เภสัชจลนศาสตร์ประชากรของยาทีโนโฟเวียร์ในผู้ป่วยติดเชื้อเอชไอวีชาวไทย

นางสาวกนกรัตน์ รุ่งทิวาสุวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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POPULATION PHARMACOKINETICS OF TENOFOVIR IN THAI HIV-INFECTED PATIENTS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Clinical Pharmacy

Department of Pharmacy Practice

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กนกรัตน์ รุ่งทิวาสุวรรณ : เภสัชจลนศาสตร์ประชากรของยาทีโนโฟเวียร์ในผู้ป่วยติดเชื้อเอชไอวีชาวไทย (POPULATION PHARMACOKINETICS OF TENOFOVIR IN THAI HIV-INFECTED PATIENTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ภญ. ดร.ธิติมา เพ็งสุภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ภญ. ดร.บราลี ปัญญาวุธโธ, พญ. ดร.อัญชลี อวิหิงสานนท์, 102 หน้า.

ภูมิหลัง: ทีโนโฟเวียร์มีความผันแปรทางเภสัชจลนศาสตร์ระหว่างบุคคลสูง ความผันแปรทางพันธุกรรมของยีนที่ขนส่งยาอาจเป็นปัจจัยหนึ่งที่ทำให้เกิดความผันแปรทางเภสัชจลนศาสตร์ระหว่างบุคคลได้ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อสร้างแบบจำลองทางเภสัชจลนศาสตร์ประชากรและศึกษาผลของปัจจัยทั้งที่เกี่ยวข้องกับพันธุกรรมและไม่เกี่ยวข้องกับพันธุกรรมต่อค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาทีโนโฟเวียร์ เพื่อใช้เป็นแนวทางในการพิจารณาขนาดยาที่เหมาะสมในผู้ป่วย

วิธีทำการศึกษา: ทำการศึกษาเชิงพรรณนาแบบย้อนหลัง โดยรวบรวมข้อมูลผู้ป่วยติดเชื้อเอชไอวีชาวไทยจากศูนย์ประสานความร่วมมือระหว่างไทย ออสเตรเลีย เนเธอร์แลนด์ เพื่อการวิจัยด้านโรคเอดส์จำนวน 342 รายเพื่อสร้างแบบจำลองทางเภสัชจลนศาสตร์ โดยวิธี nonlinear mixed effects model (NONMEM) และตรวจสอบความถูกต้องของแบบจำลองจากข้อมูลของผู้ป่วยในฐานข้อมูลของหน่วยเภสัชพันธุศาสตร์และการแพทย์เฉพาะบุคคล โรงพยาบาลรามาธิบดี จำนวน 103 รายโดยใช้การประมาณค่าแบบเบส์

ผลการศึกษา: เภสัชจลนศาสตร์ของยาทีโนโฟเวียร์สามารถอธิบายด้วยแบบจำลองเภสัชจลนศาสตร์แบบสองห้องที่มีการดูดซึมยาและการขจัดยาแปรผันตรงกับความเข้มข้นของยา อัตราการกรองของไตที่คำนวณโดยสูตร Cockcroft and Gault การได้รับยาโลปินาเวียร์/ริโทนาเวียร์ และภาวะพหุสัณฐานของยีน *ABCC4* 3463 A>G ส่งผลต่อค่าการขจัดยาทีโนโฟเวียร์ การใช้ยาโลปินาเวียร์/ริโทนาเวียร์ร่วมระหว่างการรักษาจะลดค่าการขจัดยาทีโนโฟเวียร์ร้อยละ 25.1 ผู้ป่วยที่มียีน *ABCC4* 3463A>G ผิดปกติอย่างน้อย 1 อัลลีล (AG หรือ GG) จะมีค่าการขจัดยาสูงกว่าผู้ป่วยที่มีลักษณะยีนปกติ (AA) ร้อยละ 10.5 ผลการตรวจสอบแบบจำลองโดยพิจารณาจากกราฟแบรนด์อัลท์แมนพบว่าแบบจำลองไม่แสดงอคติอย่างเป็นระบบ แต่เมื่อพิจารณาจากค่าเฉลี่ยความผิดพลาดในการทำนายพบว่ามีค่าเท่ากับ -0.00452 มิลลิกรัม/ลิตร ซึ่งแตกต่างจากศูนย์อย่างมีนัยสำคัญทางสถิติ (p=0.029) แสดงให้เห็นว่าแบบจำลองมีแนวโน้มทำนายระดับยาต่ำกว่าความเป็นจริง

สรุปผล: การทำงานของไต ยาที่ได้รับร่วมด้วย และความผันแปรทางพันธุกรรมส่งผลต่อเภสัชจลนศาสตร์ของยาทีโนโฟเวียร์ ปัจจัยดังกล่าวควรนำมาพิจารณาในการกำหนดขนาดยาทีโนโฟเวียร์ในผู้ป่วยแต่ละราย เพื่อให้แน่ใจถึงประสิทธิภาพและความปลอดภัยในการใช้ยาของผู้ป่วย

ภาควิชา เภสัชกรรมปฏิบัติ	ลายมือชื่อนิสิต .....
สาขาวิชา เภสัชกรรมคลินิก	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ปีการศึกษา 2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....
	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: TENOFOVIR / POPULALTION PHARMACOKINETICS / ABCC2 / ABCC4 / HIV / THAI

KANOKRAT RUNGTIVASUWAN: POPULATION PHARMACOKINETICS OF TENOFOVIR IN THAI HIV-INFECTED PATIENTS. ADVISOR: ASSOC. PROF. THITIMA PENGSUPARP, Ph.D., CO-ADVISOR: ASST. PROF. BARALEE PUNYAWUDHO, Ph.D., ANCHALEE AVIHINGSANON, M.D.,Ph.D., 102 pp.

Background: Tenofovir has high interindividual variability. Genetic variation of drug transporters may contribute to high interindividual variability of tenofovir. Therefore, the aims of study were to develop population pharmacokinetic model and identify factors, both genetic and non-genetic factors, influencing pharmacokinetic parameters of tenofovir in order to provide initial information for dose optimization.

Methods: This study is a retrospective descriptive study. A total of 342 Thai HIV-infected patients from clinical studies of The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) were included for the population pharmacokinetic model development using nonlinear mixed effects model (NONMEM) and 103 patients from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database were used for model validation using Bayesian estimation.

Results: Pharmacokinetics of tenofovir can be best described by a two-compartment model with first order absorption and elimination. The estimated glomerular filtration rate calculated by Cockcroft and Gault formula, concomitant use of lopinavir/ritonavir and *ABCC4* 3463A>G polymorphism were associated with apparent oral clearance (CL/F) of tenofovir. The concomitant use of lopinavir/ritonavir decreased CL/F of tenofovir by 25.1%. Patients carrying at least 1 variant allele of *ABCC4* 3463 A>G (genotype AG or GG) had tenofovir CL/F 10.5% higher than those with wild type (genotype AA). For model validation, the Bland-Altman plot showed no systematic bias. However, the mean prediction error of the final model was -0.00452 mg/l and it was significantly different from zero (p=0.029), indicating that the final model tended to underpredict the concentrations.

Conclusions: Renal function, comedication and genetic variation had impact on the pharmacokinetics of tenofovir. These factors should be considered when tenofovir is prescribed to ensure efficacy and safety of the drug.

Department:	Pharmacy Practice	Student's Signature	.....
Field of Study:	Clinical Pharmacy	Advisor's Signature	.....
Academic Year:	2014	Co-Advisor's Signature	.....
		Co-Advisor's Signature	.....

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LIST OF ABBREVIATIONS

ABC	=	Adenosine triphosphate binding cassette
AD	=	Alternate day
AIC	=	Akaike information criterion
AIDS	=	Acquire immune deficiency syndrome
ATV/r	=	Atazanavir/ritonavir
AUC	=	Area under the curve
BW	=	Body weight
CI	=	Confidence interval
CI_{cr}	=	Creatinine clearance
CL/F	=	Apparent oral clearance
C_{max}	=	Maximum concentration
C_{min}	=	Minimum concentration
CWRES	=	Conditional weighted residuals
CYP450	=	Cytochrome P450
dl	=	Deciliter
DNA	=	Deoxyribonucleic acid
DV	=	Dependent variable (observed concentration)
ELISA	=	Enzyme-linked immunosorbent assay
FOCE-I	=	First-order with conditional estimation with interaction
GFR	=	Glomerular filtration rate
GFR_{CG}	=	Glomerular filtration rate calculated by Cockcroft and Gault formula
GFR_{MDRD}	=	Glomerular filtration rate calculated by the Modification of Diet in Renal Disease Formula
GFR_{THAI}	=	Glomerular filtration rate calculated by Thai formula
HAART	=	Highly active antiretroviral therapy
HBV	=	Hepatitis B virus

HCV	=	Hepatitis C virus
HIV	=	Human immunodeficiency virus
HIV-NAT	=	The HIV Netherlands Australia Thailand Research Collobration
HPLC	=	High-performance liquid chromatography
hr	=	Hour
IIV	=	Interindividual variability
INSI	=	Integrase transfer inhibitor
IPRED	=	Individual predicted concentration
IQR	=	Interquartile range
k_a	=	Absorption rate constant
kg	=	Kilogram
KTD	=	Kidney tubular dysfunction
L	=	Liter
LPV/r	=	Lopinavir/ritonavir
LRT	=	Likelihood ratio test
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
MPE	=	Mean prediction error
MRP	=	Multidrug resistance protein
NA	=	Not applicable
ng	=	Nanogram
NNRTI	=	Non-nucleoside reverse transcriptase inhibitor
NRTI	=	Nucleoside reverse transcriptase inhibitor
NtRTI	=	Nucleotide reverse transcriptase inhibitor
OAT	=	Organic anion transporter
OFV	=	Objective function value
PE	=	Prediction error
P-gp	=	P-glycoprotein

PI	=	Protease inhibitor
PPC	=	Posterior predictive check
PRED	=	Population predicted concentration
Q/F	=	Apparent intercompartmental clearance
RMSE	=	Root mean square error
rs number	=	Reference single nucleotide polymorphism number
RUV	=	Residual unexplained variability
Scr	=	Serum creatinine
SD	=	Standard deviation
SE	=	Standard error
SLC	=	Solute carrier
SQV/r	=	Saquinavir/ritonavir
TDF	=	Tenofovir disoproxil fumarate
$t_{1/2}$	=	Half life
US FDA	=	United Stated Food and Drug Administration
V_d	=	Volume of distribution
V_c/F	=	Apparent central compartment volume of distribution
V_p/F	=	Apparent peripheral compartment volume of distribution
WHO	=	World Health Organization
WRES	=	Weighted residual

CHAPTER I
INTRODUCTION

Background and Rationale

Human immunodeficiency virus (HIV) infection is one of the major global public health issues. According to World Health Organization (WHO) report [1], there are more than 39 million HIV-infected patients around the world. About 2.1 million patients are newly infected and 1.5 million patients died from HIV-related cause. In Thailand, according to The Bureau of Epidemiology Thailand, there are 431,475 HIV-infected patients and 8,535 patients are newly infected [2, 3].

Although HIV infection cannot be cured, an effective treatment of antiretroviral drugs called highly active antiretroviral therapy, HAART, can control HIV virus and significantly improve patients' quality of life [4]. The recommended therapy is the combination of at least three antiretroviral drugs, two nucleoside reverse transcriptase inhibitors (NRTIs) and either one non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) [3, 5]. The goals of therapy include suppression of viral load, preservation and strengthening the immune system, prevention of HIV-related morbidity and mortality, limitation of adverse drug reaction and promotion of adherence [4, 6].

Tenofovir is a nucleotide reverse transcriptase inhibitor (NtRTI) antiretroviral drug approved from United States Food and Drug Administration (US FDA) for the treatment HIV infection and chronic hepatitis B [7]. According to WHO and Thai guidelines, tenofovir is one of the first line drug for the treatment of HIV infection used in combination with other antiretroviral drugs because of its high antiviral potency, low adverse drug reaction, limited drug interaction, daily dosage regimen and having a fixed-dose combination with other antiretroviral drugs [3, 7, 8].

Tenofovir disoproxil fumarate (TDF) is an oral prodrug of tenofovir. Following absorption, TDF is rapidly converted to tenofovir and is intracellularly phosphorylated to tenofovir diphosphate, an active analog, which inhibits HIV reverse transcriptase leading to terminate DNA chain elongation. Tenofovir is eliminated by a combination of

glomerular filtration and active tubular secretion. For an active tubular secretion, tenofovir is transported into kidney tubular cell by organic anion transporters (OAT) 1 and OAT3, encoded by *SLC*22A6 and *SLC*22A8, respectively at basolateral membrane. Subsequently, tenofovir is effluxed from kidney tubular cell by multidrug resistance proteins (MRP) 2 and MRP4, encoded by *ABCC*2 and *ABCC*4, respectively at apical membrane [9]. Genetic polymorphisms of these transporters may affect transportation of tenofovir at kidney tubular cell and therefore affect efficacy and toxicity of this drug.

Tenofovir plasma concentration had influence on both toxicity and efficacy of drug. Although, high tenofovir exposure was correlated with higher antiviral efficacy, it may cause higher risk of toxicity. The median steady state plasma area under the curve (AUC) of patients having virological response was significant higher than patients who did not have virological response (AUC 3,800 and 2,510 ng.hr/ml, respectively; p=0.031) [10]. Moreover, when TDF dose lower than 300 mg per day was given, tenofovir plasma concentration and the reduction of HIV-1 RNA level were lower [11]. On the other hand, the mid-dose concentration (C_{12}) more than 160 ng/ml and trough concentration more than 90 ng/ml were associated with nephrotoxicity in patients receiving tenofovir [12, 13]. Patients having tenofovir concentration more than 160 ng/ml was 4.8 times higher risk of kidney tubular dysfunction (KTD) than patients having tenofovir concentration less than 160 ng/ml [12]. These results suggested that monitoring of tenofovir plasma concentration is crucial for the optimization of dosage regimens in order to prevent renal toxicity and ensure efficacy of this drug.

Tenofovir has high variability in drug exposure [14, 15]. Many factors may contribute to its high interindividual variability. Although, the results from previous pharmacokinetic studies showed that some demographic data including body weight, renal function and concomitant medications can describe pharmacokinetic variability of tenofovir [14-17], there may be other factors including genetic variation that may influence pharmacokinetic parameters of tenofovir. Moreover, previous studies showed that the polymorphisms of *ABCC2* and *ABCC4* were associated with higher tenofovir concentration [18, 19] and KTD in patients receiving tenofovir [9, 18-20]. A study by

Kiser JJ et al found that patients with *ABCC4* 3463A>G variant had lower tenofovir renal clearance than those carrying wild type, leading to a 32% increase of tenofovir area under the curve [20]. A recent study in Thai HIV-infected population reported that patients with *ABCC2* -24 CC genotype were associated with higher tenofovir plasma concentration compared to those with CT and TT genotypes (113 ng/ml vs 93 ng/ml, respectively) [18]. Therefore, we hypothesized that genetic polymorphisms of *ABCC2* and *ABCC4* may be one of the factors that contribute to high interindividual variability of tenofovir.

Population pharmacokinetic study using nonlinear mixed effects model (NONMEM) is widely used to estimate population mean pharmacokinetic parameters, identify sources of variability and factors influencing pharmacokinetic parameters. The information obtained from the population pharmacokinetic study can be used to design and optimize dosage regimens for each individual patient [21].

However, nowadays there are a few population pharmacokinetic studies of tenofovir, especially those investigating the association between genetic polymorphisms of drug transporters and population pharmacokinetics of tenofovir. Futhermore, all of the previous studies were performed in Caucasian population [14-16], but not in Asian population. Therefore, the aims of this study were to develop the population pharmacokinetic model of tenofovir and investigate the influence of genetic factors including the polymorphisms of *ABCC2* and *ABCC4* and non-genetic factors including sex, age, body weight, renal function, hepatitis B co-infection, hepatitis C co-infection and concomitant use of drugs on pharmacokinetic parameters of tenofovir. This information would be useful for dose optimization of tenofovir in Thai and Asian HIV-infected patients.

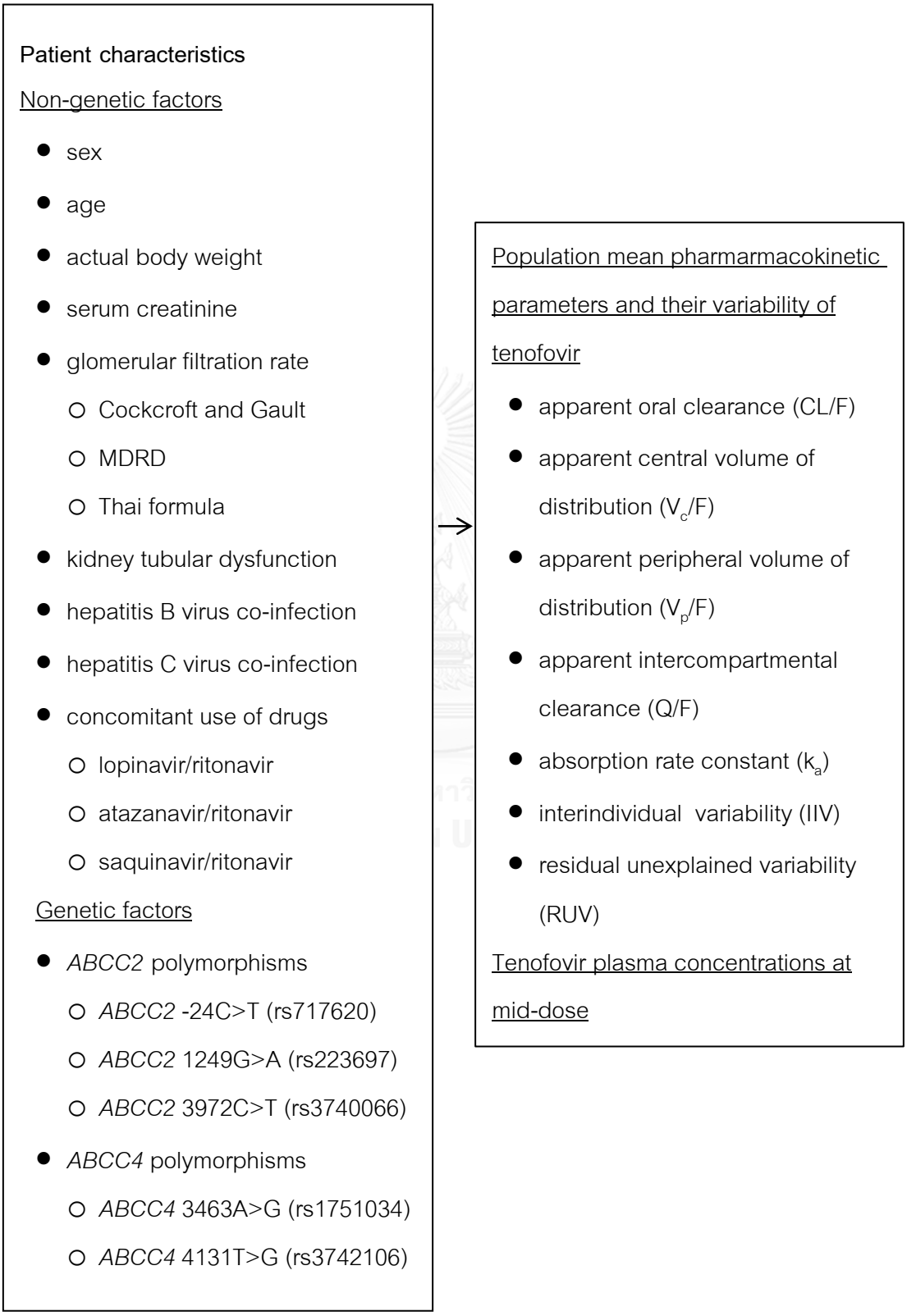
Hypothesis

Genetic factors including *ABCC2* (-24C>T, 1249G>A, 3972C>T) and *ABCC4* (3463A>G, 4131T>G) polymorphisms and non-genetic factors including sex, age, body weight, serum creatinine, glomerular filtration rate, kidney tubular dysfunction, hepatitis B co-infection, hepatitis C co-infection and concomitant use of drugs were associated with pharmacokinetic parameters and plasma concentrations of tenofovir.

Objectives

1. To develop the population pharmacokinetic model of tenofovir in Thai HIV-infected patients
2. To estimate the population mean pharmacokinetic parameters and their variability of tenofovir in Thai HIV-infected patients
3. To investigate the influence of genetic and non-genetic factors on pharmacokinetic parameters of tenofovir
4. To investigate the influence of genetic and non-genetic factors on tenofovir plasma concentrations

Conceptual framework



Scope of this study

A retrospective descriptive study of population pharmacokinetics of tenofovir was performed in Thai HIV-infected patients. Database of patients was extracted from clinical trial of The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) and Pharmacogenomics and Personalized Medicine, Ramathibidi Hospital.

