, 0.00)	0.054		
Infant age (months)	-0.078 (-0.010, -0.054)	2.6 x 10^{-9}	-0.05 (-0.079, -0.02)	0.001
Time after maternal dose (h)	0.083 (-0.008, 0.174)	0.071		
CYP3A4 -392A>G (*1B; rs2740574)	0.042 (-0.098, 0.181)	0.56		
CYP3A4 20230G>A (*1G; rs2242480)	0.18 (0.077, 0.282)	0.001	0.20 (0.096, 0.305)	3.7 x 10^{-4}
CYP2B6 516G>T (rs3745274)	0.051 (-0.059, 0.161)	0.36		
CYP2B6 983T>C (rs28399499)	0.018 (-0.20, 0.24)	0.87		
NR1I3 540C>T (rs2307424)	0.077 (-0.13, 0.29)	0.47		
NR1I3 152-1089T>C (rs3003596)	-0.071 (-0.19, 0.042)	0.22		
NR1I2 63396C>T (rs2472677)	0.054 (-0.069, 0.18)	0.38		
POR 1508C>T (*28; rs1057868)	0.11 (-0.057, 0.28)	0.19		
ABCC10 2843T>C (rs2125739)	-0.008 (-0.13, 0.11)	0.90		

$^a\beta$ is regression coefficient, which represents incremental change in log$_{10}$ NVP concentration per unit change in a patient characteristic; ^{b}only 65 mothers had paired plasma and breast milk concentrations, and 93 infants remained after excluding those less than 7 weeks with residual intra-uterine and post-exposure prophylaxis exposures; ^{c}Bonferroni correction *p* value > 0.05.
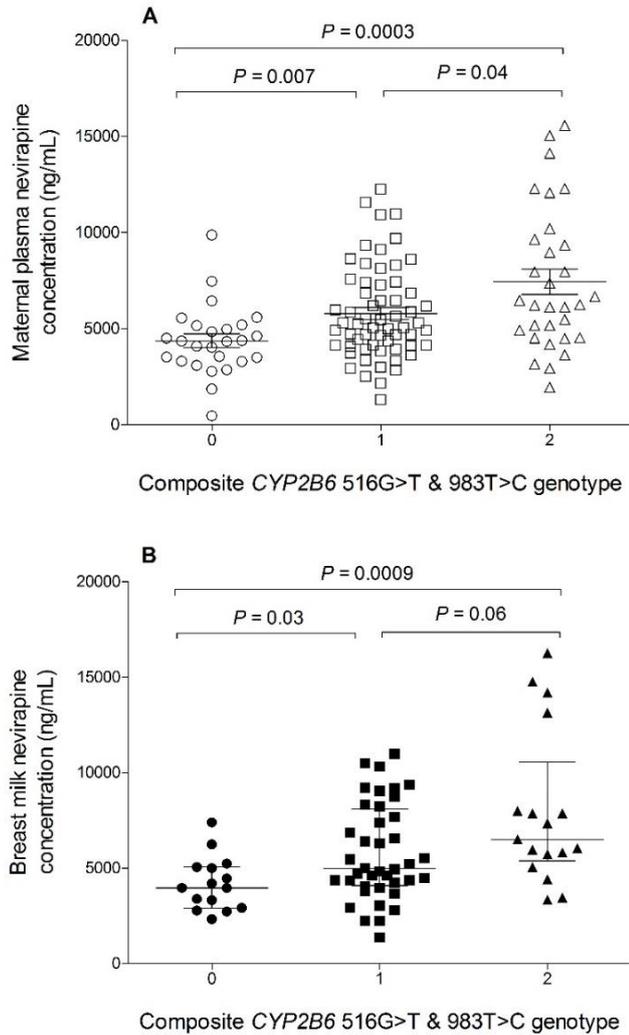
Figure 5.2 Effects of composite maternal *CYP2B6* 516G>T and 983T>C genotypes on NVP concentrations in maternal plasma (A) and breast milk (B). Bars represent median and interquartile range (IQR) and p values are for Mann-Whitney U test.

5.3.3 Intensive Pharmacokinetics of NVP in Breast Milk

The concentration-time profiles of NVP in maternal plasma and breast milk in the entire population and the three composite *CYP2B6* 516G>T and 983T>C genotype groups are presented in **Figure 5.3** and the corresponding pharmacokinetic parameters are presented in **Table 5.3**. In pooled analysis, median (range) pharmacokinetic parameters in maternal plasma versus breast milk were: AUC_{0-24}, 54700 ng.h/mL (24500-172000) versus 56300 ng.h/mL (26500-136000); C_{max}, 5980 ng/mL (3390-17000) versus 5920 ng/mL (3080-15100); and C_{min}, 4380 ng/mL
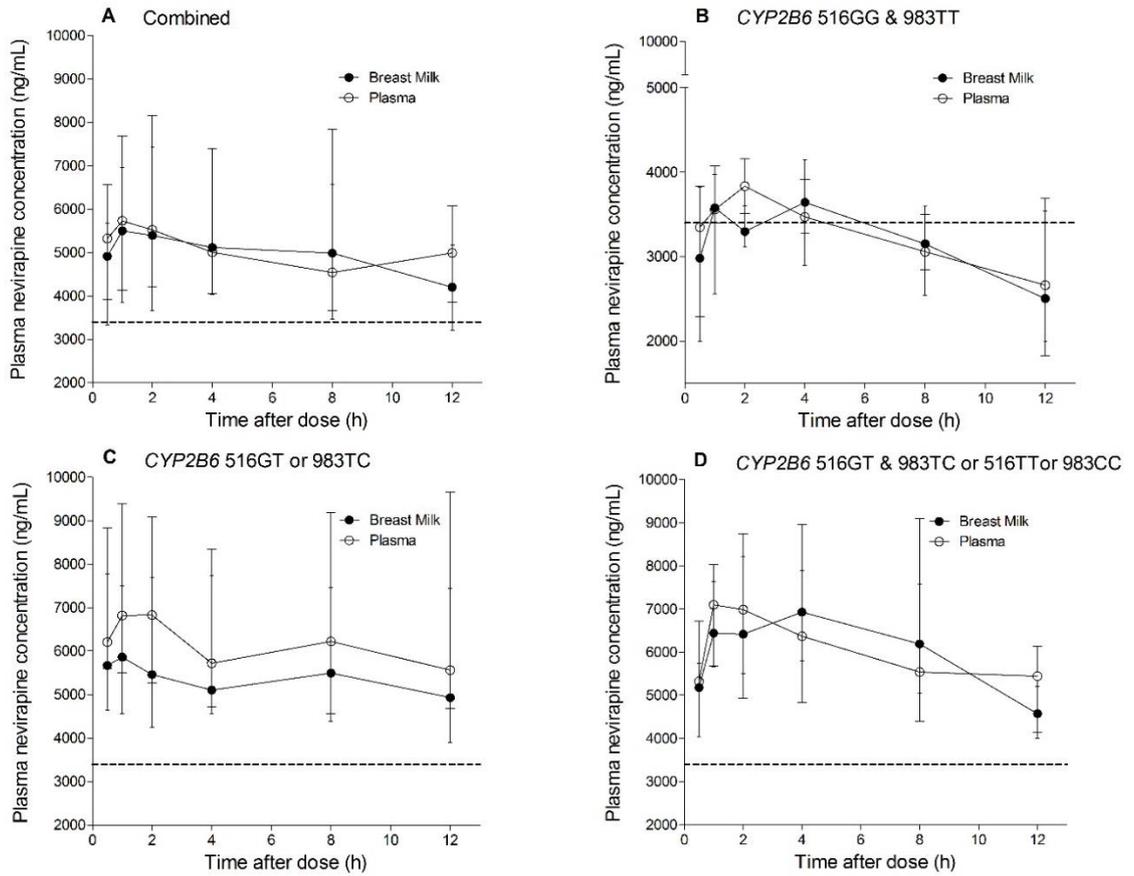
Figure 5.3 NVP concentration-time profiles in plasma and breast milk of nursing mothers: in pooled analysis (n = 28) (A); with no variant allele (n = 8) (B); one variant allele (n = 10) (C); and two variant alleles (n = 10) (D). Values are plotted as median (IQR). Details in **Table 5.3**.

(1110-13200) versus 4080 ng/mL (1190-9430). The time averaged M/P concentration ratio was 0.88 (0.74-1.2) and M/P AUC_{0-12} ratio was 0.95 (0.56-1.5). NVP AUC_{0-12}, C_{min}, and C_{max} in both plasma and breast milk were significantly lower in patients with composite *CYP2B6* 516GG & 983TT than those with at least one variant allele (Mann-Whitney U test $p < 0.001$) (**Table 5.3; Figure 5.3**). Post hoc analysis showed that there was sufficient power to detect differences in AUC_{0-12} between patients with no variant allele and those with a least one variant allele (≥ 89% power), but not between those with one compared with those with two variant alleles (5.7% power). C_{min} was below the recommended plasma target of 3400 ng/mL in breast milk of 87.5% (7/8) of women with the composite *CYP2B6* 516GG & 983TT genotypes compared with 10% (1/10) in women with at least one variant allele.

Table 5.3 NVP pharmacokinetic parameters (median, range) in breast milk and plasma of nursing mothers and breastfed infants' exposure based on composite maternal *CYP2B6* 516G>T and 983T>C genotypes.

	Pooled (n = 28)	*CYP2B6* 516GG & 983TT (n = 8)	*CYP2B6* 516GT or 983TC (n = 10)	*CYP2B6* 516GT & 983TC or 516TT or 983CC (n = 10)
Plasma pharmacokinetics[a]				
CL/F (L/h)	3.66 (1.16-8.15)	6.25 (3.86-8.15)	3.17 (1.57-4.07)	2.89 (1.16-3.85)
AUC_{0-12} (ng.h/mL)	54700 (24500-172000)	32400 (24500-51800)	63300 (49100-127000)	69800 (51900-172000)
C_{max} (ng/mL)	5980 (3390-17000)	4150 (3390-5700)	7660 (5420-11900)	7280 (5290-17000)
C_{min} (ng/mL)	4380 (1110-13200)	2720 (1110-3610)	5590 (3940-10400)	4900 (3810-13200)
Breast milk pharmacokinetics[a]				
AUC_{0-12} (ng.h/mL)	56300 (26500-136000)	37000 (26500-53400)	56300 (44300-123000)	73900 (39600-136000)
C_{max} (ng/mL)	5920 (3080-15100)	3960 (3080-5000)	6050 (4480-11700)	7360 (4170-15100)
C_{min} (ng/mL)	4080 (1190-9430)	2450 (1190-3920)	4980 (3100-9420)	4580 (3180-9430)
Time averaged M/P conc. ratio	0.88 (0.74-1.2)	0.95 (0.86-1.1)	0.87 (0.80-1.0)	0.91 (0.74-1.2)
M/P AUC_{0-12} ratio	0.95 (0.56-1.5)	1.0 (0.87-1.5)	0.89 (0.56-1.3)	0.96 (0.74-1.2)
Measures of infant exposure				
Average infant NVP dose (µg/kg/day)	704 (331-1700)	463 (331-668)	704 (554-1540)	924 (495-1700)
Maximum infant NVP dose (µg/kg/day)	888 (462-2270)	594 (462-750)	908 (672-1760)	1100 (626-2270)
EI-prophylaxis (%)	26.0 (9.93-79.1)	20.8 (9.93-26.8)	26.7 (17.4-56.9)	32.8 (17.7-79.1)
EI-treatment (%)	3.64 (1.99-9.88)	2.79 (1.99-3.35)	4.67 (2.94-7.11)	4.72 (2.34-9.88)
EI-maternal (%)	13.8 (5.77-27.7)	8.9 (5.8-12.2)	13.9 (9.7-27.6)	16.7 (7.7-27.7)
Infant plasma NVP at 2 h[b] (ng/mL)	1100 (234-4410)	860 (332-2100)	908 (234-2600)	1370 (304-4410)
Infant plasma NVP at 8 h[b] (ng/mL)	1140 (215-3700)	1050 (329-2130)	765 (215-2214)	1280 (305-3700)

