Pharmacokinetic-Pharmacogenetic-and-Pharmacodynamic Adherence Relationships in Cohort South African HIV Infected Children on Lopinavir-and Nevirapine-Based Regimens

By: Retsilisitsoe R Moholisa

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Division of Clinical Pharmacology, Pharmacometrics Laboratory, Department of Medicine

Faculty of Health Sciences, University of Cape Town

Supervisors

Professor Helen McIlleron

Division of Clinical Pharmacology, Department of Medicine

Faculty of Health Sciences, University of Cape Town

Professor Gary Maartens

Division of Clinical Pharmacology, Department of Medicine

Faculty of Health Sciences, University of Cape Town

Dr Phumla Sinxadi

Division of Clinical Pharmacology, Department of Medicine

Faculty of Health Sciences, University of Cape Town

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Name: Retsilisitsoe Moholisa

Student number: MHLRET001

Signature:

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Date:08/02/19

ABSTRACT

Background: Antiretroviral therapy (ART), notably lopinavir and nevirapine substantially reduces Human immune-deficiency virus (HIV) associated morbidity and mortality in HIV-infected children. Low concentrations of nevirapine and lopinavir have been linked to inferior virological outcomes; it is recommended that lopinavir and nevirapine concentrations are maintained above 1 mg/L and 3 mg/L, respectively, in order to maintain viral suppression. Adherence to both lopinavir and nevirapine ART, respectively has long known to be a crucial contributor to HIV treatment success. Lopinavir and nevirapine pharmacokinetics demonstrate considerable inter-individual variability, which may affect treatment outcomes. At least part of this variability may be explained by host genetic factors. Associations between human genetic variants and exposure to lopinavir and nevirapine are incompletely understood, and have not been studied in a South African paediatric population. Data in this thesis were from a clinical trial conducted at Rahima Moosa Mother and Child Hospital in Johannesburg to assess whether NVP can be re-used (**Post-randomization Phase**) among 323 children exposed to NVP for PMTCT if they are first suppressed on ritonavir-boosted lopinavir based regimen (**Pre-randomization Phase**). This thesis assessed the relationship between serial clinic visits lopinavir (**Pre-and-Post-randomization)** and nevirapine (**Post-randomization)** concentrations and/or percentage adherence(**Pre-and-Post-randomization)** and virological outcomes in children. Moreover, population pharmacokinetics models were used to characterise lopinavir and nevirapine parameters. From the final models parameters were derived and were used to assess the relationship between lopinavir and nevirapine pharmacokinetics and genetic polymorphism relevant to both drugs

Methods: Cox proportional hazard regression modelling for multiple failure events was used to estimate the crude and adjusted hazard effect of lopinavir (**Pre-and Post-randomization**) and nevirapine(**Post-randomization**) concentrations and/or percent adherence(**Pre-and Post-randomization**) of viral load>400 copies/mL (**Pre-randomization)** and >50 copies/mL (**Post-randomization**), respectively. The population means and variances of lopinavir and nevirapine pharmacokinetic parameters at steady state were estimated using non-linear mixed-effects regression. The final models of lopinavir and nevirapine were used to derive individual clearances (CL/F), minimum concentrations (C_{min}) and area under the concentration time curves (AUC). The associations between model-derived pharmacokinetic

parameters and genotypes in selected genes relevant to lopinavir or nevirapine were explored.

Results: In 237 children pre-randomization with viral loads and lopinavir concentrations, the crude and adjusted Cox models revealed significant associations between virologic failure (viral load>400 copies/mL) and both lopinavir plasma concentrations (<1/mg/L) and pre-treatment height-for-age z-scores but not percent adherence. In 99 children post-randomization, lopinavir concentrations >1 mg/L reduced the risk of viremia (viral load >50 copies/mL) with about 40%, compared to children with LPV <1 mg/L. No association was found with percent adherence in this group. In 95 children on nevirapine post-randomization, nevirapine concentrations were not significantly associated with increased hazard of viremia (viral load >50 copies/mL). Similarly, there was no significant association with percent adherence in this group. Lopinavir and nevirapine pharmacokinetics were both separately best described with a one compartment models with absorption lag time and transit compartment absorption models, respectively. There was an age driven effect on lopinavir and nevirapine relative bioavailability, respectively. After adjusting for multiple testing, there was no significant association between lopinavir CL/F, C_{min} and AUC and genetic polymorphisms in the *ABCB1*, *CYP3A4*, *CYP3A5* and *SLCO1B1*. *CYP2B6 516G→T* and *CYP2B6 983T→C* were associated with NVP CL/F. *CYP2B6 983T→C* was associated with NVP C_{min} and AUC. Additionally, polymorphisms in the *ABCB1* and *CYP3A5* were independently associated with NVP CL/F, Cmin and AUC.

Conclusions: Lopinavir concentrations <1mg/L were associated with the increased hazard of viremia (viral load >400 copies/mL or >50 copies/mL). The results suggest that lopinavir plasma concentration monitoring at a routine clinic visit may be a useful tool in identifying sub-therapeutic antiretroviral concentrations in children, and this could be used as a guide to therapeutic drug monitoring in children. There was no statistically significant association between polymorphisms in the *ABCB1*, *CYP3A4*, *CYP3A5* and *SLCO1B1* and lopinavir pharmacokinetics. Polymorphisms in the *ABCB1*, *CYP2B6 CYP3A4* and *CYP3A5* predicted nevirapine pharmacokinetics.

ABSTRACT

Keywords: Lopinavir, Nevirapine, Antiretroviral Therapy, Adherence, Pharmacokinetics, Pharmacogenetics, Pharmacodynamics, NONMEM, Therapeutic Drug Monitoring

SUMMARY

There are only few clinical trials of strategies to optimally utilize currently-approved antiretroviral drugs for the long-term treatment of human immunodeficiency virus infected children in low resource settings. Treatment is complicated by selection of drug resistance in many children whose mothers receive nevirapine (NVP) for prevention of mother to child HIV transmission (PMTCT).The NEVEREST2 (clinicaltrials.gov Identifier: NCT00117728) study was an open-labelled clinical trial conducted at Rahima Hospital Johannesburg, South Africa assessing the re-use of nevirapine amongst children exposed to NVP for PMTCT if children were first suppressed on a Lopinavir/ritonavir (LPV/r)-based regimen. As part of this trial 323 HIV-infected children (less than 24 months of age) exposed to nevirapine for PMTCT that met immunologic and clinical criteria requiring antiretroviral therapy were started on a LPV/r-based regimen. Data collected included age initiating LPV/r, sex, pre-treatment viral load, pre-treatment $CD4^+$ T lymphocyte percent, WHO stage, pre-treatment weight-for-age z-scores and pre-treatment height-for-age z-scores. During the pre-randomization phase, post LPV/r initiation treatment, children achieving and maintaining viral load <400 copies/ml for at least 3 months were eligible for randomization. Once criteria were met, 195 children entered the post-randomization phase where they either remained on the LPV/r-based regimen (LPV group) or nevirapine (NVP group) was substituted for LPV/r in their regimen. All children were followed to 76 weeks post-randomization. Data collected included age at randomization, viral load (dichotomized to viral <50 copies/mL or viral load>51-400 copies/mL), weight-for-age z-scores and height-for-age z-scores and concomitant tuberculosis therapy. Regular ultrasensitive viral loads assays were conducted to determine if virologic control was sustained in both the pre-and-post randomization phases.

Current World Health Organization guidelines recommend LPV/r as the first-line antiretroviral treatment for children. Furthermore, LPV/r plus a backbone of two nucleoside reverse

transcriptase inhibitors is the preferred antiretroviral regimen for young children (< 2 years old) previously exposed to non-nucleoside reverse transcriptase inhibitors. NVP is an inexpensive non-nucleoside reverse transcriptase inhibitor widely used in resource-limited settings for treating HIV in children. Despite its high potency, NVP has low genetic barrier for developing resistance and sub-therapeutic concentrations increase the risk of developing treatment failure and drug resistance. LPV/r has a high barrier for resistance but has poor oral palatability which might lead poor treatment adherence. Both LPV/r and NVP pharmacokinetics display considerable inter-individual variability, which may affect treatment outcomes. At least part of this variability may be explained by host genetic factors. Associations between human genetic variants and exposure to LPV and NVP are incompletely understood, and have not been studied in a South African paediatric population.

Firstly in this thesis, relationships between LPV and NVP plasma concentrations at serial clinic visit and virological outcomes were characterised using Cox proportional hazards multiple failure event models. Secondly, population pharmacokinetic models for LPV and NVP were developed using a non-linear mixed modelling approach. From the final models, individual clearances (CL/F), minimum concentrations (C_{min}), area under curves (AUC) were derived and were used to assess their relationship with genetic polymorphisms in preselected genes respective for both drugs.

During the pre-randomization phase, a total of 237 children aged 4-42 months on LPV/r oral solution were followed up for 52 weeks. LPV concentrations and viral load were measured at clinic visits 12, 24, 36 and 52 weeks. Cox proportional hazards multiple failure events models were used to estimate the crude and adjusted hazard of viral load>400 copies/mL for lopinavir concentrations and pre-determined pre-treatment variables. The hazard of viral load>400

copies/mL was increased with LPV concentrations <1mg/L compared to >1 mg/L and lower height-for-age z-scores.

During the post-randomization 99 children were randomized to remain on LPV/r regimen whereas 95 children were switched to NVP regimen. Viral load and LPV or NVP concentrations were measured at clinic visits 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post randomization. Cox proportional hazards multiple failure events models were used to estimate the crude and adjusted hazard of viral load>50 copies/mL for LPV or NVP concentrations and pre-selected variables at randomization for LPV or NVP group. In the LPV group, the hazard of viral load >50 copies/mL was increased for LPV concentrations <1 mg/L versus >1 mg/L and for children with viral loads 51-400 copies/mL at randomization. In the NVP group, there was no association between viral load >50 copies/mL and NVP concentrations or any other variable.

A population pharmacokinetic model of LPV was developed to describe LPV variability in children and relationships between LPV CL/F, C_{min} and AUC, and genetic polymorphisms in genes relevant to LPV were examined. A one compartment model with absorption lag time, first order absorption and elimination best described lopinavir pharmacokinetics. There was an age related influence on LPV bioavailability. Concomitant tuberculosis therapy increased LPV CL/F by 60%. After correcting for multiple testing, there was no statistically significant associations between LPV CL/F, Cmin and AUC and genetic polymorphisms in *ABCB1*, *CYP3A4*, *CYP3A5* and *SLCO1B1* genes.

For NVP, a population pharmacokinetic model was developed with the aim of describing NVP variability and from the final model CL/F, C_{min} and AUC were derived and were subsequently used to assess for relationships with preselected genes relevant to NVP. A one-compartment disposition model with elimination through a well-stirred liver model accounting for first-pass

effect and transit absorption best described NVP pharmacokinetics. There was an age driven effect on NVP relative bioavailability. In a univariate analysis, *CYP2B6* and genotypes were associated with NVP CL/F including *CYP2B6 516G→T* and *CYP2B6 983T→C*. *CYP2B6 983T→C* was associated with NVP C_{min} and AUC in a univariate analysis and after adjusting for *CYP2B6 516→T*.There was a significant association *CYP2B6 15582C→T* with NVP CL/F, C_{min} and AUC after adjusting for *CYP2B6 516G→T* and *CYP2B6 983T→C.* Additionally, polymorphisms in the *ABCB1* and *CYP3A5* were independently associated with NVP CL/F, C_{min} and AUC.

LIST OF ABBREVIATIONS

95%CI	95 Percent Confidence Interval
3TC	Lamivudine
ABC	ATP-Binding Cassette
ALAG1	Absorption Lag Time
ACTG	AIDS Clinical Trail Group
AIC	Akaike Information Criterion
AICw	Weighted-Akaike Information Criterion
APO	Apolipoproteins
ATV	Atazanavir
ART	Antiretroviral Therapy
ARV	Antiretrovirals
AZT	Zidovudine
BID	Bis in Die(Twice Daily)
BLQ	Below The Limit of Quantification
AUC	Area under the Concentration Time Curve
$CD4^+$	$CD4^+$ T Lymphocytes
CL/F	Clearance
CYPs	Cytochrome P450 Enzymes
CSF	Cerebrospinal Fluid
CV	Co-efficient of Variation
C_{max}	Maximum Concentration
C_{min}	Minimum Concentration
EBEs	Empirical Bayes Estimates
EC_{50}	Half Maximal Effective Concentration
EFV	Efavirenz
EMB	Expectation Maximum Likelihood Bootstrap
FM	Fast Metabolizers
FO	First Order
FOCE	First Order Conditional Estimation

LIST OF ABBREVIATIONS

FOCEI	First Conditional Estimation with Interaction
GCV	Generalized Cross Validation
GIQ	Growth Inhibitory Quotient
GOFs	Goodness of Fit Plots
GWAS	Genome Wide-Association Studies
HAZ	Height for Age Z-Scores
HIV	Human Immune-Deficiency Virus
HLA	Human Leukocyte Antigen
hr	Hour(s)
HR	Hazard Ratio
HSR	Hypersensitivity Reaction
IIV	Inter-individual Variability
IM	Intermediate Metabolizers
IOV	Inter-occasion Variability
IPREDs	Individual Predications
IQR	Interquartile Range
IWRES	Individual Weighted Residuals
KA	Absorption Rate Constant
kg	Kilogram
KTR	Transit Time Rate Constant
LAM	Lopinavir Associated Mutations
L/kg	Litres per Kilogram
LMS	Lopinavir Associated Scores
LPV	Lopinavir
LPV/r	Ritonavir-Boosted Lopinavir
PAM	Protease Inhibitor Associated Mutations
PD	Pharmacodynamics
Pg-p	P-glycoprotein
PI	Protease Inhibitor

LIST OF ABBREVIATIONS

PK	Pharmacokinetics
PREDs	Predictions
PsN	Perl Speaks NONMEM
MAR	Missing at Random
MCAR	Missing Completely at Random
mg	Milligrams
mg/kg	Milligram Per Kilogram
mg/L	Milligram Per Litre
mg/m^2	Milligram Per Metres Squared
mL	Millilitre
mL/hr/kg	Millilitre Per Hour Per Kilogram
MTT	Mean Transit Time
NPDE	Normalized Predication Errors
NONP	Nonparametric Estimation
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
PMA	Post Menstrual Age
PMA50	Post Menstrual Age Half Life
PMTCT	Prevention of Mother to Child Transmission
OAT	Organic Anion Transporter(s)
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter(s)
OFV	Objective Function Value
RTV	Ritonavir
RUV	Random Unexplained Variability
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
SM	Slow Metabolizer
TB	Tuberculosis

LIST OF ABBREVIATIONS

TDM	Therapeutic Drug Monitoring
UGTs	Uridine 5' Diphospho-Glucuronosyltransferases
USM	Ultra Slow Metabolizer
V/F	Volume of Distribution
VL	Viral Load
VPC	Visual Predictive Check
WHO	World Health Organization
WAZ	Weight for Age Z-scores