Operation definitions

- Population pharmacokinetic parameters are defined as pharmacokinetic parameters including apparent oral clearance (CL/F), apparent central volume of distribution (V_c/F), apparent peripheral volume of distribution (V_p/F), apparent intercompartmental clearance (Q/F) and absorption rate constant (k_a) that are estimated from the population pharmacokinetic model.
- Population pharmacokinetic variability is defined as pharmacokinetic variability including interindividual variability and residual unexplained variability [21, 22].
- Interindividual variability is the variability of pharmacokinetic parameter across the individuals in the population. It can be defined as the difference between individual pharmacokinetic parameter and the population value [21, 22].
- Residual unexplained variability is unexplained variability in observed data after controlling for other sources of variability. It can be defined as the difference between observed concentration and model-predicted concentration [21-23].
- Glomerular filtration rate calculated by Cockcroft and Gault formula is calculated according to equation 1.

$$GFR_{CG} = \frac{(140 - age) \times BW}{72 \times Scr} \times 0.85 \text{ (if female)} \qquad \text{.....equation 1}$$

GFR_{CG} is glomerular filtration rate calculated by Cockcroft and Gault formula (ml/min).
Scr is serum creatinine (mg/dl).
BW is actual body weight (kg).

- Glomerular filtration rate calculated by the Modification of Diet in Renal Disease formula is calculated according to equation 2.

$GFR_{MDRD} = 175 \times Scr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) [24] .....equation 2

GFR_{MDRD} is glomerular filtration rate calculated by MDRD (ml/min/1.73m^2).

Scr is serum creatinine (mg/dl).

- Glomerular filtration rate calculated by Thai formula is calculated according to equation 3.

$GFR_{THAI} = 375.5 \times Scr^{-0.848} \times age^{-0.364} \times 0.712$ (if female) [24] .....equation 3

GFR_{THAI} is glomerular filtration rate calculated by Thai formula (ml/min/1.73m^2).

Scr is serum creatinine (mg/dl).

- Kidney tubular dysfunction is defined on the basis of the presence of at least two of the following criteria [25]

1. fractional tubular absorption for phosphorus [1-{(urine phosphorus x plasma creatinine)/(plasma phosphorus x urine creatinine)}] less than 0.80 or maximum tubular for phosphate corrected for GFR(TmP/GFR){plasma phosphorus– [(urine phosphorus x plasma creatinine)/urine creatinine] less than 2.6 mg/dl
2. total daily excretion of phosphorus (urine phosphorus x urine volume) more than 1200 mg
3. fractional excretion of uric acid [1-{(urine uric acid x plasma creatinine)/(plasma uric acid x urine creatinine)}x 100] more than 15%
4. β_2 microglobulin more than 1 mg/day or β_2 microglobulin/urinary creatinine more than 0.3 mg/l
5. non-diabetic glucosuria (urine glucose > 300 mg/day or positive for urine glucose) with normal glycemic levels (plasma glucose < 100 mg/dl)

Significance of the study

The information obtained from this study including population mean pharmacokinetic parameters and factors influencing pharmacokinetic parameters can be used as initial information to optimize tenofovir dosage regimens in Thai HIV-infected patient in order to minimize toxicity, while maximize efficacy of tenofovir in this population.

CHAPTER II
LITERATURE REVIEW

Human immunodeficiency virus infection

Human immunodeficiency viruses (HIV) are lentivirus, a family of mammalian retrovirus. HIV was categorized into two groups; HIV-1 and HIV-2. HIV-1 infection is the major cause of acquired immunodeficiency syndrome (AIDS) around the world, whereas HIV-2 is found only in Soulth Africa and less severe than HIV-1 infection [26].

HIV infection can be acquired through sexual intercourse, injectable drug use, receiving of blood product and from mother to infant transmission [4]. After 6-12 weeks of infection, HIV infection can be diagnosed by detection of HIV-antibody by enzyme-linked immunosorbent assays (ELISA). However, when testing before 6 weeks, HIV-antibody may not be positive. Therefore, the detection of HIV RNA or p24 antigen test may be performed [4, 6].

In primary HIV infection, acute retroviral syndromes may be occurred during 2-4 weeks after infection. The symptoms are nonspecific including fever, fatigue, rash, headache, sore throat, muscle pain and weight loss [4, 6]. In chronic infection, HIV infection can be categorized into 4 stages according to CD4 lymphocyte level as shown in table 1.

Table 1: Stages of HIV infection [6]

Stage	CD4 lymphocyte level	Symptoms
Early-stage	> 500 cell/μl	Patients are asymptiomatic but may have lymphoadenopathy, seborheic dermatitis, chronic fungal infection of the nail and aphthous ulcer.
Mid-stage	200-500 cell/μl	Patients usually have temporary fever, weight loss, muscle pain, joint pain and chronic sinusitis. The skin symptoms and mouth ulcers may be advanced.
Advanced-stage	50-200 cell/μl	The immune system of patients in this stage is decreased resulting in higher risk of opportunistic infection. Patients usually have papulopruritic skin eruption. Some patients may have nervous system symptoms such as mononeuritis, myelitis, cranial nerve palsies, idiopathic peripheral neuropathy and HIV-1 retinopathy.
End-stage	<50 cell/μl	Patients are usually thin (called HIV wasting syndrome) and have severe opportunistic infection.

Although, HIV infection cannot be cured, early treatment of potent combination of antiretroviral drugs can reduce metastasis of virus in the body, decrease the destruction of immune system and decrease latent reservoirs of HIV virus [6]. The recommended antiretroviral therapy is the combination of at least three antiretroviral drugs that is usually combined between two NRTIs and one NNRTI [3, 5]. However, if patients cannot tolerate to NNRTI, protease inhibitor is recommended [3]. The preferred first-line and alternative antiretroviral regimen are presented in table 2.

Table 2: Preferred first-line and alternative antiretroviral drug regimens according to WHO and Thai guideline [3, 5, 27]

WHO guideline 2013

When to start antiretroviral drugs

Start antiretroviral drugs for all patients with CD4 ≤ 500 cell/mm^3 and initiate immediately regardless of CD4 for children aged ≤ 5 years, patients with active tuberculosis or co-infected with hepatitis B virus with severe chronic liver disease and patients living with HIV in serodiscordant partnership

Recommended antiretroviral drugs

Preferred : TDF+3TC (or FTC)+ EFV as a fixed dose combination

Alternative : AZT+3TC+EFV

AZT+3TC+NVP

TDF+3TC (or FTC)+NVP

Thai guideline 2014

When to start antiretroviral drugs

Initiate antiretroviral drugs in HIV-infected patients at all CD4 level especially in patients with CD4 <500 cell/mm^3

Recommended antiretroviral drugs

NRTIs		**NNRTIs**		**PIs**
Preferred		*Preferred*		*Preferred*
TDF/FTC*		EFV	If patients cannot	LPV/r
TDF/3TC*	Plus	or	→ tolerate NNRTs	or
Alternative		RPV		ATV/r
ABC+3TC		NVP		
AZT+3TC				

*fixed dose combination regimen is preferred

ABC, abacavir; ATV/r, atazanavir/ritonavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NVP, nevirapine; PIs, protease inhibitors, RPV; rilpivirine; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine

Tenofovir

Indication [7, 28]

Tenofovir, a nucleotide reverse transcriptase inhibitor, was approved from US FDA for the treatment of HIV-1 infection in combination with other antiretroviral drugs in adults and pediatric patients ≥ 2 years of age and for the treatment of chronic hepatitis B in adults and pediatric patients ≥ 12 years of age.

Dosage regimen [28]

- Children 2 years to less than 12 years of age: TDF 8 mg per kg body weight (up to maximum 300 mg) once daily
- Children ≥ 12 years and body weight ≥ 35 kg: TDF 300 mg once daily
- Adult patients with normal renal function (creatinine clearance ≥ 50 ml/min): TDF 300 mg once daily
- Adult patients with moderate renal impairment (creatinine clearance 30-49 ml/min): TDF 300 mg every 48 hours
- Adult patients with severe renal impairment (creatinine clearance 10-29 ml/min): TDF 300 mg every 72 to 96 hours
- Hemodialysis patients: TDF 300 mg every 7 days or after 12 hours of dialysis

Mechanism of action [29]

Due to poor absorption of tenofovir, an oral prodrug (tenofovir disoproxil fumarate; TDF) was developed in order to increase bioavailability. Following absorption, TDF is rapidly hydrolyzed to tenofovir by enzyme esterase, and then is intracellularly phosphorylated to tenofovir diphosphate, an active analog, which is a competitive inhibitor of HIV reverse transcriptase and terminates the growing of DNA chain.

Pharmacokinetics [29-31]

Absorption

TDF is rapidly absorbed into blood circulation and then converted to tenofovir. An oral bioavailibility of TDF is 25% in fasted state and 39% after a high-fat meal. After administration TDF 300 mg single dose, the maximum concentration (C_{max}) is 300 ng/ml and the area under the curve (AUC) is 2,290 ng.hr/ml. The pharmacokinetics of tenofovir is dose-proportional in the dose range of 75-600 mg.

Distribution

Plasma protein binding of tenofovir is less than 0.7%. After administration TDF 300 mg once daily, the volume of distribution (V_d) at steady state is 0.8 L/kg.

Metabolism

Neither TDF nor tenofovir are substrate or inhibitor of cytochrome P450 (CYP450) enzyme. However, an in vitro study showed that TDF can slightly induce CYP 1A1 and 2B, but without any evidence of clinical significance. Therefore, drug interactions of tenofovir mediated via CYP450 are minimal.

Elimination

Tenofovir is mainly eliminated as an unchanged form in urine. The elimination of tenofovir is combined with glomerular filtration and active tubular secretion. Tenofovir is transported into kidney tubular cell by OAT1 and OAT3, encoded by *SLC22A6* and *SLC22A8*, respectively, at basolateral membrane. Subsequently, tenofovir is secreted to the tubular lumen by MRP2 and MRP4, encoded by *ABCC2* and *ABCC4*, respectively, at apical membrane. The terminal elimination half life ($t_{1/2}$) of tenofovir is approximately 12-18 hours.

Drug interaction [29-32]

Because TDF and tenofovir are not substrate or inhibitor of CYP450, drug interactions mediated via hepatic enzyme are minimal. Tenofovir is eliminated via active tubular secretion by drug transporters. Therefore, co-administration with other drugs that compete or inhibit drug transporters may have drug interactions with tenofovir.

NRTIs

When concomitantly use tenofovir with didanosine or abacavir, C_{max} and AUC of didanosine and abacavir are increased. Some adverse events such as pancreatitis, hyperlactatemia and lactic acidosis have been reported. On the other hand, the interaction between tenofovir and lamivudine, stavudine or emtricitabine has not been observed.

PIs

PIs are usually combined with low dose ritonavir in order to increase bioavailibility and drug concentration. Ritonavir is a potent inhibitor of p-glycoprotein (P-gp) and MRP2 drug transporter. Inhibition of efflux transport by ritonavir can increase concentration of tenofovir. Previous pharmacokinetic studies showed that C_{max} and AUC of tenofovir were increased when concomitantly use with lopinavir/ritonavir, atazanavir/ritonavir, darunavir/ritonavir or saquinavir/ritonavir [32]. The effects of PIs on tenofovir pharmacokinetic parameters are presented in table 3.

Table 3: Effects of PIs on the plasma pharmacokinetics of tenofovir when TDF was given 300 mg once daily [32]

PIs	Dosage regimen (mg)	% Change of tenofovir		
		AUC	C_{max}	C_{min}
atazanavir/ritonavir	300/100 OD	↑37	↑34	↑29
brecanavir/ritonavir	300/100 BID	↑32	↑24	Not report
indinavir	800 TID	↔	↑14	Not report
darunavir/ritonavir	300/100 BID	↑22	↑24	↑37
lopinavir/ritonavir	400/100 BID	↑32	↑15	↑51
saquinavir/ritonavir	1000/100 BID	↑14	↑15	↑23

Other drugs

The use of acyclovir, cidofovir, foscanet, ganciclovir and amphotericin B in combination with tenofovir should be avoided. Although these interactions were not proved, these drugs may compete with tenofovir for renal tubular secretion resulting in an increasing tenofovir plasma concentration.

Adverse drug reactions

Tenofovir is a well-tolerated drug [30, 31]. The effect of tenofovir on blood lipid, fat accumulation and mitochondria toxictiy is found to be less than other NRTIs. Gastrointestinal symptoms including diarrhea (11-16%), nausea (8-11%) and vomiting (4-7%) are the most common adverse effects of drug [33]. The other adverse events include hypophosphatemia, decreased bone mass, headache, fatigue, rash and neuropathy [33].

There were some reports of tenofovir induced nephrotoxicity especially proximal tubular dysfunction and fanconi syndrome [34]. Factors including older age, low body weight, preexisting renal impairment, concomitant use of nephrotoxic drugs or protease inhibitors, polymorphisms of *ABCC2* and *ABCC4* and high tenofovir plasma concentration were associated with renal toxicity in patients receiving tenofovir [35-37].

The mechanism of tenofovir induced nephrotoxicity is still unclear. However, two mechanisms have been proposed; 1) tenofovir inhibits DNA polymerase γ, leading to the decrease of mitochondria DNA and causing mitochondria toxicity, similar to adefovir and cidofovir. 2) tenofovir may interfere normal function of tubular cells due to the interaction between tenofovir and transporter proteins located at the renal tubule [36].

According to the HIV Medicine Association of Infectious Disease Society of America guideline, patients with GFR lower than 90 ml/min/1.73m^2, having other comorbid diseases or using protease inhibitors as comedication should monitor renal function, serum phosphorus and proteinuria at least every 6 months due to potential risk of nephrotoxicity [35]. However, in the first year of therapy, patients may be monitored more frequently (e.g. every 3 months) in order to detect any renal dysfunction [37].

Association between tenofovir plasma concentration and toxicity

High tenofovir plasma concentration was found to be associated with nephrotoxicity. A study by Rodriguez-Novoa S et al [12] showed that the median tenofovir plasma concentration at mid dose (10-14 hours after last dose) in patients with KTD was significantly higher than those without KTD (182 ng/ml and 106 ng/ml, respectively; p=0.001). Patients having tenofovir plasma concentration more than 160

ng/ml were 4.8 times higher risk of KTD than patients having tenofovir concentration less than 160 ng/ml. Thus, the cut-off tenofovir plasma concentration at mid dose greater than 160 ng/ml was proposed to discriminate the risk of KTD. Futhermore, a study by Poizot-Martin et al [13] showed that tenofovir trough concentration greater than 90 ng/ml was associated with a significantly decrease of GFR from baseline ($p<0.001$).

Association between tenofovir plasma concentration and efficacy

Tenofovir exposure was also associated with antiviral efficacy. Median steady state TDF AUC of patients having virological response (>0.5 $\log_{10}$ HIV copies/ml decline) was higher than those who did not have virological response (AUC 3,800 and 2,510 ng.hr/ml respectively; $p=0.031$) [10]. When TDF dose lower than 300 mg per day was given, tenofovir plasma concentration and the reduction of HIV-1 RNA concentration were lower [11].

Organic anion transporters (OATs) and multidrug resistance proteins (MRPs)

Membrane transporters are found at endothelial and epithelial barriers including blood brain barrier, intestinal epithelial cells, hepatocytes and renal tubular cells [38]. Nowadays, it is accepted that membrane transporters have a major impact on the absorption, distribution and excretion of several drugs and toxin [39].

In general, drug transporters can be classified into two groups; [38]

1. Uptake solute carrier (*SLC*) transporters including organic anion transporting polypeptide (OATP) and organic anion transporter (OAT).
2. Efflux adenosine triphosphate binding cassette (*ABC*) or multidrug resistant (MDR) transporters including *ABCB1* (p-glycoprotein or MDR1), *ABCC* (MRP) and *ABCG2* (breast cancer resistance protein or BRCP).

OATs are encoded by *SLC*22 gene subfamily. Most of the OATs are occurred at renal proximal tubules, the site of active drug secretion, with an exception for human OAT7 that is expressed at liver cells [40]. In human kidneys, OAT1, OAT2 and OAT3 are located at basolateral membrane, whereas OAT4 and OAT10 are located at apical membrane of proximal cells [40].

OAT1 is predominantly expressed at human kidney cell, but it is also detected at brain, skeletal muscle and placenta. OAT1 has an important role in secretion of p-aminohippurate (PAH) and several therapeutic drugs including beta-lactam antibiotics, loop diuretics, non-steroidal anti-inflammatory drugs and antiviral nucleoside analogs [41]. However, the association between genetic polymorphisms of this transporter and pharmacokinetics of drugs was limited. Previous studies found that genetic polymorphisms of *SLC*22A6 (encode OAT1) were not associated with renal clearance of adefovir and torsemide [41, 42].

OAT3 is mainly located at the kidney, and less expressed at liver, brain and eye. The gene for OAT3 (*SLC*22A8) is located on chromosome 11q123, where is close to *SLC*22A6, the gene coding for OAT1 [43]. Therefore, the substrates transported by these two transporters are overlapping [43]. OAT3 recognized a broad spectrum of substrates including PAH, estrone sulfate and various drugs including beta-lactam antibiotics, loop diuretics, antiviral drugs and HMG-CoA reductase inhibitors [40, 43]. Similar to OAT1, the association between polymorphisms of *SLC*22A8 (encoded OAT3) and pharmacokinetics of drugs was limited. Previous studies found that polymorphisms of *SLC22A8* were not found to be associated with renal clearance of pravastatin and torsemide [42, 44].

MRPs are encoded by *ABCC* subfamily. Nowadays, there are 9 MRPs which differ in structures, substrate specificities and intracellular locations [39]. Most of drug transporters are located at tissues with a barrier function, such as intestine, liver, brain capillaries, placenta and kidney [45].

MRP2 is predominantly expressed at hepatocyte canalicular membrane and less expressed at gallbladder epithelial cell and apical membrane of proximal tubular cell [39]. MRP2 plays an important role in detoxification and chemoprotection by transporting a wide range of compounds, especially conjugates of lipophilic substances with glutathione, glucuronate and sulfate. Moreover, MRP2 also transports exogenous compounds including anticancer drugs (doxorubicin, methotrexate, cisplatin, irinotecan), antiviral drugs (ritonavir, indinavir, saquinavir, adefovir, cidofovir, tenofovir),

antibiotics (ampicillin, ceftriaxone, azithromycin) and HMG-CoA reductase inhibitors (pravastatin) [39].

Previous studies showed that the polymorphisms of *ABCC2* (encode MRP2) were associated with pharmacokinetics of several drugs including irinotecan and methotrexate [46-48]. A study by Hagleitner MM et al found that patients with *ABCC2* 3972C>T genotype TT had methotrexate concentration higher than those with genotype CC [47]. Furthermore, a recent study showed that genetic polymorphism of *ABCC2* -24C>T can describe interindividual variability of CL/F and V_c/F of methotrexate [46]. Patients carrying *ABCC2* -24C>T variant allele (genotype CT or TT) had a 30% increase of CL/F and a 40% increase of V_c/F of methotrexate.

MRP4 is generally located at basolateral membrane, whereas it is expressed at apical membrane of renal proximal cell and the luminal side of brain capillary endothelium [45]. MRP4 transports endogenous compounds including cyclic nucleotides, ADP, urate and conjugated steroid hormones, which have an important role in cellular communication and signaling [45]. Moreover, MRP4 also transports several drugs including antiviral drugs (adefovir, tenofovir, ganciclovir, lamivudine), antibiotics (cephalosporins), cardiovascular drugs (loop diuretics, thiazides, angiotensin II receptor antagonists) and cytotoxic agents (methotrexate, 6-mercaptopurine) [45].

The association between genetic polymorphisms of *ABCC4* (encode MRP4) on pharmacokinetics of drug have been reported. A study by Anderson P et al found that patients carrying *ABCC4* 4131 TG or GG genotype had a 20% increase of intracellular lamivudine concentrations compared with those carrying TT genotype [49].

Effect of genetic polymorphisms of drug transporters on pharmacokinetics and renal toxicity of tenofovir

Tenofovir is transported into kidney tubular cell by OAT1 and OAT3 encoded by *SLC22A6* and *SLC22A8*, respectively. Subsequently, it is secreted to the tubular lumen by MRP2 and MRP4 encoded by *ABCC2* and *ABCC4*, respectively. Previous studies showed that the polymorphisms of *ABCC2* and *ABCC4* were associated with pharmacokinetics and renal toxicity of tenofovir as shown in table 4. However, the genetic polymorphisms of *SLC22A6* and *SLC22A8* were not found to be associated with the pharmacokinetics of tenofovir and several drugs including adefovir, pravastatin and torsemide [19, 20, 41, 42, 50]. Therefore, it is possible that transportation of drug across apical membrane (from cell to tubular lumen) may be rate limiting step for kidney elimination of tenofovir.

Table 4: Association between genetic polymorphisms of drug transporters and pharmacokinetics and renal toxicity of tenofovir

Study	Gene (protein)	SNP (rs number)	Association between genetic polymorphisms and pharmacokinetics/ renal toxicity of tenofovir
Rodriguez S [9] Manosuthi W[18] Kiser JJ [20] Izzedine H [51]	*ABCC2* (MRP2)	-24 C>T (rs717620)	- Patients with genotype CT had tenofovir renal clearance higher than genotype CC. - Patients with genotype CT or TT had tenofovir plasma concentration and incidence of KTD less than genotype CC.
Izzedine H [51]	*ABCC2* (MRP2)	1249 G>A (rs2273697)	Patients with genotype GA or AA had incidence of KTD more than genotype GG.
Rodriguez S [9] Izzedine H [51]	*ABCC2* (MRP2)	3563 T>A (rs8187694)	Patients with genotype TA or AA had incidence of KTD less than genotype TT.