Table 5.3 continued

Abbreviations: NVP, nevirapine; AUC_{0-12}, area under the concentration-time curve during the dosing interval; CL/F, apparent clearance; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; EI, exposure index (expressed as percentages of prophylaxis, treatment, and weight-adjusted maternal doses); M/P, milk-to-plasma. [a]NVP plasma and breast milk AUC_{0-12}, C_{max}, and C_{min} were significantly lower in patients with composite *CYP2B6* 516GG & 983TT than those with at least one variant allele (Mann-Whitney U test $p < 0.001$); [b]time after maternal dose (including exposure through post-exposure prophylaxis).

5.3.4 Infants' Exposure to NVP from Breast Milk/Prophylaxis

In combined analysis, the average and maximum NVP doses from breast milk were 704 µg/kg/day (331-1700) and 888 µg/kg/day (462-2270), respectively. Calculated as percentages of paediatric, maternal and PEP weight-adjusted doses, the maximum exposure indices were 3.64% (1.99-9.88), 13.8% (5.77-27.7) and 26.0% (9.93-79.1), respectively. When stratified according to maternal composite *CYP2B6* 516G>T & 983T>C genotypes, NVP dose from breast milk and the corresponding exposure indices were lowest in infants whose mothers had no variant allele (**Table 5.3**).

Plasma NVP concentrations in infants were 1100 ng/mL (234-4410) at 2 hours and 1140 ng/mL (215-3700) at 8 hours after maternal dose in combined analysis (**Figure 5.4A**). The highest plasma concentrations were observed in infants whose mothers had two variant alleles (**Table 5.3**). After excluding infants still taking PEP, maternal *CYP3A4* 20230G>A (*1G; rs2242480) and infant age were independently associated with infant plasma NVP concentration (**Table 5.2**). After excluding infants less than 7 weeks old with residual intra-uterine and PEP exposures, those whose mothers were non-carriers (n = 56), heterozygotes (n = 20), or homozygotes (n = 12) for this SNP had plasma concentrations of 497 ng/mL (141-3090), 851 ng/mL (120-2680), and 1410 ng/mL (673-2600), respectively (**Figure 5.4B**). The association was more pronounced after adjusting for infant age (**Table 5.2**).

Additionally, increasing infant age was associated with decrease in plasma NVP concentration in infants (**Figure 5.4C**). Differences in infant plasma NVP concentrations between those exposed through breast milk alone, PEP alone, and a combination of breast milk and PEP were explored. Breastfed infants with no additional exposure through PEP (n = 65) had plasma concentration of 660 ng/mL (104-3090) compared with 1020 ng/mL (401-3325) in infants taking PEP and whose mothers were on efavirenz-based regimen (n = 10), and 2720 ng/mL (1360-7290) in infants with both breast milk and PEP exposures (**Figure 5.4D**). The median infant age was 19.4 weeks (range:

1.4-73.9) and residual NVP from intra-uterine exposure was considered completely eliminated in most infants by age 7 days.
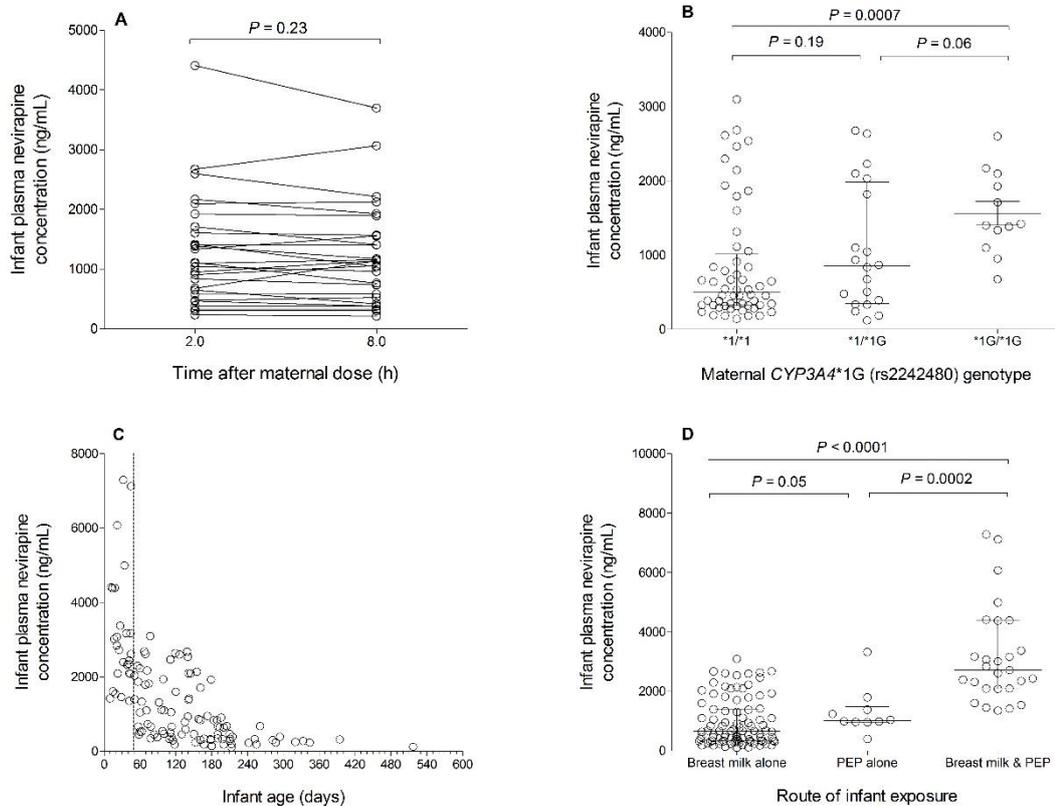
Figure 5.4 Comparison of infant plasma concentrations: at different time-points after maternal dose (A); based on maternal *CYP3A4* 20230G>A (*1G; rs2242480) genotype (B); based on infant age (dashed line is at 49 days when residual intra-uterine and post-exposure prophylaxis exposures are assumed to have been cleared) (C); and between infants exposed through breast milk or post-exposure prophylaxis alone versus both breast milk and post-exposure prophylaxis (D).

5.4   DISCUSSION

The influence of genetic polymorphisms in NVP disposition genes on its breast milk concentrations, its pharmacokinetics in breast milk during an entire dosing interval, and infant exposure through breast milk, PEP, or dual breast milk and PEP have been described for the first time. The *CYP2B6* 516G>T and 983T>C SNPs, previously associated with higher plasma NVP concentrations in studies conducted across different populations, were shown to be associated

with higher concentrations in breast milk. The time-averaged M/P concentration ratio of 0.88 (0.74-1.2) observed in the present study show that NVP penetrates well into human breast milk. Breast milk exposure alone resulted in plasma concentration of 660 ng/mL (104-3094) in infants, influenced by maternal *CYP3A4* 20230G>A (*1G; rs2242480) and infant age.

Similar to previous studies, we observed high NVP concentrations in breast milk. The time-averaged M/P concentration ratio reported here is similar to the 0.82 previously reported by Rezk et al. for single time points[98] but higher than 0.58-0.75 in other studies.[94,95,309] This reflects the complexity associated with drug quantification in breast milk and the differences in methods used in these studies. Here we used a fully validated method that quantifies NVP in whole milk.[242] The higher M/P AUC_{0-12} ratio of 0.95 (0.56-1.5) is a more accurate reflection of infant exposure. Additionally, for the first time we incorporated a pharmacogenetic analysis and showed that most women with the composite *CYP2B6* 516GG and 983TT genotypes had plasma and breast milk concentrations below the recommended 3400 ng/mL (**Table 5.3**).[261] It remains unclear if this plays any role in the emergence of resistance in a subset of women receiving ART for PMTCT. The NVP concentration in breast milk required to prevent localised HIV replication is unknown. However, patients with plasma NVP concentrations below 3000 ng/mL are five times more likely to experience virological failure than those with higher concentrations.[332] Interestingly, Chaix et al. associated slower clearance of NVP following intrapartum single dose with genotypic resistance detection at four weeks postpartum.[333] This is consistent with the *CYP2B6* slow metaboliser genotypes associated with reduced clearance and higher concentration in the present study, but will result in prolonged exposure to subtherapeutic concentrations after single dose NVP.

Plasma NVP concentrations in infants with dual exposure from breast milk and PEP were 4-fold higher than in those exposed through breast milk alone and 2.7-fold higher than in those exposed through PEP alone. All infants, including those exposed through breast milk alone,

achieved plasma concentrations above the 46 ng/mL protein-adjusted IC$_{50}$ and the 100 ng/mL recommended target for PEP.[306] Observed concentrations in infants with dual exposure were not above the range reported for HIV infected infants treated with fixed-dose NVP containing regimens.[334,335] However, it raises concerns about the potential for the emergence of resistant HIV strains where infants became infected, and early infant diagnosis and treatment initiation are delayed. In fact, prolonged exposure to subtherapeutic infant NVP concentrations has been linked with emergence of resistance in infants testing positive with wild-type HIV.[309] The effects of maternal *CYP3A4* 20230G>A (*1G; rs2242480) and infant age on infant plasma NVP concentration as a result of breast milk exposure suggest a role for the infant CYP3A4 metabolic pathway which increases rapidly after birth, reaching 30-40% of the adult activity after one month.[336] Unfortunately, we did not directly assess the impact of infant *CYP3A4* 20230G>A (*1G; rs2242480) or any other genetic factors and this should be considered a limitation.

In conclusion, the data presented here support previous findings that NVP is present in human breast milk at high concentrations. Substantially higher infant plasma NVP concentrations resulted from dual breast milk and PEP exposures compared with breast milk or PEP alone. As with plasma, breast milk and the resulting infant plasma NVP concentrations are influenced by genetic polymorphisms in drug disposition genes. The long-term consequences of this exposure, including potential emergence of resistant HIV strains, warrant further investigation. Retrospective pharmacogenetic analysis of samples from previous studies with postpartum transmitters may reveal important associations that highlight sources of residual transmissions in ART era and potential treatment intensification strategies.