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PUBLICATIONS

This Thesis is based on the following Manuscripts

1. **Retsilisitsoe R. Moholisa**, Michael Schomaker, Louise Kuhn, Sandra Meredith, Lubbe Wiesner, Ashraf Coovadia, Renate Strehlau, Leigh Martens, Elaine Abrams, Gary Maartens, Helen McIlleron. Plasma lopinavir concentrations predict virological failure in a cohort of South African children initiating a protease-inhibitor based regimen. *Antivir Ther*. 2014; 19(4): 399–406

2. **Retsilisitsoe R. Moholisa**, Michael Schomaker, Louise Kuhn, Sandra Castel, Lubbe Wiesner, Ashraf Coovadia, Renate Strehlau, Faeezah Patel, Francoise Pinillos, Elaine J. Abrams, Gary Maartens, Helen McIlleron. Effect of Lopinavir and Nevirapine Concentrations on Viral Outcomes in Protease Inhibitor-Experienced HIV-Infected Children. *Pediatr Infect Dis J* 2016;35:e378–e383

3. **Retsilisitsoe R. Moholisa**, Tim R. Cressey, Phumla Sinxadi, Louise Kuhn, Emile R. Chimusa, Sandra Meredith, Lubbe Weisner, Ashraaf Coovadia, Renate Strehlua, Elaine J. Abrams, Gary Maartens, Helen McIlleron, David Haas. Associations between Lopinavir Pharmacokinetics and Genetic Variants in ABCB1, CYP3A4, CYP3A5 and SLCO1B1 in a Cohort of South-African Children. **Manuscript Prepared**

4. **Retsilisitsoe R. Moholisa**, Tim R. Cressey, Phumla Sinxadi, Louise Kuhn, Emile R. Chimusa, Sandra Meredith, Lubbe Weisner, Ashraaf Coovadia, Renate Strehlua, Elaine J. Abrams, Gary Maartens, Helen McIlleron, David Haas. Effect of Genotypes in the CYP2B6, CY3A4, CYP3A5 and ABCB1 Genes on Nevirapine Pharmacokinetics in a Cohort of HIV-Infected South African Children. **Manuscript Prepared**

DECLARATION OF WORK FOR THE PUBLISHED MANUSCRIPTS

RR Moholisa

08-04-2018

Manuscript 1

Title "Effect of Lopinavir and Nevirapine Concentrations on Viral Outcomes in Protease Inhibitor-experienced HIV-infected Children".

Moholisa RR, Schomaker M, Kuhn L, Castel S, Wiesner L, Coovadia A, Strehlau R, Patel F, Pinillos F, Abrams EJ, Maartens G, McIlleron H. Pediatr Infect Dis J. 2016;**35**:e378-e383."

i) I was responsible for the data handling, processing and analysis under the guidance of Doctor Michael Schomaker

ii) I wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures under the guidance of Professors Gary Maartens, Louise Kuhn and Helen McIlleron and Dr Michael Schomaker.

Manuscript 2

Title "Plasma lopinavir concentrations predict virological failure in a cohort of South African children initiating a protease-inhibitor-based regimen".

Moholisa RR, Schomaker M, Kuhn L, Meredith S, Wiesner L, Coovadia A, Strehlau R, Martens L, Abrams EJ, Maartens G, McIlleron H. Antivir Ther. 2014;**19**:399-406. "

i) I was responsible for data handling, processing and analysis with supervision from Doctor Michael Schomaker and Professors Gary Maartens, Louise Kuhn and Helen McIlleron

ii) I wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures. I was guided by Professors Gary Maartens, Louise Kuhn and Helen McIlleron and Dr Michael Schomaker.

PERMISSSION FROM CO-AUTHORS

Dear Ray

I fully support publication, as part of your thesis, of both of the manuscripts you have detailed below.

Best wishes

Helen

Dear Ray

The attached declaration and content is fine with me.

Please go ahead!

Michael

This is fine with me

Louise

I agree as well.

Elaine

No problem by me.

Kind Regards

Adjunct Professor Ashraf Coovadia

Academic Head of Paediatrics and Child Health

School of Clinical Medicine

Faculty of Health Sciences

University of The Witwatersrand

Rahima Moosa Mother and Child Hospital

PERMISSSION FROM CO-AUTHORS

Hi Ray

Please see attached the declaration with some comments.

Fine by me to include in your thesis.

Regards

Dr. Faeezah Patel

ESRU

Rahima Moosa Hospital

I agree to both

Gary Maartens

I am also in agreement.

Renate

Hi Ray

I support both

Kind regards

Sandra

Dear Ray

I agree with your declaration.

Best Regards

Dear Ray

I agree with your declaration.

Best Regards

Lubbe

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Chapter 1

Literature Review

Introduction

Human immunodeficiency virus (HIV) is one of the most serious paediatric disease affecting an estimated 3.4 million children under the age of 15 years worldwide[1,2]. HIV in children is predominately acquired through mother to child transmission (MTCT) of the virus from HIV-infected women during pregnancy, labour and/or breastfeeding[3]. Combination Antiretroviral therapy (ART) significantly reduces mortality and morbidity associated with HIV and thereby facilitating normal growth and development, and improved survival and quality of life in children [4]. Significant progress has been made in early infant diagnosis of HIV and early paediatric ART initiation, however ART coverage in adults is still twice that of children[5,6]. ART requires maintenance of adequate drug exposure and, high levels of adherence in order to prevent viral resistance and ART failure[4]. However, both lopinavir (LPV) and nevirapine (NVP) concentrations display a high degree of variability even after observed doses.

1.1 Antiretroviral Therapy in Children

The main goal of ART is to maintain maximum virologic suppression and immune reconstitution[2]. Currently available antiretroviral (ARVs) drugs either block replication within the infected cell or prevent entry into the cell[2]. The efficacy of ART in the management of HIV in children and adolescents is measured by maintenance of virologic suppression below detectable thresholds or log10 drop in viral load (VL) as well as improvement or reservation of $CD4^+$ T lymphocyte count and/or percentage[2,3]. These defined laboratory measurements are assessed at baseline and repeated after durations ranging mostly from 24 or 48 weeks post initiation ART[2]. Despite significant differences in immunologic function and responses to HIV between children and adults, thresholds for defining immunodeficiency and severity of VL are similar[1,2]. Thus both pharmacokinetic (PK) and pharmacodynamic (PD) targets for children have been largely derived from adult data and paediatric ART studies, which have always been after adult drug approval, are aimed meeting the same PK and PD targets[2].

Chapter 1

Literature Review

1.1.1 **Classes of Antiretroviral Drugs Used for Antiretroviral Therapy**

In order to understand how ARVs work, an understanding of HIV life cycle is required. **Figure 1** depicts the HIV life cycle with ARV targets**.** HIV is an RNA virus primarily infecting the $CD4^+$ lymphocytes by attaching and binding to the CD4 receptor and specific chemokine co-receptors (CXR5 and/or CCR4) and thus resulting in fusion of the virus and host cell membranes and thereby entry of HIV RNA into the target cell[7] . The HIV RNA then undergoes reverse transcription from RNA to DNA, which is then transported into the nucleus integrating with the host DNA where multiple copies of full length and spliced HIV RNA are made and exported from the nucleus[7].

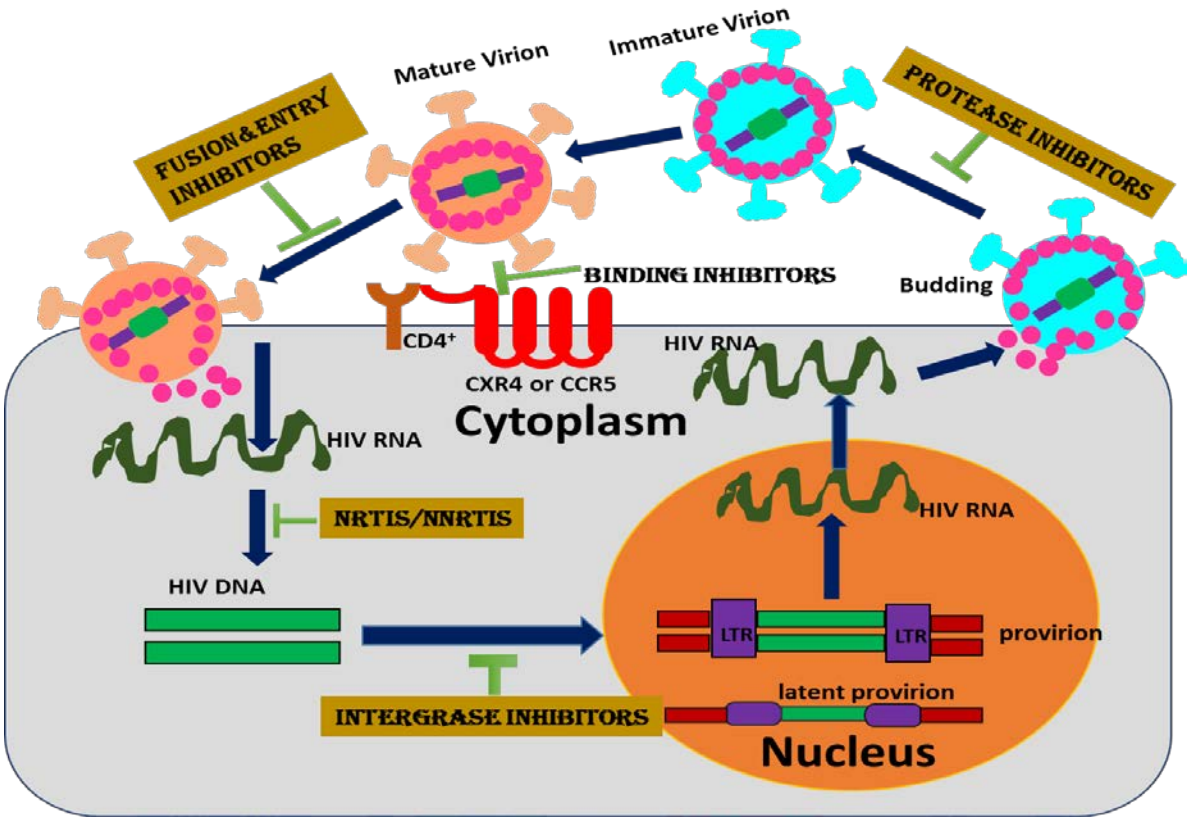

Figure 1: HIV life cycle with antiviral targets. CCR4, chemokine receptor type 4; CCR4 , chemokine receptor type 4; CXR5, chemokine receptor type 5; $CD4^+$, $CD4^+$ T lymphocyte receptor; HIV DNA, human immunodeficiency virus deoxyribonucleic acid; HIV RNA, human immunodeficiency virus ribonucleic acid; LTR, long terminal repeat; NRTIS, nucleoside/nucleotide reverse transcriptase inhibitors; NNRTIS, non-nucleoside reverse transcriptase inhibitors. Adapted from Volberding *et al* 2010

Chapter 1

Literature Review

ART utilizes five classes of ARVs, namely: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); non-nucleoside reverse transcriptase inhibitors (NNRTIs); protease inhibitors (PIs); entry and fusion inhibitors and intergrase inhibitors (**Table 1**). Combination ART regimens, uses three ARVs from at least two major classes so as to achieve maximal HIV replication suppression and immune function preservation affected by the HIV disease[8]. Moreover, ART has the added benefit of reducing HIV transmission from one person to another including the vertical transmission of the virus from the mother to her foetus, newborn and infant[8,9].

Table 1: Different classes and types of antiretroviral drugs

Antiretroviral Class	Site of Action	Representative Antiretroviral Drug
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Interrupt the HIV replication cycle via competitive inhibition of HIV reverse transcriptase and termination of the DNA chain	Lamivudine(3TC),Zidovudine(AZT),Didanosine(DDI),Stavudine (4DT), Abacavir(ABC), Tenofovir(TDF), Entricitabine
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Bind to the p66 subunit at a hydrophobic pocket distant from the active site of the enzyme. This noncompetitive binding induces a conformational change in the enzyme that alters the active site and limits its activity	Nevirapine(NVP), Efavirenz(EFV), Etravirine, Rilpivirine
Protease Inhibitors(PIs)	Function as competitive inhibitors that directly bind to HIV protease and prevent subsequent cleavage of polypeptide	Lopinavir(LPV), Atazanavir(ATV) Fosamprenavir, Ritonavir(RTV) Tipranavir, Saquinavir (SQV), Indinavir(IDV)
Fusion or Entry Inhibitors	Act extracellularly to prevent the fusion or entry of HIV to the CD4 or other target cell	Enfuvirtide Maraviroc
Integrase Inhibitors	Competitively inhibit the strand transfer reaction by binding metallic ions in the active site of the enzyme Intergrase	Elvitegravir Dolutegravir Raltegravir

Chapter 1

Literature Review

1.1.2 **Current Recommendations for Antiretroviral Therapy in Children**

The current South African guidelines[10] indicate that children younger than 3 years or older but weighing less than 10kg should be on first line ART including a combination of two NRTIs (ABC and 3TC) plus LPV/r. Children between 3-10 years of age and weighing 10kg should be started on a combination including two NRTIs (as with the children <3 years) plus EFV. In adolescents <15 years and <40 kg, the regimen should be two NRTIs and EFV whereas in adolescents ≥15 years and ≥40kg their regimen should be TDF plus 3TC and EFV.

The world health organization (WHO) guidelines[6] similarly recommend that for children <3years LPV/r plus two NRTIs should be used as first line. For HIV infected infants previously exposed to single dose NVP or maternal NNRTIs-containing ART, PI-based regimen should be used. It is recommended that infants should start ART soonest regardless on immune status. Results in a recent study indicate that a LPV/r-based regimen is preferred to NVP-based regimen even in infants not exposed to ART.

1.2 **Drugs of Interest in This Thesis**

LPV (MW= 628.81 g/mol, EC_{50}=6.5nM) is a second generation PI used both in drug naïve and drug experienced patients. LPV (**Figure 2** was developed by Abbot Laboratories USA and is marketed co-formulated with ritonavir (LPV/r) under the name Kaletra® or Aluvia® since 2000. LPV inhibits HIV-1 protease with an EC_{50} of 17nM[11] and is dosed at 400mg with low dose RTV of 100mg in adults twice daily and is available as 80ml oral suspension for children.