Table 4: Association between genetic polymorphisms of drug transporters and pharmacokinetics and renal toxicity of tenofovir (cont)

Study	Gene (protein)	SNP (rs number)	Association between genetic polymorphisms and pharmacokinetics/ renal toxicity of tenofovir
Izzedine H [51]	*ABCC2* (MRP2)	4544 G>A (rs88187710)	Patients with genotype GA or AA had incidence of KTD less than genotype GG.
Izzedine H [51]	*ABCC4* (MRP4)	669 C>T (rs899494)	Patients with genotype CT or TT had incidence of KTD less than genotype CC.
Kiser JJ [19]	*ABCC4* (MRP4)	3463 A>G (rs1751034)	Patients with genotype AG or GG had intracellular tenofovir diphosphate concentration higher than genotype AA.
Kiser JJ [20]	*ABCC4* (MRP4)	3463 A>G (rs1751034)	Patients with genotype AG or GG had tenofovir renal clearance less than genotype AA.

Overall, according to previously study reviews, the genetic polymorphisms that may have influence on pharmacokinetics of tenofovir can be summarized in table 5.

Table 5: Genetic polymorphisms that may have influence on pharmacokinetics of tenofovir (based on variant allele frequency, functional significance and previously study results) [18, 20, 47, 49, 51-53]

SNP (location)	Variant allele frequency	Functional significance	Previously study results
ABCC2 -24C>T (promoter)	26.9 (Thai) 21.4% (Chinese)	Altered promoter function	Associated with tenofovir plasma concentration and risk of KTD
ABCC2 1249 G>A (exon 10)	6.0% (Thai) 10.1% (Chinese)	In membrane spanning domain, could alter substrate specificity	Associated with KTD in patients receiving tenofovir
ABCC2 3972 C>T (exon 28)	26.7% (Chinese)	May alter protein expression and play an important role in MRP2 translational regulation	Associated with methotrexate concentration
ABCC4 3463 A>G (exon 26)	18.3% (Thai) 18.1% (Chinese)	Increased probability of mRNA splicing and altered protein expression based on exonic splicing enhancer analyses	Associated with tenofovir renal clearance and intracellular tenofovir diphosphate concentration
ABCC4 4131 T>G (3'UTR)	48.9% (Thai) 53.6% (Chinese)		Associated with intracellular lamivudine concentration

Population pharmacokinetics

Population pharmacokinetic study

Population pharmacokinetics is the study of sources and correlates of variability in plasma drug concentrations between individuals who are the target population [17, 54]. The main objectives of population pharmacokinetic study are to estimate population mean pharmacokinetic parameters, identify sources of pharmacokinetic variability and identify factors influencing the pharmacokinetic parameters.

The advantages and disadvantages of population pharmacokinetics are presented in table 6.

Table 6: Advantages and disadvantages of population pharmacokinetics [21, 55]

Advantages
• Pharmacokinetic data may not be rich data (many observations per subject), but it may be sparse data (few observations per subject). • The data may be irregularly sampled time data. • Can integrate data from different sources. • Can be applied to special populations including neonates, elderly, critical care patients, patients with AIDS and patients with cancer in which a limited number of samples can be obtained because of ethical and medical concerns. • Important covariates which explain pharmacokinetic variability can be identified. • Information obtained from the study can be used for individual dose prediction.
Disadvantages
• The method is difficult to understand. • There are few experts for consultation. • The process may take a long time. • The result of population pharmacokinetic study has less power than phase I study.

Population pharmacokinetic model

Population pharmacokinetic model developed by nonlinear mixed effects modeling approach (NONMEM) consists of a structural model, variance model and covariate model.

1. Structural or base model [21, 56, 57]

The first step to develop population pharmacokinetic model is to identify structural or base model which describes pharmacokinetics of drug. In this step, the different models including one-, two- and three-compartment models with different absorption and elimination model are tested. The best structural model is chosen based on the goodness of fit plots and significant statistics such as likelihood ratio test (LRT) and akaike information criterion (AIC).

2. Variance model or statistical model [21, 56, 57]

Variance model is composed of interindividual variability and residual unexplained variability models.

2.1 Interindividual variability (IIV) model

IIV is the variability of pharmacokinetic parameter across different individuals in the population. IIV model can be developed using three different models.

Additive model:

$$P_i = P_{pop} + \eta_i$$

Proportional model:

$$P_i = P_{pop} \times (1+ \eta_i)$$

Exponential model:

$$P_i = P_{pop} \times \exp (\eta_i)$$

P_i is value of pharmacokinetic parameter for i^{th} individual.

P_{pop} is population mean of pharmacokinetic parameter.

η_i is the deviation between individual pharmacokinetic parameter and the population mean value, which is assumed to be normally distributed with a mean of zero and variance of ω^2.

2.2 Residual unexplained variability (RUV) model

RUV is an unexplained variability in observed data after controlling for other sources of variability. RUV model can be developed using four different models.

Additive model:

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij}$$

Proportional model:

$$C_{obs,ij} = C_{pred,ij} \times (1+ \varepsilon_{ij})$$

Exponential model:

$$C_{obs,ij} = C_{pred,ij} \times \exp(\varepsilon_{ij})$$

Combined additive and proportional model:

$$C_{obs,ij} = C_{pred,ij} \times (1+ \varepsilon_{1,ij}) + \varepsilon_{2,ij}$$

$C_{obs,ij}$ is observed concentration in i^{th} individual at time j^{th}.

$C_{pred,ij}$ is predicted concentration in i^{th} individual at time j^{th}.

ε_{ij} is the difference between observed concentration and model-predicted concentration in i^{th} individual at time j^{th} or RUV, which is assumed to be normally distributed with a mean of zero and variance of σ^2.

3. Covariate model [21, 23, 58]

After the structural model was identified, the covariate model is then developed using stepwise approach. The likelihood ratio test is normally used to compare the objective function value (OFV) of the two models: the base model and the model with specific covariate. The significance of each covariate can be determined by comparing the difference of OFV between the two models using a chi-square distribution. Covariate factors can be tested as continuous or categorical covariate.

3.1 Continuous covariate

For continuous covariate, it can be added into the model as follows:

Linear model:

$$CL = \theta_1 + \theta_2 \times COV$$

Exponential model:

$$CL = \theta_1 \times \exp(\theta_2 \times COV)$$

Power model:

$$CL = \theta_1 \times (COV)^{\theta_2}$$

θ_1 is the typical value of pharmacokinetic parameter.

θ_2 is the change of pharmacokinetic parameter per one unit change of covariate.

COV is continuous covariate.

3.2 Categorical covariate

For categorical covariate, it can be added into the model as follows:

Additive model:

$$CL = \theta_1 + \theta_2 \times COV$$

Fractional change model:

$$CL = \theta_1 \times (1 + \theta_2 \times COV)$$

Exponential model:

$$CL = \theta_1 \times \exp(\theta_2 \times COV)$$

θ_1 is the typical value of pharmacokinetic parameter.

θ_2 is the change of pharmacokinetic parameter when covariate is presented.

COV is categorical covariate.

Model evaluation and validation

Pharmacokinetic models can be either descriptive or predictive model [59]. The descriptive model can be used to explain the pharmacokinetic variability in the population and the predictive model can be used to predict drug concentration and determine dosing regimens for patients [59, 60]. For descriptive purpose, the goodness of fit, reliability and stability of the model should be assessed. For predictive purpose, the validation of the model should be evaluated [59, 60].

1. Goodness of fit

Goodness of fit of the model can be determined by graphical evaluation. The typical diagnostic plots for population pharmacokinetic model development are presented in table 7.

Table 7: Diagnostic plots for population pharmacokinetic model development [22, 61-63]

Plot	Usage	Interpretation
DV vs PRED	Assess ability of model prediction in measuring observed data	The distribution of the data around the line of identity indicates no major bias in population pharmacokinetic model.
DV vs IPRED	Assess ability of individual model prediction in measuring individual data	The distribution of the data around the line of identity indicates that the structural model can describe most of the individual data.
WRES or CWRES vs TIME	Use for an examination of structural model	The scattering of the data around the zero line indicates no major bias in structural model.
WRES or CWRES vs PRED	Use for an examination of residual error model	- The scattering of the data around the zero line indicates no major bias in residual error model. - For an adequate model (stable model with reliable parameter estimates and no major bias), the mean of WRES should be distributed around zero and most observations should be within ± 4.

CWRES, conditional weighted residuals; DV, observed concentration; IPRED, individual predicted concentration; PRED, population predicted concentration; WRES, weighted residual

2. Reliability

Parameter reliability can be considered from standard error (SE) or confidence interval (CI) of parameter estimates. For the population analysis, the percent relative standard error (%RSE) for fixed effects and for random effects should less than 30% and 50%, respectively [60]. Other approaches for determining parameter reliability include the jackknife, bootstrapping and profile likelihood method [59].

3. Stability

Model stability can determine whether the covariates should be retained in the population pharmacokinetic model [64]. Bootstrapping is one of the practical methods that can be used to evaluate the stability of the model.

4. Model validation

Model validation is the method used to evaluate predictive performance of the developed model [65]. Model validation can be classified into two types: external validation and internal validation. Although external validation is the most conventional type of validation, the obtaining of external data set is time consuming, costly and difficult. When the external population data is not available, the internal validation including data splitting, resampling techniques (cross-validation, bootstrapping) and the posterior predictive check (PPC) may be considered [65].

Population pharmacokinetic studies of tenofovir

Jellien V et al [15] developed the population pharmacokinetic model of tenofovir in 193 HIV-infected patients receiving TDF 300 mg/day (equivalent to tenofovir disoproxil 245 mg). The study showed that the pharmacokinetics of tenofovir can be described by a two-compartment model with both absorption rate constant (k_a) and distribution rate constant (α) are equal. Interpatient variability and residual unexplained variability was best described by exponential and additive error model, respectively. The results from covariate testing showed that body weight per serum creatinine ratio (BW/Scr), concomitant with lopinavir/ritonavir and tubular dysfunction had influence on

CL/F of tenofovir. The final model of CL/F of tenofovir can be described by the following equation:

$$CL/F\ (l/h) = 90.9 \cdot ([BW/Scr]/0.77)^{0.83} \cdot L/T$$

BW = Body weight (kg); Scr= serum creatinine (μmol/l);

L =0.86 if concomitantly use lopinavir/ritonavir; T = 2.3 if tubulopathy is occurred.

Gagnieu MC et al [14] investigated the population pharmacokinetics of tenofovir in 175 HIV-infected patients. Among patients with creatinine clearance more than 50 ml/min, tenofovir disoproxil 245 mg once daily was given. Patients with creatinine clearance less than 50 ml/min received tenofovir disoproxil 245 mg every 48 hours. The study showed that the pharmacokinetics of tenofovir can be described by a two-compartment model with first order absorption and elimination. After accounting for the variability from body weight and serum creatinine, interpatient variability and inter-occasion variability was 20% and 30%, respectively. CL/F of tenofovir can be described by the following equation:

$$CL/F\ (l/h) = 36.2 + 135 \times (BW/Scr)$$

BW =Body weight (kg); Scr = serum creatinine (μM)

Ramanathan S et al [16] pooled data from 7 studies (4 were phase I studies and 3 were phase II and III studies). All the patients received TDF 300 mg/day. Intensive and sparse pharmacokinetic samplings were done in 190 healthy subjects and 396 HIV-infected patients, respectively. A two-compartment model with both zero and first order absorption and lag time was the best model to describe tenofovir pharmacokinetics. GFR calculated by Cockcroft and Gault formula was associated with CL/F as the following equation:

$$CL/F\ (l/h) = 42.3 \times \exp (CG/116)^{0.497}$$

CG = GFR calculated by Cockcroft and Gault formula (ml/min)

Baheti G et al [17] investigated the population pharmacokinetics of tenofovir in 55 HIV-infected patients receiving TDF 300 mg once daily. The study showed that the pharmacokinetics of tenofovir can be best described by a two-compartment model with first order absorption. Interpatient variability and residual unexplained variability were

described by exponential and proportional error model, respectively. GFR calculated by Cockcroft and Gault formula was associated with both CL/F and V_c/F of tenofovir.

According to previous population pharmacokinetic studies, tenofovir had high interindividual variability in drug exposure. Although, some demographic data including body weight, renal function and concomitant medications can describe part of the variability of drug, there may be other factors including genetic variation that have an influence on pharmacokinetic paremeters of tenofovir. Therefore, further studies investigating the influence of both genetic and non-genetic factors on the pharmacokinetics of tenofovir would be beneficial for optimizing dosage regimen of this drug in HIV-infected patients.

Factors influencing pharmacokinetics of tenofovir

The previous pharmacokinetic and population pharmacokinetic studies showed that several factors can influence pharmacokinetic parameters of tenofovir.

1. Age

Previous pharmacokinetic studies showed that age was significantly associated with trough concentration and renal clearance of tenofovir [20, 66]. For every 10-years increase of age, tenofovir renal clearance decreased by 20% [20]. However, in the population pharmacokinetic studies, a significant association between age and CL/F of tenofovir was not presented [14, 15].

2. Gender

Rodriquez-Novoa S et al [12] studied the influence of factors including age, gender, body weight, serum creatinine, disease and concomitant medications on tenofovir plasma concentration by multiple linear regression analysis. The results showed that female was independently associated with high tenofovir plasma concentration (odd ratio 71; 95%CI 33-111; $p<0.01$).

3. Body weight

There were several pharmacokinetic studies showing the association between body weight and pharmacokinetic parameters of tenofovir. Population pharmacokinetic

studies in adults showed that body weight per serum creatinine ratio was associated with CL/F of tenofovir [14, 15]. In children 5-18 years of age, CL/F and dosage regimen of tenofovir were also dependent on body weight [67]. A recent pharmacokinetic study in Thai HIV-infected patients showed that mean tenofovir plasma concentration in patients with body weight less than 55 kg was significantly higher than those with body weight greater than 55 kg [25].

4. Body mass index

Calcagno A et al [66] showed that in univariate analysis, body mass index (body weight/height^2) was significantly associated with tenofovir trough concentration (p=0.0025). When body mass index increased, tenofovir trough concentration was found to be increased. However, the association between body mass index and trough concentration was not statistically significant in multivariate analysis.

5. Renal function

Tenofovir is mainly excreted via kidney by a combination of glomerular filtration and active tubular secretion. Therefore, a change of tenofovir renal excretion is expected when renal function is altered. Tenofovir renal clearances in patients with normal renal function (Cl_{cr} > 50 ml/min), moderate renal dysfunction (Cl_{cr} 30-49 ml/min) and severe renal dysfunction (Cl_{cr} 10-29 ml/min) were 1043.7 ± 115.4 ml/min, 444.4 ± 209.8 ml/min and 117.0 ± 97.1 ml/min, respectively [28]. Previous population pharmacokinetic studies showed that GFR calculated by Cockcroft and Gault [16, 17] and tubular dysfunction [15] were associated with CL/F of tenofovir. When GFR decreased, CL/F of tenofovir was found to be decreased.

6. Comorbid diseases

A recent pharmacokinetic study in Thai HIV-infected patients [25] showed that hepatitis C co-infection was associated with higher tenofovir plasma concentration. On the other hand, other comorbidities including diabetes mellitus, hypertension and chronic hepatitis B infection were not found to be associated with tenofovir plasma concentration.

7. Comedications

The increment of AUC and C_{max} of tenofovir was reported when tenofovir was concomitantly used with atazanavir/ritonavir, darunavir/ritonavir or lopinavir/ritonavir [29-31]. Moreover, previous population pharmacokinetic study showed that concomitant lopinavir/ritonavir use was associated with a decrease of tenofovir CL/F [15]. After controlling for estimated GFR, patients receiving lopinavir/ritonavir had tenofovir renal clearance 17.5% lower than patients who did not use lopinavir/ritonavir [20].

8. Genetic polymorphisms

Tenofovir is transported into renal tubular cell by OAT1 and OAT3, and is then effluxed from tubular cell by MRP2 and MRP4. Genetic polymorphisms of these transporters may affect pharmacokinetic of tenofovir. Previous study showed that polymorphisms of *ABCC2* (encode MRP2) and *ABCC4* (encode MRP4) had an influence on tenofovir renal clearance. A study by Kiser JJ et al [20] showed that patients with *ABCC2* -24C>T genotype CT excreted 19% more of tenofovir than those with wild type (genotype CC), whereas patients with *ABCC4* 3463A>G variant (genotype AG or GG) had tenofovir renal clearance lower than those with wild type, leading to a 32% increase of tenofovir area under the curve. However, the polymorphism of *SLC22A6* (encode OAT1) was not associated with the pharmacokinetics of tenofovir.

CHAPTER III
PATIENTS AND METHODS

Study design

A retrospective descriptive study was performed in Thai HIV-infected patients extracted from clinical trial of The HIV Netherlands Australia Thailand Research Collobration (HIV-NAT) and Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database (Appendix B).

For data analyses, there were two parts of the study. The first study aimed to identify factors influencing tenofovir plasma concentrations at mid-dose. In this study, patients from HIV-NAT database who received TDF 300 mg once daily and had record of tenofovir plasma concentration at mid-dose were included. The second study aimed to develop and validate the population pharmacokinetic model of tenofovir. In this study, patients from HIV-NAT database were used to develop the population pharmacokinetic model and patients from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database were used to validate the population pharmacokinetic model.

Study patients

Study 1: The influence of genetic and non-genetic factors on tenofovir plasma concentrations at mid-dose

In the first study, Thai HIV-infected patients receiving tenofovir as part of the antiretroviral therapy enrolled in the study “Incidence and predictor of TDF associated nephrotoxicity and pharmacokinetic of TDF in HIV-1 infected Thai patients” at HIV-NAT during 1 January to 1 September 2012 were included.

Inclusion criteria

1. Patient aged 18 years and older
2. Patient had been receiving TDF 300 mg once daily for the treatment of HIV-1 infection at least 2 weeks before plasma blood samples were collected

3. Patients who had record of tenofovir plasma concentration at mid-dose (10-14 hour after last dose) in the database
4. Patients who had record of *ABCC2* (-24C>T, 1249G>A, 3972C>T) and *ABCC4* (3463A>G, 4131T>G) genotyping in the database

Exclusion criteria

1. Patients with incomplete record of demographic data including age, sex, body weight, serum creatinine, time of blood samples, hepatitis B virus co-infection, hepatitis C virus co-infection and concomitant antiretroviral drugs

Sample size

The main objective of this study was to compare mean tenofovir plasma concentrations between 2 groups of patients (group1: homozygous wild type, group2: at least one variant allele). Therefore, the sample size for this study was calculated as the following equation:

$$N = \frac{(r+1)(Z_{\alpha/2} + Z_{1-\beta})^2 \sigma^2}{rd^2} \quad [68]$$

Where; Z_{α} is the normal deviate at a level of significance (Z_{α} = 1.96 for 5% level of significance).

$Z_{1-\beta}$ is the normal deviate at 1-β% power ($Z_{1-\beta}$ =0.84 at 80% power).

r = n1/n2 is the ratio of sample size required for 2 groups.

$$= \frac{\text{number of homozygous wild type patients}}{\text{number of patients that have at least one variant allele}}$$

σ is the pooled standard deviation of mean tenofovir plasma concentrations between 2 groups.

d is the difference of mean tenofovir plasma concentrations between 2 groups.

The sample sizes of patients for each polymorphism are shown in table 8.

Table 8: The sample sizes of patients for each polymorphism

Genetic polymorphism	$Z_{\alpha/2}$	$Z_{1-\beta}$	σ^a	r^a	d^a	Sample size (N)
ABCC2 -24C>T	1.96	0.84	27.29	64/42 = 1.52	82.76-88.26 =-5.5	321
ABCC2 1249G>A	1.96	0.84	27.39	90/16 = 5.63	85.70-80.69 = 5.01	276
ABCC2 3972C>T	1.96	0.84	NA	NA	NA	NA
ABCC4 3463A>G	1.96	0.84	26.83	72/34 =2.12	88.87-76.62 = 12.25	56
ABCC4 4131T>G	1.96	0.84	26.85	22/84 = 0.26	73.91-87.83 = -13.92	142

NA; not applicable

[a] obtained from study of Mitruk S et al [69]

Therefore, the overall sample size in the first study should be at least 321 patients in order to be able to detect the difference of all the polymorphisms. However, due to the limitation of obtained data and ethical concern, there were only 150 patients for this data analyses.