CITATIONS

Olagunju A, Amara A, Waitt C, Else L, Penchala SD, Bolaji O, Soyinka J, Siccardi M, Back D, Owen A, Khoo S. Validation and clinical application of a method to quantify NVP in dried blood

spots and dried breast milk spots [in press]. *Journal of Antimicrobial Chemotherapy*. 2015; DOI:

10.1093/jac/dkv174.

CHAPTER 6

PREDICTION OF INFANT EXPOSURE TO MATERNAL DRUGS THROUGH BREAST MILK USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING

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6.1 INTRODUCTION

The re-enactment of The Best Pharmaceuticals for Children Act (BPCA), The Pediatric Research Equity Act (PREA) in the United States[337,338] and The Paediatric Regulation in the European Union[339] in 2007 were significant steps in paediatric health promotion. The BPCA and PREA were subsequently made permanent by the FDA Safety and Innovation Act in 2012, mandating necessary paediatric studies.[340] Although these legal frameworks do not remove the ethical and logistical challenges of conducting research in paediatric patients, they reinforced that children be protected through research, not from it, giving impetus to clinical studies in this population. For instance, between September 27, 2007 and November 18, 2013 about 470 paediatric studies (involving more than 178,000 patients and 160 drugs) were completed under BPCA and PREA in the United States,[341,342] reducing off-label paediatric drug use from over 80% to about 50%.

However, paediatric drug exposure is not limited to those administered for specific paediatric indications. More than 90% of nursing mothers take at least one drug in the early postnatal period, 17% until 4 months after delivery, and 5% receive drugs for chronic conditions.[84] For most drugs, the extent of excretion into breast milk and the potential effects on the breastfed infant are unknown. Though the FDA 2005 guidance on clinical lactation studies contain recommendations,[343] there is still no legislation requiring drug companies to conduct clinical research in nursing mother-infant pairs to evaluate infant exposure through breast milk. It is apparent that to avoid legal liability, most drugs are thus labelled not to be used during lactation despite a lack of data. However, this is not practical in many cases, especially for nursing mothers being treated for chronic conditions. For instance, HIV positive nursing mothers take antiretroviral drugs for the entire duration of breastfeeding (Option B) or for life (Option B+) for their own health and/or for prevention of mother-to-child transmission of HIV.[13] Understandably, conducting clinical pharmacokinetic studies in nursing mother-infant pairs is fraught with ethical and logistical challenges.

Physiologically-based pharmacokinetic (PBPK) modeling is increasingly being used in paediatric studies, with significant regulatory support.[344,345] Indeed, the US Food and Drug Administration (FDA) Advisory Committee for Pharmaceutical Science and Clinical Pharmacology unanimously voted in support of modeling and simulation for paediatric drug development.[346] Interestingly, the advances in PBPK modeling now allow for integration of compartments and parameters representing the anatomical and physiological features of a nursing woman (system parameters) with physicochemical, in vitro, preclinical, and clinical data (drug parameters) to generate predictions of drug-specific pharmacokinetics. In addition, system-specific parameters can be modified for extrapolations across different age groups. They also allow for integration of maternal and infant anatomy and physiology to simulate complex scenarios of infant exposure to substances through lactation. However, a cursory literature search indicates that the application of PBPK modeling in the study of infant exposure to xenobiotics through breast milk has predominantly been limited to environmental risk assessments.[347-351] There is no report of its application to describe infant exposure to maternal drugs through breast milk.

The aim of the present study was to develop a bespoke PBPK model to predict infant exposure to maternal drugs through breast milk. Published clinical data on infant exposure to the antiretroviral drug efavirenz (EFV)[331] presented in Chapter 4 was used for model validation.

6.2 METHODS

6.2.1 Model Structure and Parameterisation

The human breastfeeding model integrates a whole-body PBPK maternal model with a whole-body PBPK infant model (**Figure 6.1**). The maternal model was based on a previously validated adult model of orally administered EFV adapted for intramuscular long-acting nanoformulations,[352] with appropriate adjustments to exclude male-specific system parameters and an additional compartment introduced to represent the mammary gland. As previously
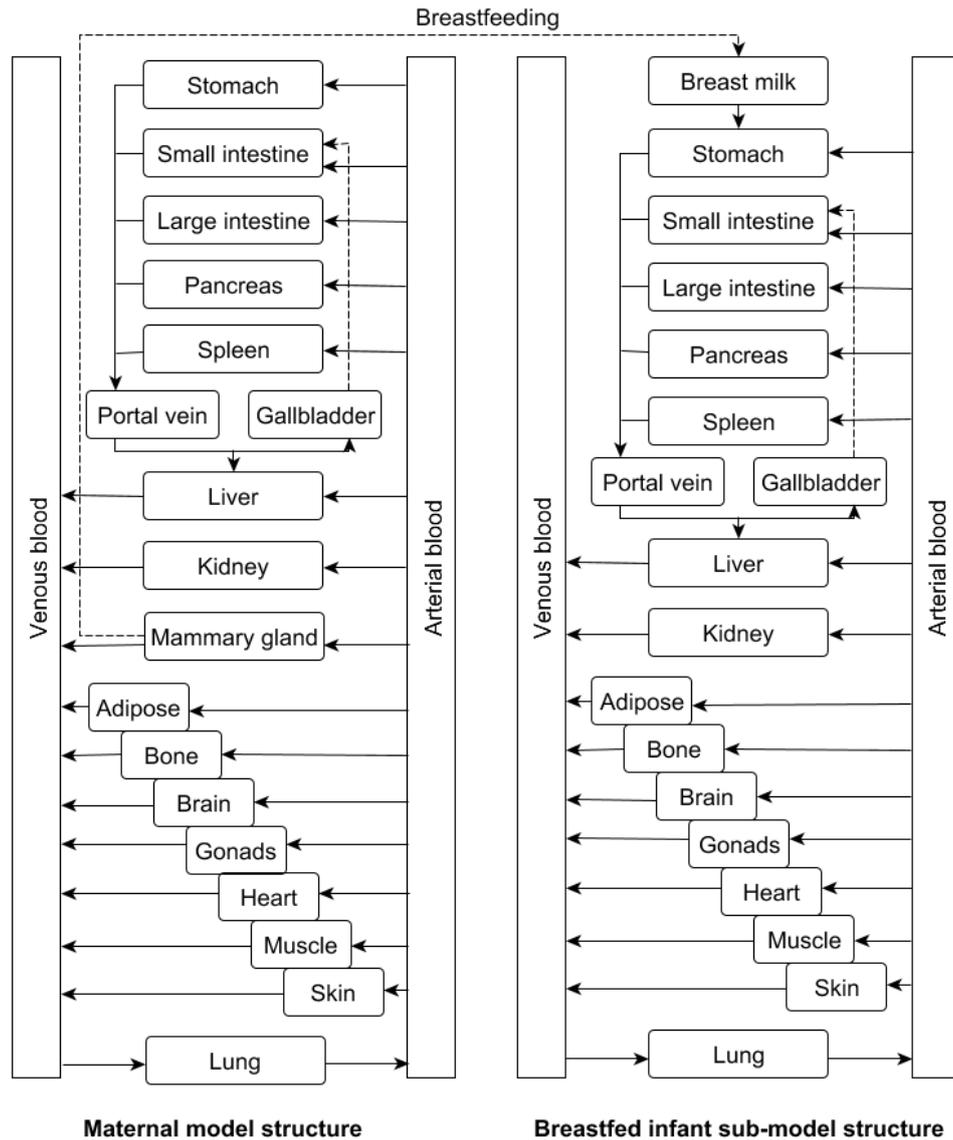
Figure 6.1 Schematic representation of the breastfeeding PBPK model.

described,[353] individual organ weights and blood flows were predicted from anthropometric characteristics (age, height, weight, body mass index, and body surface area), based on values in the HIV positive breastfeeding cohort described in Chapter 4.[331] The infant sub-model was scaled from the maternal model for different age groups (10 days-1 month, 1-3 months, 3-6 months, and 6-12 months) to account for age-dependent anatomical and physiological changes in system parameters. These included organ/tissue volumes and blood flows. Infants less than

10 days old were excluded because of residual intrauterine EFV exposure.[301,331] EFV-specific

parameters included in the model are presented in **Table 6.1**.

Table 6.1 Drug-specific physicochemical properties and in vitro data for efavirenz.

Drug properties	Description	Values
MW	Molecular weight	316
LogP	Octanol-water partition coefficient	4.60
pKa	Acid dissociation constant	10.2
R	Oral bioavailability	0.74
PSA	Polar surface area	38.33
HBD	Hydrogen bond donor	1
K (mg/mL)	Water solubility	0.00855
M/P ratio[331]	Milk/plasma AUC_{0-24} ratio	1.13
Fu	Fraction unbound in plasma	0.01
Vd (L/kg)	Volume of distribution at steady state	3.6
P_{eff} (cm/s)	Effective permeability (Caco-2)	2.5×10^{-6}
CL_{int} (µL/min/pmol)[249]	Intrinsic hepatic clearance	
rCYP1A2 CL_{int}		0.008
rCYP2A6 CL_{int}		0.05
rCYP2B6 CL_{int}		0.55
rCYP3A4 CL_{int}		0.007
rCYP3A5 CL_{int}		0.03
Ind_{CYP} (µM)[354]	Hepatic CYPs induction	
CYP2B6 Ind_{max}		5.76
CYP3A4 Ind_{max}		6.45
CYP2B6 Ind_{50}		0.82
CYP3A4 Ind_{50}		3.93

6.2.2 Modeling Absorption, Distribution, Metabolism and Elimination

A compartmental absorption and transit model incorporating both gastric emptying and small

intestinal transit flow was used to describe drug absorption. Fraction of dose absorbed (F_a) was

described using effective permeability (P_{eff}) derived from Caco-2 permeability as shown in

equations 6.1 and 6.2.[355]

$$F_a = 1 - (1 + 0.54 \times P_{eff})^{-7} \tag{6.1}$$

$$P_{eff} = 10^{(0.6836 \times (\log Caco-2)) - 0.5579)} \tag{6.2}$$

Intestinal drug clearance (CL_{gut}) was calculated from bioavailability (R), in vitro CYP3A4 intrinsic clearance (rCLint), and intestinal CYP3A4 abundance (Ab_{CYP3A4}) using equation 6.3. The fraction of drug escaping gut metabolism (F_g) was calculated using equation 6.4, where Q_{gut} and $f_{u,gut}$ are intestinal blood flow and fraction unbound in the intestine, respectively.