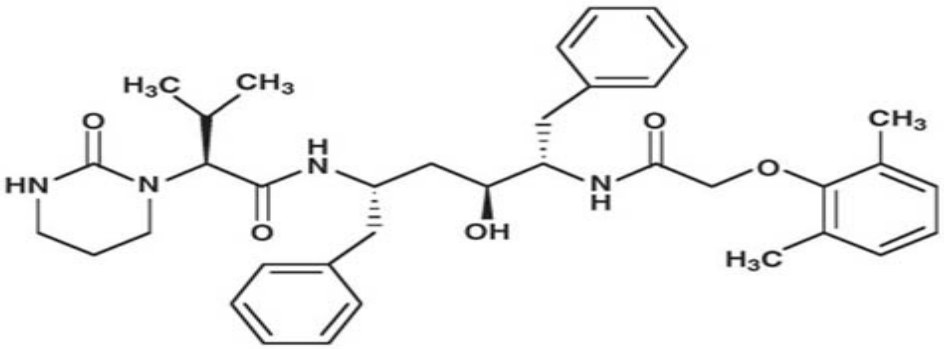

Figure 2: Chemical Structure of Lopinavir

NVP(MW=266.30 g/mol, EC_{50}=1.5nM) is a benzodiazepine derivative with a molecular weight of 266.3 grams/mole chemically synthesized as a non-nucleoside inhibitor of HIV-1 reverse transcriptase[12] (**Figure 3).** NVP is slightly soluble in water at neutral pH and is insoluble in nonpolar solvents[13]. Currently NVP is marketed as Viramune® (,Boehringer Ingelheim, Germany) and it is available as a 200mg tablet and a 10 mg/ml oral suspension[14].

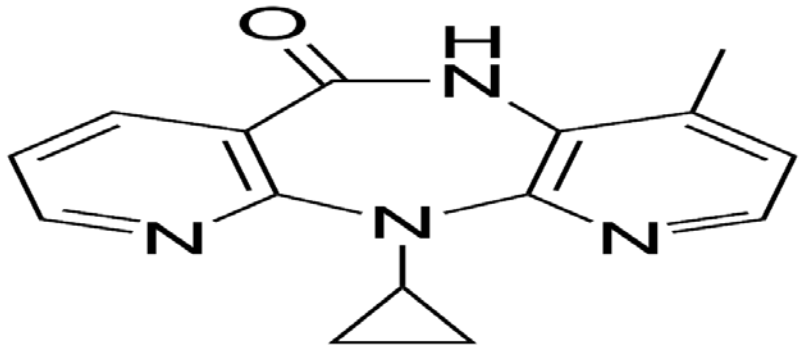

Figure 3: Structure of Nevirapine

1.2.1 **Mechanism of Action of Lopinavir**

LPV is a highly potent and specific inhibitor of HIV-1 protease[15,16]. HIV-1 protease is an aspartyl protease responsible for post-translational processing of viral gag and gag-pol proteins into functional and active moieties[17,18]. This process occurs concomitantly with or instantaneously after the budding of an immature virion on the surface on an infected cell and is essential for production of mature infectious viral particles[19]. HIV PIs, including LPV, prevent cleavage of gag and gag-pol proteins leading to maturation arrest, thus resulting in blockage of infectivity of nascent virions[20,21]. The main antiviral activity of PIs is to prevent infection of susceptible cells. LPV showed good antiviral activity against HIV strains in lymphoblastic cell lines and clinical HIV-1 isolates in peripheral blood lymphocytes in vitro[15]. LPV mean EC_{50} ranged from 4-11nmol/L in the absence of serum against several isolates of HIV-1 subtype B[22]. LPV combined with other ART agents in vitro demonstrated additive to antagonistic activity against nelfinavir (NFV) and additive to synergistic activity with fosamprenavir(FSP), atazanavir(ATZ), indinavir(IDR), saquanavir(SQR) and tipranavir(TPR)[22]. Moreover, 0.5nmol/L LPV inhibited 93% of the wild-type virus protease activity and displayed a $\geq 10^5$-fold specificity to HIV protease more than mammalian proteases renin and cathespin D and E in vitro[15]. RTV

has low plasma concentrations and 10-fold lower antiviral activity than that of LPV; hence, the antiviral activity of fixed-dose combination of LPV and RTV is attributable to LPV[22].

1.2.2 **Mechanism of Action of Nevirapine**

NVP is a non-competitive HIV-1 reverse transcriptase inhibitor with high specificity and minimal activity against HIV-2 reverse transcriptase inhibitor[23,24]. NVP inhibits HIV-1 reverse transcriptase by directly binding tyrosine residues at positions 181 and 188 on the p66 subunit close the catalytic side of the enzyme[25]. This binding results in reduced catalytic activity of the enzyme. NRTIs such as zidovudine (ZDV), lamivudine (3TC) etc. inhibit HIV replication by undergoing intracellular phosphorylation whereas NVP inhibits HIV-1 reverse transcriptase in its active state without requiring intracellular metabolism[12]. NVP penetrates cell free virions and thus inactivates virion-associated reverse transcriptase *in situ*[26]. The inactivation of cell free virions by NVP in maternal genital tract and breast milk is beneficial for preventing mother-to-child transmission of HIV[12]. NVP has been shown to inhibit many HIV-1 strains in vitro[27]. Human T-lymphocyte cultures display 10 µg/L 50% inhibitory concentration (IC_{50})[27]. NVP has also been shown to be active against strains that are resistant to NRTIs[28]. NVP has been shown to have synergistic antiviral activity when combined with PIs and/or NRTIs[28]. NVP resistant HIV-1 isolates have been shown to have 100-250 fold decreased susceptibility to NVP in vitro[29]. HIV-1 reverse transcriptase single amino acid substitutions lead to resistance to NVP and drug resistant strains might exist at low frequencies prior NVP exposure[30,31]. Thus, resistant strains develop, when NVP is as monotherapy, within weeks[32]. Combination of NVP with at least 2 other ARVs lead to sustained viral suppression and prevention of the development of resistance can achieved in many individuals[14,33].

1.3 **Pharmacodynamics of Antiretroviral Drugs**

Pharmacodynamics(PD) is broadly defined as “what the drug does to the body” [34] and seeks to quantify mechanisms of drug action and/or the relationship between drug concentration and effect. The science of PD was defined early in the 1960s with the work of Levy and others illustrating correlation between reversible drug effects and drug concentrations[35]. It thus becomes very important to incorporate the knowledge of ARV PD to` optimise their use.

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Though the goal of ART is good health, however in clinical practice it is difficult to measure this endpoint due to the overall efficacy of ARVs and a distant time-to-event horizon[1,36]. Nonetheless, the primary surrogate markers for antiretroviral studies typically include virological response (e.g. patients with viral load (VL) of <50 copies/mL), and/or immunological response (e.g. change in $CD4^+$ lymphocyte count or increase in $CD4^+$ lymphocyte percentage), which are all measured at baseline and after a defined period of treatment[1]. Immunological surrogates are usually tied to prediction of opportunistic infections and survival[37], whereas virological markers are used to predict treatment success or failure[8]. Typically, both these markers are measured only after 12, 24 or 48 weeks post initiation therapy[1].Consequently, numerous strategies have been proposed to predict ART outcomes even before the first dose, including therapeutic target concentrations[38] and inhibitory quotients[39] (e.g. phenotypic inhibitory quotient [PID] or genotypic inhibitory quotient [GIQ]). Due to ARV's viral molecular targets, a major assumption for paediatric therapy is that the PK-PD behaviour should be the same in children as in adults. Indeed, all ARVs PK studies in children have been designed to find the dose that is associated with exposures (e.g. Maximum plasma drug concentrations [C_{max}], area under the concentration time curve [AUC], and trough plasma concentrations [$C_{trough/min}$]) similar to those found in adults[1]. Thus there are fewer children infected with HIV needing ART compared to adults, and therefore the majority of HIV therapeutic studies in children take advantage of this assumption and are small phase II or phase IIb trials instead of large phase III trials[1].

1.3.1 **Pharmacodynamics of Lopinavir in Children**

The target LPV C_{min} of ≥1.0 mg/L required to achieve virological suppression for wild type HIV has been established in adults and confirmed in several paediatric studies[40–42]. Studies in children, have shown that low LPV C_{min} (<1.0 mg/L) is associated with increased risk of virologic failure[43]. Similar to adult data, minimum target plasma C_{min} of LPV and other PIs are recommended for treatment-naïve children, and higher target trough concentrations are required in PI-experienced paediatric patients[44]. A LPV population PK model of treatment experienced children aged 4-18 years found a median C_{min} of 5.9 mg/L lower than that found reported in adults, even though the children received 20% higher doses recommended in

children[44]. Nonetheless, simulations based on the model, showed that 90% of the children given the standard dose of LPV/r, achieved therapeutic LPV concentrations against the wild type virus[44]. Remarkably, a study of reduced LPV/r dose (70% of the recommended standard dose) illustrated adequate LPV exposure and virological suppression (VL<50 copies/mL) in 83% of the children, compared to 50% in children receiving standard doses[45]. However, these 24 children were PI naïve, strengthening the conclusions from the LPV population PK model that children with viral populations naïve to PIs are likely to achieve more than adequate lopinavir concentrations to maximize chances of virological suppression with standard or modestly reduced dosing; however, up to 10% may not do so[44,45]. Furthermore, low-dose LPV/r is inappropriate for PI-experienced children[1,2]. In addition to PK targets, several methods that incorporate both patient-specific drug exposure and HIV susceptibility have been established to improve predictions and virological suppression outcomes for LPV and other PIs. These include genotypic inhibitory quotients (GIQ) which incorporates both patient-specific PK parameters (such as Cmin) and ARV susceptibility of the dominant strain expressed as a ratio of C_{min} to IC_{50} (concentration of drug required for 50% inhibition of viral replication in vitro)[1]. The virtual GIQ uses the fold-change in virtual IC_{50} (derived from the genotype), multiplied by a reference wild-type protein-adjusted IC_{50} and the normalized GIQ is the patient-specific GIQ is divided by a reference GIQ calculated as the ratio of typical C_{min} for a given dose and wild-type viral IC_{50}, normalizing the GIQ target across PIs to a ratio of >1. Another tool for predicting virological response to specific ARV drugs (including PIs) has been introduced and is defined as the instantaneous inhibitory potential (IPP)[46]. IPP measures ARV activity by using the slope of the dose-response curve, directly quantifying the log inhibition of single-round infectivity at clinical concentrations. To date limited quantitative analysis has not shown advantage of the IPP to the GIQ. Nonetheless, assessment of the dose-response curve slope for various ARV drugs must be further investigated[47,48]. Various GIQ targets have been proposed in adults for LPV and other PIs, and have been shown to be practical when evaluating exposure-virological suppression in children[1]. However, the clinical usefulness of this approach is restricted by limited data on their clinical application, lack of standardized methods for calculations, high intra-and-interpatient variability in the PK of ARV drugs, and challenges in adherence to ART and importantly there is limited experience and expertise in

combining both virological and pharmacological data for the therapeutic dose adjustment[2]. Except for poor palatability and gastrointestinal intolerance, LPV is well tolerated by children[2]. Nonetheless, concerns for adverse effects in paediatric HIV care are focused on PI-associated changes in lipids and the unknown long-term effects of elevated cholesterol and triglycerides during childhood on the development cardiovascular disease in adulthood[2]. Recently, a study of 156 children on mean duration of treatment 4.2±0.7 years, where 85 were randomized to the LPV-based regimen and 71 to NVP-based regimen , showed that children on LPV-based regimen, had significantly lower mean high density lipoprotein and higher mean total cholesterol, low density lipoprotein, and triglycerides compared to those on NVP-based regimen[49].

1.3.2 **Pharmacodynamics of Nevirapine in Children**

The relationship between plasma NVP concentrations and efficacy and toxicity has been well established in children and adults[50–52]. Maintaining NVP C_{min} above 3.0 mg/L has been associated with long term virological suppression[53]. Recently, as study in 322 African children showed that children with a C_{min} <3.0 mg/L had increased hazard of virological non-suppression[54]. Moreover, some children and adult data showed that a higher NVP C_{min} of >4.3 mg/L reduced emergence of resistance mutations compared with lower concentrations (3-4.3 mg/L)[51,55,56]. Interestingly, achieving efficacious concentrations for NVP and other NNRTIs, is mostly important during the first weeks and months of therapy and becomes less relevant in the later stages of therapy, because high level single mutation resistance is not repressed by increased dose and exposure. A recent study of 31 children, showed that ART initiation in young children using the dose escalation strategy for NVP resulted in significant sub-therapeutic (<4.0 mg/L) NVP concentrations during the lead-in period compared to the steady state period[57]. Sub-therapeutic nevirapine concentration were more pronounced in children <8 years of age[57]; supporting the evidence that younger children metabolized NVP more rapidly than older children[58–60]. However, there was no clinically relevant effect of NVP concentration on virological outcome(viral load≥200 copies/mL)[57]. Amazingly, in a study of 323 children initiated on LPV/r whereby half (96) were later switched to NVP, there was no sex difference in the risk of confirmed virological failure (viral load> 1000 copies/mL),

however girls tended to more robust CD4 count compared to boys[61]. The most common side effect associated with NVP concentrations is skin rash, whereas NVP-associated hepatotoxicity is not associated with NVP plasma concentrations[62–66].

1.4 **Pharmacokinetics of Lopinavir in Children**

Several studies have been completed that assessed the PK of LPV/r in a capsule formulation[67–69]. The corrected protein binding steady-state concentrations of LPV required to inhibit HIV replication by 50% (EC_{50}) were shown to be 0.07 µg/mL in two studies with different dosing regimens[70,71]. LPV/r table formulation displays similar bioequivalence to both liquid and capsule formations in both the 400/100mg twice-daily and 800/100mg once daily regimens[72]. Both the soft-gelatin capsule and the tablet formulations comparatively displayed serum PK C_{min} values of 5.17 µg/mL and 5.64 µg/mL respectively as well as respective C_{max} values of 6.97 µg/mL and 10.26 µg/mL. However, this differences were not statistically significant[72].

LPV/r capsule or liquid formulation has increased bioavailability(F) following moderate to high fat diet and thus its recommended to take this type of meals along with both formulations during prescription[22]. Evaluation of PK differences between the two formulations showed that the tablet formulation led to more consistent LPV and RTV exposure compared to the capsule or liquid formulations and that the ingestion of meal had no significant impact on LPV/r bioavailability[73]. Hence, LPV/r tablet formulation can be taken with or without food.

Both LPV and RTV are highly protein bound at steady-state, 98-99% bound to plasma proteins albumin and α_1 acid glycoprotein[74]. The mean plasma C_{max} of LPV is 9.8±3.7 µg/mL, occurring approximately 4 hours post dose[69,75]. The mean trough concentration is 5.2 µg/mL with the mean elimination half-life($T_{1/2}$) of 2-3 hours after single dose administration and 4-6 hours with an apparent oral clearance (CL/F) of 6-7L/h following multiple doses[67,69]. Examining the penetration of drugs such as LPV is important due to the fact that HIV replication occurs at intracellular level[74]. LPV is insoluble in water and accumulates mostly in peripheral blood mononuclear cells and penetrates the cerebrospinal fluid and significantly reduces viral load in CSF[70]. A study found that following 400/100mg LPV/r capsules, the plasma and peripheral

blood mononuclear cells, EC_{50} was reached with a C_{max} of 8.44 µg/mL and 13.40 µg/mL, respectively[70].