Study 2: Population pharmacokinetics of tenofovir

In the second study, the population pharmacokinetic model were developed and validated. The data used for population pharmacokinetic model building were Thai HIV-infected patients receiving tenofovir as part of the antiretroviral therapy enrolled in the study "Incidence and predictor of TDF associated nephrotoxicity and pharmacokinetic of TDF in HIV-1 infected Thai patients" at HIV-NAT during 1 January to 1 September 2012. The samples for model validation were patients enrolled in the study "*ABCC2**1C and plasma tenofovir concentration are correlated to decreased glomerular filtration rate in patients receiving a tenofovir-containing antiretroviral regimen" at Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital during 2009 to 2011.

Inclusion criteria

1. Patient aged 18 years and older
2. Patient had been receiving tenofovir for the treatment of HIV-1 infection at least 2 weeks before plasma blood samples were collected
3. Patients who had record of tenofovir plasma concentration in the database
4. Patients who had record of *ABCC2* (-24C>T, 1249G>A, 3972C>T) and *ABCC*4 (3463A>G, 4131T>G) genotyping in the database or had genomic DNA samples for genetic testing

Exclusion criteria

1. Patients with incomplete record of demographic data including age, sex, body weight, serum creatinine, time of blood samples, hepatitis B virus co-infection, hepatitis C virus co-infection and concomitant antiretroviral drugs

Sample size

Sample size in the second study was calculated from the equation:

$$N \geq 15p \quad [70]$$

Where; N is sample size of the patients.

p is number of covariates that were tested in this study including sex, age, body weight, serum creatinine, GFR calculated by 3 formulas: Cockcroft and Gault (GFR_{CG}), the Modiciation of Diet in Renal Disease (GFR_{MDRD}) and Thai formula (GFR_{THAI}), KTD, hepatitis B virus (HBV) co-infection, hepatitis C virus (HCV) co-infection, *ABCC*2 polymorphisms (-24C>T, 1249G>A, 3972C>T), *ABCC*4 polymorphisms (3463A>G, 4131T>G) and concomitant antiretroviral drugs (lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r), saquinavir/ritonavir (SQV/r)).

Therefore, p in this study equals to 18.

$$N \geq 15(18)$$

$$N \geq 270$$

Then, the sample size of the second study is at least 270 patients.

Study protocol

1. Review literatures and design research protocol.
2. Submit protocol for approval from The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University.
3. Select patients in databases according to the inclusion and exclusion criteria.
4. Identify factors influencing tenofovir plasma concentrations at mid-dose from patients in HIV-NAT database (study 1).
5. Develop the population pharmacokinetic model of tenofovir from patients in HIV-NAT database and validate the population pharmacokinetic model of tenofovir from patients in Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database (study 2).
6. Discussion and conclusion.

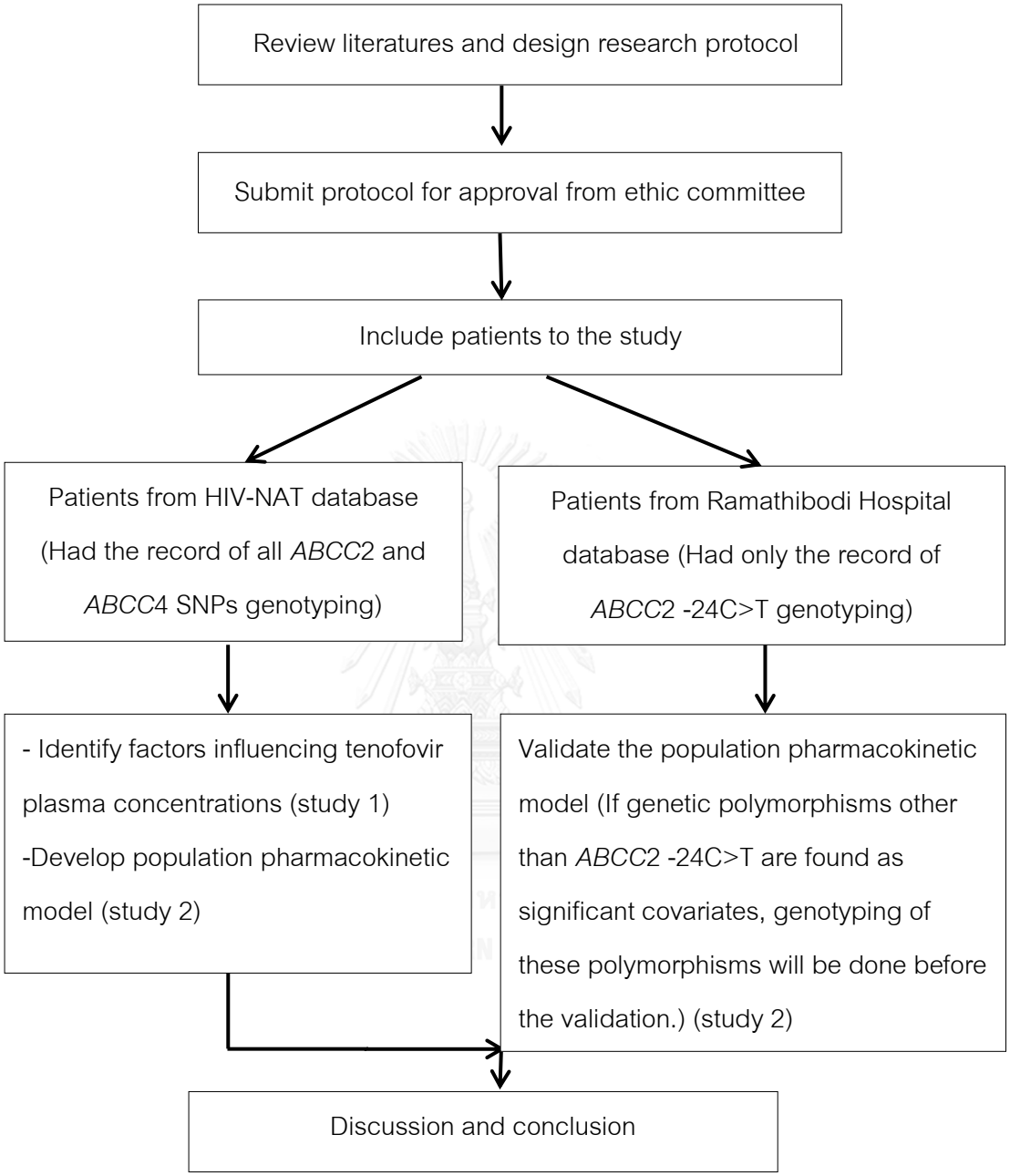


Figure 1: Study protocol

Tenofovir plasma concentration determination

Tenofovir plasma concentrations from HIV-NAT and Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database were determined at the laboratory of HIV-NAT by validated high-performance liquid chromatography (HPLC) assay with a fluorimetric detector by modified method according to Droste JA et al [71]. The lower limit of quantification was 0.015 mg/l. The tenofovir calibration curve was linear over the concentration range of 0.015 to 1.50 mg/l. The within-run and between-run coefficient of variation (precision) was less than 10% and the accuracy of tenofovir was between 95-105%.

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***ABCC2* and *ABCC4* genotyping assay**

The polymorphisms of *ABCC2* -24C>T (rs717620), *ABCC2* 1249G>A (rs2273697), *ABCC2* 3972C>T (rs3740066), *ABCC4* 3463A>G (rs1751034) and *ABCC4* 4131T>G (rs3742106) from HIV-NAT database were analyzed at Chula Medical Research Center and Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University using Taqman allelic discrimination assay (Applied Biosystems Califonia, USA) [72] according to previously published thesis “Association between polymorphisms of tenofovir transporters and tenofovir plasma levels in Thai HIV-infected patients” [69].

The polymorphisms of *ABCC2* and *ABCC4* of patients from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database that have influence on population pharmacokinetic parameters of tenofovir in the population pharmacokinetic model were further genotyped at Laboratory for Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital using Taqman allelic discrimination assay (Applied Biosystems Califonia, USA) [72].

Data analysis

Study 1: The influence of genetic and non-genetic factors on tenofovir plasma concentrations at mid-dose

Demographic data of patients were presented as frequency and percent for categorical data and mean ± standard deviation (SD) or median ± interquartile range (IQR) for continuous data. The association between tenofovir plasma concentrations and continuous covariates including age, weight, serum creatinine, GFR_{CG}, GFR_{MDRD} and GFR_{THAI} were tested by correlation coefficient analysis. The association between tenofovir plasma concentrations and categorical covariates including sex, KTD, HBV co-infection, HCV co-infection and concomitant drugs (ritonavir-boosted protease inhibitor, LPV/r, ATV/r, SQV/r) were tested by independent t-test. Due to a small number of patients in some groups of genotyping, the mean tenofovir plasma concentrations for *ABCC2* -24C>T, *ABCC2* 1249G>A, *ABCC2* 3972C>T and *ABCC4* 3463A>G polymorphisms were compared between patients with homozygous wild type and patients with at least one variant allele by independent t-test. For *ABCC4* 4131T>G polymorphisms, the mean tenofovir plasma concentrations were compared among three groups (genotype TT, TG and GG) by one-way ANOVA. All analyses were performed by Statistical Package for Social Sciences (SPSS version 17, SPSS Co., Ltd, Bangkok, Thailand) software. The p-value < 0.05 was considered statistically significant.

Study 2: Population pharmacokinetics of tenofovir

Demographic data of patients were analyzed by the Statistical Package for Social Sciences software (SPSS version 17, SPSS Co., Ltd., Bangkok Thailand). Categorical data were presented as frequency and percent. Continuous data were presented as mean ± SD or median ± IQR. Baseline demographic characteristics of patients from HIV-NAT and Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital databases were compared using Chi-square test or Fisher's exact test for categorical data and Independent t-test or Mann-Whitney U test for continuous data.

Population pharmacokinetic model of tenofovir was developed by nonlinear mixed effects model using NONMEM software program (version VII, Icon Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation with interaction (FOCE-I) was used for all analyses. Graphical assessment of goodness of fit was performed using R program (version 2.8.0, R Development Core Team; www.r-project.org) and Xpose.

1. Structural model

One-compartment model with first order absorption and elimination (ADVAN 2 TRANS 2) and two-compartment model with first order absorption and elimination (ADVAN 4 TRANS 4) were investigated. The best structural model was chosen based on parameter estimates with standard error, objective function value (OFV), akaike information criterion (AIC) and goodness of fit plots.

2. Variance model

2.1 Interindividual variability (IIV) model was investigated by three difference models.

Additive model:

$$P_i = P_{pop} + \eta_i$$

Proportional model:

$$P_i = P_{pop} \times (1+ \eta_i)$$

Exponential model:

$$P_i = P_{pop} \times \exp (\eta_i)$$

P_i is value of pharmacokinetic parameter for i^{th} individual.

P_{pop} is population mean of pharmacokinetic parameter.

η_i is the deviation between individual pharmacokinetic parameter and the population mean value, which is assumed to be normally distributed with a mean of zero and variance of ω^2.

2.2 Residual unexplained variability (RUV) model was investigated by four different models.

Additive model:

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij}$$

Proportional model:

$$C_{obs,ij} = C_{pred,ij} \times (1+ \varepsilon_{ij})$$

Exponential model:

$$C_{obs,ij} = C_{pred,ij} \times \exp(\varepsilon_{ij})$$

Combined additive and proportional model:

$$C_{obs,ij} = C_{pred,ij} \times (1+ \varepsilon_{1,ij}) + \varepsilon_{2,ij}$$

$C_{obs,ij}$ is observed concentration in i^{th} individual at time j^{th}.

$C_{pred,ij}$ is predicted concentration in i^{th} individual at time j^{th}.

ε_{ij} is the difference between observed concentration and model-predicted concentration in i^{th} individual at time j^{th} or RUV, which is assumed to be normally distributed with a mean of zero and variance of σ^2.

The IIV and RUV models were chosen based on parameter estimates with standard error, OFV, AIC and goodness of fit plots.

3. Covariate model

For covariate model development, patient characteristics including sex, age, body weight, serum creatinine, GFR_{CG}, GFR_{MDRD}, GFR_{THAI}, KTD, HBV, HCV, genetic polymorphisms (*ABCC2* -24C>T, *ABCC2* 1249G>A, *ABCC2* 3972C>T, *ABCC4* 3463A>G, *ABCC4* 4131T>G) and concomitant antiretroviral drugs (LPV/r, ATV/r, SQV/r) were tested as covariates.

The influence of covariates on pharmacokinetic parameters was investigated by stepwise forward inclusion and backward deletion approach. During forward inclusion, each covariate was added into the base model one at a time, the covariates that decreased OFV of at least 3.84 (χ^2, $p \leq 0.05$, df=1) were included into the base model to obtain the full model. During backward deletion, each covariate was deleted from the

full model one at a time, the covariates that increased OFV of at least 6.63 (χ^2, $p \leq 0.01$, df=1) were retained into the final model.

4. Model validation

The data from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital were used for model validation using Bayesian estimation method. The bias and precision of the final model in predicting tenofovir plasma concentrations was considered from mean prediction error (MPE) and root mean square error (RMSE), respectively according to the following equations:

$$MPE = \frac{\sum PE_i}{n} \quad [58]$$

$$RMSE = \sqrt{\frac{\sum (PE_i)^2}{n}} \quad [58]$$

Where; PE is prediction error (the difference between observed concentration and predicted concentration).

n is number of pairs of observed and predicted concentrations.

CHAPTER IV
RESULTS

Study 1: The influence of genetic and non-genetic factors on tenofovir plasma concentrations at mid-dose

Demographic data

In this study, a total of 150 patients providing 150 blood samples were included. The summary of patient characteristics is presented in table 9. Among 150 patients, 101 (67.3%), 48 (32.0%) and 1 (0.7%) patients received tenofovir in combination with NNRTIs, ritonavir boosted-protease inhibitors and integrase inhibitor, respectively. The mean tenofovir plasma concentration at mid-dose was 0.100 ± 0.052 mg/l.

Table 9: Demographic data of study patients (n=150)

Demographics and laboratory	Frequency (%) or Mean±SD (range)
Sex;	
Male	85 (56.7)
Female	65 (43.3)
Age (years)	43.9 ± 7.2 (25.4-61.4)
Body weight (kg)	60.3 ± 11.9 (38-105.3)
Serum creatinine (mg/dl)	0.9 ± 0.2 (0.4-1.2)
GFR_{CG} (ml/min)	90.8 ± 22.0 (57.2-189.9)
GFR_{MDRD} (ml/min/1.73m^2)	90.3 ± 18.0 (55.7-168.8)
GFR_{THAI} (ml/min/1.73m^2)	97.6 ± 16.2 (64.4-156.4)
KTD; yes	17 (11.3)
Hepatitis B virus antigen; positive	60 (40.0)
Hepatitis C virus antibody; positive	12 (8.0)
Tenofovir sampling time after last dose (hours)	11.9 ± 0.8 (10.1-13.8)
Tenofovir plasma concentration (mg/l)	0.100 ± 0.052 (0.018-0.497)

Genetic polymorphisms of *ABCC2* and *ABCC4* of patients are shown in table 10. All polymorphisms were in Hardy-Weinberg equilibrium (χ^2, $p \geq 0.05$). Patients who had at least one variant allele of *ABCC2* -24C>T, *ABCC2* 1249G>A, *ABCC2* 3972C>T, *ABCC4* 3463A>G and *ABCC4* 4131T>G were 34.7%, 18.0%, 37.3%, 36.0% and 73.3%, respectively.

Table 10: Genotype frequencies of the *ABCC2* and *ABCC4* polymorphisms (n=150)

Genetic polymorphism	Genotype			Allele		p-value[a]
	Genotype	Frequency	%	Allele	%	
ABCC2 -24C>T	CC	98	65.3	C	80.7	0.968
	CT	46	30.7	T	19.3	
	TT	6	4.0			
ABCC2 1249G>A	GG	123	82.0	G	90.7	0.976
	GA	26	17.3	A	9.3	
	AA	1	0.7			
ABCC2 3972C>T	CC	94	62.7	C	78.3	0.562
	CT	47	31.3	T	21.7	
	TT	9	6.0			
ABCC4 3463 A>G	AA	96	64.0	A	80.7	0.590
	AG	50	33.3	G	19.3	
	GG	4	2.7			
ABCC4 4131T>G	TT	34	22.7	T	49.3	0.713
	TG	80	53.3	G	50.7	
	GG	36	24.0			

[a] p-value compared to Hardy Weinberg Equilibrium

Association between covariates and tenofovir plasma concentrations

The association between covariates and tenofovir plasma concentrations at mid-dose are shown in table 11 and table 12.

Table 11: Association between tenofovir plasma concentrations and continuous covairates (n=150)

Demographic data	Correlation coefficient with tenofovir plasma concentration	p value [a]
Age	0.025	0.760 [a]
Weight	-0.207	0.011 [a*]
Serum creatinine	0.201	0.013 [a*]
GFR_{CG}	-0.390	<0.001 [a*]
GFR_{MDRD}	-0.330	<0.001 [a*]
GFR_{THAI}	-0.332	<0.001 [a*]

[a] Pearson correlation *p<0.05

Table 12: Association between tenofovir plasma concentrations and categorical covairates (n=150)

Demographic data	Category	Mean tenofovir plasma concentration (mg/l) ± SD	p value
Sex	Male (n=85)	0.094 ± 0.044	0.107 [a]
	Female (n=65)	0.108 ± 0.061	
KTD	No (n=133)	0.099 ± 0.053	0.421 [a]
	Yes (n=17)	0.110 ± 0.051	
HBV co-infection	No (n=90)	0.105 ± 0.057	0.164 [a]
	Yes (n=60)	0.093 ± 0.045	
HCV co-infection	No (n=132)	0.100 ± 0.054	0.986 [a]
	Yes (n=12)	0.100 ± 0.035	
ABCC2 -24C>T	Genotype CC (n=98)	0.101 ± 0.057	0.706 [a]
	Genotype CT or TT (n=52)	0.098 ± 0.043	

Table 12: Association between tenofovir plasma concentrations and categorical covairates (n=150) (cont)

Demographic data	Category	Mean tenofovir plasma concentration (mg/l) ± SD	p value
ABCC2 1249G>A	Genotype GG (n=123)	0.100 ± 0.056	0.984 [a]
	Genotype GA or AA (n=27)	0.101 ± 0.037	
ABCC2 3972C>T	Genotype CC (n=94)	0.103 ± 0.057	0.455 [a]
	Genotype CT or TT (n=56)	0.096 ± 0.045	
ABCC4 3463A>G	Genotype AA (n=96)	0.105 ± 0.057	0.177 [a]
	Genotype AG or GG (n=54)	0.093 ± 0.043	
ABCC4 4131T>G	Genotype TT (n=34)	0.086 ± 0.031	0.168 [b]
	Genotype TG (n=80)	0.108 ± 0.064	
	Genotype GG (n=36)	0.097 ± 0.038	
Concomitant with ritonavir boosted PI	No (n=102)	0.088 ± 0.034	0.001 [a*]
	Yes (n=48)	0.128 ± 0.073	
Concomitant with LPV/r	No (n=133)	0.095 ± 0.041	0.068 [a]
	Yes (n=17)	0.143 ± 0.101	
Concomitant with ATV/r	No (n=141)	0.100 ± 0.053	0.511 [a]
	Yes (n=9)	0.112 ± 0.046	
Concomitant with SQV/r	No (n=131)	0.098 ± 0.054	0.166 [a]
	Yes (n=19)	0.116 ± 0.040	

[a] Independent t-test [b] One-way ANOVA *p<0.05

The results showed that factors including weight (p=0.011), serum creatinine (p=0.013), GFR (p<0.001) and concomitant use with ritonavir-boosted protease inhibitor (p<0.001) were significantly associated with tenofovir plasma concentrations at mid-dose. On the other hand, sex (p=0.107), age (p=0.760), HBV co-infection (p=0.164), HCV co-infection (p=0.986) and genetic polymorphisms (*ABCC2* -24C>T, *ABCC2* 1249G>T, *ABCC2* 3972C>T, *ABCC4* 3463A>G and *ABCC4* 4131T>G) were not found to be associated with tenofovir plasma concentrations.

Study 2: Population pharmacokinetics of tenofovir

Demographic data

A total 342 patients with 643 plasma concentrations from HIV-NAT database and 103 patients with 103 plasma concentrations from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database were included in this study.

Patients from HIV-NAT database consisted of 182 males and 160 females. About 42 (12.3%) patients were diagnosed as KTD. The average age of patients was 45.8 ± 8.1 years. The mean GFR calculated by Cockcroft and Gault formula was 85.9 ± 22.2 ml/min. Most of the patients received TDF 300 mg once daily, but 19 (5.6%) patients received TDF 300 mg every 48 hours. The combination of tenofovir with NNRTIs, ritonavir boosted-protease inhibitors and integrase inhibitors was found in 155 (45.3%), 184 (53.8%) and 3 (0.9%) patients, respectively.

Patients from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database consisted of 80 males and 23 females. The average age of patients was 36.4 ± 8.6 years. The mean GFR calculated by Cockcroft and Gault formula was 102.3 ± 22.6 ml/min. All of the patients received TDF 300 mg once daily in combination with lamivudine and efavirenz.