$$CL_{gut} = (R \times (CYP3A4 \; rCL_{int}/0.34) \times Ab_{CYP3A4} \times 1000 \times 60)/1000000 \qquad (6.3)$$

$$F_g = \frac{Q_{gut}}{(Q_{gut} + f_{u,gut} \times CL_{gut}} \qquad (6.4)$$

Systemic circulation was defined as a function of the rate of blood flow to tissues (perfusion-limited) and by a mechanism-based approach using tissue composition-based equations 6.5 and 6.6. Volume of distribution (V_{ss}) was calculated using equation 6.7.[356,357]

$$P_{t:p,nonadipose} = \frac{[P_{o:w} \times (V_{nlt} + 0.3 \times V_{pht})] + [1 \times (V_{wt} + 0.7 \times V_{pht})]}{[P_{o:w} \times (V_{nlp} \; 0.3 \times V_{php})] + [1 \times (V_{wp} + 0.7 \times V_{php})]} \times \frac{f_{u,p}}{f_{u,t}} \qquad (6.5)$$

$$P_{t:p,adipose} = \frac{[D_{vo:w} \times (V_{nlt} + 0.3 \times V_{pht})] + [1 \times (V_{wt} + 0.7 \times V_{pht})]}{[D_{vo:w} \times (V_{nlp} \; 0.3 \times V_{php})] + [1 \times (V_{wp} + 0.7 \times V_{php})]} \times \frac{f_{u,p}}{1} \qquad (6.6)$$

$$V_{ss} = (\Sigma V_t * P_{t:p}) + (V_e * E:P) + V_p \qquad (6.7)$$

$P_{t:p}$ is the non-adipose and adipose tissue:plasma partition coefficients, $P_{o:w}$ is the n-octanol:buffer partition coefficient of the nonionized species at pH 7.4, $D_{vo:w}$ is the olive oil:buffer partition coefficient of both the nonionized and ionized species at pH 7.4, V is the fractional tissue volume content of neutral lipids (nl), phospholipids (ph), and water (w), t is tissue, p is plasma, and fu is unbound fraction. In equation 6.7, V is the fractional body volume of a tissue (t), erythrocyte (e), and plasma (p), E:P is the erythrocyte:plasma ratio. All parameters are defined and calculated as previously described.[356]

The abundances of cytochrome P450 (CYP) enzymes in nursing mothers were based on reported in vivo adult data.[358,359] CYP2B6 abundances for different infant age groups were based on data from human liver microsomal samples obtained from 102 infants previously reported by Croom

et al.[307] A plot of CYP2B6 expression in individual tissue samples from birth to 1 year was digitised using Plot Digitizer to provide the raw input data. Samples with levels below the limit of detection (0.25 pmol/mg protein) were excluded.[307] The mass of microsomal protein per gram of liver (MMPGL), intrinsic clearance (CL_{int}), CYP2B6 induction (Ind_{CYP2B6}), total intrinsic clearance (TCL_{int}), total apparent clearance (CL_{app}), systemic clearance (CL), and fraction escaping first-pass metabolism (F_h) were calculated using previously described equations 6.8 to 6.14.[352]

$$\text{MMPGL} = 10^{(1.407+0.0158 \text{ x Age} - 0.00038 \text{ x Age}^2 + 0.0000024 \text{ x Age}^3)} \tag{6.8}$$

$$\text{CL}_{int} = (\text{Ind x } (rCL_{int}/0.34) \text{ x } Ab_{CYP} \text{ x } Ab_{CYP} \text{ x MMPGL x } 1000 \text{ x } 60 \text{ x } Wt_{liver}))/$$
$$1000000 \tag{6.9}$$

$$\text{Ind}_{CYP2B6} = 1 + (5.7 \text{ x } [EFV]_{plasma})/(0.252 + [EFV]_{plasma}) \tag{6.10}$$

$$\text{TCL}_{int} = (\text{CL}_{int} \text{ x } Ab_{CYP} \text{ x } Wt_{liver} \text{ x MMPGL x } 1000 \text{ x } 60)/1000000 \tag{6.11}$$

$$\text{CL}_{app} = \sum_{n=1}^{n} \text{TCL}_{int} \tag{6.12}$$

$$\text{CL} = \frac{Q_{hv} \text{ x } f_u \text{ x } CL_{app}}{Q_h + CL_{app} \text{ x } f_u} \tag{6.13}$$

$$F_h = 1 - \text{CL}/Q_{hv} \tag{6.14}$$

6.2.3 Population Variability

Variability in system and drug-specific parameters in both maternal model and the infant sub-model was introduced mainly through anthropometric characteristics as previously described. Variability in infant age was introduced using the MATLAB® linspace function to generate equally spaced values within each group. Where available clinical data were used, MATLAB® rule expressions incorporating the mean, standard deviation, minimum and maximum parameter values, were used to introduce variability. Some of the parameters thus varied were absorption constants, microsomal protein per gram of liver, CYP enzyme abundance and intrinsic clearance.

6.2.4 Modeling Breastfeeding

Breastfeeding was described by four hourly oral doses of maternal breast milk regulated by the milk production/infant suckling rate.[360] The corresponding infant EFV dose per feeding session (EFV Dose$_{milk}$) was calculated from breast milk volume (V_{milk}) and EFV concentration in breast milk ($[EFV]_{milk}$) according to equations 6.15 and 6.16.

$$\text{EFV Dose}_{milk} = V_{milk} \times [EFV]_{milk} \tag{6.15}$$

$$[EFV]_{milk} = M/P_{AUC_{0-24}} \times [EFV]_{plasma} \tag{6.16}$$

where $[EFV]_{plasma}$ is the simulated plasma EFV concentration and M/P_{AUC} is the reported milk-to-plasma AUC_{0-24} ratio (median: 1.13; range: 0.50-1.93).[331] In addition, previously described log-transformed phase distribution[361] model for predicting the M/P ratio was evaluated for possible integration into the current model to make it more predictive, especially for drugs with no in vivo breast milk data. Infant suckling rate was assumed to be equal to milk production rate reported by Gentry et al. from birth to 6 months of age.[360] Suckling rate at 6 months was retained for older infants up to 12 months of age to reflect reduced breast milk intake following the introduction of alternative foods when exclusive breastfeeding ends at 6 months.

6.2.5 Model Simulation and Evaluation

The model was built and simulated using the SimBiology® (version 5.1, MATLAB® 2014b, MathWorks Inc., Natick, MA, USA). Virtual populations of nursing mother-infant pairs (n = 100 per infant age group: 10 days-1 month, 1-3 months, 3-6 months, and 6-12 months) were simulated. All model simulations were run using female anatomical and physiological parameters to simulate EFV pharmacokinetics during lactation and breastfed infants were simulated as females because of the expected similarities between males and females at this early age. Simulated mothers were on a daily dose of 600 mg EFV and infants were not taking any medication.

The validity of model estimations was confirmed by comparison with reference values from the literature, with 2-fold difference acceptance limit. For organ weights and blood flows, data from Coppoletta et al. and Pryce et al. were used.[362,363] Pharmacokinetic parameters were evaluated at steady state and AUC_{0-24} was calculated using the trapezoidal rule. The most comprehensive published clinical data of EFV pharmacokinetics in human breast milk and exposure of breastfed infants (Chapter 4)[331] were used to validate the estimates of pharmacokinetic parameters.

6.3 RESULTS

6.3.1 Breastfed Infant Sub-Model Validation

The validation of the adult model has been previously described.[352] Key anatomical and physiological parameters predicted with the breastfed infant sub-model, including body weight, organ weights, regional blood flow, and CYP enzyme expression, were within 50% difference of available data for all four age groups. For instance, predicted cardiac output calculated as a function of body weight was 44 and 91 L/h in 10 days-1 month and 6-12 month old infants, respectively, comparable to the reference values of 36 and 72 L/h in newborn and 12 month old infants.[364] Predicted infant body weight, organ weights, and blood flows calculated as fractions of cardiac output are presented in **Table 6.2**.[307,362,363]

6.3.2 Predicted Breast Milk Pharmacokinetics and Breastfed Infants' Exposure

The simulated concentration-time profiles of EFV in breast milk and plasma of nursing mothers (n = 400) are shown in **Figure 6.2**. The model adequately described EFV pharmacokinetics, with over 90% of all individual (n = 29) observed data points within the predictive interval (**Figure 6.2**). Predicted versus observed breast milk AUC_{0-24}, C_{max} and C_{min} were 69.4 (17.2-317.0) versus 68.5 µg.hr/mL (26.3-257), 4.1 (1.2-15.4) versus 5.4 µg/mL (1.4-18.4),

Table 6.2 Key simulated anatomical and physiological parameters (mean, SD) for infant sub-model.

	10 days-1 month	1-3 months	3-6 months	6-12 months
Age (y)	0.06 (0.02)	0.18 (0.04)	0.38 (0.07)	0.80 (0.12)
Weight (kg)	3.89 (0.58)	4.81 (0.65)	6.40 (0.80)	8.65 (1.04)
Organ Weights (kg)				
Adipose	1.14 (0.57)	1.38 (0.56)	1.94 (0.61)	2.59 (0.75)
Blood	0.40 (0.00)	0.40 (0.00)	0.43 (0.01)	0.50 (0.03)
Bones	0.21 (0.04)	0.26 (0.06)	0.34 (0.07)	0.48 (0.11)
Brain[1]	0.40 (0.03)	0.46 (0.04)	0.58 (0.04)	0.82 (0.07)
Heart[1]	0.02 (0.00)	0.03 (0.01)	0.04 (0.01)	0.10 (0.03)
Intestines	0.06 (0.05)	0.08 (0.04)	0.09 (0.05)	0.12 (0.05)
Kidneys[1]	0.04 (0.00)	0.04 (0.00)	0.05 (0.00)	0.06 (0.00)
Liver[1]	0.18 (0.03)	0.22 (0.03)	0.27 (0.03)	0.34 (0.04)
Lungs[1]	0.06 (0.00)	0.07 (0.01)	0.08 (0.01)	0.10 (0.01)
Muscle	0.75 (0.46)	0.47 (0.38)	1.42 (0.73)	3.14 (0.95)
Pancreas[1]	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)
Remaining	0.28 (0.03)	0.32 (0.04)	0.39 (0.04)	0.51 (0.06)
Skin	0.29 (0.06)	0.30 (0.05)	0.34 (0.06)	0.40 (0.05)
Spleen[1]	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)	0.02 (0.00)
Stomach	0.10 (0.05)	0.10 (0.05)	0.11 (0.06)	0.11 (0.05)
Thymus[1]	0.01 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)
Total weight	4.57 (0.10)	4.72 (0.12)	4.99 (0.14)	5.51 (0.18)
Organ Blood Flows (L/h)				
Cardiac output	44.66 (5.88)	53.96 (6.47)	69.60 (7.80)	91.05 (9.70)
Adipose	1.74 (0.23)	2.10 (0.25)	2.71 (0.30)	3.55 (0.38)
Brain	14.11 (1.86)	17.05 (2.04)	21.99 (2.46)	28.77 (3.07)
QCC	15.19 (1.08)	15.00 (1.01)	15.12 (0.77)	15.10 (0.97)
Gonads	0.22 (0.03)	0.27 (0.03)	0.35 (0.04)	0.46 (0.05)
Gut	3.89 (0.51)	4.69 (0.56)	6.06 (0.68)	7.92 (0.84)
Qha	2.90 (0.38)	3.51 (0.42)	4.52 (0.51)	5.92 (0.63)
Qhv	3.90 (0.38)	4.51 (0.42)	5.52 (0.51)	6.92 (0.63)
Kidneys	4.11 (0.54)	4.96 (0.60)	6.40 (0.72)	8.38 (0.89)
Lungs	0.54 (0.07)	0.65 (0.08)	0.84 (0.09)	1.09 (0.12)
Muscle	1.74 (0.23)	2.10 (0.25)	2.71 (0.30)	3.55 (0.38)
Pancreas	0.54 (0.07)	0.65 (0.08)	0.84 (0.09)	1.09 (0.12)
Qpv	6.42 (0.58)	7.34 (0.64)	8.89 (0.77)	11.01 (0.96)
Qre	2.23 (0.29)	2.70 (0.32)	3.48 (0.39)	4.55 (0.49)
Skin	1.16 (0.15)	1.40 (0.17)	1.81 (0.20)	2.37 (0.25)
Spleen	0.89 (0.12)	1.08 (0.13)	1.39 (0.16)	1.82 (0.19)
Stomach	0.40 (0.05)	0.49 (0.06)	0.63 (0.07)	0.82 (0.09)