LPV is primarily metabolised by cytochrome (CYP) 3A4 and 3A5 whereas RTV is a potent inhibitor of CYP3A4 and thus co-administration of LPV with RTV leads to increased plasma concentrations of LPV. When administered together, RTV is given at a lower dose than when administered as monotherapy and acts as boosting agent with LPV producing the antiretroviral activity[76,77]. LPV together with its metabolites is primarily eliminated faecally. After 400/100mg LPV/r dose, 82.6% of the dose is found in faeces and 10.4% excreted in urine[78].

In paediatric patients, administration of LPV/r liquid suspension result in similar PK profiles to that shown in adults[45,79]. LPV undergoes extensive hepatic metabolism and thus patients with hepatic impairment might have increased concentrations, therefore great care should be taken when administering LPV/r to such patients[80]. Interestingly, in renally impaired patients, it is estimated that LPV/r concentrations will not be affected due minimal renal excretion[81].

1.5 **Pharmacokinetics of Nevirapine in Children**

PK is characterised by rapid absorption and rapid distribution throughout the body and prolonged elimination[32]. Following oral administration of a tablet or liquid syrup, NVP bioavailability exceeds 90%[82].NVP reaches plasma C_{max} after 4 hours post dose[83]. Concomitant administration with food or antacids delays the rate but no the extent of NVP absorption, therefore no dose adjustment of NVP is required[83]. NVP is highly lipophilic at physiological pH and the mean apparent volume of distribution of NVP exceeds total body water and is significantly higher in females (1.54 L/kg) compared to males (1.38 L/kg)[12]. Plasma protein binding of NVP is approximately 60%. Interestingly, a study in 6 children showed NVP concentrations in children CSF samples being 45% that of plasma concentrations, equivalent to free fraction in plasma[33]. Moreover, another study in adults, showed that NVP concentrations in CSF ranged from 3.36-27.81 mg/mL well in excess of the IC_{50} for wild type HIV-1. In this study, the average ratio between plasma and CSF NVP concentration was estimated to be 29%[12,84].

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NVP is mainly eliminated is via hepatic metabolism followed by renal excretion. Urine excretion of NVP is about 80% whereas faecal excretion is 10% after an administered dose[85]. NVP is extensively bio transformed by hydroxylation and glucuronidation into hydroxylated metabolites. In vitro studies have shown that NVP is primarily metabolised by CYP3A4/5 and CYP2B6 and to a lesser extent CYP2D6 and CYP2C9[13,85]. NVP elimination half-life ($T_{1/2}$) after single dosing is 45 hours (range 22 to 84 hours), and 25-30 hours during steady state dosing[32]. Long term therapy with NVP leads to induction of its elimination pathway. NVP auto-induction results in 1.5-2 fold increased NVP clearance after the first 2 weeks of treatment[32,86]. Therefore, NVP is initiated at 200mg daily dose, and increased to 200mg twice daily after two weeks on treatment.

NVP plasma concentrations and PK are associated with significant sex differences. Regazzi *et al*, showed that women had 44% higher C_{max} compared to males[87]. Moreover, NVP PK is not significantly affected by age (range 18-68 years)[88]. However, NVP PK is significantly associated with race, with black people having 39% higher concentrations that Caucasians[89].

In newborn infants, NVP washout elimination is extensive and highly variable with median $T_{1/2}$ of 64.9 hours in two studies[90,91]. During the first days of life, elimination increases. In the same studies, after administration of 2mg/kg oral dosing at 48-72 hours following NVP birth, the median NVP $T_{1/2}$ was 43.6 hours (range 23.6 to 81.6 hours) and the median CL/F was 36.1 ml/h/kg (range 22-40 ml/h/kg)[90,91].In new-borns, absorption was variable and extensive with T_{max} of 8.2 hours (range 2-26.1 hours)[90]. In older infants, NVP clearance is rapid, averaging around 120 ml/h/kg, during the first 2 years of life[92]. Thereafter, NVP clearance decreases gradually to an average of 60ml/h/kg by 8-10 years of age[92].

1.6 **Therapeutic Drug Monitoring in Children**

Therapeutic drug monitoring (TDM) refers to the measurement of drug concentrations in a biologic matrix (e.g. serum, plasma or urine etc.) to assess correlation between patient's clinical condition and whether there is need for adjustment for dose or dose intervals[93,94]. The process of TDM is based on the assumption that there is a precise relationship between dose and plasma drug concentrations, and between concentration and therapeutic effects. TDM

criteria in children is almost the same as that of adults, though some additional factors must be considered: (i) In neonates, infants and children major rapid age-related physiologic and biochemical changes occur, more especially in the first year of life and thus resulting in clinically dissimilar PK and PD parameters from that of an adult[95]; (ii) Gastrointestinal absorption of drug in children greatly differs from that of adults, before the age of 5 years, the stomach pH in children is higher than that of adults[96]; (iii) gastric emptying time changes with age. In neonates, gastric emptying time is slower than that of adults, whilst in infants and older children, gastric emptying is faster than that of adults[97,98]. Furthermore, neonates have reduced intestinal motility and biliary function[97];(IV) Elimination of drugs in children is influenced by age related changes in hepatic enzyme activity and kidney maturation. In the liver, drug metabolism occurs through 2 hepatic enzyme phases: phase 1 reaction (oxidation, reduction and hydrolysis) and phase 2 reaction (reduction)[99,100]. In neonates, the CYP450 mixed-function oxidation system activity is 20-70% of adult values[101]. This increases to adult levels by 6-12 months, and exceeds that of adults by 1-4 years and declines to that of adults at the end of puberty[101]. With regards to the phase 2 enzyme-mediated system, glucuronidation is diminished at birth and reaches adult levels after 3 years.[100] Drug elimination is influenced by age[102]. The glomerular filtration rate and tubular secretion are both reduced at birth and reach adult levels at during the first year of life[95,103]. Renal function reaches peak at 3-5 years and decline to average levels over time. Thus drug administration to children must account for age-related changes in drug absorption, distribution, metabolism and clearance in order to optimize efficacy and avoid toxicity. Moreover, drug administration compliance by parents at the appropriate time interval my further complicate non-adherence in the paediatric population.

For ART, TDM has another layer of complexity: incomplete suppression during therapy may lead to HIV mutations resulting in changes in drug susceptibility becoming a moving target[104]. This is unique compared to other diseases where TDM is applied whereby target concentration ranges remain the same throughout therapy[104]. HIV treatment uses concomitant ARVs to achieve durable viral suppression; however, TDM usually monitors only a single drug concentration[45,105–108]. Prospective randomized clinical trials have confirmed

the effectiveness of TDM in achieving virologic end points consistent with treatment efficacy and/or decreasing incidences of toxicity in treatment-naïve patients[109,110].

In the literature, TDM of ART in children and adolescents is limited especially PK data in paediatrics compared to adults, multiple drug-drug and drug food interactions, a narrow margin between therapeutic and toxic

1.5.1 **Therapeutic Drug Monitoring of Lopinavir**

The HIV-NAT017 prospective study was conducted whereby 20 children were enrolled on 230/57.5 mg/m^2 LPV/r regimen BID plus SQV 50mg/kg. In 19 children, the PK showed a median C_{min} of 5.9mg/L. Furthermore, 2 children with LPV <1.0 mg/L had VL>400 copies/mL compared with one child in a group of 17 with LPV C_{min}>1.0 mg/L. Though the study found a similar cut-off of 1.0 mg/L previously proposed from in vitro data, nonetheless, SQV could have lowered the cut-off value due to synergism[40].

Another prospective study of 126 PI-experienced patients was done to explore the utility of GIQ by predicting virological response to LPV therapy[40,111]. Included in the GIQ model were HIV protease resistance mutations at positions 10, 20, 24, 30, 32, 33, 36, 46, 47, 48, 50, 53, 54, 63, 71, 73, 77, 82, 84, 88, and 90. Virological response at 3 months was defined as VL<50 copies/mL. The median (IQR) number of resistance mutations was shown to be 4(2-7), the median LPV C_{trough} was 6.2(2.1-8.6) mg/L and the GIQ positively correlated with virological response. Furthermore, receiver operating characteristic (ROC) curves were used to find the LPV cut-off values. It was shown that patients with GIQ cut-off of >0.70 mg/L/mutation significantly achieved virological response than those with GIQ<0.70 mg/L/mutation.[111]

Recently, a retrospective study on GIQ whereby 95 patients were treated with LPV/r evaluated the cut-offs based on different sets of mutations[40,112]. Included were PI-associated mutations (PAM), lopinavir associated mutations (LAM) and lopinavir mutation score (LMS), consisting of mutations at positions: 10, 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90. In this study 76% of patients showed virological response with median (IQR) LPV C_{trough} of 5.2(3.7-6.3) mg/L. The median number of respective mutations for PAM, LAM and LMS were 4(2-7), 3(1-6) and 3(1-6). ROC curves were used to find optimal cut-offs and the cumulative GIQ including mutations found in patient's previous genotypic tests had the strongest association with

response. The cut-off values found for PAM, LAM and LMS, respectively, were 0.9 mg/L/mutation, 1.1 mg/L/mutation and 1.3 mg/L/mutation. Sensitivity was shown to be 0.74 for all cut-offs whereas specificity was 0.78 for PAM cut-off and 0.83 for both LAM and LMS cut-offs[112].

1.5.2 **Therapeutic Drug Monitoring of Nevirapine**

A prospective study was done where NVP plasma concentrations were measured in an unselected cohort of 189 patients[40,53]. In this study, patients were divided into two groups based on NVP concentrations below and equal to or above 3mg/L. Virologic failure was defined as VL >500 copies/mL for two consecutive occasions , >10 000 copies/mL for a single occasion or failure to achieve VL below 500 copies/mL for six months after commencing NVP treatment. The results revealed that 12% (22) and 7% (13) respectively, of patients had NVP concentrations <3mg/L. A multivariate analysis showed that the risk of virological failure was increased in patients NVP concentrations <3mg/L[53].

Another prospective study evaluated the efficacy of NVP in relation to plasma levels in 74 patients[50]. All patients were PI-experienced with a baseline VL of <20 copies/mL and virological failure was defined as having VL>1000 copies/mL or 2 consecutive intermittent viremia episodes between 20 and 1000 copies/mL. The study showed that 14 patients had viremia at the same point during the study, versus 45 patients that remained suppressed for the duration of the study. The mean plasma NVP concentrations were 4.6 and 2.6mg/L, respectively (p=0.003), in responders compared to non-responders. A NVP cut-off of 3mg/L was shown to be efficacious, however the positive predictive value of the cut-off was 55% whereas the negative predictive value was 88%.

In the PK sub-study of the 2NN trial, the risk of viremia was increased in patients with NVP C_{min} <3.1 mg/L in 511 patients included in the study[51]. However, the results were not significant and the sensitivity parameter was 28%. Nonetheless, the negative predictive value was 78%, suggesting reasonable success with the determined NVP C_{min}. Previous guidelines were based on the median C_{min} value found in the INCAS trail[113], however findings the studies by Vries-Sluijs *et al*, Duong *et al* and the 2NN trail suggest the C_{min} cut-off to be 3.0mg/L[40,50,53].

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1.6 **Adherence Monitoring**

Adherence to ART directly correlates with clinical and virological outcomes.[114,115] With more potent regimens, the adherence threshold required to achieve robust viral suppression has declined. Studies have shown that patients can achieve undetectable viral loads at adherence proportions as low as 70%[116–119]. Therefore, full and sustained benefit of ART can only be derived from high levels of adherence. Furthermore, it has been shown that adherence in Sub-Saharan African is comparable or even superior to that in Western countries, however retention in programmes is a serious challenge[120,121]. Both in high and low income countries, medication side effects and complexity of drug regimens, psychiatric of lack of social support are barriers of adherence[122–125]. Moreover, in Sub-Saharan Africa conditions of extreme poverty and livelihood insecurities add a further dimension to adherence costs[125]. Another factor that compounds to this problem include non-disclosure due to fear of stigmatization[122,124]. Medication related barriers to adherence include pill burden, dosing frequency, dietary restrictions and side effects. Currently, most first line regimens are one or two pills daily, however second regimens are more complex adding to complexity in adherence. Side effects related to ART use also greatly affects adherence with studies having shown that when patients experience side effects, they tend to stop taking treatment or irregularly take their medication[126].

In paediatric populations, maintaining adherence is even more challenging, especially over long periods. Factors relating to children, caregivers, medications and the interaction thereof all good adherence very challenging[127]. Currently, paediatric ART formulations are limited. Some have poor palatability, require high liquid volume or pill burden, frequent dosing or, dietary restrictions. Side effects also impact on the regular intake of medications[128]. In children, successful adherence to treatment requires commitment and involvement of the caregiver.

Currently, there is no gold standard for the measurement of adherence. However, approaches such as patient self-reports, pill counts, and pharmacy refill records are used to monitor and evaluate medication adherence. Viral load and CD4 cell count are biological markers used to monitor for suboptimal adherence, however the approaches are largely influenced by viral

resistance, prior treatment failure or poor absorption of drugs. In the literature, discordance has been shown between viral suppression and adherence in those contexts[129]. Currently, no approach produces completely valid and reliable measure adherence and therefore, a new feasible and reliable method would be highly beneficial.

1.7 **Pharmacogenetics**

Pharmacogenetics is the science of discerning genetic variability between subjects to study host drug response and hence predict optimal treatment regimens, thus reducing expenses and often harmful trial and error methods currently used.[130] Single nucleotide polymorphisms (SNPs) are defined as sequence variations occurring in human DNA as a single nucleotide changes at allele frequencies greater 1%[131] . Nucleotide changes occurring at rate less this are referred to as mutations. Advances in genetic analyses technologies are generating great prospects in unveiling the role of sequence variations in the human genome that influence drug disposition, metabolism, efficacy and toxicity of ARVs. Interestingly, performing genome-wide analyses has disclosed new possibilities in this area of research[132]. However, current research mostly focuses on employing the hypotheses driven candidate gene approach to study phenotype-genotype relationships. In most studies, the hypotheses are to determine a plausible link between genetic variations that have possible impact on drug metabolism and/or drug toxicity and phenotype under study. Moreover, using the hypothesis-driven candidate gene approach in host genome variability influencing ARV efficacy and tolerability, relationships between genetic factors involved in immunological and PK determinants of response are explored (**Figure 2**).