Baseline characteristics of the patients from both databases are presented in table 13. Due to the difference sources of study patients, most of the patient characteristics between two groups were significantly different. The proportion of male and mean GFR of patients in HIV-NAT database was lower than Ramathibodi Hospital database (53.2% vs 77.7%; $p<0.001$ and 85.9 vs 102.3 ml/min; $p<0.001$, respectively). The mean age and duration of TDF treatment of patients from HIV-NAT was higher than Ramathibodi Hospital database (45.8 vs 36.4 years; $p<0.001$ and 3.46 vs 0.40 years; $p<0.001$, respectively).

In HIV-NAT database, patients who had at least one variant allele of *ABCC2* -24C>T, *ABCC2* 1249G>A, *ABCC2* 3972C>T, *ABCC4* 3463A>G and *ABCC4* 4131T>G were 38.8%, 15.5%, 43.6%, 34.5% and 74.9%, respectively. All polymorphisms were in Hardy-Weinberg equilibrium (χ^2, $p \geq 0.05$). From Ramathibodi Hospital database, only

ABCC4 3463A>G polymorphism of patients were further genotyped in this study and the number of patients who had at least one variant allele of *ABCC4* 3463A>G was 30.0%. The genetic polymorphism of *ABCC4* 3463A>G from two databases was not significantly different (p=0.693).

Table 13: Baseline characteristics of patients

Patient Characteristics	HIV-NAT database Frequency (%)	Ramathibodi database Frequency (%)	p-value
Number of patients	342	103	
Number of concentrations	643	103	
Sex			<0.001 [a*]
Male	182 (53.2)	80 (77.7)	
Female	160 (46.8)	23 (22.3)	
Hepatitis B virus			<0.001 [a*]
Positive	102 (29.8)	7 (6.8)	
Negative	240 (70.2)	96 (93.2)	
Hepatitis C virus			0.036 [a*]
Positive	24 (7.0)	14 (13.6)	
Negative	318 (93.0)	89 (86.4)	
Kidney tubular dysfunction			NA
Yes	42 (12.3)	NA	
No	300 (87.7)	NA	
TDF dosage regimen			0.010 [b*]
300 mg once daily	323 (94.4)	103 (100.0)	
300 mg every 48 hours	19 (5.6)	0 (0.0)	

Table 13: Baseline characteristics of patients (cont)

Patient Characteristics	HIV-NAT database Frequency (%)	Ramathibodi database Frequency (%)	p-value
Comedications			
Lamivudine	245 (71.6)	103 (100.0)	<0.001 [a*]
Emtricitabine	82 (24.0)	0 (0.0)	<0.001 [b*]
Zidovudine	15 (4.4)	0 (0.0)	<0.001 [b*]
Nevirapine	40 (11.7)	0 (0.0)	<0.001 [b*]
Efavirenz	114 (33.3)	103 (100.0)	<0.001 [a*]
Rilpivirine	1 (0.3)	0 (0.0)	0.769 [b]
Lopinavir/ritonavir	50 (14.6)	0 (0.0)	<0.001 [b*]
Darunavir/ritonavir	11 (3.2)	0 (0.0)	0.053 [b]
Atazanavir/ritonavir	36 (10.5)	0 (0.0)	<0.001 [b*]
Saquinavir/ritonavir	87 (25.4)	0 (0.0)	<0.001 [b*]
Raltegravir	3 (0.9)	0 (0.0)	0.663 [b]
Patient characteristics	**Mean ± SD (range)**	**Mean ± SD (range)**	**p-value**
Age (years)	45.8 ± 8.1 (24.2-73.2)	36.4 ± 8.6 (20-59)	<0.001 [c*]
Weight (kg)	59.9 ±11.9 (37.6-117.7)	58.0 ± 9.3 (35.1-85.4)	0.098 [c]
Serum creatinine (mg/dl)	0.9 ± 0.2 (0.4-1.8)	0.8 ± 0.2 (0.5-1.8)	0.002 [c*]
GFR_{CG} (ml/min)	85.9 ± 22.2 (28.5-189.9)	102.3 ± 22.6 (48.1-186.6)	<0.001 [c*]
GFR_{MDRD} (ml/min/1.73m^2)	86.2 ± 17.9 (30.2-168.8)	106.3 ± 23.6 (44.2-196.6)	<0.001 [c*]
GFR_{THAI} (ml/min/1.73m^2)	93.1 ± 16.4 (40.2-156.5)	118.0 ± 23.6 (63.5-198.4)	<0.001 [c*]
Duration of TDF treatment (yrs)	3.46 ± 2.01 (0.21-7.85)	0.40 ± 0.02 (0.36-0.46)	<0.001 [c*]

Table 13: Baseline characteristics of patients (cont)

Genetic polymorphisms	HIV-NAT database Frequency (%)	Ramathibodi database Frequency (%)	p-value
ABCC2 -24C>T			0.545 [a]
Genotype CC	209 (61.1)	60 (58.2)	
Genotype CT	113 (33.0)	39 (37.9)	
Genotype TT	20 (5.8)	4 (3.9)	
ABCC2 1249 G>A			NA
Genotype GG	289 (84.5)	NA	
Genotype GA	50 (14.6)	NA	
Genotype AA	3 (0.9)	NA	
ABCC2 3972 C>T			NA
Genotype CC	193 (56.4)	NA	
Genotype CT	124 (36.3)	NA	
Genotype TT	25 (7.3)	NA	
ABCC4 3463 A>G			0.693 [a]
Genotype AA	224 (65.5)	72 (69.9)	
Genotype AG	105 (30.7)	28 (27.1)	
Genotype GG	13 (3.8)	3 (2.9)	
ABCC4 4131 T>G			NA
Genotype TT	86 (25.1)	NA	
Genotype TG	164 (48.0)	NA	
Genotype GG	92 (26.9)	NA	

NA; not applicable

[a] Chi-square test [b] Fisher's exact test [c] Independent t-test

*p<0.05

Population pharmacokinetics model development

A total of 342 patients in HIV-NAT database were used for the population pharmacokinetic model building. About 323 (94.4%) patients received TDF 300 mg once daily and 19 (5.6%) patients received TDF 300 mg every 48 hours.

Among 643 tenofovir plasma concentrations included for analyses, most of the concentrations were sparse data. However, intensive samplings (at 0, 1, 2, 4, 6, 8, 10, 12 and 24 hours post-dose) were obtained from 16 patients. The plots of tenofovir plasma concentration vs time after dose are shown in figure 2.

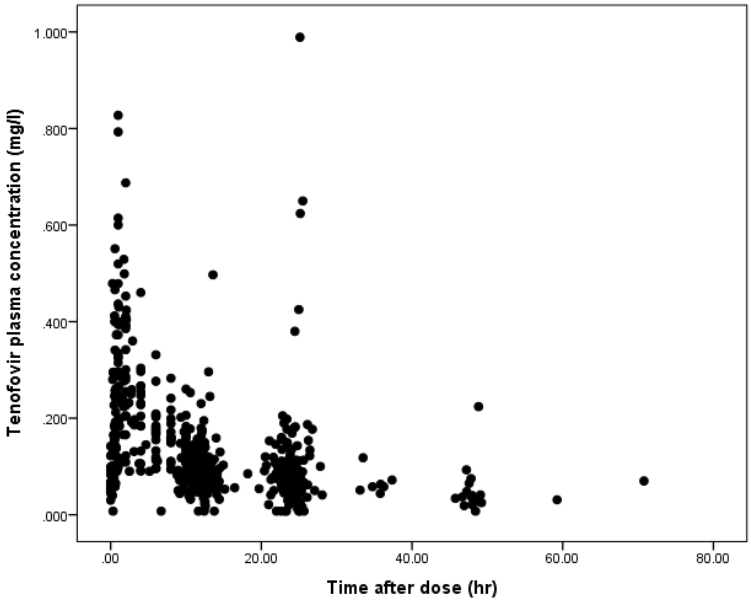

Figure 2: The plots of tenofovir plasma concentration vs time after dose

Base model

One-compartment model and two-compartment model with first order absorption and elimination were investigated in order to describe pharmacokinetics of tenofovir. However, when two-compartment model was tested for characterizing the pharmacokinetic model of tenofovir, the model cannot be minimized successfully. Therefore, some of the parameters were not estimated when the two-compartment model was fitted to the data. The results of pharmacokinetic parameter estimates and OFV from all the investigated models are shown in table 14 and table 15.

Table 14: Results of one compartment model with different IIV and RUV model

IIV model	RUV model	OFV	AIC	Parameter estimate (SE%)						
				CL (L/hr)	V (L)	k_a (hr^{-1})	ω_{CL}	ω_v	ω_{Ka}	σ
Additive	Additive	-2326.806 [a]	-2312.806	42.4	345	1.07	0.559	0.545	0.709	0.098
	Proportional	-2676.723 [a]	-2662.723	44.7	748	2.52	0.627	0.604	1.265	0.638
	Combined	-2917.354 [b]	-2091.354	59 (3.2)	1120 (6.8)	3.5 (16.2)	15.133 (7.9)	474.342(10.0)	0.935 (39.1)	Prop 0.411 (4.3) Add 0.012 (19.8)
	Exponential	-2912.678 [b]	-2896.678	58.8 (2.9)	1110 (3.2)	3.37 (16.2)	15.033 (7.8)	465.833 (3.5)	0.844 (39.0)	0.422 (4.5)
Proportional	Additive	-2605.576 [b]	-2591.576	54.7 (3.9)	993 (12.0)	2.89 (10.8)	0.241 (11.0)	0.498 (7.4)	0.595 (9.0)	0.063 (11.3)
	Proportional	-2914.065 [a]	-2900.065	58.7	1120	3.43	0.252	0.406	0.258	0.427
	Combined	-2904.584 [a]	-2888.584	56.5	944	1.94	0.257	0.396	1.114	Prop 0.359 Add 0.017
	Exponential	-2891.611 [b]	-2877.611	56.6 (3.4)	972 (8.9)	1.93 (12.3)	0.250 (6.6)	0.378 (6.0)	1.136 (9.5)	0.399 (4.5)
Exponential	Additive	-2616.192 [b]	-2602.192	55.3 (4.2)	1230 (17.4)	2.99 (22.0)	0.295 (15.0)	0.974 (11.8)	0.918 (18.6)	0.061 (12.5)
	Proportional	-2902.158 [b]	-2888.158	58.1 (4.1)	1070 (14.0)	1.59 (5.5)	0.308 (8.2)	0.622 (15.2)	2.119 (14.6)	0.378 (5.1)
	Combined	-2920.020 [b]	-2904.02	58.6 (3.9)	1130 (14.3)	1.8 (16.8)	0.329 (9.1)	0.696 (13.1)	2.035 (12.9)	Prop 0.336 (6.8) Add 0.016 (11.4)
	Exponential	-2902.158 [b]	-2888.158	58.1 (4.1)	1070 (14.0)	1.59 (5.5)	0.308 (8.2)	0.622 (15.2)	2.119 (14.6)	0.378(5.1)

[a] Minimization terminated [b] Minimization successful

Table 15: Results of two compartment model with different IIV and RUV model

IIV model	RUV model	OFV	AIC	Parameter estimate (SE%)										
				CL	V_c	V_p	Q	k_a	ω_{CL}	ω_{Vc}	ω_{Vp}	ω_{Q}	ω_{Ka}	σ
Additive	Additive	-2489.729 [d]	-2467.729	43.5	296	674	90.7	0.789	10.000	0.554	0.548	0.555	0.435	0.084
Additive	Proportional	- [e]		-	-	-	-	-	-	-	-	-	-	-
Additive	Combined	-2348.553 [d]	-2324.553	41.2	457	92.5	91.6	2.250	0.567	0.548	0.548	0.548	1.661	Prop 0.117 Add 0.088
Additive	Exponential	- [e]		-	-	-	-	-	-	-	-	-	-	-
Proportional	Additive	-2590.639 [d]	-2568.639	46.7	91.5	50600	90.7	0.214	0.294	0.228	0.345	0.005	0.678	0.066
Proportional	Proportional	- [e]		-	-	-	-	-	-	-	-	-	-	-
Proportional	Combined	-2973.702 [d]	-2949.702	56.7	710	509	112	1.270	0.265	0.534	0.009	0.310	0.792	Prop 0.332 Add 0.028
Proportional	Exponential	- [e]		-	-	-	-	-	-	-	-	-	-	-
Exponential [a]	Additive	-2676.527 [f]	-2654.527	52.9 (4.0)	535 (26.0)	718 (23.5)	145 (19.0)	0.804 (17.4)	0.302 (15.1)	1.105 (16.4)	0.268 (117.8)	0.837 (19.7)	1.100 (18.1)	0.055 (15.8)
Exponential [b]	Additive	-2676.422 [f]	-2656.422	52.9 (3.9)	521 (22.8)	710 (21.0)	147 (21.4)	0.786 (17.2)	0.303 (15.0)	1.105 (17.8)	-	0.828 (16.9)	1.095 (16.2)	0.055 (15.5)
Exponential [c]	Additive	-2649.838 [f]	-2631.838	53.9 (8.1)	940 (53.4)	335 (29.6)	51.9 (30.6)	1.600 (73.1)	0.308 (18.3)	1.005 (30.2)	-	-	1.015 (58.7)	0.058 (14.3)
Exponential [a]	Proportional	-2989.428 [g]	-2967.428	55.2	376	825	137	0.506	0.279	1.319	0.005	0.269	0.362	0.390

Table 15: Results of two compartment model with different IIV and RUV model (cont)

IIV model	RUV model	OFV	AIC	Parameter estimate (SE%)										
				CL	V_c	V_p	Q	k_a	ω_{CL}	ω_{Vc}	ω_{Vp}	ω_{Q}	ω_{Ka}	σ
Exponential [b]	Proportional	-2989.429 [f]	-2969.429	55.2	376	825	137	0.506	0.279	1.319	-	0.269	0.362	0.390
Exponential [c]	Proportional	-2981.706 [f]	-2963.706	55.3 (5.9)	535 (81.7)	660 (80.8)	121 (114.0)	0.729 (73.1)	0.272 (33.4)	0.774 (189.5)	-	-	1.208 (387.0)	0.389 (16.3)
Exponential [a]	Combined	-2996.722 [g]	-2972.722	56.6	656	549	118	0.891	0.312	0.833	0.005	0.409	1.549	Prop 0.324 Add 0.015
Exponential [b]	Combined	-2996.723 [f]	-2974.723	56.7 (4.2)	656 (23.3)	549 (17.1)	118 (18.5)	0.891 (12.9)	0.312 (10.9)	0.833 (14.8)	-	0.409 (61.1)	1.549 (18.8)	Prop 0.324(9.0) Add 0.015(14.4)
Exponential [c]	Combined	-2995.524 [f]	-2975.524	56.3 (3.3)	667 (11.4)	531 (12.2)	103 (11.4)	0.916 (0.1)	0.311 (10.5)	0.814 (8.1)	-	-	1.559 (14.5)	Prop 0.327(7.8) Add 0.015(14.4)
Exponential [a]	Exponential	-2989.428 [g]	-2967.428	55.2	376	825	137	0.506	0.279	1.319	0.005	0.269	0.362	0.390
Exponential [b]	Exponential	-2989.429 [h]	-2967.429	55.2 (4.7)	376 (50.5)	825 (21.3)	137 (24.2)	0.506 (51.4)	0.279 (12.6)	1.319 (28.3)	-	0.269 (76.1)	0.362 (62.2)	0.390 (6.4)
Exponential [c]	Exponential	-2981.706 [f]	-2961.706	55.3 (5.9)	535 (81.7)	660 (80.8)	121 (114.0)	0.729 (73.1)	0.272 (33.4)	0.774 (189.5)	-	-	1.208 (387.0)	0.389 (16.3)

[a] estimated IIV of all parameters

[b] did not estimate IIV of V_p

[c] did not estimate IIV of V_p and Q

[d] Minimization terminated

[e] Estimation omitted

[f] Minimization successful

[g] Minimization successful, however parameter estimate is near its boundary

[h] Minimization successful but covariance step aborted

When pharmacokinetic parameter estimates with standard error, OFV, AIC and goodness of fit plots were considered, the pharmacokinetics of tenofovir can be best described by a two-compartment model with first order absorption and elimination. The interindividual variability of CL/F, V_c/F and k_a were best described by an exponential model. Residual unexplained variability was best described by a combined additive and proportional model. The OFV of the base model was -2995.524.

Basic goodness of fit plot of selected base model is shown in figure 3.

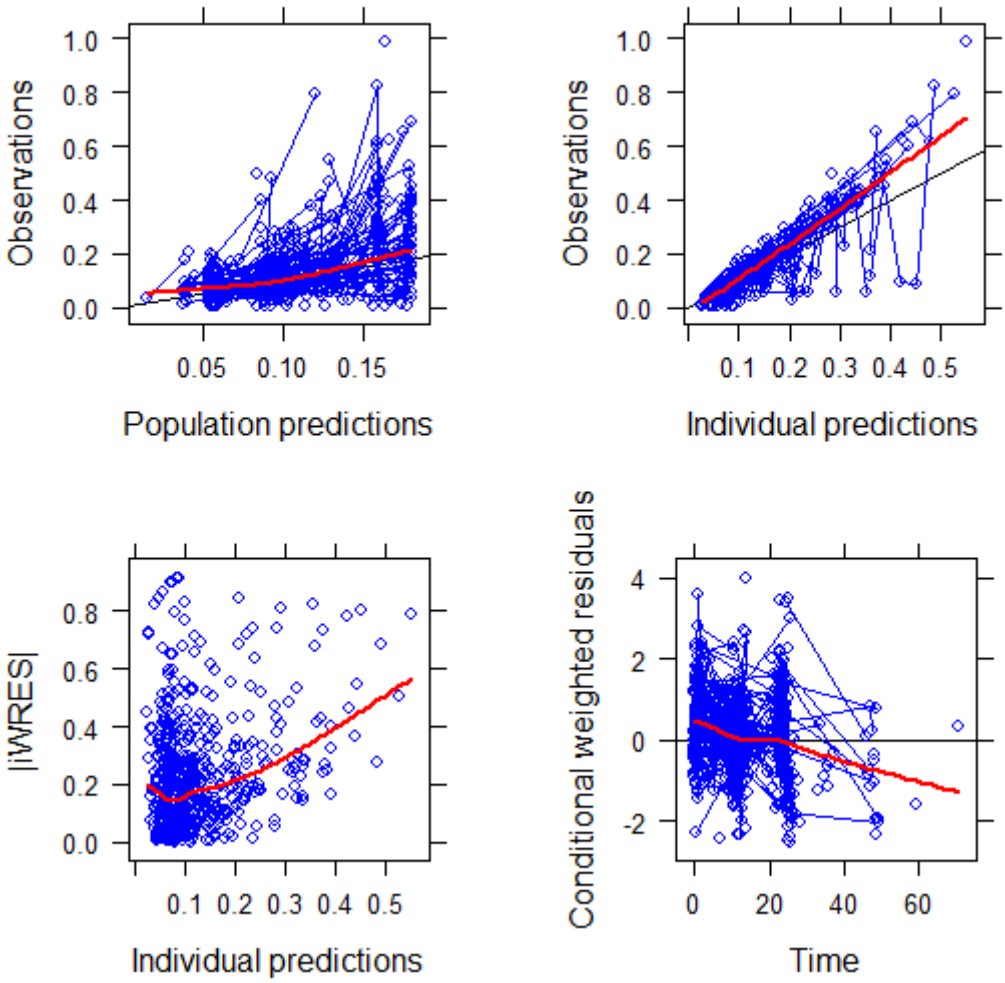

Figure 3: Basic goodness of fit plot of base model

Covariate model

The influence of covariates on pharmacokinetic parameters was investigated by stepwise forward inclusion and backward deletion approach. The continuous covariates including age, weight, serum creatinine, GFR_{CG}, GFR_{MDRD} and GFR_{THAI} were tested with linear, exponential and power model. The categorical covariates including sex, KTD, HBV, HCV, genetic polymorphisms (*ABCC2* -24C>T, *ABCC2* 1249G>A, *ABCC2* 3972C>T, *ABCC4* 3463A>G, *ABCC4* 4131T>G) and concomitant protease inhibitors (LPV/r, ATV/r, SQV/r) were tested with fractional change model. Due to a small number of patients in each genotype group, all SNPs except *ABCC4* 4131T>G were categorized to dichotomous covariate (homozygous wild type (wild type group) and at least one variant allele (variant group)). For genetic polymorphisms of *ABCC4* 4131T>G, the population pharmacokinetic parameters were investigated by comparing 3 genotype groups: homozygous wild-type, heterozygous variant and homozygous variant.

The relationship between various demographic data and individual predicted CL/F of tenofovir are shown in figure 4. For continuous covariates, the plots showed that a linear relationship between individual predicted CL/F and age or body weight was observed, whereas the relationship between CL/F and other covariates including serum creatinine, GFR_{CG}, GFR_{MDRD} and GFR_{THAI} tended to be a nonlinear manner. The relationship between categorical covariates and individual predicted CL/F of tenofovir was not clearly seen. However, a lower individual predicted CL/F was observed when LPV/r was used as comedication.