[1]Reference values are available from Pryce et al. and/or Coppoletta et al.; with the exception of pancreas in the 10 days-1 month stratum, infant organ weights and blood flows were within 50% fold difference of reference values.[362-364]
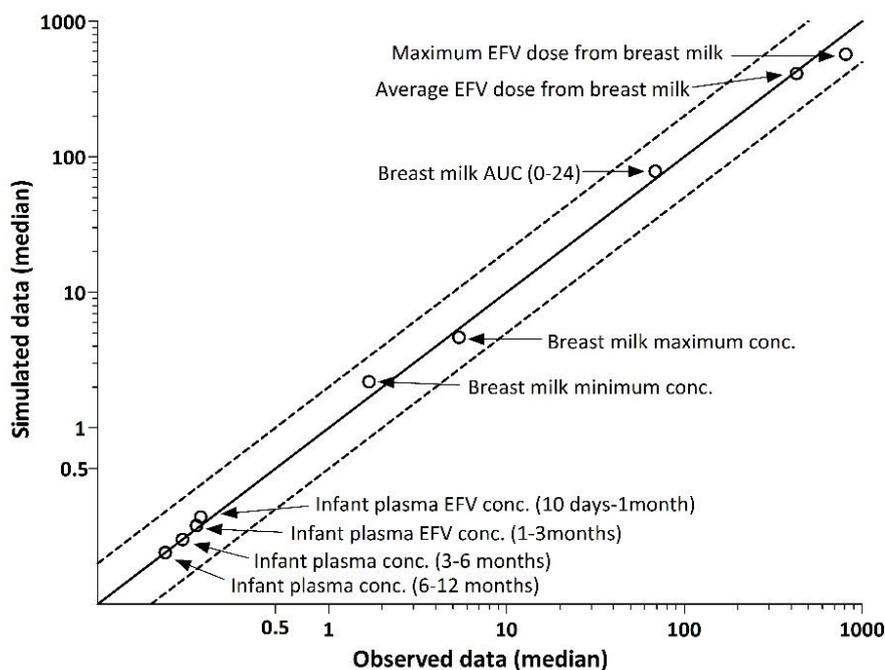
Figure 6.2 Simulated versus observed breast milk pharmacokinetic parameters and infant exposure indices. All predictions were within 2-fold difference (dotted lines) of the observed values (solid line).

and 2.02 (0.382-11.9) versus 1.68 µg/mL (0.316-9.57), respectively (**Table 6.3**). Compared with clinical data, all parameters were within 0.5-fold difference (**Figure 6.3**). No reasonable predictions of the M/P ratio were obtained by the log-transformed phase distribution model, considered to be the most reliable of those evaluated by Fleishaker et al.[361] Therefore, only the clinically derived M/P ratios were used within the final model.

Model predictions for parameters relating to breastfed infant exposure were also comparable to clinical data. Predicted versus observed average and maximum infant EFV dose from breast milk were 412 (82.3-2170) versus 428 µg/kg/day (164-1610), and 571 (131-2430) versus 809 µg/kg/day (215-2760), respectively. The resulting simulated concentration-time profiles in infants of different age groups were relatively flat (**Figure 6.4**), with average infant plasma concentration highest in the 10 days-1 month old at 0.22 µg/mL (0.061-0.77), 0.19 µg/mL (0.037-0.81) in 1-3 month old, 0.15 µg/mL (0.035-0.52) in 3-6 month old, and 0.12 µg/mL (0.03-0.60) in

Table 6.3 Predicted versus observed pharmacokinetic parameters of efavirenz in breast milk and plasma (median, range).

Parameters	Predicted (n = 400[a])	Observed (n = 29[a])[331]
Breast milk $AUC_{(0-24)}$ (µg.hr/mL)	78.2 (15.3-335)	68.5 (26.3-257)
Breast milk C_{max} (µg/mL)	4.65 (1.15-18.0)	5.39 (1.43-18.4)
Breast milk C_{min} (µg/mL)	2.19 (0.283-13.0)	1.68 (0.316-9.57)
Plasma $AUC_{(0-24)}$ (µg.hr/mL)	70.7 (21.0-336)	60.7 (26.8-177)
Plasma C_{max} (µg/mL)	4.26 (1.71-17.5)	4.63 (2.05-9.76)
Plasma C_{min} (µg/mL)	1.97 (0.371-12.6)	2.03 (0.755-6.74)
Average infant EFV dose from breast milk (µg/kg/day)	412 (82.3-2170)	428 (164-1610)
Maximum infant EFV dose from breast milk (µg/kg/day)	571 (131-2430)	809 (215-2760)
Infant plasma EFV conc., 10 days-1 month old (µg/mL)[c]	0.22 (0.061-0.77)	0.19 (0.071-0.705)
Infant plasma EFV conc., 1-3 months old (µg/mL)[c]	0.19 (0.037-0.81)	0.18 (0.036-0.504)
Infant plasma EFV conc., 3-6 months old (µg/mL)[c]	0.15 (0.035-0.52)	0.15 (0.052-0.33)
Infant plasma EFV conc., 6-12 months old (µg/mL)[c]	0.12 (0.026-0.60)	0.12 (0.038-0.590)

[a]A total of 400 mothers and 100 infants per group were simulated; [b]observed maternal data were obtained from 29 mothers and 96 infants (n = 10, 26, 29 and 29, respectively; infants < 10 days and > 12 months old were excluded); [c]predicted infant plasma EFV concentrations did not change significantly during the dosing interval. Median of predicted values, averaged for each infant, are presented.

6-12 month old infants. This is similar to the observed decrease from 0.19 µg/mL (0.52–0.71) in 9 days-3 month old, to 0.15 µg/mL (0.052–0.33) in > 3–6 month old, and 0.10 µg/mL (0.041–0.59) in > 6 month old infants in our clinical cohort.[331]
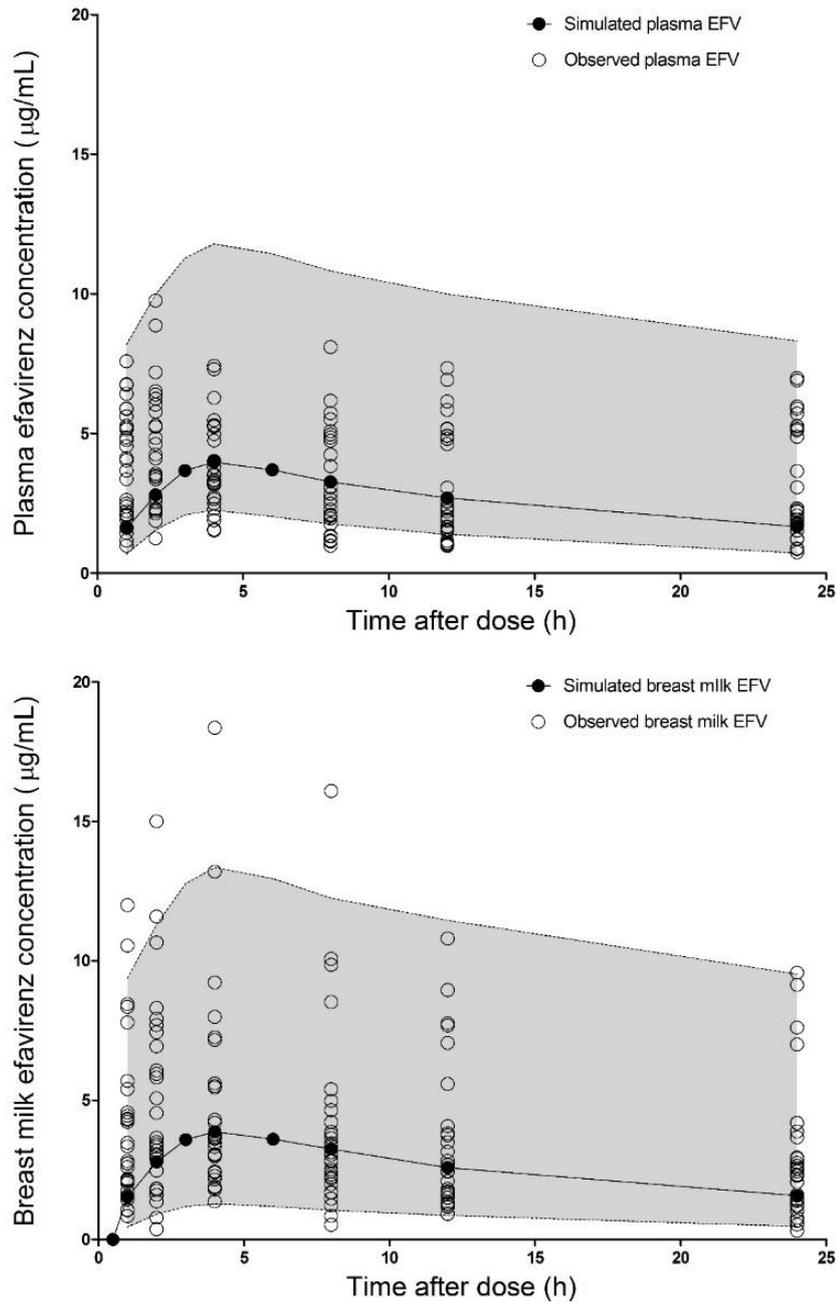
Figure 6.3 Observed plasma (A) and breast milk (B) efavirenz data (open circles) and simulated concentration-time profiles (solid lines, median; dotted lines, range). The model adequately described efavirenz pharmacokinetics, with over 90% of all individual (n = 29) observed data points within the predictive interval.
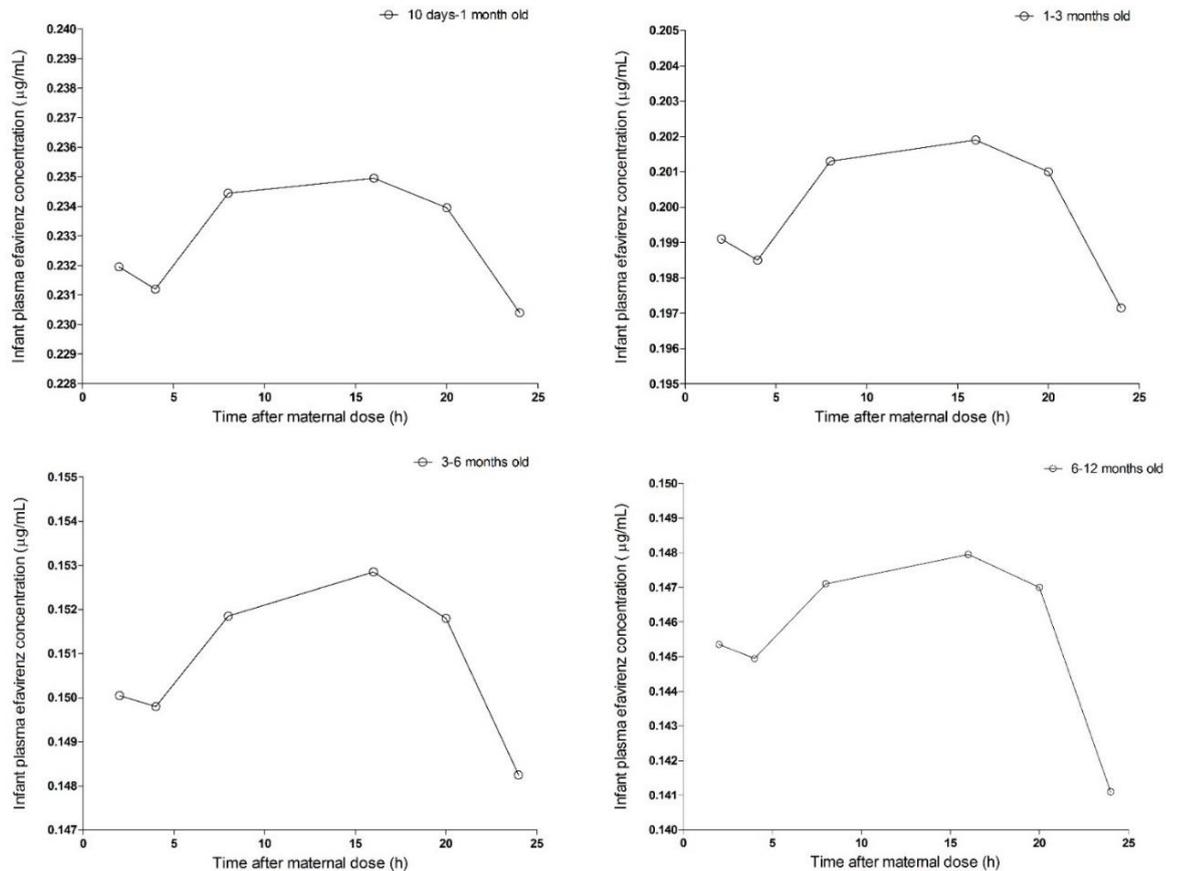
Figure 6.4 Infant plasma efavirenz concentration-time profiles. Simulated concentration-time profiles were relatively flat in infants, highest in the 10 days-1 month old and lowest in 6-12 months old.