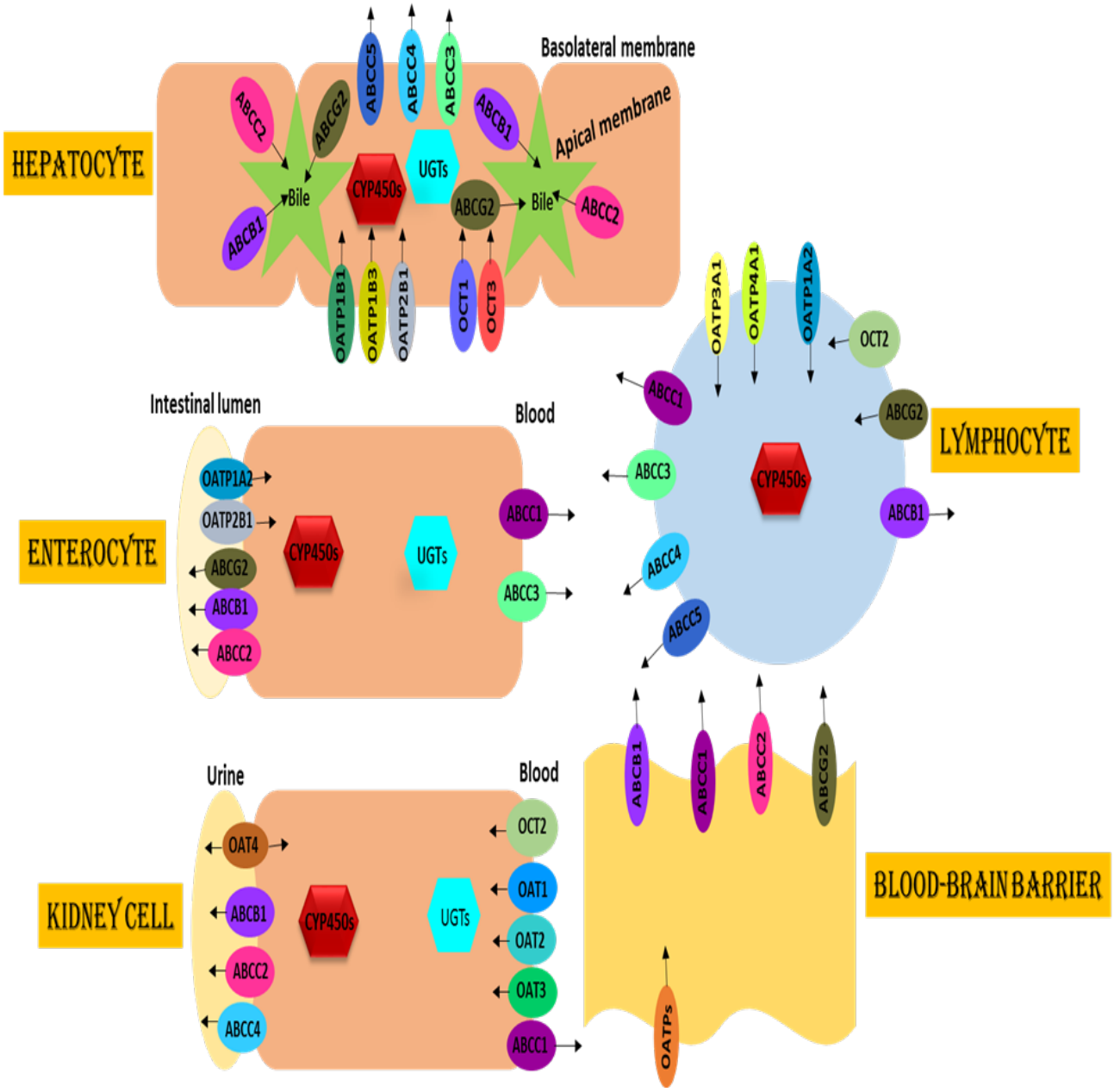

Figure 2: Schematic representation of proteins involved antiretroviral drugs metabolism and disposition in various sites in the body. ABC, ATP-binding cassette transporters; CYP450s, cytochrome P450 enzymes; OAT, organic anion transporters; OATP, organic anion transporter polypeptide; OCT, organic cation transporters; UGTs, uridine diphosphate glucuronosyltransferase enzymes. Adapted from Michaud *et al* 2014 and Owen *et al* 2006

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A great number of associations between host genetic variations and responses to ARVs have been reported. These include PK-PD, hypersensitivity reaction syndromes, hepatotoxicity, central nervous system side effects, hyperbilirubinemia, peripheral neuropathy, lipodystrophy, hyperlipidaemia, pancreatitis and renal toxicity[130]. Nonetheless, it remains important to note that numerous barriers exist in translating this body of knowledge into the ultimate goal represented by individualization of ART. Moreover, the risk of false discoveries due to multiple testing is a well-known phenomenon in statistical genetics and thus caution is needed in consideration of early reports on genotype-phenotype association studies. To date, most studies in the literature have significant limitations represented by small sample size, inadequate statistical power and selection bias. Additionally, very often the carriage of a variant is linked to ethnicity and as a consequence the risk of ethnic bias exists in most genotype-phenotype association studies. Thus, in order to successfully introduce pharmacogenetic testing into routine clinical practice several prerequisites must be met. Firstly, the test must be clinically relevant with high specificity and sensitivity. Secondly, there should be evidence on genotype-phenotype association ideally based on randomized, double-blind, prospective studies involving patients of varying ethnicities. Finally, the genotypic test should be rapid, simple to interpret and cost effective.

1.7.1 **Pharmacogenetics of Protease Inhibitors**

PIs show marked inter-individual variability in bioavailability and plasma PK explainable by drug metabolism. PIs are metabolised by CYP3A4 but also inhibit CYP3A[133], hence the impact of polymorphisms in these genes on PI disposition is difficult to predict (**Table 2**). PIs are also substrates of drug transporter Pg-p[134], expressed extensively in human cells of different tissues like liver, kidney, central nervous system, small intestine and lymphoid tissue (**Table 2**). The impact of Pg-p variants on PI disposition has been widely studied.

Additionally, polymorphisms in the apolipoproteins (APO) have been associated with hyperlipidaemia and cardiovascular events in the general population. Polymorphisms in APO have been studied broadly in PI-associated metabolic and morphological abnormalities[135,136].

Table 2: Genetic polymorphisms and the clinical relevance of proteins involved in PI metabolism and disposition

Drug	Gene(Protein)	Variant	PK Effect
LPV	CYP3A4	**22*	TT:53% Lower CL/F[137]
	SLCO1B1(OATP1B1)	*521T→C*	CT/TT: High plasma levels[138]
ATV	CYP3A5	**1*	GG/GT: Lower CL/F[139]
	UGT1A1	**28*	High risk of hyperbilirubinemia[140,141]
	ABCB1(Pg-p)	*3435T→C*	TT: Risk of sub-therapeutic levels[140,142]
		2677G→T	Risk of sub-therapeutic levels[140,142]
	SLCO1B1(OATP1B1)	*521T→C*	CT/TT: High plasma levels[143]
	NR1I2(PXR)	*63396C→T*	TT: Risk of sub-therapeutic levels[143–145]
IDV	CYP2C19	**2*	AA: 44% faster oral CL/F[146]
	MRP2	*-24C→T*	CT/TT: Faster CL/F[147]

1.7.1.1 **Pharmacogenetics of Lopinavir**

LPV is mainly metabolised by CYP3A enzymes and is a substrate of efflux transporters Pg-p, ABCC1 and ABBC2 genes, contributing to its low and variable oral bioavailability[148–150].

A common SNP in the SLCO1B1 (*521T→C*) gene leads to increased plasma LPV levels, however the clinical relevance remains controversial in the literature and thus further studies are required to confirm this association and to assess impact on LPV PK[151–156]. Similarly, a link between the 4544G>A polymorphism in the ABCC2 gene and accumulation of LPV in the peripheral mononuclear cells has been shown in a small cohort study of HIV-infected patients[157]. Nonetheless, more studies are required to confirm this findings and to explore the real PD impact.

1.7.2 **Pharmacogenetics of Non-Nucleoside Reverse Transcriptase Inhibitors**

NNRTIs are predominantly metabolized in the liver by CYP enzymes and are absorbed and distributed mainly by P-glycoprotein (P-gp)[149]. Both NVP and EFV are extensively metabolised by highly polymorphic *CYP2B6* enzyme[158]. The *CYP2B6* gene has numerous SNPs and

associated haplotypes with genetic variability being assessed in different ethnicities leading to a number of functional variants being discovered. Interestingly, more than 28 variants have been described and more than 100 SNPs have been determined for the *CYP2B6* gene[149]. Amid various alleles, the *CYP2B6*6* haplotype (*516 G→T* and *785 A→G*) reduces enzymatic catalytic activity and significantly decreases protein expression. The *CYP2B6*6* allele has been shown to vary between different ethnicities with 15 to 40% in Asians, 25% in Caucasians and more than 50% in African Americans and black Africans[159–162]. Moreover, though the *CYP2B6*16* (*785 A→G*, *983 T→C*) or *CYP2B6*18*(*983 T→C*) polymorphisms are common in black populations and leads to decreased protein expression, they do not affect its intrinsic catalytic activity[163].

NVP is predominantly metabolized by *CYP3A4* and *2B6* with a minor contribution from *CYP3A5* to its hydroxynevirapine metabolites[164]. EFV is primarily *CYP2B6* with a minor contribution from *CYP3A4*[165]. Thus, there is considerable inter-individual variability in the metabolism and disposition of NNRTIs. Hence, the *CYP2B6*, *CYP3A4* and *CYP3A5* genes have been extensively studied with regards to PK-PD, treatment response and toxicity of both NVP EFV (**Table 3**). P-gp encoded by the *MDR1*(*ABCB1*) gene affects the oral absorption and tissue penetration of NNRTIs[134,166].

Table 3: Genetic polymorphisms and the PK effect of proteins involved in NNRTI metabolism and disposition

Drug	Gene(Protein)	Variant	PK Effect
NVP	**CYP3A4**	**1B or -392A→G*	*1/*1B: Higher C_{min}[146] Not predictive of hepatoxicity[167]
	CYP3A5	**1 or 6986A→*	Higher AUC[168] No association with C_{min}[167] Not predictive of hepatoxicity[167]
	CYP2B6	*516G→T*	Increased plasma levels[164,169]
		983T→C	Increased plasma levels[170,171]
		1459C→T	Decreased plasma levels[146]
	ABCB1(Pg-p)	*3435TC→T*	TT: Higher C_{min}[172] Decreased risk of hepatoxicity[173,174]
	MRP7(ABCC10)	*c.28473T→C*	CC: Lower plasma levels[175]
EFV	**CYP3A4**	**1B or -392A→G*	GG: higher EFV AUC[146]
	CYP3A5	**1 or A6986*	No association with plasma levels[160]
	CYP2B6	*516C→T*	TT: High plasma levels[136] Increased risk of CNS adverse events[176,177]
		785A→G	High plasma levels[178–180] Increased risk of CNS adverse events[160]
		983T→C	High plasma levels[163,181,182] Increased risk of CNS adverse events[176]
	ABCB1(Pg-p)	*3435TC→T*	Dispute in the literature regarding influence on plasma EFV levels[183,184] Decreased likelihood of virological failure and decrease emergence of resistant virus[185]
		2677G→T	TT: affect plasma levels[146]

1.7.2.1 **Pharmacogenetics of Nevirapine**

NVP is widely prescribed especially in resource limited settings for HIV-1 infection treatment for pregnant women and their children. Nonetheless, it has limited use due to drug related-adverse effects appearing frequently in the first 6 weeks of treatment and has low barrier for developing drug resistance mutations[186]. The main adverse effects accompanying using NVP are rash, affecting 15% of patients initiating NVP, increased transaminases above 5 times the normal range in 20% of patients, fever and immune mediated hypersensitivity that may manifest as hepatotoxicity[187,188]. The mechanism involved in NVP associated adverse events has not been described. Nevertheless, the cutaneous effects might be mediated MHC class I influenced by *CYP2B6* metabolism, whereas hepatotoxicity is most likely mediated by MHC class II and unaffected by *CYP2B6* polymorphism.[189] Interestingly, several human leukocyte class I and class II antigens have been associated with rash and/or hepatitis reactions development. The concurrent presence of *HLA-DRB1*01:01* variant and $CD4^+$ T lymphocyte count greater than 25% prominently increases the risk of developing NVP associated HSR and hepatotoxicity[190,191]. Similarly, other HLA class alleles such as *HLA*B14:02*, *HLA-Cw08* and *HLA-B*35:05* have been associated with NVP associated drug reactions[192–194]. Until recently, the majority of studies were largely focused on white populations, however Phillips et al published the first study carried out in black population, where the need for HLA studies being HLA variants are predominant[195]. Interestingly, a GWAS study was conducted in Thai population whereby genetic variations in the *CCHCR1* gene were strongly associated with NVP-induced rash[196].

NVP is metabolized into its major metabolites 2- and 3-hydroxynevirapine, respectively, mainly by CYPs *3A4* and *2B6*[136,164]. In the literature, several studies have shown that the *516 G→T* and *983T→C* polymorphisms in the *CYP2B6* gene are associated with NVP PK in ethnically diverse populations[136,162,171,168,197]. However, the clinical impact of such findings remains controversial since the association between NVP exposure and toxicity has not been fully elucidated. Nonetheless, NVP clearance was shown to be reduced significantly in Cambodian patients homozygous for the *516 G→T* polymorphism, 1.86 L/h compared to 2.95 L/h in patients with wild type allele[198]. Additionally, Mahungu et al showed that the *516 G→T* significantly predicted NVP trough concentrations[169]. Interestingly, recent results have shown

that the *CYP2B6 983 T→C* polymorphism heterozygosity was associated with significantly higher plasma NVP concentrations in black patients[172]. Furthermore, a population PK model was used to examine relationships between EFV and NVP exposure, weight and *CYP2B6 516 G→T* and 983 *T→C* polymorphisms[199]. The results confirmed the significant effect of the *983 T→C* variant heterozygosity with 40% decreased oral clearance. Moreover, a recent population PK multicentre study in African children revealed differences in evening C_{min} based on metabolizer status of the *CYP2B6 516/983* haplotypes (Ultraslow[USM], Slow[SM], Intermediate[IM] and Fast[FM]) and weight[200]. The results showed that NVP doses in children belonging to USM and SM groups should be reduced by 50% and children weighing <6kg belonging to the IM and FM groups should receive the same dose as those weighing 6-10kg in order to achieve homogenous exposures[200]. Polymorphisms affecting Pg-p activity have been postulated to influence intracellular concentrations and might be related to toxicity. Interestingly, the *ABCB1 3435C→T* shows decreased risk of hepatotoxicity related to NVP therapy[197,174], however, such results are paradoxical since lower Pg-p expression would lead to increased NVP concentrations in hepatocytes and thus further studies are required to ascertain this findings.