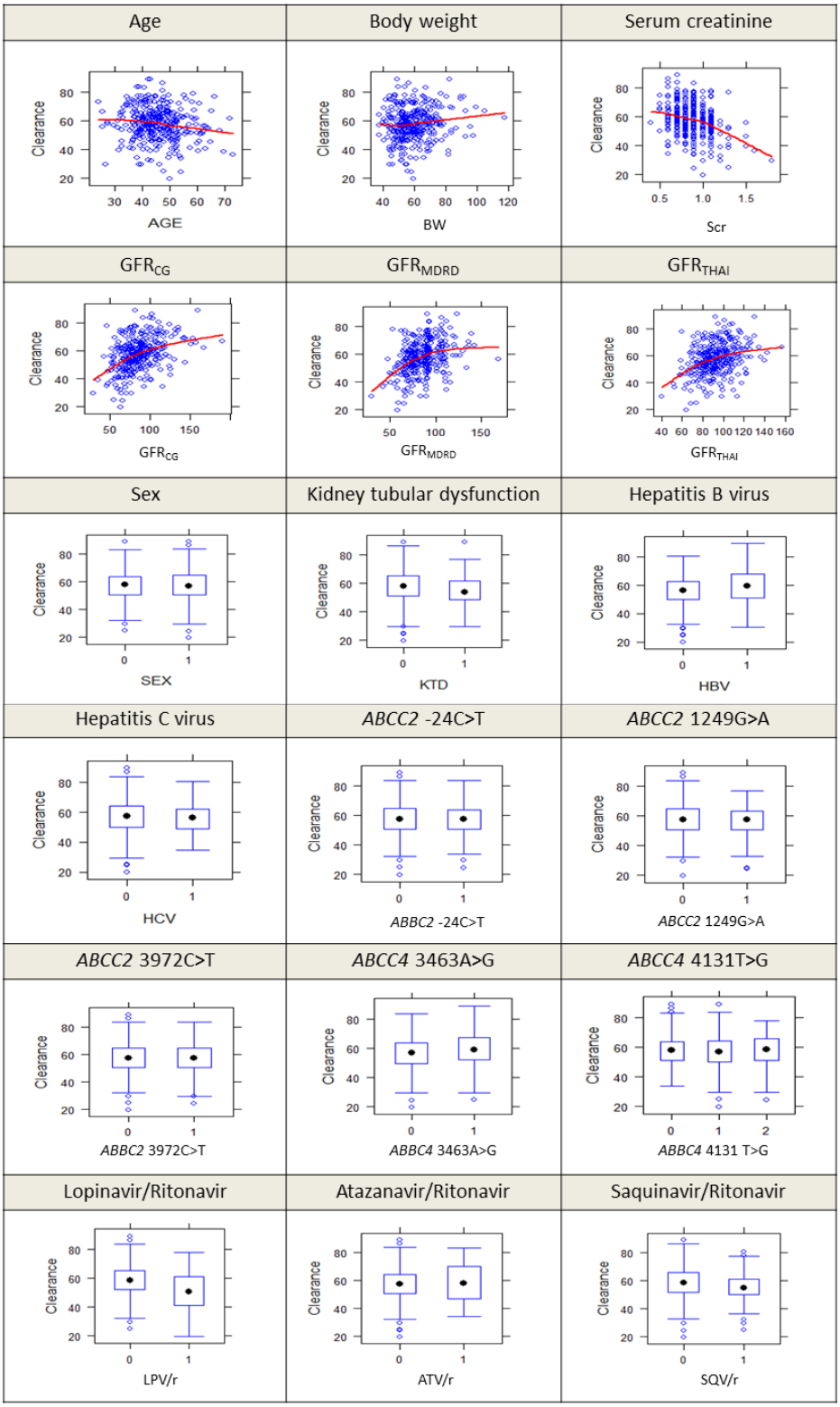

Figure 4: Graphical displays of the relationship between covariates and CL/F of tenofovir

Stepwise forward inclusion

The results from stepwise forward inclusion are shown in table 16-20.

Table 16: Results of stepwise forward inclusion (univariate analysis)

Added covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1$	-2995.524	
AGE (Linear)	$CL/F = \theta_1 + \theta_2 \times AGE$	-3001.664	-6.140*
AGE (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times AGE)$	-3001.545	-6.021*
AGE (Power)	$CL/F = \theta_1 \times AGE^{\theta_2}$	-3001.009	-5.485*
BW (Linear)	$CL/F = \theta_1 + \theta_2 \times BW$	-3001.894	-6.370*
BW (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times BW)$	-3002.063	-6.539*
BW (Power)	$CL/F = \theta_1 \times BW^{\theta_2}$	-3001.381	-5.857*
Scr (Linear)	$CL/F = \theta_1 + \theta_2 \times Scr$	-3034.666	-39.142*
Scr (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times Scr)$	-3033.625	-38.101*
Scr (Power)	$CL/F = \theta_1 \times Scr^{\theta_2}$	-3029.214	-33.690*
GFR_{CG} (Linear)	$CL/F = \theta_1 + \theta_2 \times GFR_{CG}$	-3057.114	-61.590*
GFR_{CG} (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times GFR_{CG})$	-3050.520	-54.996*
GFR_{CG} (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2}$	-3059.176	-63.652*
GFR_{MDRD} (Linear)	$CL/F = \theta_1 + \theta_2 \times GFR_{MDRD}$	-3049.721	-54.197*
GFR_{MDRD} (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times GFR_{MDRD})$	-3043.468	-47.944*
GFR_{MDRD} (Power)	$CL/F = \theta_1 \times GFR_{MDRD}^{\theta_2}$	-3051.098	-55.574*
GFR_{THAI} (Linear)	$CL/F = \theta_1 + \theta_2 \times GFR_{THAI}$	-3041.839	-46.315*
GFR_{THAI} (Expo)	$CL/F = \theta_1 \times \exp(\theta_2 \times GFR_{THAI})$	-3037.669	-42.145*
GFR_{THAI} (Power)	$CL/F = \theta_1 \times GFR_{THAI}^{\theta_2}$	-3042.488	-46.964*
SEX	$CL/F = \theta_1 \times (1 + \theta_2 \times SEX)$	-2995.525	-0.001
KTD	$CL/F = \theta_1 \times (1 + \theta_2 \times KTD)$	-2995.525	-0.001
HBV	$CL/F = \theta_1 \times (1 + \theta_2 \times HBV)$	-2997.183	1.659
HCV	$CL/F = \theta_1 \times (1 + \theta_2 \times HCV)$	-2995.571	-0.067

Table 16: Results of stepwise forward inclusion (univariate analysis) (cont)

Added covariate	Model	OFV	dOFV
ABCC2 -24C>T	CL/F = θ_1x (1+ θ_2 x *ABCC2* -24C>T)	-2995.715	-0.191
ABCC2 1249G>A	CL/F = θ_1x (1+ θ_2 x *ABCC2* 1249G>A)	-2996.120	-0.596
ABCC2 3972C>T	CL/F = θ_1x (1+ θ_2 x *ABCC2* 3972C>T)	-2995.571	-0.047
ABCC4 3463A>G	CL/F = θ_1x (1+ θ_2 x *ABCC4* 3463A>G)	-3000.854	-5.330*
ABCC4 4131T>G	CL/F = θ_1x (1+ θ_2 x *ABCC4* 4131TT) x (1+ θ_3 x *ABCC4* 4131TG) x (1+ θ_4 x *ABCC4* 4131GG)	-2997.371	-1.847
LPV/r	CL/F = θ_1x (1+ θ_2 x LPV/r)	-3027.681	-32.157*
ATV/r	CL/F = θ_1x (1+ θ_2 x ATV/r)	-2996.048	-0.524
SQV/r	CL/F = θ_1x (1+ θ_2 x SQV/r)	-2998.383	-2.859

*OFV decreased at least 3.84 (χ^2, df=1, p$\leq$0.05)

The addition of GFR_{CG} resulted in the largest drop of OFV. Therefore, GFR_{CG} was the first covariate that was added into the base model. For the next step, other covariates were added into the GFR_{CG} base model one at a time. However, serum creatinine, GFR_{MDRD} and GFR_{THAI} had a high correlation with GFR_{CG}, they were not further tested in the covariate model.

Table 17: Results of stepwise forward inclusion (GFR_{CG} was added)

Added covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2}$	-3059.176	
AGE (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} + \theta_3 \times AGE$	-3059.264	-0.088
AGE (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times \exp(\theta_3 \times AGE)$	-3059.263	-0.087
AGE (Power) [a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times AGE^{\theta_3}$	-3059.308	-0.132
BW (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} + \theta_3 \times BW$	-3063.823	-4.647*
BW (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times \exp(\theta_3 \times BW)$	-3063.875	-4.699*
BW (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times BW^{\theta_3}$	-3064.058	-4.882*
SEX	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times SEX)$	-3059.826	-0.650
KTD	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times KTD)$	-3059.622	-0.446
HBV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times KTD)$	-3059.841	-0.065
HCV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times KTD)$	-3060.390	-1.214
ABCC2 -24C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC2* -24C>T)	-3059.217	-0.041
ABCC2 1249G>A	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC2* 1249G>A)	-3059.185	-0.009
ABCC2 3972C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC2* 3972C>T)	-3059.347	-0.171
ABCC4 3463A>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC4* 3463A>G)	-3063.576	-4.400*
ABCC4 4131T>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC4* 4131TT) x $(1+ \theta_4 \times$ *ABCC4* 4131TG) x $(1+ \theta_5 \times$ *ABCC4* 4131GG)	-3061.757	-2.581
LPV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r)$	-3084.504	-25.328*
ATV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times ATV/r)$	-3059.865	-0.689
SQV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times SQV/r)$	-3059.225	-0.049

[a] minimization successful but covariance step aborted

*OFV decreased at least 3.84 (χ^2, df=1, $p \leq 0.05$)

Table 18: Results of stepwise forward inclusion (GFR_{CG} and LPV/r were added)

Added covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r)$	-3084.504	
AGE (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r)+ \theta_4 \times AGE$	-3088.195	-3.691
AGE (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times \exp(\theta_4 \times AGE)$	-3088.192	-3.688
AGE (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times AGE^{\theta_4}$	-3088.276	-3.772
BW (Linear) [a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r)+\theta_4 \times BW$	-3091.277	-6.773*
BW (Expo) [b]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times \exp(\theta_4 \times BW)$	-3088.995	-4.491*
BW (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+\theta_3 \times LPV/r) \times BW^{\theta_4}$	-3091.474	-6.970*
SEX	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times SEX)$	-3085.571	-1.067
KTD	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times KTD)$	-3088.108	-3.604
HBV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times HBV)$	-3088.140	-3.636
HCV [b]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times HCV)$	-3090.869	-6.365*
ABCC2 -24C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC2* -24C>T)	-3085.037	-0.533
ABCC2 1249G>A	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC2* 1249G>A)	-3084.978	-0.474
ABCC2 3972C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC2* 3972C>T)	-3084.717	-0.603

Table 18: Results of stepwise forward inclusion (GFR_{CG} and LPV/r were added) (cont)

Added covariate	Model	OFV	dOFV
ABCC4 3463A>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G)	-3094.157	-9.653*
ABCC4 4131T>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 4131TT) x $(1+ \theta_5 \times$ *ABCC4* 4131TG) x $(1+ \theta_6 \times$ *ABCC4* 4131GG)	-3085.571	-1.067
ATV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times ATV/r)$	-3092.792	-8.288*
SQV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times SQV/r)$	-3086.843	-2.339

[a] minimization terminated

[b] minimization successful but covariance step aborted

*OFV decreased at least 3.84 (χ^2, df=1, $p \leq 0.05$)

Table 19: Results of stepwise forward inclusion (GFR_{CG}, LPV/r and *ABCC4* 3463A>G were added)

Added covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G)	-3094.157	
AGE (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $+ \theta_5 \times AGE$	-3094.159	-0.002
AGE (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times \exp(\theta_5 \times AGE)$	-3091.060	3.097
AGE (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times AGE^{\theta_5}$	-3094.190	-0.033
BW (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $+ \theta_5 \times BW$	-3098.372	-4.215*
BW (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times \exp(\theta_5 \times BW)$	-3098.421	-4.264*
BW (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5}$	-3098.437	-4.280*
SEX	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times SEX)$	-3095.261	-1.104
KTD	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times KTD)$	-3091.061	3.096
HBV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times HBV)$	-3094.428	-0.271
HCV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times HCV)$	-3096.805	-2.648
ABCC2 -24C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times$ *ABCC2* -24C>T)	-3094.245	-0.088

Table 19: Results of stepwise forward inclusion (GFR_{CG}, LPV/r and *ABCC4* 3463A>G were added) (cont)

Added covariate	Model	OFV	dOFV
ABCC2 1249G>A	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+\theta_5 \times$ *ABCC2* 1249G>A)	-3091.293	2.864
ABCC2 3972C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times$ *ABCC2* 3972C>T)	-3094.346	-0.189
ABCC4 4131T>G[a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times$ *ABCC4* 4131TT) $\times (1+ \theta_6 \times$ *ABCC4* 4131TG) $\times (1+ \theta_7 \times$ *ABCC4* 4131GG)	-3096.107	-1.950
ATV/r[a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times$ ATV/r)	-3098.097	-3.940*
SQV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times (1+ \theta_5 \times$ SQV/r)	-3092.603	1.554

[a] minimization terminated

*OFV decreased at least 3.84 (χ^2, df=1, $p \leq 0.05$)

Table 20: Results of stepwise forward inclusion (GFR_{CG}, LPV/r, *ABCC4* 3463A>G and BW were added)

Added covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5}$	-3098.437	
AGE (Linear)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} + \theta_6 \times$ AGE	-3100.096	-1.659
AGE (Expo)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times \exp(\theta_6 \times$ AGE$)$	-3100.069	-1.632
AGE (Power)	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times$ AGE$^{\theta_6}$	-3100.248	-1.811
SEX	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times (1+ \theta_6 \times$ SEX$)$	-3098.458	-0.021
KTD	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times (1+ \theta_6 \times$ KTD$)$	-3098.442	-0.005
HBV [a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times (1+ \theta_6 \times$ HBV$)$	-3098.851	-0.414
HCV	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times (1+ \theta_6 \times$ HCV$)$	-3100.846	-2.409
ABCC2 -24C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5} \times (1+ \theta_6 \times$ *ABCC2* -24C>T$)$	-3098.560	-0.123

Table 20: Results of stepwise forward inclusion (GFR_{CG}, LPV/r, *ABCC4* 3463A>G and BW were added) (cont)

Added covariate	Model	OFV	dOFV
ABCC2 1249G>A	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5} \times (1+\theta_6 \times$ *ABCC2* 1249G>A)	-3098.569	-0.132
ABCC2 3972C>T	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5} \times (1+ \theta_6 \times$ *ABCC2* 3972C>T)	-3098.686	-0.249
ABCC4 4131T>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5} \times (1+ \theta_6 \times$ *ABCC4* 4131TT) $\times (1+ \theta_7 \times$ *ABCC4* 4131TG) $\times (1+ \theta_8 \times$ *ABCC4* 4131GG)	-3100.753	-2.316
ATV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5} \times (1+ \theta_6 \times ATV/r)$	-3101.083	-2.646
SQV/r[a]	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G) $\times BW^{\theta_5} \times (1+ \theta_6 \times SQV/r)$	-3099.973	-1.536

[a] minimization successful but covariance step aborted

Therefore, covariates that were included in the full model were GFR_{CG}, LPV/r, *ABCC4* 3463A>G and body weight.

Backward deletion

The results from backward deletion showed that body weight failed to reach the significant level (dOFV > 6.63, χ^2, df=1, $p \leq 0.01$). Therefore only GFR_{CG}, LPV/r and *ABCC4* 3463A>G were retained in the final model as shown in table 21.

Table 21: Results of backward deletion

Deleted covariate	Model	OFV	dOFV
Base model	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_5}$	-3098.437	
BW	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times (1+ \theta_4 \times$ *ABCC4* 3463A>G$)$	-3094.157	4.280
ABCC4 3463A>G	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times LPV/r) \times BW^{\theta_4}$	-3091.474	6.963*
LPV/r	$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1+ \theta_3 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_4}$	-3069.069	29.368*
GFR_{CG}	$CL/F = \theta_1 \times (1+ \theta_2 \times LPV/r) \times (1+ \theta_3 \times$ *ABCC4* 3463A>G$) \times BW^{\theta_4}$	-3040.033	58.404*

*OFV increased at least 6.63 (χ^2, df=1, $p \leq 0.01$)

Final model

The influence of covariates on OFV and IIV of CL/F of tenofovir is shown in table 22. The results showed that IIV of CL/F obtained from final model (when GFR_{CG}, LPV/r and *ABCC4* 3463A>G were added) was lower than the value obtained from the base model (19.2% and 31.0%, respectively).

Table 22: The influence of covariates on OFV and IIV of CL/F of tenofovir

Model	OFV	ΔOFV [a]	IIV	ΔIIV [b]
Base model (Model without covariate)	-2995.524	-	31.0%	-
Model with covariate				
GFR_{CG} was added	-3059.176	-63.652	23.2%	-7.8%
GFR_{CG} and LPV/r were added	-3084.504	-88.980	19.7%	-11.3%
GFR_{CG}, LPV/r and *ABCC4* 3463A>G were added (final model)	-3094.157	-98.633	19.2%	-11.8%

[a] ΔOFV = OFV of covariate model – OFV of base model

[b] ΔIIV = IIV of covariate model – IIV of base model

The final model of tenofovir CL/F can be described by the following equation:

$$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1 + \theta_3 \times LPV/r) \times (1 + \theta_4 \times ABCC4\ 3463A>G)$$

$$CL/F = 3.77 \times GFR_{CG}^{0.601} \times [1-(0.251 \times LPV/r)] \times [1 + (0.105 \times ABCC4\ 3463A>G)]$$

Where; GFR_{CG} = GFR calculated by Cockcroft and Gault formula (ml/min)

LPV/r = 1 when lopinavir/ritonavir was concomitantly used

LPV/r = 0 when lopinavir/ritonavir was not concomitantly used

ABCC4 3463A>G = 1 when *ABCC4* 3463A>G genotype AG or GG (variant type)

ABCC4 3463A>G = 0 when *ABCC4* 3463A>G genotype AA (wild type)

The final model of CL/F of tenofovir showed that low GFR_{CG} and concomitant use with LPV/r were associated with low CL/F of tenofovir, whereas *ABCC4* 3463A>G variant was associated with higher tenofovir CL/F. GFR_{CG} was related to CL/F of tenofovir by a power function with an exponent 0.601. Concomitant use with LPV/r decreased CL/F of

tenofovir by 25.1%. *ABCC4* 3463A>G variant type (genotype AG or GG) increased CL/F of tenofovir by 10.5%.

The population pharmacokinetic parameter estimates of the final model are shown in table 23.

Table 23: Pharmacokinetic parameter estimates of final model

Parameter	Estimation value	(95%CI)[a]
CL/F (L/hr) = θ_1 x $GFR_{CG}^{\theta_2}$ x (1+ θ_3 x LPV/r) x (1+ θ_4 x *ABCC4* 3463A>G)		
θ_1	3.77	(1.71, 5.83)
θ_2	0.601	(0.589, 0.613)
θ_3	-0.251	(-0.341, -0.161)
θ_4	0.105	(0.009, 0.201)
V_c/F (L)	483	(173.16, 792.84)
V_p/F (L)	607	(379.76, 834.24)
Q (L/hr)	119	(87.28, 150.72)
k_a (hr^{-1})	0.656	(0.409, 0.903)
IIV of CL/F (%CV)	19.2	(11.8, 26.6)
IIV of V_c/F (%CV)	65.3	(41.4, 89.2)
IIV of k_a ((%CV)	127.7	(49.4, 206.0)
RUV proportional model (%CV)	36.7	(28.9, 44.5)
RUV additive model (mg/l)	0.010	(0.002, 0.018)

[a] Calculated as estimates ± 1.96 x standard error

CI, confidence interval; CL/F, apparent oral clearance; CV, coefficient of variation; k_a, absorption rate constant; IIV, interindividual variability; Q/F, apparent intercompartmental clearance; V_c/F, apparent central compartment volume of distribution; V_p/F, apparent peripheral compartment volume of distribution; RUV, residual unexplained variability

The goodness of fit plots of the base and the final model are presented in figure 5-6. All the plots showed no systematic bias of the model. The plot of CWRES vs PRED of the final model showed that most of observed concentrations were scattered around the zero line and were within ± 4, indicating that the final model was deemed adequate. The goodness of fit plots of the final model showed an improvement from the base model. Therefore the addition of covariates including GFR_{CG}, LPV/r and *ABCC4* 3463A>G into the model can explain the interindividual variability of the pharmacokinetic parameters of tenofovir.