6.4 DISCUSSION

PBPK modelling was applied for the prediction of plasma and breast milk pharmacokinetics of the antiretroviral drug EFV in nursing mothers and the exposure of breastfed infants. To our knowledge this is the first time this approach has been utilised for prescription drugs. The model integrates a previously validated whole-body oral adult PBPK model[352] with a whole-body breastfed infant PBPK sub-model. System and drug-specific parameters for the infant sub-model were either obtained from the literature or scaled from the adult model, and variability was introduced to reflect in vivo observations. Plasma and breast milk profiles of EFV were successfully simulated. Breastfeeding was described by repeated ingestion of a volume of breast milk controlled by infant suckling rate.[365]

PBPK models have been used to describe plasma and intracellular EFV pharmacokinetics following oral and intramuscular administration, respectively.[352,366] In addition to accurately predicting plasma pharmacokinetics as in previous models, breast milk pharmacokinetics predicted by the current model are very similar to those observed from clinical data.[331] Previous applications of PBPK models in human lactation studies have been limited to environmental risk assessments. Such models have been used in environmental risk assessments to quantitatively describe the transfer of inhaled contaminants during lactation[347] for trichloroethylene and its metabolite,[348] tetrachloroethylene and associated cancer risk for breastfed infants,[349,350] perchlorate and iodide including inhibition of iodide thyroidal uptake by perchlorate.[351] An extensive review by Corley et al. describes the underlying assumptions, model structures, data and methods used in the development and validation of these previous PBPK models.[367] Similar models have been described for polychlorinated biphenyls,[368] co-exposure to polychlorinated biphenyls and methyl mercury,[369] persistent organic pollutants (including an initial infant body burden to represent intrauterine exposure),[370] manganese,[365] and perfluoroalkyl carboxylates and sulfonates.[371,372]

Replicating the ontogeny of drug metabolism enzymes is one of the major challenges in the development of paediatric PBPK models. Children often display developmentally unique differences in drug disposition compared to adults, making simple scaling using anthropometric characteristics unreliable. For instance, paediatric doses of EFV derived from adult dose using simple allometric scaling have been reported to result in subtherapeutic and higher variability in plasma concentrations compared to adults.[373] Hepatic CYP2B6 accounts for over 90% of EFV metabolism and polymorphisms in the *CYP2B6* gene are known to underpin significant inter-individual variability in CYP2B6 enzyme expression and activity, resulting in variability in the metabolism of substrate drugs. We used the paediatric CYP2B6 protein expression data available in the literature,[307] and replicated in vivo variability using MATLAB rule expression that incorporated the mean, standard deviation, minimum and maximum values for each age

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stratum. Further variability in the resulting CYP2B6 intrinsic clearance was introduced through simple linear interpolation of age which modulates its abundance through liver weight. In view of their minimal role in EFV metabolism, CYP2A6, CYP3A4, and CYP3A5 intrinsic clearances were scaled from adult values through infant age, and variability in each age stratum was introduced by linear interpolation.

This approach adequately described infant plasma EFV concentrations resulting from breastfeeding in the presence of maternal EFV for the different age groups, demonstrating the robustness of age-related changes in model parameters as well as the associated scaling and variability. As with the clinical data, model predictions indicate that infants are exposed to maternal EFV from breast milk. The implications for possible development of drug resistance in infants who become infected underscore the need for further clinical investigation. The model can be used for other drug classes and in other therapeutic areas, provided the requisite drug-specific parameters are available or predictable from other known parameters.

A major limitation of the current model is the absence of a milk-to-plasma ratio prediction component. Although the log-transformed phase distribution model did not yield reasonable predictions for EFV, other approaches have been described, including an artificial neural network model.[274] In addition, the model did not consider the potential role of drug transporters in breast milk excretion because EFV is not a known substrate of any transporter in humans.[181,374] However, this could be incorporated for drugs with known active transport mechanisms in mammary gland. This was successfully done for OATP1B1/1B3-mediated hepatic uptake of irbesartan.[275] For instance, ABCG2 is known to be highly expressed in lactating human mammary gland,[179] involved in the secretion of its substrates into breast milk,[375,376] and can be affected by polymorphisms in the *ABCG2* gene.[377] Integrating such approaches with the current model have the potential to extend its application to drugs with no available breast milk data and may have application during the drug development process.

In conclusion, the breastfeeding PBPK model described here provides opportunities for expanding the understanding of infant exposure to maternal drugs through breast milk, including during the drug development process. Its application can help in bridging existing gaps and pave the way for evidence-based recommendations for drug use during lactation.

CHAPTER 7

GENERAL DISCUSSION

An HIV infected woman can become pregnant at any stage of infection and a woman can become HIV infected at any stage of pregnancy or postpartum. Timely initiation of life-long or breastfeeding-limited antiretroviral therapy (ART) before or as early as possible during pregnancy can prevent mother-to-child transmission (MTCT) by reducing viral load in the blood, cervicovaginal fluid and breast milk (**Figure 7.1**).[378,379] Achieving sustainable virological suppression requires optimal plasma concentrations of antiretroviral (ARV) drugs, a surrogate for target site concentrations. The data presented in chapters 2 and 3 of this thesis provide evidence of significant pregnancy-induced changes in the pharmacokinetics of efavirenz (EFV) and nevirapine (NVP), key components of currently
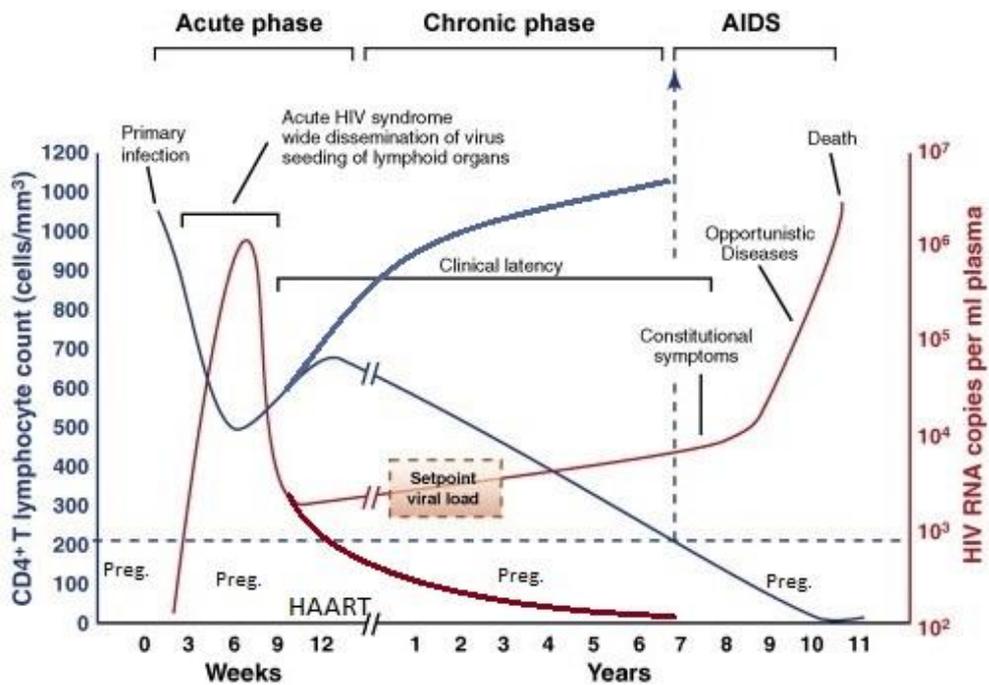
Figure 7.1 Natural course of HIV infection without treatment and with ART (thick lines). A woman can become pregnant before primary infection or at any of the later stages. The acute phase after primary infection lasts for 6-12 weeks with flu-like symptoms, peak viral load and drop in CD4+ T cells. Chronic phase is asymptomatic and lasts 7-10 years; viral replication reaches a steady level known as the 'set point'. AIDS onset is associated with further increase in viral replication and declining CD4+ cell counts to < 200/mm^3. Time to AIDS onset varies between individuals from 2 years to more than 15 years after seroconversion. ART initiated before or during pregnancy reverses these trends and prevents MTCT. Adapted from An et al.[380]

recommended first-line regimens for pregnant women.[13] The differential impact of pregnancy on pharmacokinetics in women stratified by polymorphisms in drug disposition genes was observed, indicating a potential role for pharmacogenetics in treatment optimisation strategies. Chapters 4 and 5 describe the intensive breast milk pharmacokinetics of EFV and NVP for the first time and detail different measures of breastfed infants' exposure. The novel application of PBPK modeling to predict infant exposure to maternal drugs from breast milk presented in chapter 6 extends our ability to evaluate the safety of drug use during lactation in virtual populations of nursing mother-infant pairs, and may constitute an invaluable tool in the absence of clinical data.