1.8 **Pharmacometrics**

Pharmacometrics can be defined as "the science of developing and applying mathematical and statistical methods to characterize, understand and predict drug's PK-PD behaviour; and quantify uncertainty of information about such behaviour; and rationalize data-driven decision making in drug development process and pharmacotherapy"[201]. Initially, pharmacometrics was developed to facilitate more efficient development and use of pharmaceuticals by applying mathematical and statistical models to clinical data. The nonlinear mixed-effects models are the most commonly used in population pharmacometric approaches, which particularly useful in application to heterogeneous biological data by their ability to characterise sources and levels of variability[202]. The model approaches are used to integrate prior knowledge and pool data across studies and therefore used to predict dosing and dosage regimens; to extrapolate to other target populations; to improve study design

using optimal design theory and clinical trial simulations; and to investigate optimal dosage for population and individual treatments[154,203].

1.8.1 **Population Pharmacokinetic Modelling**

Population pharmacokinetic (PopPK) modelling involves using nonlinear mixed effects model(s) to simultaneously evaluate data from individuals in a population[204,205](**Table 4** presents advantages and disadvantages of PopPK). "Nonlinear" can be defined as dependent variable (e.g. drug concentration) being nonlinearly related to model parameters and independent variable(s). "Mixed effects" refers to model parameterization: "Fixed effects" are defined as parameters that do not vary across individuals whereas "random effects" are parameters that vary across individuals, including inter-individual variability (IIV); inter-occasion variability (IOV), and residual unexplained variability (RUV). The general structure of a mixed effects is written as follows:

$$Yijk = f(Xijk, Pjk) \quad \text{.....Equation(1)}$$

Where Y_{ijk} is the jth observation of the dependent variable (usually drug concentrations) at the kth occasion in an individual *i*. Y_{ijk} is described by a vector of individual parameters P_{ik} and a vector independent variables x_{ijk} (e.g. time and dose).

There are five major facets of PopPK model: (i) the data, (ii) structural model, (iii) statistical model, (iv) covariate models, and (v) modelling software[205]. The structural model describes the time course of drug concentrations within a population. The statistical models describes "unexplainable" variability of drug concentrations within a population (e.g. IIV, IOV, RUV etc.). Covariate model account for variability explained by subject specific characteristics (e.g. gender, age, weight etc.). The modelling software brings the data and all models together and implements an estimation method for finding parameters for the structural, statistical, and covariate models that best describes the data[206].

In comparison with traditional methods, PopPK is a powerful tool for summarizing large amounts of data and quantifying interactions. The model can be seen as repository of integrated knowledge and information about the biological system, disease and drug properties thereby achieving a collated picture. New knowledge can be obtained by

integrating new information to confirm previous findings as well as further model refinement. Furthermore, PopPK does not require "rich" data (many observations [>5 samples post dose] or many subjects), as with single-subject data analysis, as well as no need for structured sampling schedules. Using "sparse" data (few observations [<3 samples post dose]/few subjects) or combination of both "rich" and "sparse" can be done with the PopPK approach.

The typical value of the population and individual random effect is defined as an individual parameter. The individual parameter is assumed to be log-normally distributed such that it only takes positive values as follows:

$$Pjk = \theta p * e^{\eta j + kj}$$

.....**Equation(2)**

where P_{ik} is the individual parameter at the k occasion, is the typical parameter estimate and η_i and k_i are the random effects that describe IIV and IOV, respectively. The variables are assumed to be normally distributed with mean zero and variance. Parameters are assumed to be log normally distributed and hence both BSV and BOV are described exponentially.

A covariate model describes the relations between covariates and parameters. Covariates are characteristics describing the patient, conditions drug of treatment or other factors potentially influencing the outcome. Subject specific covariates, such as age, gender, weight, genetics, liver or kidney function, etc. often explains part of the variability between individual. Therefore, the typical parameter value will in part be a function of the individual parameter value to explain part of the IIV.

The difference between the individual prediction and the observed value is described by RUV. RUV may occur due to mis-recording of the time of sampling, mistreatment of samples, error due induced by analytical methods, model misspecification[207] etc. In PopPK, RUV is investigated using additive and/or proportional models. The j^{th} individual observation can be expressed in the general equation 1 as follows:

$$Yijk = f(Xijk) + \varepsilon ijk$$ ……**Equation(3)**

where Y_{ijk} represents the j^{th} observation of the dependent variable at k^{th} occasion in an individual. The individual predication $f(....)$ is described by the independent x_{ijk} and individual parameters P_{ik}, ε_{ijk} is the residual error term defining the difference between observed value and individual prediction. The ε is assumed to be normally distributed with mean zero and variance δ^2.

Table 4: Presents the advantages and disadvantages of population pharmacokinetic modelling

Advantages	**Disadvantages**
pharmacokinetic analysis is usually conducted in patients taking the drug	relatively large numbers of patients are required (typically >40)
can accommodate flexible study designs which occur during treatment	complex mathematical and statistical analyses
only a few samples are needed from each patient	requires collection, compilation and verification of large amounts of data
opportunistic sampling has the potential to be cost-effective	model building may be tedious, labour intensive and time-consuming
screening and quantification of covariates for explaining variability	model diagnostics are often complex and time-consuming
can distinguish between interindividual and intraindividual variability	difficulties with handling missing data (e.g. all covariates in all patients)
modelling software is widely available (e.g. NONMEM, MONOLIX)	

1.8.2 **Model Estimation**

Estimating PopPK can be done is a number of software packages, all of which are based on hierarchical nonlinear mixed effects modelling[208,209]. In this thesis, NONMEM[210] was used for the analysis, which is based on parametric maximum likelihood method for parameter estimation. The parameters of a model are estimated by maximization of the probability of

the data under the model. In NONMEM, parameters are estimated by minimizing the extended least objective function (OFV), which is approximately proportional to minus 2 the logarithm of the likelihood (-2LL) of the data. The difference between 2 nested models is approximately chi-squared distributed under the assumption that the model is correct and errors are normally distributed. The likelihood ratio test can be computed and nested models can be compared whereby a difference in OFV of 3.84 corresponds to a significance level of $p<0.05$[211–214]. Standard errors of parameters estimates are also obtained through maximum likelihood estimation.

It is a challenge to specify and evaluate the explicit likelihood function due to the entrance of random effects in the model nonlinearly[215]. Nonetheless, in NONMEM, this handled by using approximations of the nonlinear model and also involve linearization of the random effects[216]. There are several alternative parametric methods available to approximate in NONMEM including first order method (FO), first order conditional method (FOCE), first order conditional with interaction method (FOCEI) and Laplacian second order estimation method (LAPLACE). Nonparametric estimation methods (NONP) are also available in NONMEM, whereby no assumption is made about the distribution shape, but only define the parameter space is defined[217,218]**.** Although they relax distribution assumptions, NONP approaches also preserve mathematical and statistical consistency. Though powerful, NONP is associated with some drawbacks, such as increased computation time, no imprecision of measurements and the impossibility to estimate residual variability. In order to circumvent this drawbacks, a two stage estimation can be performed, where the first estimation step is parametric (FO or FOCE) and the second estimation step NONP[218].

1.8.3 **Model Validation**

Model validation is always used throughout the model building process to evaluate the adequacy, accuracy and robustness of the model. The main objective of model validation is to assess whether the model best describes the validation dataset in terms of its behaviour and of the application proposed. Graphical and statistical techniques are the most widely used and help in understanding the data and lead to proficient analysis of the data. Graphic diagnostics are intuitive and provide powerful approaches in interpreting the mode[219]l. There is numerous diagnostic graphical approaches, some of which rely on simulation to evaluate

different aspects of model adequateness[220]. Moreover, each diagnostic approach has assumptions, strengths and weaknesses[220,221]. As previously mentioned (1.8.2), OFV is the most used numerical diagnostic. OFV provides information on model robustness and identify poor model fit (SE of parameters).

Typically the model predictions (PRED) and individual predications (IPRED) versus observations are routinely used as good of fits plots (GOFs). Both PRED and IPRED assess the fit of the data along the line of identity and outliers can be identified visually[220–223]. This plots follow trends of individuals and can be used to indicate bias with the use of a regression line. Residual based diagnostics such as individual weighted residuals (IWRES) and conditional weighted residuals (CWRES) are also used commonly as part of GOFs. Both IWRES and CWRES are used to assess model misspecification, CWRES may also improve model accuracy. IWRES are calculated using the FO method whereas CWRES are calculated using FOCE approximation[220]. Normalised prediction errors (NPDE) can also be used as part of GOFs. NPDE are not true residuals but are rather simulated based on the rank order of the observations of the original dataset in relation to the model[224]. Generally, the residual based diagnostics should be normally distributed with mean 0 across any independent variable.

Visual predictive check (VPC) is a powerful tool to assess models. VPC simulates data and computes 95% prediction intervals[225]. Simulated predictions include fixed and random (both IIV and IOV) effects variability as well as residual error. A plot is then generated displaying prediction intervals and the observed data. The model robustness is then assessed by comparing observations to simulations for a particular prediction interval, making VPC a powerful tool for model validation.

Bootstrapping is resampling technique also commonly used in model validation[226]. Resampling generates multiple samples from the original dataset and calculates quantities based on the estimations obtained from each new set of data. Means, standard errors, and 95% confidence intervals obtained from the bootstrap are compared with ones from the original dataset. Bootstrapping thus provides measures of the stability of the final parameter estimates as well as final model robustness.

Chapter 1

Literature Review

1.9 **Thesis Rationale and Aims**

There are only few clinical trials strategies that optimally utilize currently approved drugs for long-term treatment of HIV-infected children in low resource settings.Treatment is complicated by selection of drug resistance in many children whose mothers receive NVP for prevention of mother to child transmission (PMTCT). A clinical trial was thus conducted at Rahima Moosa Mother and Child Hospital in Johannesburg to assess whether NVP can be re-used (**Post-randomization Phase**) among 327 children exposed to NVP for PMTCT if they are first suppressed on ritonavir-boosted lopinavir based regimen (**Pre-randomization Phase**). Data on treatment response has been published[227,228].

This is a retro-prospective study of lopinavir (LPV) and nevirapine (NVP) pharmacokinetics (PK) in a cohort of children infected with HIV. Both LPV and NVP PK demonstrate considerable inter-individual variability, which may affect treatment outcomes. At least part of this variability may be explained by host genetic factors. In children, however associations between human genetic variants and LPV and NVP exposure are incompletely understood.

The specific aims of the thesis were to:

i) Firstly, assess the relationship between serial clinic visit LPV concentrations and virologic outcomes in the pre-randomization phase using Cox proportional hazard multiple failure event analysis.

ii) Secondly, use Cox proportional hazard multiple failure event modeling to evaluate the relationships between serial clinic visit LPV and NVP concentrations and virologic outcomes in the post-randomization phase.

iii) Thirdly, use a population pharmacokinetic-pharmacogenetic model to explore the relationship LPV and genetic polymorphisms in preselected drug metabolizing enzymes and drug transporters

iv) Lastly, explore the effect of genetic polymorphisms in predetermined drug metabolizing enzymes and drug transporters on NVP population pharmacokinetics.

Chapter 1

Literature Review

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Chapter 2

General Methods

2.1 **Study Population**

A randomized, open label trial involving a total of 195 children infected with HIV was conducted at one site in Johannesburg, South Africa. The randomized children were accumulated from an initial cohort of 323 NVP-exposed children who met clinical and immunologic criteria for treatment when younger than 24 months of age. Data of the children in the study population included data from two studies about immune reconstitution inflammatory syndrome and initial response to PI-based antiretroviral therapy, respectively.

2.1.1 **Study Design**

Women with HIV-infected children younger than 24 months of age who reported that nevirapine was used for prevention of mother-to-child transmission were identified and referred from inpatient wards and paediatric HIV clinics to one research site for a period between 8 April 2005 to 10 July 2007. Children were evaluated for eligibility for treatment based on South African guidelines. Eligibility criteria for treatment included World Health Organization (WHO) stage III or IV disease, $CD4^+$ percentage less than 25 if younger than 12 months or less than 20 if 12 months or older, or recurrent(>2times/year) or prolonged (>4weeks) hospitalization for HIV related complications. Children needing acute treatment for opportunistic infections (except tuberculosis) or tumours were excluded. These children were considered as candidates for ART initiation but were not eligible to be enrolled in the trial. For most children (n = 254) enrolled, treatment was initiated under supervision of the study team. A further 69 children were enrolled after initiating PI-based therapy elsewhere (other local paediatric antiretroviral treatment services) but who otherwise met all study eligibility criteria except that pre-treatment blood samples could not be stored for resistance testing. The 69 children all initially began receiving ritonavir-boosted lopinavir, stavudine, and lamivudine, but not administered by our study team. During the pre-randomization phase, 323 children were first initiated onto LPV/r, lamivudine and stavudine and during phase were followed with regular viral load tests until they suppressed. From this cohort, those who achieved a viral load < 400 copies/ml and sustained this level over a 3 month or longer period were eligible for randomization. A total of 195 children were randomized. Half were randomized (n=99) to remain on the LPV/r-based regimen (LPV Group) and the other half (n=96) were

randomized to substitute nevirapine (NVP Group) for the LPV/r. The NRTI backbone remained the same in both groups. The randomized children were then followed for an additional 76 weeks with regular viral load monitoring. The schedule of blood sampling is shown below.

PRE-RANDOMIZATION (n=263 children or 789 samples): Blood sampling schedule for HIV-infected children

Time (months) after treatment →			0.5	1[1]	2	3[1]	6[1]	9[1] (if needed)	12[1] (if needed)
Weeks →	Time –1	Time 0	2	4	8	12	24	36	52
Blood sampling	Yes	Yes	No	Yes	No	Ye	Yes	Yes	Yes
Toxicity	ALT/FBC/ Lipids	No	No	No	No	No	FBC diff**	FBC diff**	FBC diff**
CD4 counts	No	Yes	No	No	No	No	Yes	Yes	Yes
HIV RNA quantity	No	Yes (std)	No	No	No	Yes (std)	Yes (ultra*)	Yes (ultra*)	Yes (ultra*)
Plasma & cell pellets stored	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes

ALT, alanine transferase**;** FBC diff**, differential full blood count; HIV RNA, human immunodeficiency virus ribonucleic acid

POST-RANDOMIZATION (n=195 children or 780 samples): Blood sampling schedule for both LPV Group and NVP Group

Months after randomized treatment [1] started -→		0.5	1	2	4	6	9	12	15	18
Weeks -→	Time 0-R	2	4	8	16	24	36	52	64	76
Blood sampling	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Toxicity	ALT/FBC/ Lipids	ALT	FBC diff		ALT	ALT	Lipids/ALT	ALT	ALT	ALT
CD4 counts	No	No	No		Yes	Yes	Yes	Yes	Yes	Yes
HIV RNA quantity	No	No	Y(ultra*)		Y(ultra*)	Y(ultra*)	Y(ultra*)	Y(ultra*)	Y(ultra*)	Y(ultra*)
Plasma & cell pellets stored	Yes	Yes	Yes		Yes	Yes	Yes	Yes	Yes	Yes

ALT, alanine transferase**;** FBC diff**, differential full blood count; HIV RNA, human immunodeficiency virus ribonucleic acid

Additionally, weight and height measurements were recorded at each visit as well as clinical information concerning illness, hospitalization or other events since the last visit and concomitant medications including TB treatment. At each visit, the medication bottles were weighed by the pharmacist to determine the amount of liquid consumed and the percentage "adherence" was calculated based on the syrup reconciliation. Questions were also asked of caregivers concerning reported adherence.