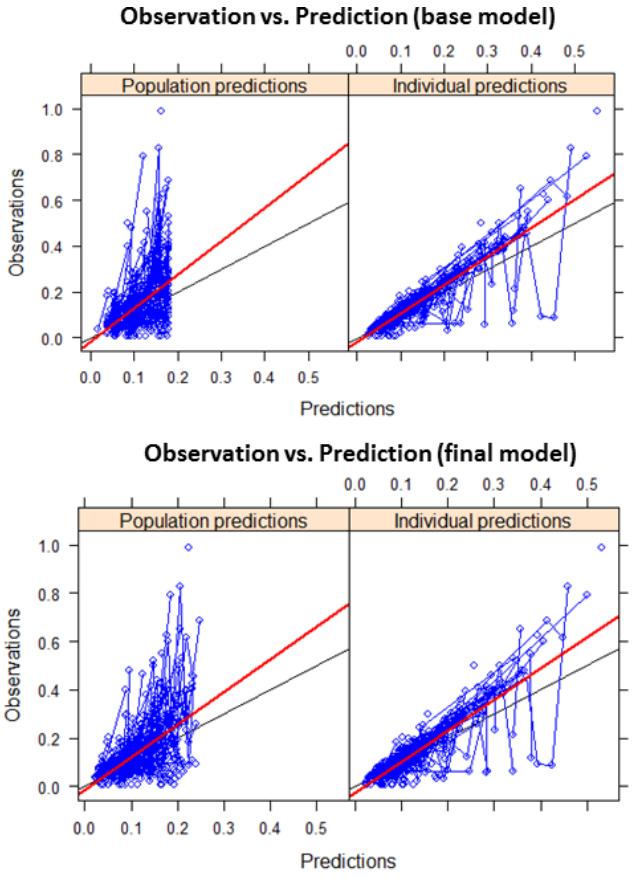

Figure 5: The goodness of fit plots of the base model and the final model; observed concentrations (DV) vs population predicted concentrations (PRED) and observed concentrations (DV) vs individual predicted concentrations (IPRED)

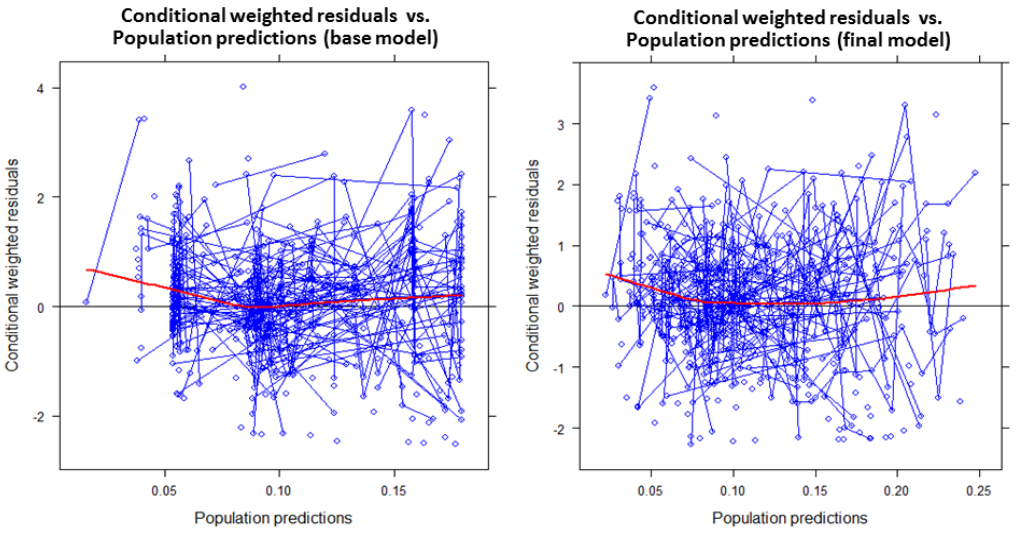

Figure 6: The goodness of fit plots of the base model and the final model; conditional weighted residuals (CWRES) vs population predicted concentration (PRED)

Model validation

A total of 103 patients and 103 plasma concentrations from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database were used for model validation. The individual predicted concentrations of each subject in validation group were obtained using the “posthoc” option in NONMEM without estimation step (MAXEVAL=0) by setting mean parameter values, IIV and RUV to the values obtained from the final model. The individual predicted concentrations were then compared to the observed concentrations (Appendix C).

Bias and precision of the model were described by mean prediction error (MPE) and root mean square error (RMSE), respectively.

$$MPE = \frac{\sum PE_i}{n}$$

$$= \frac{-0.466}{103}$$

$$= -0.00452 \text{ mg/l}$$

$$RMSE = \sqrt{\frac{\sum (PE_i)^2}{n}}$$

$$= \sqrt{\frac{0.045944}{103}}$$

$$= 0.0211 \text{ mg/l}$$

Where; PE = prediction error

= predicted concentration - observed concentration

n = number of pairs of observed and predicted concentrations

The MPE and RMSE were -0.00452 mg/l and 0.0211 mg/l, respectively. The results from one-sample t-test (Table 24) showed that MPE was different from zero (MPE= -0.00452 mg/l; p=0.029) indicating that final model tended to underpredict tenofovir concentrations.

Table 24: One-sample t-test of MPE compared to zero

One sample t-test						
Test value = 0						
	t	df	Sig (2-tailed)	Mean Difference	95% CI	
					Lower	Upper
MPE	-2.212	102	0.029	-0.00452	-0.00858	-0.00047

An agreement between individual predicted concentrations and observed concentrations was described by a Bland-Altman plot as shown in figure 7. The mean of difference between predicted concentrations and observed concentrations was near zero, the plots were equally distributed and most of them were within mean ± 2SD. Therefore, the results showed that final model was fairly adequate.

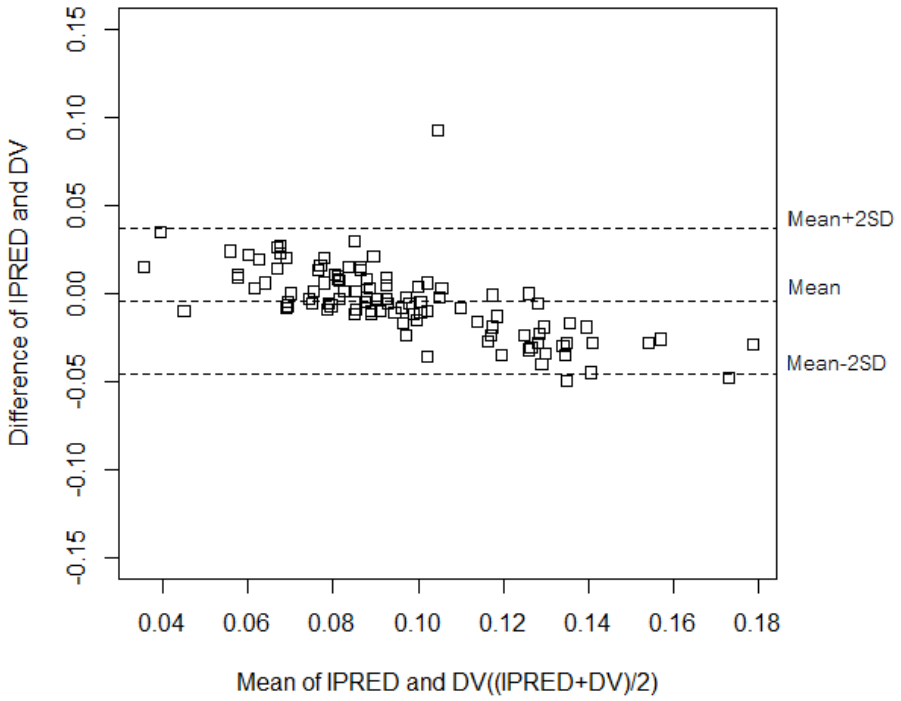

Figure 7: Bland-Altman plot described an agreement between individual predicted concentrations (IPRED) and observed concentrations (DV)

Comparison of pharmacokinetic parameters of tenofovir among different studies

Pharmacokinetic parameters and factors influencing pharmacokinetic parameters of tenofovir from different studies are shown in table 25. The results showed that all pharmacokinetic parameter estimates of tenofovir in our study were comparable to the previous studies.

Table 25: Comparison of pharmacokinetic parameters of tenofovir among different studies

	Jullien V [15]	Gagnieu MC [14]	Ramanathan S [16]	Baheti G [17]	This study
Population	Adult HIV-infected patients in Europe (n=193)	Adult HIV-infected patients in Europe (n=175)	Healthy subjects and HIV-infected patients in America (n=586)	Adult HIV-infected patients in America and other (n=55)	Adult HIV-infected patients in Thailand (n=342)
Dose TDF	300 mg/day	Clcr>50; 300 mg/day Clcr<50; 300 mg AD	300 mg/day	300 mg/day	300 mg/day (n=323) 300 mg AD (n=19)
K_a (IIV); hr^{-1}	NA	0.59 [a] (49)	2.48 (103)	1.05	0.656 (127.7)
CL/F (IIV); liters/hr	50.5[a] (26)	36.2+135x(BW/Scr)[b] (19)	42.3 (21)	42.0 (33.5)	54.8 (19.2)
V_c/F (IIV); liters	297 [a] (61)	495 [a] (51)	354 (46)	277 (41.1)	483 (65.3)
V_p/F (IIV); liters	848 [a]	1382 [a] (58)	530	436 (46.5)	607
Q/F (IIV); liters/hr	80 [a]	273 [a] (81)	150	182	119
Factors influencing CL/F	BW/Scr lopinavir/ritonavir tubular dysfunction	BW/Scr	GFR (Cockcroft and Gault)	GFR (Cockcroft and Gault)	GFR (Cockcroft and Gault) lopinavir/ritonavir *ABCC4* 3463A>G

AD, alternate day; BW/Scr, body weight per serum creatinine ratio; Clcr, creatinine clearance; CL/F, apparent oral clearance; K_a, absorption rate constant; IIV, interindividual variability; NA, not applicable; mg, milligrams; Q/F, intercompartmental clearance; V_c/F, apparent central compartment volume of distribution; V_p/F, apparent peripheral compartment volume of distribution

[a] population pharmacokinetic parameters after correct dose of tenofovir disoproxil with tenofovir

[b] CL/F of tenofovir disoproxil

CHAPTER V
DISCUSSION AND CONCLUSION

Tenofovir is one of the first line drugs for the treatment of HIV infection [8]. Due to its simple regimen as once daily dosing and coformulation with emtricitabine (Truvada®) or with emtricitabine and efavirenz (Atripla®) as a single pill tablet, high antiviral potency, low adverse drug reaction and limited drug interaction, tenofovir is a recommended backbone in naïve and experienced-treatment HIV-infected patients [73]. The effective treatment of drug can control HIV virus, preserve immune system, prevent HIV-related morbidity and significantly improve patients' quality of life [4, 9].

Tenofovir is transported into kidney tubular cells by OAT1 and OAT3, and then is secreted to tubular lumen by MRP2 and MRP4 [9]. The genetic variation of these transporters may affect transportation of tenofovir in kidney tubular cell and then affect pharmacokinetics of tenofovir. Previous studies have been shown that genetic polymorphisms of *ABCC2* (encode MRP2) and *ABCC4* (encode MRP4) were associated with higher tenofovir concentration [18, 19] and higher tenofovir concentration also correlated with renal toxicity [12, 13]. The cut-off values of mid-dose concentration (C_{12}) more than 160 ng/ml and trough concentration more than 90 ng/ml were associated with higher risk of renal toxicity in pateints receiving tenofovir [12, 13]. These results suggested that genetic variation of tenofovir transporter genes may lead to an overexposure of tenofovir resulting in renal cell damage.

Tenofovir exposure was also correlated with antiviral efficacy. Although, a clear cut-off value of tenofovir concentration associated with antiviral efficacy was not established, the plasma AUC of tenofovir was found to be associated with its efficacy [10]. Median steady state AUC of patients having virological response (>0.5 $\log_{10}$ HIV copies/ml decline) was higher than patients who did not have virological response (3,800 and 2,510 ng.hr/ml respectively; p=0.031) [10]. Moreover, when TDF dose lower than 300 mg per day was given, tenofovir plasma concentration and the reduction of HIV-1 RNA concentration were lower [11].

All of the mentioned above showed that tenofovir plasma concentration had an influence on both efficacy and toxicity of the drug. Although, high tenofovir exposure was correlated with higher antiviral efficacy, it may cause higher risk of toxicity. Futhermore, the pharmacokinetics of tenofovir is highly variable between individual [14, 15, 17]. Several factors may contribute to high interindividual variability of tenofovir. Therefore, the study investigating the influence of genetic and non-genetic factors on tenofovir pharmacokinetics is crucial for optimizing tenofovir dosage regimens to ensure efficacy and safety of this drug.

In this study, we investigated the influence of genetic and non-genetic factors on tenofovir plasma concentrations at mid-dose. Additionally, the population pharmacokinetics of tenofovir was developed. The population mean pharmacokinetic parameters and their variability were estimated. Factors influencing population pharmacokinetic parameters of tenofovir were also investigated. This information is important in order to optimize individual tenofovir dosage regimen in Thai-HIV infected patients.

In the first part of this study, a total of 150 patients and 150 plasma concentrations at mid-dose (10-14 hours after last dose) from HIV-NAT database were used for analysis. The results showed that factors including body weight, serum creatinine, GFR and concomitant use with ritonavir-boosted protease inhibitor were associated with tenofovir plasma concentrations ($p<0.05$). On the other hand, other factors including age, sex, KTD, hepatitis B co-infection and hepatitis C co-infection were not associated with tenofovir plasma concentrations. Due to a small number of patients using lopinavir/ritonavir, atazanavir/ritonavir and saquinavir/ritonavir, the significant influence of each ritonavir-boosted protease inhibitor on tenofovir plasma concentrations was not detected. Interestingly, the association between genetic polymorphisms (all SNPs of *ABCC2* and *ABCC4*) and tenofovir plasma concentrations were not found. This could be due to a small number of patients and the use of univariate analysis in this study. Therefore, the impact of other factors that may confound

the relationship between genetic polymorphisms and tenofovir plasma concentrations were not controlled.

In the second part of this study, a total of 342 patients with 643 plasma concentrations from HIV-NAT database were included in order to develop a population pharmacokinetic model of tenofovir. The pharmacokinetics of tenofovir was best described by a two-compartment model with first order absorption and elimination which is consistent with previous studies [14, 17]. The IIV and RUV model was described by an exponential and a combined additive and proportional error model, respectively. Due to a spase characteristic of the data, the IIV of V_p and Q cannot be estimated. For covariate model development, factors including GFR calculated by Cockcroft and Gault formula, concomitant use of lopinavir/ritonavir and *ABCC4* 3463 A>G polymorphism were associated with CL/F of tenofovir.

Tenofovir is mainly eliminated by renal excretion. When renal dysfunction is occurred, the elimination of tenofovir also decreases and then elevates tenofovir plasma concentration. In this study, the effect of three different formulas of estimated GFR (Cockcroft and Gault, MDRD and Thai formula) on tenofovir pharmacokinetics were investigated. In univariate analysis, all the estimated GFR formulas had significant influence on CL/F of tenofovir ($p<0.05$). But, the most significant one was GFR calculated by Cockcroft and Gault. When GFR calculated by Cockcroft and Gault was added into the model, the IIV of CL/F and OFV was decreased 7.8% and 63.562, respectively.

Concomitant use of other drugs may have influence on CL/F of tenofovir. Although tenofovir is not a substrate or inhibitor of cytochrome P450, it is eliminated via active tubular secretion transported by drug transporters. Concomitant with drugs that compete for renal excretion or inhibit drug transporter of tenofovir may alter pharmacokinetics of tenofovir [29, 30]. This study showed that concomitant use of lopinavir/ritonavir had significant influence on CL/F. On the other hand, other protease inhibitors including atazanavir/ritonavir and saquinavir/ritonavir did not show statistical significance. The effect of each protease inhibitor on pharmacokinetics of tenofovir may

be different. It depends on an ability of each drug for 1) inhibition of TDF hydrolysis in intestine tissue 2) inhibition of p-glycoprotein (p-gp) mediated efflux of tenofovir and 3) induction of p-gp expression [32]. In vitro study showed that lopinavir can inhibit p-gp more than atazanavir and saquinavir [32]. Futhermore, in this study, lopinavir is usually combined with ritonavir 100 mg twice daily (equal to ritonavir 200 mg/day), whereas atazanavir and saquinavir are usually combined with ritonavir 100 mg/day. A higher dose of ritonavir in lopinavir combination may result in a greater influence on tenofovir renal clearance than atazanavir or saquinavir combination. This could be the reason why lopinavir/ritonavir was found to be the only protease inhibitor that affects CL/F of tenofovir in our study. Interestingly, the effect of lopinavir/ritonavir on tenofovir CL/F found in our study was higher than those previously reported in European patients (25.1% vs 14.0%) [15]. It could be due to a higher plasma concentration of lopinavir and ritonavir observed in Thai patients [74, 75]. Previous studies showed that the plasma AUC of lopinavir in Thai children was approximately 30% higher than Caucasian [75]. Moreover, the different of genetic variation among races could be one of the factors influencing pharmacokinetics of tenofovir and lopinavir/ritonavir.

In our study, the significant effect of genetic variation of drug transporter on tenofovir CL/F was found only for *ABCC4* 3463A>G. However, the effect of *ABCC2* -24C>T on pharmacokinetics of tenofovir has been reported. Kiser JJ et al showed that patients with *ABCC2* -24C>T genotype TT had tenofovir renal clearance 19% higher than those carrying wild type [20]. Manosuthi W et al also found that the mean tenofovir plasma concentration in patients with *ABCC2* -24C>T variant was less than those with wild type (93 ng/ml vs 113 ng/ml, respectively) [18].

The influence of *ABCC4* 3463A>G on pharmacokinetics of tenofovir is controversy. In this study, we found that patients with *ABCC4* 3463 genotype AG or GG had CL/F of tenofovir 10.5% higher than those with genotype AA, resulting in a lower tenofovir plasma concentration. The result was similar to the study of Mitruk S et al which found that the mean tenofovir plasma concentration in patients with *ABCC4* 3463A>G variant was lower than those with wild type (76.6 ng/ml vs 88.9 ng/ml, respectively;

p<0.05) [69]. However, the result was in conflict with the study of Kiser JJ et al which found that after controlling for race and GFR, patient with *ABCC4* 3463 A>G variant had tenofovir renal clearance, on average, 15% lower than those with wild type [20]. The inconsistency of the impact of *ABCC4* 3463A>G polymorphism on tenofovir pharmacokinetics could be due to a small number of patients in the previous study (n=30) and different ethnicity between studies. However, it was shown that an intracellular concentration of tenofovir diphosphate was found to be higher in patients with *ABCC4* 3463 A>G variant compared to those with wild type [19]. Therefore, the influence of *ABCC4* 3463 A>G on the pharmacokinetics of tenofovir requires further investigation.

Based on the results from our study, the population mean CL/F of tenofovir in patients with *ABCC4* 3463A>G wild type, not using lopinavir/ritonavir as comedication and having an estimated GFR of 85.9 ml/min was 54.8 L/hr which was similar to the value previously reported (42.0-50.5 L/hr) [14-17]. The IIV of CL/F was 19.2% which was similar to the study by Gagnieu MC et al (19%) [14] but tended to be lower than other studies (21-33.5%) [15-17]. After including all significant covariates, the IIV of CL/F decreased by 11.8%. Therefore, the study was shown that the IIV of CL/F could partly be explained by GFR calculated by Cockcroft and Gault, concomitant use of lopinavir/ritonavir and *ABCC4* 3463A>G polymorphism.

The goodness of fit plots were used to assess the adequacy of the final model. The plot of PRED vs DV and CWRES vs PRED of the final model were superior to the base model. The mean of CWRES was distributed around the zero line and were within ±4. This confirmed that the final model was appropriate and had no major bias.

For model validation, a total 103 patients with 103 plasma concentrations from Ramathibodi Hospital database were used. The results showed that MPE was -0.00452 mg/l and the RMSE was 0.0211 mg/l. The results from one-sample t-test showed that MPE was significantly different from zero (95%CI -0.00858 to -0.00047; p=0.029), indicating that our final model tended to underpredict tenofovir concentration. There were some explanations for these results. First, the data used for model building and

model validation were extracted from different sources and found to have different characteristics. The different of baseline characteristics of patients may have influence on pharmacokinetics of tenofovir. Futhermore, there could be other factors influencing tenofovir pharmacokinetics in patients from Ramathibodi Hospital which may not be able to detect from HIV-NAT data. Second, some laboratory values such as serum creatinine from HIV-NAT and Ramathibodi Hospital database were determined from different laboratories. This different can cause an error when GFR was estimated and have impact on model validation.

Although MPE from the final model was statistically different from zero, the MPE was small and the value is near zero which may not be clinically important. Moreover, the Bland-Altman plot showed that most of the MPE were within two SD, indicating that the final model was deemed adequate. Therefore, regardless of the difference of patient's characteristics between the data, the final model should be sufficient for guiding individual tenofovir dosage regimens in this population.

In conclusion, the population pharmacokinetic of tenofovir was successfully developed. Factors including low GFR calculated by Cockcroft and Gault, concomitant use with lopinavir/ritonavir and *ABCC4* 3463A>G genotype AA were associated with lower CL/F of tenofovir. Although MPE obtained from model validation was statistical different from zero, the MPE was small and results from the Bland-Altman plot showed that the final model was deemed adequate. Therefore, this population pharmacokinetic model developed in this study could be useful to individualize tenofovir dosage regimen in Thai HIV-infected patients in order to ensure efficacy and safety of patients.