Subtherapeutic exposure to ARVs results in virological failure and this is well documented for plasma concentrations of EFV < 1000 ng/mL and NVP < 3400 ng/mL in non-pregnant adults.[176,381] Many components of the preferred first-line regimens have lower exposure during pregnancy compared with postpartum (see Sections 1.3.4 to 1.3.8). However, the limitations of currently recommended pharmacokinetic targets highlight the need for robust systems to evaluate their adequacy prior to their wide application. Though the lack of pharmacodynamic endpoints precludes any conclusions about the effects of observed reduced EFV and NVP exposures during pregnancy on MTCT risk in the studies reported here, it can be speculated that this may influence viral decay dynamics in patients genetically predisposed to plasma concentrations below the recommended targets. This may affect the probability of virological suppression at delivery and the risk of MTCT in those initiating therapy late in pregnancy at high baseline viral loads (**Figure 7.2**).

Suboptimal virological suppression in HIV positive pregnant women results in detectable viral load at delivery and increases the risk of MTCT. This has been reported in a number of studies.[382-385] For instance, viral load > 1000 copies/mL near delivery was associated with a 12-fold increased risk of MTCT in a European Collaborative Study.[385] In a large multicentre US cohort
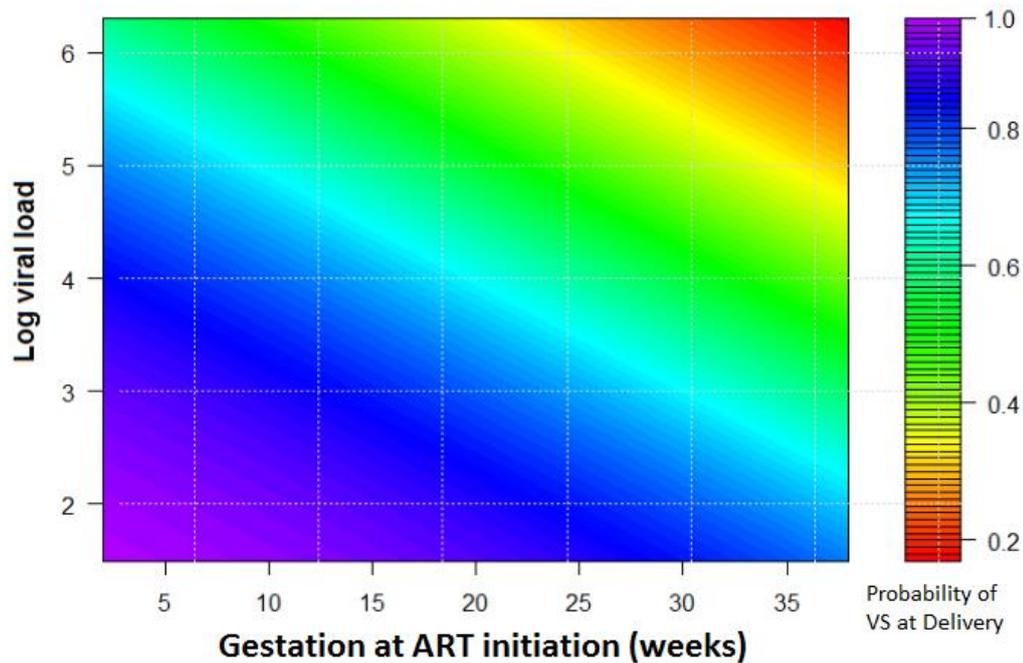
Figure 7.2 Contour plot from probit model predicting probabilities of virological suppression (VS) at delivery according to pre-ART viral load (log10 copies/mL) and gestation at ART initiation. Red indicates < 20% (highest risk of MTCT) and purple indicates > 90% (lowest risk of MTCT) probability of VS at delivery. Adapted from Meyer et al.[286]

study, Katz et al. reported detectable viral load at delivery in 13% of women who initiated ART during pregnancy (n = 671), increasing to 23.9% when started at third trimester, and identified gestational age at ART initiation, poor medication adherence, and treatment interruptions as factors increasing risk.[291] Among South African women who started ART before pregnancy (n = 574), 13% reportedly had viral load > 1000 copies/mL.[386] Although the use of ART has reduced MTCT to less than 5% from the baseline 25-40% without intervention, about 250 000 infants are still infected every year.[7] Therefore, lack of virological suppression at delivery appears to be a major contributor to residual MTCT in the era of ART and the role of pregnancy-induced changes in pharmacokinetics warrants further investigation. **Figure 7.3** presents hypothetical scenarios of what such investigation may reveal.
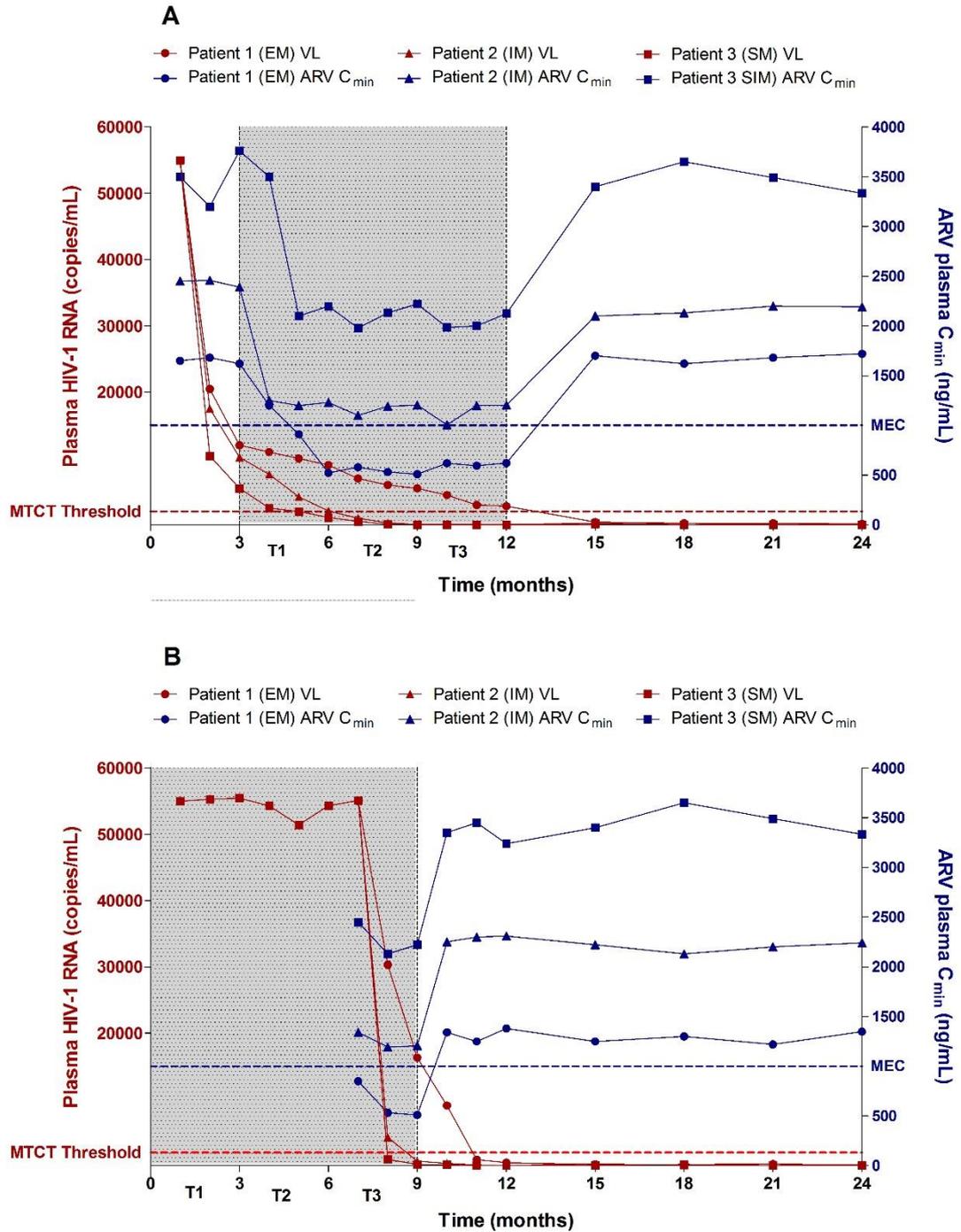
Figure 7.3 Hypothetical scenarios of pregnancy-induced changes in antiretroviral drug pharmacokinetics and potential consequences on viral decay dynamics and MTCT risk. The shaded region indicate the gestational period; red line, hypothetical threshold above which the risk of MTCT increases; blue line, hypothetical minimum effective concentration (MEC). Abbreviations: EM, extensive metaboliser; IM, intermediate metaboliser; SM, slow metaboliser; VL, viral load.