2.1.2 **Ethical Consideration**

The study was approved by the Ethic Committee of the University of the Witwatersrand and Columbia University, New York. All care-givers signed informed consent for participation in the trial (Appendix I).

2.1.3 **Laboratory Methods**

2.1.3.1 **Pharmacokinetic Assays**

The samples collected as described above were tested for concentrations of lopinavir and nevirapine depending on the regimen the child is receiving. This testing was done at UCT. The time the sample was collected and the time of the last dose were recorded for most of the samples. Stored plasma samples were assayed for LPV and NVP by existing validated liquid chromatography mass spectrometry methods which have quantitative sensitivity to concentrations 0.1 mg/L or lower. The laboratory, at which the concentrations were assayed, participates in the International Inter-laboratory Pharmacology Quality Control Program, the AIDS Clinical Trial Group. Assays were performed in batch mode and results were provided electronically to the NEVEREST2 Data Management Centre. The Data Management Centre merged the results with clinically relevant data. Results were not be reported back to the patients or to the clinicians providing care for the patients.

2.1.3.2 **DNA Extraction**

Human DNA was extracted from buffy coats using the QIAsymphony (QIAGEN, Hilden, Germany) DNA midi kit. The QIAsymphony DNA midi Kit is designed for automated isolation and purification of total DNA from human whole blood, buffy coat, human and animal tissues, cultured cells, and bacterial cultures as well as viral DNA from human whole blood. Purified

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DNA is free of proteins, nucleases and other impurities. Up to 96 samples were processed in a single run. QIAsymphony technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples, and comprises 4 steps: lyse, bind, wash, and elute (**Figure 1**).The patient's sample (buffy coat) was 400µl and smaller sample with volumes were adjusted to 400µl final volume with physiological saline before loading to the QIAsymphony machine. The elution volume chosen was 200µL. DNA was transferred from the 96 well plate into pre-labelled Eppendorf tubes. The DNA concentration in the Eppendorf tube was determined using nano drop spectrophotometer. The tube was stored at stored at -20^{o}C before further analysis.

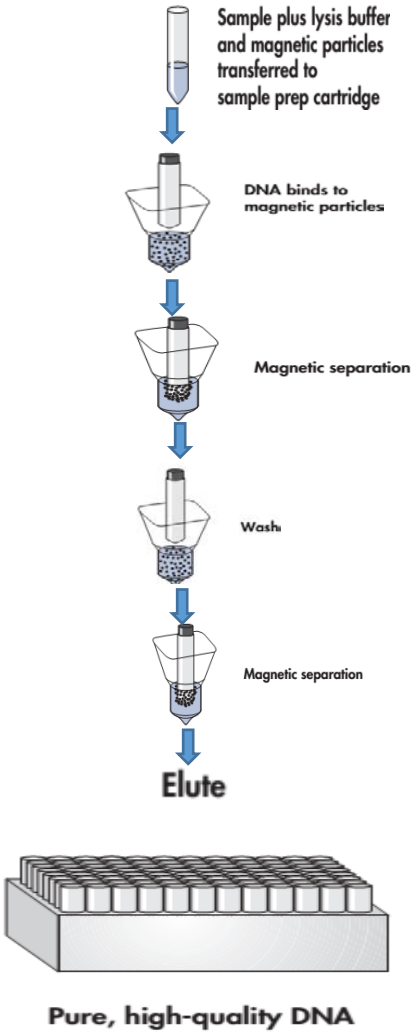

Figure 1: Flow chart of the DNA purification using the QIAsymphony Automated Technology

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2.3 **Statistical Modelling**

The general purpose this section is outlined below, with more specific issues being found in the appropriate sections of the following chapters.

2.3.1 **Multiple Imputation in R Package Amelia II (Paper 1 and Paper 2)**

Amelia II performs multiple imputation, a general-purpose approach to data with missing values[1]. Multiple imputation reduces bias and provides increased efficiency compared to list-wise deletion. Moreover, ad-hoc methods of imputation, for example mean imputation, lead to serious bias in variances and covariances. However, due to the technical naturel of algorithms involved, creating multiple imputations can be cumbersome. Amelia simply provides a way to create and implement an imputation model, generate imputed datasets, and check its fit using diagnostics. Furthermore, expectation maximum likelihood bootstrap (EMB) algorithm included in Amelia II imputes many variables, with more observations, in a short amount of time. The simplicity and power of the EMB algorithm makes it possible to write Amelia II so that it virtually never crashes, which it unique among all existing multiple imputation software and is much faster than the alternatives. Additionally, Amelia II has features to make valid and much more accurate imputations for cross-sectional, time-series, and time-series-cross-section data, and allows the incorporation of observation and data-matrix-cell level prior information. Furthermore, Amelia II provides diagnostic functions that help in checking the validity of the imputation model. The Amelia II software implements the ideas developed by Honaker and King[2].

2.3.1.1 **How Amelia Works**

Multiple imputation involves creating *m* completed data sets by imputing *m* values for each missing cell in the data matrix. Across completed data sets, the observed values are the same, whereas missing values are filled in with a distribution of imputations that reflect the uncertainty about the missing data. After imputation with Amelia II's EMB algorithm, any statistical method can be applied as if there had been no missing values to each of the *m* data sets, and a simple procedure is used to combine the results. Normally, imputation is done once and the *m* imputed data sets can be analyzed as many times and for as many purposes

wished. The advantage of Amelia II is that it combines the comparative speed and ease-of-use of the EMB algorithm with the power of multiple imputation. Unless the rate of missingness is very high, *m* = 5 (the program default) is probably adequate

2.3.1.2 **Assumptions in Amelia**

The imputation model in Amelia II assumes multivariate normal distribution for complete data (includes both observed and unobserved). If the (*n* × *k*) dataset are denoted as D (with observed part D^{obs} and unobserved part D^{mis}), then this assumption is

$$D \sim \mathcal{N}\kappa(\mu, \sum) \quad \text{.....(1)}$$

Stating that D has a multivariate normal distribution with mean vector μ and covariance matrix Σ. The multivariate normal distribution is a crude approximation of the true distribution of the data. It has been shown that this model works as well as other, more complicated models even in the face of categorical or mixed data[3,4]. Furthermore, transformations of many types of variables can often make this normality assumption more plausible (transformations include; ordinal, nominal, natural log, square root, and logistic). Essentially, the problem of imputation is that only D^{obs} is observed, not the entirety of D. In order to gain traction, the usual assumption in multiple imputation that the data are missing at random (MAR) is made. This assumption means that the pattern of missingness only depends on the observed data D^{obs} and not the unobserved data D^{mis}. Let M to be the missingness matrix, with cells *mij* = 1 if $dij \in D^{mis}$ and *mij* = 0 otherwise. Simply, *M* is a matrix that indicates whether or not a cell is missing in the data. With this, MAR assumption can be defined as:

$$\rho(M|D) = \rho(M|D^{obs}) \quad \text{.....(2)}$$

Importantly, MAR includes the case when missing values are created randomly, but it also includes many more sophisticated missingness models. When missingness is not dependent on the data at all, then data are missing completely at random (MCAR). Amelia requires both the multivariate normality and the MAR assumption (or the simpler special case of MCAR). Additionally, MAR assumption can be made more plausible by including additional variables

in the dataset *D* in the imputation dataset than just those eventually envisioned to be used in the analysis model.

2.3.1.3 **The Amelia Algorithm**

Multiple imputation is concerned with the complete-data parameters, θ = (μ, Σ). When writing down a model of the data, the observed data is actually D^{obs} and *M*, the missingness matrix. Thus, the likelihood of the observed data is $p(D^{obs}, M|\theta)$. Using the MAR assumption, this can be broken up as:

$$\rho(D^{obs}, M\big|\theta) = \rho(M|D^{obs})\rho(D^{obs}|\theta) \quad (3)$$

Because inference on the complete data parameters is important, the likelihood can be written as:

$$L(\theta\big|D^{obs}) \propto \rho(D^{obs}|\theta) \quad (4)$$

which can be rewritten using the law of iterated expectations as:

$$\rho(D^{obs}\big|\theta) = \int \rho(D|\theta)dD^{mis} \quad (5)$$

With this likelihood and a flat prior on θ, then the posterior is

$$\rho(\theta\big|D^{obs}) \propto \rho(D^{obs}\big|\theta) \quad (6)$$

The main computational difficulty in the analysis of incomplete data is taking draws from this posterior. The EM algorithm approach is computationally simplified to finding the mode of the posterior[5]dde (**Figure 2**). Amelia II's EMB algorithm combines the classic EM algorithm with a bootstrap approach to take draws from this posterior. For each draw, data are bootstrapped to simulate estimation uncertainty and then run the EM algorithm to find the mode of the posterior for the bootstrapped data, giving fundamental uncertainty as well[1]. Once posterior of the complete-data parameters is drawn, imputations are made by drawing values of D^{mis} from its distribution conditional on D^{obs} and the draws of θ, which is a linear regression with parameters that can be calculated directly from θ.

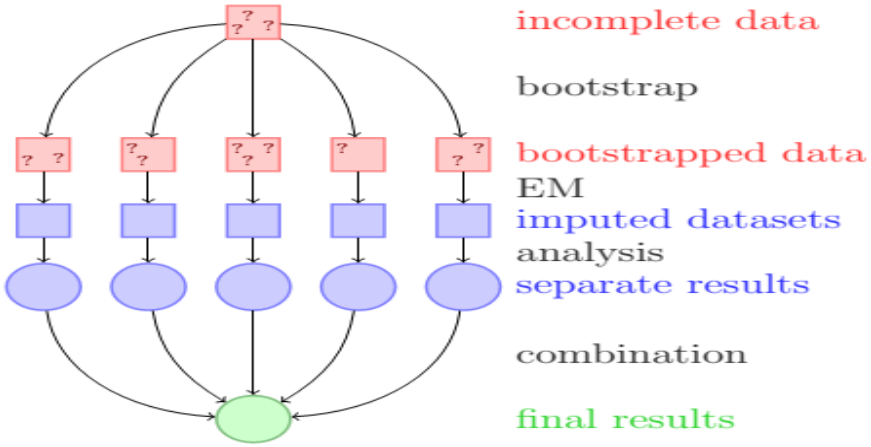

Figure 2: Schematic of Multiple Imputation Approach with the EMB Algorithm, Adapted from Honaker *et al* 2011

2.3.2 **Cox Proportional Hazards Model for Multiple Failure Events (Paper 1 and Paper 2)**

Survival analysis wherein time from exposure to outcome is analyzed, is considered a powerful and flexible approach[6]. Nevertheless, in studies where the outcome of interest occurs multiple times in one individual, this approach prohibits measurement of the exposure effect on repeated occurrences and thus becomes inefficient. Cox's proportional hazards model provides reliable estimates of survival times, as well as the relative risk associated with time-to-event occurrence[7]. As a semiparametric model, it does not have any constraints on distributional assumptions, making it more attractive than a fully parametric model. Nonetheless, survival time in the standard Cox model terminates at an event and discards any information past that point. A solution is to use multiple failure times instead whereby not only the first, but the event time within individuals are correlated[6]. However, the assumption of independence is violated using the standard Cox regression model, and this introduces statistical complications. To avoid error resulting from analysing correlated repeated events, the time to first event is commonly used for events that occur repeatedly.

Survival analysis typically examines the relationship of the survival distribution to covariates. Most commonly, this examination entails the specification of a linear-like model for the log hazard. The parametric model based on the exponential distribution is written as:

$$loghi(t) = \alpha + \beta 1\mathrm{X}i + \beta 2\mathrm{X}i2 + \cdots + \beta k\mathrm{X}ik \quad \text{.....(7)}$$

or equivalently,

$$hi(t) = \exp(\alpha + \beta 1\mathrm{X}i + \beta 2\mathrm{X}i2 + \cdots + \beta k\mathrm{X}ik)$$

that is, as a linear model for the log-hazard or as a multiplicative model for the hazard. Where i is the individual observation, x covariates, α log-baseline hazard, since log hi(t) = α [or hi(t) = e^{α}] when all of the covariates(x) are 0. In contrast, the Cox model leaves the baseline hazard function α(t) = log h0(t) unspecified:

$$loghi(t) = \alpha(t) + \beta 1\mathrm{X}i1 + \beta 2\mathrm{X}i2 + \cdots + \beta k\mathrm{X}ik \text{..............................(8)}$$

or equivalently,

$$hi(t) = h0(t)\exp(\alpha + \beta 1\mathrm{X}i1 + \beta 2\mathrm{X}i2 + \cdots + \beta k\mathrm{X}ik) \text{...................(9)}$$

The model is semi-parametric because whilst the baseline hazard takes any form, the covariates enter the model linearly. Consider, now, two observations i and i0 that differ in their x-values, with the corresponding linear predictors is independent of time t.

$$\eta i = \beta 1\mathrm{X}i1 + \beta 2\mathrm{X}i2 + \cdots + \beta k\mathrm{X}i\mathrm{k} \text{..(10)}$$

And

$$\eta i' = \beta 1\mathrm{X}i'1 + \beta 2\mathrm{X}i'2 + \cdots + \beta k\mathrm{X}i'\mathrm{k} \text{..(11)}$$

The hazard ratio for these two observations,

$$\frac{hi(t)}{hi(t)\prime} = \frac{h0(t)e^{\eta i}}{h0(t)e^{\eta i'}} \text{..(12)}$$

$$= \frac{e^{\eta i}}{e^{\eta i\prime}}$$

The time to virological failure was used as the endpoint for the multiple-failure models. Thus, a person with one virological failure at a point in time is considered the same as someone with more than one episode of virological failure at that point in time. In multiple-failure models, multiple observations per individual are used, depending on the number of events

(episodes) they have had during the study period. The Andersen-Gill approach was used to model repeated virological failure episodes for each person as separate observations, with the risk set not constrained by the number of events occurring within an individual, and makes a strong assumption of independence among multiple observations per person over time[8]. Nonetheless, a robust sandwich covariance matrix structure for the intra-individual correlation is used to overcome this assumption. Survival time for the Andersen-Gill model is calculated as the time since the beginning of the study to the first episode and the time between episodes thereafter. It uses a common baseline hazard function for all events and estimates a global parameter for the intervention. In this thesis Cox proportional-hazards regression model was fitted in R with the **coxph** function (located in the survival package)[9].