Limitations of study

1. The polymorphisms of other transporter genes involving tenofovir influx transport, such as *SLC22A6* and *SLC22A8* were not investigated in this study. However, there is evidence that genetic polymorphisms of these transporters were not associated with the pharmacokinetics of several drugs and the

transportation of drug across apical membrane (from cell to tubular lumen) by MRPs may be rate limiting step for drug secretion in the kidney.

2. Due to sparse data of tenofovir plasma concentrations, some pharmacokinetic parameters including the IIV of V_p/F and Q could not be estimated.
3. Due to incomplete data of Ramathibodi Hospital database, the data from HIV-NAT and Ramatibodi Hospital were not combined for model building. The data from Ramatibodi Hospital were separately used for model validation. With the different characteristics between data, some of the covariates that may be significant for patients in validation dataset may not be included in the model. This could lead to a poor prediction of tenofovir plasma concentration from our final model.

Recommendation

This population pharmacokinetic model should be further validated in group of patients that have baseline characteristics comparable to model building group to re-confirm the study results.

Application for clinical practice

Although, the results from population pharmacokinetics of tenofovir showed that GFR calculated by Cockcroft and Gault, concomitant use of lopinavir/ritonavir and *ABCC4* 3463 A>G polymorphism were associated with CL/F of tenofovir, the genotyping of *ABCC*4 3463A>G polymorphism may not be done in clinical practice. Therefore, the alternative model developed using only GFR calculated by Cockcroft and Gault and lopinavir/ritonavir may be used (Appendix D).

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APPENDIX

APPENDIX A

Certificate of Approval

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
อาคารสถาบัน 2 ชั้น 4 ซอยจุฬาลงกรณ์ 62 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์: 0-2218-8147 โทรสาร: 0-2218-8147 E-mail: eccu@chula.ac.th

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COA No. 146/2557

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 122/57 : เภสัชจลนศาสตร์ประชากรของยาทีโนโฟเวียร์ในผู้ป่วยติดเชื้อเอชไอวีชาวไทย

ผู้วิจัยหลัก : นางสาวกนกรัตน์ รุ่งทิวาสุวรรณ

หน่วยงาน : คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม.....
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)
ประธาน

ลงนาม.....
(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
กรรมการและเลขานุการ

วันที่รับรอง : 7 สิงหาคม 2557 **วันหมดอายุ** : 6 สิงหาคม 2558

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ผู้วิจัย

คณะกรรมการจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1
เลขที่โครงการวิจัย 122/57
วันที่รับรอง - 7 ส.ค. 2557
วันหมดอายุ - 6 ส.ค. 2558

เงื่อนไข

1. *ข้าพเจ้ารับทราบว่าเป็นการผิดจริยธรรม หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ*
2. *หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องชุดิ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย*
3. *ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด*
4. *ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารเชิญเข้าร่วมวิจัย (ถ้ามี)* ***เฉพาะที่ประทับตราคณะกรรมการเท่านั้น***
5. *หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ*
6. *หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ*
7. *โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น*

AF 02-12

The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University
Institute Building 2, 4 Floor, Soi Chulalongkorn 62, Phyat hai Rd., Bangkok 10330, Thailand,
Tel: 0-2218-8147 Fax: 0-2218-8147 E-mail: eccu@chula.ac.th

COA No. 146/2014

Certificate of Approval

Study Title No.122/57 : **POPULATION PHARMACOKINETICS OF TENOFOVIR IN THAI HIV-INFECTED PATIENTS**

Principal Investigator : MS. KANOKRAT RUNGTIVASUWAN

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP) and/or Code of Conduct in Animal Use of NRCT version 2000.

Signature: ..... Signature: .....

(Associate Professor Prida Tasanapradit, M.D.) (Assistant Professor Dr. Nuntaree Chaichanawongsaroj)

Chairman Secretary

Date of Approval : 7 August 2014 Approval Expire date : 6 August 2015

The approval documents including

1) Research proposal
2) Researcher

Protocol No. 122/57
Date of Approval -7 AUG 2014
Approval Expire Date -6 AUG 2015

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (ECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the ECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the ECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the ECCU for any serious adverse events within 5 working days*
5. *Report to the ECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

APPENDIX B

Study Population

The study population of this study was extracted from HIV-NAT and Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital database. The summary data from two databases are presented in table A.

Table A: The summary data from HIV-NAT and Ramathibodi Hospital database

	HIV-NAT database	Ramathibodi Hospital database
Study title	"Incidence and predictor of TDF associated nephrotoxicity and pharmacokinetic of TDF in HIV-1 infected Thai patients"	"ABCC2*1C and plasma tenofovir concentration are correlated to decreased glomerular filtration rate in patients receiving a tenofovir-containing antiretroviral regimen"
Enrollment period	1 January to 1 September 2012	between 2009 and 2011
Inclusion criteria	- aged ≥ 18 years - HIV RNA < 50 copies/ml	- age 18-60 year - naïve to antiretroviral therapy - having a CD4 count < 350 cell/mm^3
Exclusion criteria	- history of Tc-99m DTPA allergy - malnutrition (BMI <18m^2) - amputation - bed-ridden - currently taking cotrimoxazole or cimetidine - acute deterioration of renal function within the last 3 months - pregnant/lactating	- serum creatinine level > 2xthe upper limit of the normal range - AST and ALT levels > 5xthe upper limit of the normal range -lost to follow-up before week 12 -receiving nephrotoxic drugs -being pregnant

Appendix C

Validation of Population Pharmacokinetic Model

The data from Pharmacogenomics and Personalized Medicine, Ramathibodi Hospital were used for model validation using Bayesian estimation method. A total of 103 patients and 103 plasma concentrations were included. The individual predicted concentrations were compared to the observed concentrations as shown in table B.

Table B: Results of observed concentrations (DV) and individual predicted concentrations (IPRED) obtained from Bayesian estimation

ID	IPRED	DV	Prediction Error	ID	IPRED	DV	Prediction Error
1	0.091	0.094	-0.003	16	0.095	0.090	0.005
2	0.126	0.126	0.000	17	0.100	0.070	0.030
3	0.071	0.049	0.022	18	0.086	0.076	0.010
4	0.083	0.095	-0.012	19	0.090	0.087	0.003
5	0.092	0.100	-0.008	20	0.110	0.142	-0.032
6	0.105	0.099	0.006	21	0.065	0.073	-0.008
7	0.072	0.053	0.019	22	0.085	0.069	0.016
8	0.086	0.075	0.011	23	0.076	0.082	-0.006
9	0.072	0.053	0.019	24	0.083	0.070	0.013
10	0.095	0.101	-0.006	25	0.079	0.056	0.023
11	0.043	0.028	0.015	26	0.149	0.197	-0.048
12	0.092	0.107	-0.015	27	0.057	0.022	0.035
13	0.130	0.149	-0.019	28	0.081	0.054	0.027
14	0.127	0.155	-0.028	29	0.119	0.149	-0.030
15	0.097	0.088	0.009	30	0.090	0.096	-0.006

Table B: Results of observed concentrations (DV) and individual predicted concentrations (IPRED) obtained from Bayesian estimation (cont)

ID	IPRED	DV	Prediction Error
31	0.151	0.058	0.093
32	0.120	0.139	-0.019
33	0.076	0.075	0.001
34	0.091	0.076	0.015
35	0.063	0.052	0.011
36	0.076	0.083	-0.007
37	0.127	0.144	-0.017
38	0.081	0.075	0.006
39	0.086	0.085	0.001
40	0.084	0.120	-0.036
41	0.080	0.054	0.026
42	0.164	0.193	-0.029
43	0.093	0.080	0.013
44	0.074	0.060	0.014
45	0.089	0.092	-0.003
46	0.100	0.079	0.021
47	0.088	0.105	-0.017
48	0.104	0.106	-0.002
49	0.106	0.122	-0.016
50	0.096	0.098	-0.002
51	0.118	0.163	-0.045
52	0.095	0.106	-0.011
53	0.040	0.050	-0.010
54	0.085	0.078	0.007
55	0.144	0.170	-0.026
56	0.110	0.160	-0.050
57	0.073	0.076	-0.003
58	0.089	0.100	-0.011
59	0.109	0.149	-0.040
60	0.074	0.083	-0.009
61	0.067	0.061	0.006
62	0.068	0.044	0.024
63	0.125	0.131	-0.006
64	0.072	0.078	-0.006
65	0.084	0.094	-0.010
66	0.085	0.109	-0.024
67	0.107	0.104	0.003
68	0.070	0.070	0.000
69	0.108	0.127	-0.019
70	0.103	0.130	-0.027
71	0.088	0.068	0.020
72	0.085	0.090	-0.005
73	0.105	0.129	-0.024
74	0.079	0.091	-0.012
75	0.080	0.083	-0.003
76	0.113	0.147	-0.034

Table B: Results of observed concentrations (DV) and individual predicted concentrations (IPRED) obtained from Bayesian estimation (cont)

ID	IPRED	DV	Prediction Error
77	0.092	0.084	0.008
78	0.066	0.073	-0.007
79	0.102	0.098	0.004
80	0.114	0.142	-0.028
81	0.081	0.090	-0.009
82	0.106	0.114	-0.008
83	0.117	0.118	-0.001
84	0.087	0.089	-0.002
85	0.093	0.105	-0.012
86	0.098	0.103	-0.005
87	0.085	0.077	0.008
88	0.094	0.079	0.015
89	0.062	0.053	0.009
90	0.117	0.152	-0.035
91	0.097	0.107	-0.010
92	0.113	0.137	-0.024
93	0.086	0.096	-0.010
94	0.140	0.168	-0.028
95	0.102	0.137	-0.035
96	0.117	0.140	-0.023
97	0.063	0.060	0.003
98	0.121	0.149	-0.028
99	0.112	0.125	-0.013
100	0.067	0.072	-0.005
101	0.083	0.082	0.001
102	0.079	0.059	0.020
103	0.111	0.142	-0.031

Appendix D

Population Pharmacokinetic Model of Tenofovir for Clinical Practice

The result from the final model showed that GFR_{CG}, LPV/r and *ABCC4* 3463A>G polymorphism were associated with CL/F of tenofovir. However, the genotyping of *ABCC4* 3463A>G polymorphism is complex and expensive that may not be used in clinical practice. Therefore, in case of patients who cannot genotype this SNP, the model developed using only GFR_{CG} and LPV/r may be used. The parameter estimates of this model are shown in table C.

Table C: Parameter estimates of population pharmacokinetic model of tenofovir (GFR_{CG} and LPV/r were added)

Parameter	Estimation value	(95%CI)
CL/F (L/hr) = θ_1x $GFR_{CG}^{\theta_2}$x (1+ θ_3 x LPV/r)		
θ_1	3.45	(3.19,3.71)
θ_2	0.631	(0.631,0.631)
θ_3	-0.247	(-0.335,-0.157)
V_c/F (L)	540	(291.28,788.72)
V_p/F (L)	587	(405.22, 768.78)
Q (L/hr)	115	(78.26, 151.74)
k_a (hr^{-1})	0.725	(0.508, 0.942)
IIV of CL/F (%CV)	19.7	(12.5, 26.9)
IIV of V_c/F (%CV)	66.1	(32.8, 99.4)
IIV of k_a ((%CV)	143.2	(71.9, 214.5)
RUV proportional model (%CV)	36.3	(30.1, 42.8)
RUV additive model (mg/l)	0.011	(0.004, 0.179)

CI, confidence interval; CL/F, apparent oral clearance; CV, coefficient of variation; k_a, absorption rate constant; IIV, interindividual variability; Q/F, apparent intercompartmental clearance; V_c/F, apparent central compartment volume of distribution; V_p/F, apparent peripheral compartment volume of distribution; RUV, residual unexplained variability

Therefore, CL/F of tenofovir can be described by following equation:

$$CL/F = \theta_1 \times GFR_{CG}^{\theta_2} \times (1 + \theta_3 \times LPV/r)$$

$$CL/F = 3.45 \times GFR_{CG}^{0.631} \times [1-(0.247 \times LPV/r)]$$

Where; GFR_{CG} = GFR calculated by Cockcroft and Gault formula (ml/min)

LPV/r = 1 when lopinavir/ritonavir was concomitantly used

LPV/r = 0 when lopinavir/ritonavir was not concomitantly used

The results from this model showed that low GFR_{CG} and concomitant use with LPV/r were associated with low CL/F of tenofovir. GFR_{CG} was related to CL/F of tenofovir by a power function with an exponent 0.631. Concomitant use with LPV/r decreased CL/F of tenofovir by 24.7%.

The goodness of fit plots of this model were compared to the base model (model without covariate) and the final model (GFR_{CG}, LPV/r and *ABCC*4 3463A>G were added) as shown in figure A and figure B. The results showed that this model was superior to the base model but was slightly inferior to the final model.

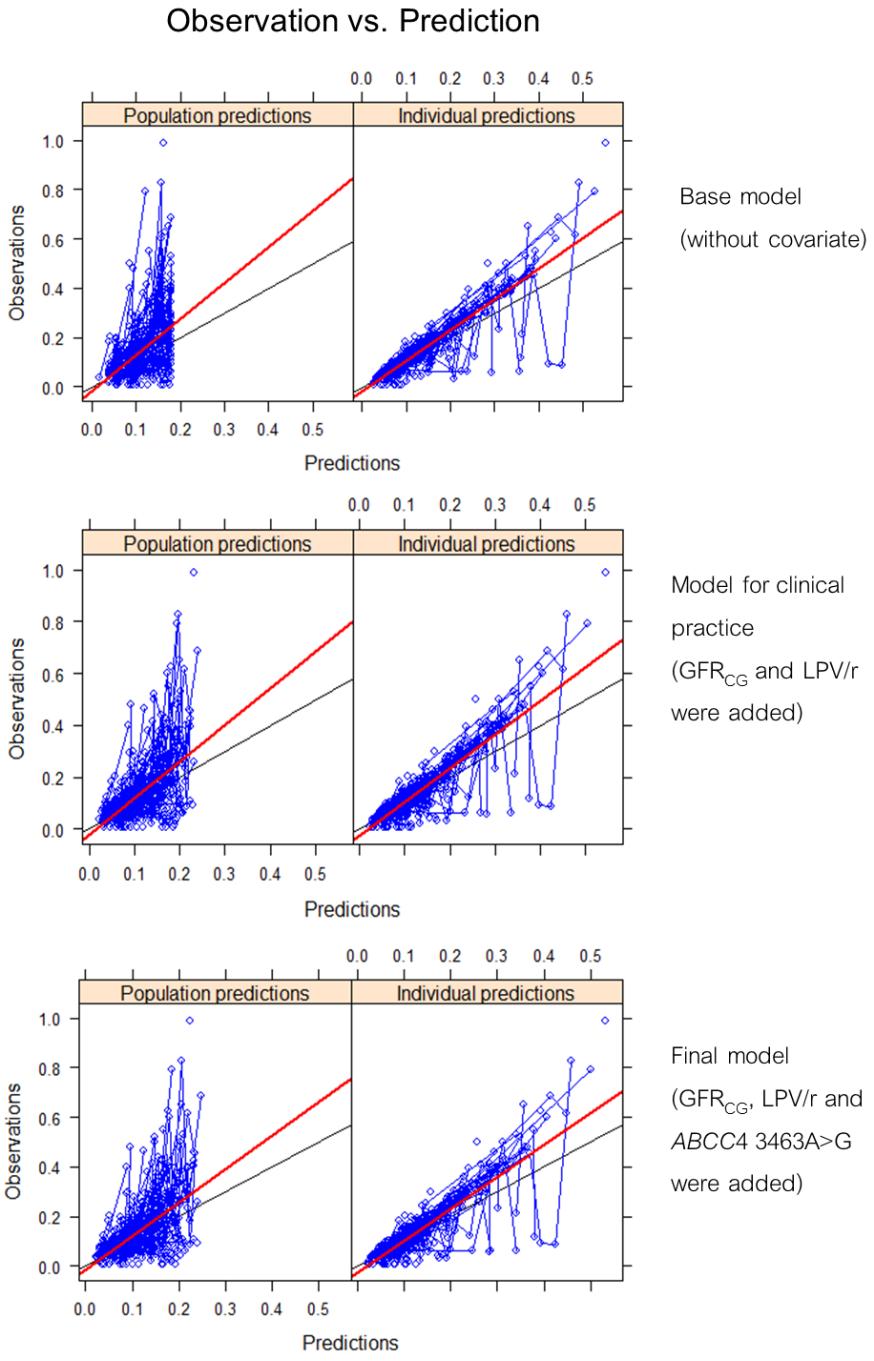

Figure A: The goodness of fit plots of the base model, the model for clinical practice and the final model; observed concentrations (DV) vs population predicted concentrations (PRED) and observed concentrations (DV) vs individual predicted concentrations (IPRED)

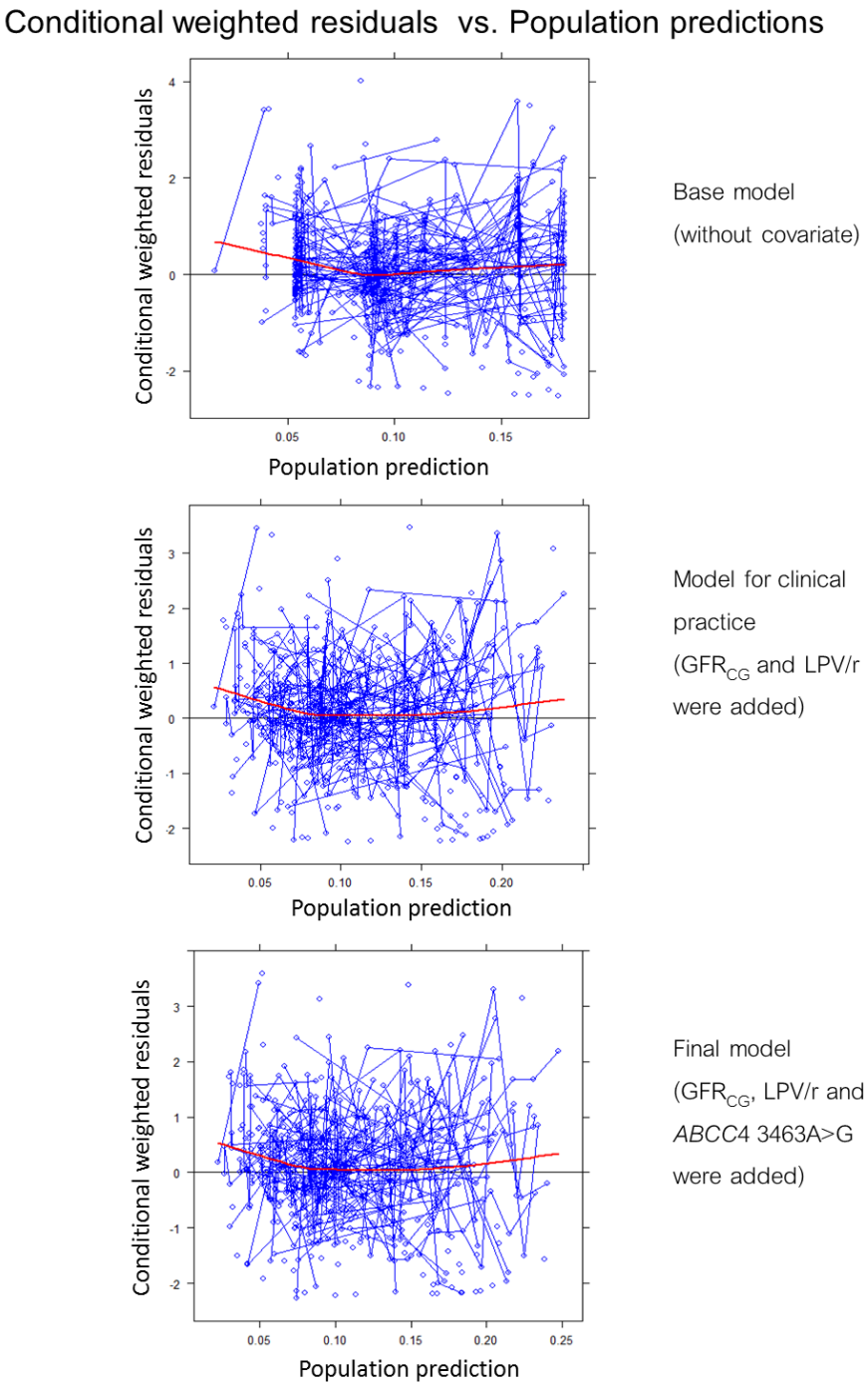

Figure B: The goodness of fit plots of the base model, the model for clinical practice and the final model; conditional weighted residuals (CWRES) vs population predicted concentration (PRED)

VITA

Ms. Kanokrat Rungtivasuwan was born on 25 January 1988 in Bangkok. She got a Bachelor of Science in Pharmacy (1st degree honors) from Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 2011. She has been working as clinical pharmacist at the Somdech Phra Debaratana Medical Center, Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand since 2011. She had been enrolled in Master degree program of the Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand since 2013.