Additionally, lower exposure to EFV and NVP in pregnant women have some important policy implications. The ENCORE1 trial demonstrated the non-inferiority of 400 mg daily dose of EFV compared with the standard 600 mg dose when used in combination with emtricitabine and tenofovir in non-pregnant adults. The percentage of patients with viral load < 200 copies/mL at 96 weeks was 90·0% (289/321) with EFV 400 mg versus 90·6% (280/309) with EFV 600 mg (P = 0·72). In addition, incidence of EFV-related adverse events was significantly lower in the 400 mg group (38% versus 48%, P = 0·01).[265,387] A follow up pharmacokinetic-pharmacodynamic analysis showed that virological suppression was comparable between the two groups despite significantly lower exposure in the 400 mg group, including in patients with plasma concentrations < 1000 ng/mL and when stratified according to genotypes.[388] The authors argue that these findings challenge the currently recommended 1000 ng/mL threshold and recommend the 400 mg reduced dose. Despite the inadequacy of currently applied cut-off, our findings highlight the need for a cautious evaluation of this dose in pregnant women, with close monitoring of those with *CYP2B6* extensive metaboliser genotype, before widespread implementation.[240]

As discussed in Chapter 3, NVP is still a major component of first-line ART recommended as an alternative to EFV.[13] Our findings raise questions about the appropriateness of current dosing recommendations for NVP (200 mg once daily for 14 days followed by 200 mg twice daily) in pregnant women with *CYP2B6* extensive metaboliser genotype, especially when therapy is initiated late in pregnancy. In this regard, a number of lessons can be learned from studies that investigated NVP dose optimisation strategies to overcome reduced exposure when co-administered with rifampicin. Ramachandran et al. reported successful restoration of therapeutic concentrations with 300 mg twice daily dose with no clinical or laboratory evidence of adverse events.[389] However, the same strategy was associated with increased risk of NVP hypersensitivity reaction in another study.[390] The two-week 200 mg daily dose escalation strategy was associated with minimum plasma concentration (C_{min}) below target in 79%

compared with 19% at 200 mg twice daily at week 2 in the latter study. Additionally, C_{min} was 7300 ng/mL (range 5600-11002) at the time of hypersensitivity compared to 5900 ng/mL (5020–7400) at week 4 in the 600 mg/day group.[390] This is consistent with the slow metaboliser phenotype and suggests a genetic component to the observed hypersensitivity. Interestingly, the *CYP2B6* 516G>T and 983T>C SNPs have been associated with NVP hypersensitivity, with odds ratios of 2.52 (P = 0.0005) and 4.2 (P = 0.0047) reported for 983T>C in two separate studies.[391,392] Therefore, an increased dose of NVP in patients observed to be genetically predisposed to suboptimal exposure during pregnancy in the present study (composite *CYP2B6* 516GG and 983TT genotypes) warrants further investigation. The study by Ciccacci et al. showed that this composite genotype conferred a protective effect against NVP hypersensitivity.[392]

The uncertainties about the possible effect of pregnancy-induced pharmacokinetic changes on MTCT risks highlight the need to better characterise or re-characterise the minimum effective concentration (MEC) for certain ARVs. The theoretical effective concentration at which virus replication is inhibited by 50%, 90%, or 95% (IC_{50}, IC_{90}, or IC_{95} for biochemical or subcellular assays; EC_{50}, EC_{90}, or EC_{95} for cell-based assays) is determined for susceptible virus strains using quantitative assays.[393] These are corrected for plasma protein binding and reduced susceptibility of certain virus strains to obtain a parameter which would theoretically correspond to in vivo MEC. In addition, the combination antiviral activity of new ARVs with those from other classes is evaluated for antagonistic, synergistic, or additive effects where future combination therapy is anticipated.[393] The goal is to select doses for phase II trials that will provide exposures expected to exceed, by several-fold, the protein binding-adjusted in vitro EC_{50} value of the drug for the relevant virus strain and subtype.[393] A number of authors have highlighted the usefulness of the inhibitory quotient (IQ) in evaluating efficacy.[394-397] The IQ relates in vivo drug exposure to viral susceptibility, usually C_{min} divided by IC_{95}, indicating the number of times the C_{min} is greater than the IC_{95}. Interestingly, Acosta et al previously reported protein-corrected IC_{95} of 126 and 366 ng/mL for efavirenz and nevirapine, corresponding to IQ_{95} of 14 and 12, respectively.[306]

However, these values are for individual drugs and do not account for the influence of partner drugs in combination therapy or virus strains with reduced susceptibility. The inclusion of target IQs in regulatory guidance for ARV development will be more useful than the current non-specific recommendations.

Therefore, it can be argued that the MEC should be re-evaluated for new drug combinations. For instance, efavirenz is now used in combination with NRTIs that are more efficacious than those used when the current dose of 600 mg and the 1000 ng/mL C_{min} target were established. Though lessons can be learned from preclinical and clinical oncology research about the design and analysis of combination therapy studies,[398-400] the field is still developing and controversies persist.[401,402] Additionally, limitations on study designs are imposed by the potential for resistance development if HIV infected patients are exposed to subtherapeutic drug concentrations.[393] The FDA recommend the inclusion of wide enough range of doses in phase II dose ranging studies to adequately populate exposure- or dose-response curves and facilitate a reliable estimate of optimal drug exposure targets.[393,403] The use of mechanistic modeling is recommended as the most appropriate approach to characterise the relationship between drug exposure and viral kinetics.[393] However, the simplified approach of relating the proportion of virologically suppressed patients with an appropriate exposure variable is often used to justify dose selection in most dose ranging or dose comparison trials. Additionally, this is often done in phase III trials where all participants receive the same dose or assigned to a few doses within a narrow range. This limits opportunities for identifying the minimum optimal pharmacokinetic targets and potential savings in cost. This is likely to become more important as we transition to universal ART for HIV infected patients from 2016.[404] Therefore, studies aimed at developing better in vitro assays for evaluating the antiviral activity of combination therapy, and robust statistical methods for designing and analysing dose finding phase II trials for combination therapy are warranted.

A novel approach incorporating genetically-defined maternal and infant metabolic capacity was used to evaluate the exposure of breastfed infants to maternal EFV and NVP from breast milk. The data presented in chapters 4 and 5 indicate that the levels of exposure observed are mostly within the acceptable limit of 10% of therapeutic dose. The relatively high plasma concentrations EFV and NVP observed during the neonatal period support the recommendation of a population-tailored approach to paediatric ADR assessment.[405,406] However, assessment of ADR (especially CNS) in paediatric populations as a result of breast milk drug intake is difficult for drugs not used in particular age groups. For instance, EFV is not recommended for use in infants < 3 months old and weighing < 3.5 kg. Therefore, using the adult-derived 1000-4000 ng/mL as the effective-toxic plasma concentration range is an oversimplification as the toxicity threshold could be lower in neonates.

Reports about the emergence of resistant HIV strains in breast milk in parallel with the clearance of intrapartum single dose NVP highlight another clinical importance of the presence of ARVs in human breast milk. Aizire et al. reported a reversal of the virological suppression observed after single dose NVP following its clearance from plasma and breast milk.[407] The presence of NVP at subtherapeutic concentrations creates a selective pressure that gives rise to the emergence of persistent NVP-resistant HIV detected in up to 65% of breast milk samples.[408-410] The divergent resistance mutation patterns observed in plasma versus breast milk in many of these studies is indicative of possible compartmentalisation of HIV variants within the mammary gland.[312,409-412] Up to 87% of infants who became infected when single dose NVP failed to prevent MTCT have been shown to have resistant HIV strains that often differed from those in maternal plasma.[411,413-415] All infants in both EFV and NVP cohorts used six-week extended NVP prophylaxis; similar findings have been reported when it is used without maternal ART.[411]

Unlike single dose NVP prophylaxis for PMTCT, long-term ART under Option B or B+ ensures persistently high ARV concentrations in breast milk throughout the breastfeeding period. The

importance of this in prevention of resistance emergence and transmission is unclear. The plasma concentrations of EFV and NVP observed in studies reported here are well above the IC_{50} for wild-type HIV and, combined with co-administered ARVs that are also excreted into breast milk,[89] may play a role in PMTCT. However, the concentrations are mostly subtherapeutic after the first week of life and this raises concerns about the potential for the emergence of resistance in infants who may become infected. This further highlights the importance of early infant diagnosis and treatment. In the Kisumu Breastfeeding Study, HIV-infected pregnant women took zidovudine, lamivudine, and either NVP or nelfinavir from gestation week 34-36 until 6 months postpartum while the infants received single-dose NVP at birth.[310] The cumulative MTCT or death rate was 15.7% at 24 months and genotypic resistance mutations were detected in most infected infants between 2 weeks and 6 months postpartum.[310,416] However, the Mitra plus Study recently reported increasing postpartum virological failure and drug resistance in parallel with declining adherence,[417] indicating a possible role for suboptimal adherence in postpartum emergence of resistant HIV in the presence of ART. Interestingly, Palombi et al. did not observe resistant HIV strains in breast milk and plasma of mothers who initiated ART during pregnancy.[309]

Pregnant women and nursing mothers have historically been excluded from drug development studies as a result of the practical and ethical challenges involved in conducting research in these populations. This has often led to the adoption of adult dosing recommendations in pregnant women and lack of practical recommendations on drug use during lactation.[248] Conducting the clinical studies described in this thesis necessitated the adoption of novel strategies to circumvent the challenges presented by traditional pharmacokinetic sampling techniques.[242,243] The breastfeeding PBPK model described in chapter 6 further highlights how novel approaches may have utility to generate data to inform clinical decisions in real life situations. Only two other groups have used a population pharmacokinetic modeling approach to describe drug excretion into breast milk and/or transfer to breastfed infants and further development in this area is required.[418,419] Recent advances in PBPK modeling now allow for incorporation of the

complex changes that happen during pregnancy for accurate prediction of pharmacokinetics. As with the breastfeeding model, a pregnancy PBPK model is first validated in non-pregnant adults and then extended to pregnant populations by inclusion of certain physiological and anatomical parameters changes associated with pregnancy and extra compartments to represent the fetoplacental unit, uterus and mammary glands.[420]

In most cases, the prerequisite drug and system data for parameterising such models already exist in the literature. Abdujalil et al. recently used a meta-analysis of published data to generate a comprehensive list of equations describing these changes.[421] These equations were included in a pregnancy PBPK model to predict changes to the pharmacokinetics of substrates for CYP1A2, CYP2D6 and CYP3A4 during pregnancy using caffeine, metoprolol and midazolam as model drugs.[422] Prior to this, a PBPK model to predict the pharmacokinetics and enzyme induction sites of CYP3A-metabolised drugs midazolam, nifedipine and the ARV indinavir during the third trimester were described.[423] Interestingly, the pregnancy-induced changes in AUC, C_{max} and C_{min} of indinavir predicted by the PBPK model were similar to clinically observed changes.[424] Incorporation of pregnancy-induced CYP1A2 suppression and CYP2D6 induction into the same model allowed for accurate prediction of the disposition of theophylline (CYP1A2 substrate), paroxetine, dextromethorphan and clonidine (CYP2D6 substrates) during pregnancy.[425] Such models can be validated with available clinical data and then used to simulate dose optimisation strategies for drugs with clinically significant pharmacokinetic alterations during pregnancy. Additionally, a PBPK model can be coupled with a pharmacodynamic model to predict the effects of pharmacokinetic changes on treatment outcomes.[426] However, caution needs to be applied when using modeled data for clinical decisions because a mathematical model is only as good as the input data and significant but unknown variables are usually unaccounted for.

In conclusion, for drugs with significant relative genetic contribution to pharmacokinetic variability, an enrichment design in which patients are stratified based on their genotypes is

recommended to unravel important pharmacokinetic changes during pregnancy. The novel data presented in this thesis highlight how this approach reveal the effects of host genetics on the magnitude of pregnancy-induced changes in EFV and NVP pharmacokinetics. Early diagnosis and treatment in infants who become infected with HIV in utero, during labour/delivery, or during the breastfeeding period will reduce the likelihood of resistance development as a result of exposure to subtherapeutic concentrations of ARVs in breast milk. Where clinical research in pregnant women and nursing mothers is impractical or precluded, novel applications of pharmacokinetic modeling strategies may help generate surrogate data.

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