The Cox proportional hazard model was used in this thesis because it accommodates repeated events over time for a single measurement of the dependent variable (viral load) in an individual, allows for estimation of the effects of an intervention on the hazard of continued events[10,11]. Like time series, series hazard modelling relies upon the variation in activity for one unit. Unlike time series, it relies on the duration between activities instead of artificially aggregating the activities to multiple time periods. It only works with event data that record discrete incidents for one unit over time and include the exact date of all events so that the dependent variable can be calculated as the duration until the next event. A second criterion is that the events must occur with relative frequency to produce enough statistical power to efficiently estimate parameters.

2.4 **Pharmacometric Analyses**

The aim this section is to give a general outline below, with more specific issues being found in the appropriate sections of the following chapters.

2.4.1 **Population Pharmacokinetic Modelling (Paper 3 and Paper 4)**

The nonlinear mixed effects modelling implemented in the software NONMEM version7.3 (ICON Development Solutions, Ellicott City, MD, USA) was used for parameter estimation. A cluster of LINUX operating machines using Intel fortran compiler was used to operate NONMEM. Perl speaks NONMEM (PsN) (version 4.6.8) was used for executing estimations,

covariate modelling building and bootstrap procedures as well as calculations of the VPC. Concentration-time data was fitted using a two-step estimation method. In the first step, NONMEM simply runs a parametric method, first order conditional estimation with interaction (FOCEI) whereby the empirical Bayes estimates (EBEs) are computed. The EBEs are reserved as the support points of the nonparametric distribution. Once NONMEM has obtained support points, it proceeds to the second step where maximum likelihood estimates of the probability associated with each support point are obtained. From this joint probability, the marginal cumulative probability for each parameter is calculated. Model parameters were generally added stepwise starting from the base model. A decrease in the goodness-of-fit criterion, the objective function value (OFV) of at least 3.84 points ($p< 0.05$) when comparing 2 hierarchical models was regarded as statistically significant for the addition of a single model parameter.

Various model structures and features were evaluated: one- and two-compartment disposition; zero- and first-order absorption; and absorption with lag time and a series of transit compartments as proposed by Savic[12]. Absorption lag (ALAG) and Mean transit time (MTT) was used to describe the drug absorption delay, respectively. The transit absorption rate (ktr) was used to describe the transit absorption rate between different transit compartments. The calculation of ktr is indicated as follows:

$$\text{ktr} = \frac{n+1}{MTT} \quad \text{.....(13)}$$

where n is the number of transit compartments.

The IIV and IOV of the pharmacokinetic parameters of lopinavir and nevirapine were modelled using log-normal distribution i.e.:

$$Pij = TV(Pij) * EXP^{\eta iP} \quad \text{.....(14)}$$

where Pij is the jth pharmacokinetic parameter for the ith individual; TV(Pij) is the typical value of the jth population parameter, and ηij represents a random variable for the ith individual in the jth parameter(P) distributed with a mean of zero and variance of ωij. Using a

first-order approximation, the variability of the lognormal distributions is reported as % coefficient of variation (CV).

Different error structures describing of the residual unexplained variability (RUV) were tested: additive, proportional, combined error models. A combined proportional and additive error model (the difference between the observed and predicted concentrations) was described as follows:

$$Cij = Cij' * (1 + \epsilon 1) + \epsilon 2 \quad \text{.....(15)}$$

where Cij and Cij' are the jth observed and predicted blood concentrations for the ith individual, respectively. ε1 and ε2 are random variables distributed with a mean of zero and variances of σ1 and σ2, respectively.

For concentrations below the limit of quantification (BLQ), the Beal M5 method was used[13]. For this method, whenever a measurement was recorded as being BLQ in the dataset, the observation was replaced with BLQ/2 and the likelihood of the observation being below BLQ was maximised. The M5 method is particularly useful when there are very few observations e.g. one early observation and one late observation.

Covariates were added to the base model using a forward inclusion and backward elimination stepwise approach. Selection of a covariate was guided by a decrease in standard error of the parameters, reduction in IIV, IOV and RUV, and goodness-of-fit plots (GOFs). Available continuous covariates in this thesis included age, body weight and, sex (Male=0, Female=1) and concomitant TB therapy (No=0, Yes=1) as a categorical covariates.

Graphical diagnostics of the model fit was performed using Xpose (version 4.5.0) implemented in R. The graphical diagnostics included GOFs, individual plots, scatterplots, boxplots and VPC plots. GOFs include observed concentrations versus population predicted concentration (PRED), observed concentrations versus individual predicted concentration (IPRED), IWRES versus IPRED, and normalised prediction errors (NPDE) versus time as explained in the introduction. The regression lines in these plots represent equal predicted concentrations to observed ones and thus, indicate how close the predicted concentrations are close to the observed ones; the even and close distribution along the regression line would

be a good evidence for model predicted profiles. VPC was used diagnose model adequacy. The purpose of these plots is to compare if the 5th, 50th, 95th percentile of observed data (solid and dotted lines) are agreement with the 95% confidence interval of each percentile of simulated data (shaded areas) based on the model. This approach is based on simulation and is a more robust approach for model validation.

The minimum concentrations(C_{min}) and area under curves (AUC_{0-12}) were derived for each individual from the final models were calculated as follows:

$$\mathrm{AUC} = \frac{AMT*BIO}{CL} \quad \text{.....(16)}$$

$$\mathrm{Cmin} = \frac{(BIO*\frac{AMT}{2})*1/\tau}{((V-(\tau-k)*((1/(1-Exp(-K*12))-((\frac{1}{1}-Exp(-\tau*12))} \quad \text{.....(17)}$$

Where AUC is the area under curve from 0-12 hours, AMT is the dose, CL is the clearance. Cmin is the minimum concentration, V is the volume of the central compartment, K is the elimination rate constant, τ is the inter-dosing interval

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Author Contributions

Plasma Lopinavir Concentrations Predict Virological Failure in a Cohort of South African Children Initiating a Protease-Inhibitor-Based Regimen

[1]Retsilisitsoe R. Moholisa, [2]Michael Schomaker, [3]Louise Kuhn, [1]Sandra Meredith, [1]Lubbe Weisner, [4]Ashraaf Coovadia, [4]Renate Strehlua, [4]Leigh Martens, [5]Elaine J. Abrams, [1,6]Gary Maartens, [1,6]Helen McIlleron.

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town,[2]Centre for Infectious Diseases Epidemiology and Research, School of Public Health and Family, University of Cape Town,[3]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York,[4]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [5]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[6] Institute of Infectious Diseases and Molecular Medicine, University of Cape Town

RR Moholisa: Responsible for the data handling, processing and analysis under; Wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures

M Schomaker: Guided the analysis strategy and provided guidance from the intital draft of the manuscript especially the statistical analysis section and the discussion

L Kuhn, A Coovadia, R Strehlau, F Patel, F Pinillos and EJ Abrams: Responsible for the overall study design and collecting all the data.

S Castel and L Weisner: performed the laboratory analysis of measuring lopinavir concentrations

G Maartens and H McIlleron: Oversaw overall analysis strategy planning and mentoring, guided the writing of the manuscript from beginning to end

Chapter 3

Title: Plasma Lopinavir Concentrations Predict Virological Failure in a Cohort of South African Children Initiating a Protease-Inhibitor-Based Regimen

[1]Retsilisitsoe R. Moholisa, [2]Michael Schomaker, [3]Louise Kuhn, [1]Sandra Meredith, [1]Lubbe Weisner, [4]Ashraaf Coovadia, [4]Renate Strehlua, [4]Leigh Martens, [5]Elaine J. Abrams, [1,6]Gary Maartens, [1,6]Helen McIlleron.

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town,[2]Centre for Infectious Diseases Epidemiology and Research, School of Public Health and Family, University of Cape Town,[3]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York,[4]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [5]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[6] Institute of Infectious Diseases and Molecular Medicine, University of Cape Town

3.1 **Abstract**

3.1.1 **Background:** Poor adherence to antiretroviral therapy contributes to pharmacokinetic variability and is the major determinant of virologic failure. However, measuring treatment adherence is difficult, especially in children. We investigated the relationship between plasma lopinavir concentrations, pre-treatment characteristics and viral load >400 copies/mL.

3.1.2 **Methods:** Two-hundred and thirty seven HIV-infected children aged 4-42 months on lopinavir/ritonavir oral solution were studied prospectively and followed for up to 52 weeks. Viral load and lopinavir concentration were measured at clinic visits 12, 24, 36 and 52 weeks after starting treatment. Cox multiple failure events models were used to estimate the crude and adjusted effect of lopinavir concentrations on the hazard of viral load >400 copies/mL.

3.1.3 **Results:** The median (IQR) pre-treatment CD4% was 18.80 (12.70, 25.35) and 53% of children had a pre-treatment viral load higher than 750 000 copies/mL. The median (IQR) weight-for-age and height-for-age z-scores were -2.17 (–3.35, -2.84) and -3.34 (-4.57, -3.41) respectively. Median lopinavir concentrations were 8.00 (IQR: 4.11, 12.42) mg/L a median 3.50 (IQR: 2.67, 4.25) hours after the dose. The hazard of a viral load >400 copies/mL increased with lower lopinavir concentrations (crude and hazard ratios: 4% [95% CI: 2-7%] for each mg/L lopinavir; 2.3 [95% CI: 1.63-3.26] for lopinavir concentration <1.0 mg/L vs

≥1.0mg/L) and height-for-age z-scores in relationships that were preserved in adjusted models.

3.1.4 **Conclusions:** Low lopinavir concentrations (<1.0 mg/L) are associated with viremia in children. This measure could be used as a proxy for adherence and to determine which children are more likely to fail.

3.2 **Introduction**

Approximately 20-50% of children on antiretroviral therapy (ART) do not achieve viral suppression during first year of treatment[1–3] . Failure to achieve virological suppression may be due to the presence of HIV quasispecies resistant to antiretroviral drugs[4] or inadequate adherence, amongst other factors.

A first-line ART regimen, including ritonavir-boosted lopinavir (LPV/r), is recommended for children exposed to non-nucleoside reverse transcriptase inhibitors (NNRTI) used to prevent mother-to-child transmission (PMTCT) of HIV [5,6]. LPV/r has a high barrier for the development of resistance. However, the oral suspension of LPV/r has poor palatability[7,8], which may result in poor adherence. Most children with virologic failure on a first line LPV/r regimen do not have protease inhibitor (PI) mutations, suggesting that adherence rather than resistance is the cause of failure[9]. Establishing that adherence rather than resistance is the reason for virologic failure will reduce inappropriate ART switches and expenditure on resistance testing. In a small study of South African adults, low lopinavir concentrations were shown to be associated with virologic failure[10]. However, wide inter-individual variability is observed in the concentrations of lopinavir even after observed doses and few data exist on the relationship between lopinavir concentrations and virologic failure in children.

We measured lopinavir concentrations in plasma samples collected at the same time as viral load tests in a cohort of children initiated on a first line LPV/r-based ART regimen and followed them prospectively to determine whether plasma lopinavir concentrations measured in the first 52 weeks after starting therapy are associated with virological response.

Chapter 3

3.3 **Methods**

3.3.1 **Study Participants**

Plasma lopinavir concentrations were retrospectively analyzed in samples collected at clinic visits during the pre-randomization period from participants of the Neverest2 trial[11,12]. The Neverest2 trial was a randomized open-label clinical trial investigating treatment options for nevirapine exposed children who initiated PI-based ART when less than 24 months of age. Treatment responses during the pre-randomization phase have been previously described[13]. The study population included HIV infected children attending the Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa. Treatment eligibility criteria included WHO stage III or IV disease, $CD4^+$ lymphocyte percentage (CD4%) of less than 25% if younger than 12 months or less than 20% if older than 12 months, or recurrent (more than twice yearly) or prolonged (>4 weeks) admission to hospital for HIV related complications. Children being treated for opportunistic infections including tuberculosis were excluded from this analysis. All children received 230/57.5 mg/m^2 LPV/r (Kaletra® oral solution, Abott laboratories, USA), 1 mg/kg stavudine and 4 mg/kg lamivudine as oral solutions 12 hourly. At each visit, drug doses were adjusted according to growth. The caregivers of the children were provided with comprehensive counseling about treatment adherence. Treatment doses were typically taken in the morning prior to the clinic visit. The time of dosing was as reported by the caregiver and the time of sample collection was recorded**.**

Data collected included age at starting LPV/r therapy, sex, pre-treatment viral load (VL), pre-treatment CD4% and WHO stage. Pre-treatment weight-for-age z-score (WAZ) and height-for-age z-score (HAZ) were calculated using WHO software[14]. Blood samples were collected pre-treatment and at clinic visits 12, 24, 36 and 52 weeks after starting treatment, and at unscheduled clinic visits. Caregivers were requested to return medication bottles at each visit. The bottles were weighed, and the contents reconciled with the expected usage of each medication to determine the extent of adherence. Adherence was defined as returning less than 20% of the expected volume of any of the three drugs whereas returning more than 20% was defined as non-adherence. Children exited the pre-randomization phase of the study when they maintained viral suppression (VL ≤400 copies/mL) for two consecutive visits and

were followed as part of the post-randomization study (not analyzed here). Some children were retained for longer than the planned 52 weeks in an attempt to achieve viral suppression. These children were not eligible for randomization but were included in this analysis.

3.3.2 **Laboratory Methods**

Plasma HIV-1 RNA measurement (Roche Amplicor assay version 1.5; Roche, Branchburg, New Jersey, quantification range, 400-750 000 copies/mL) and $CD4^+$ cell counts were determined on pre-treatment samples. The ultrasensitive assay (quantification range 50-150 000 copies/mL) was used for VL determination post ART initiation.

Plasma lopinavir concentrations were assayed using validated liquid chromatography tandem mass spectrometry methods developed in the Division of Clinical Pharmacology, Cape Town, South Africa. An AB Sciex 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode. The assay was validated over the concentration range of 0.16-20 mg/L. Inter- and intra-day coefficients of variation were below 10% for all quality control concentrations. The laboratory participates in the International Inter-laboratory Control Program Therapeutic Drug Monitoring in HIV Infection (KKGT; Hague, Netherlands) and the AIDS Clinical Trial Group (ACTG), Pharmacology Quality Control Program.

3.3.3 **Statistical Analysis**

Children with a pre-treatment WAZ below -3 (i.e. >3 standard deviations below the average weight of comparable children in the reference population) were categorized as severely underweight; a WAZ from -3 to -2 was defined as moderate underweight and a WAZ higher than -2 was regarded as normal. HAZ below -3, from -3 to -2, and more than-2 were defined respectively as severe stunting, moderate stunting and normal. Pre-treatment immunity was categorized as low (CD4% less than 25%) or high (CD4% greater than or equal to 25%). Pre-treatment VL was expressed on a log scale and categorized as low or high for $\log_{10}$ VL greater than or less than or equal to 5 respectively. We defined WHO stages 1 and 2 as early disease and stages 3 and 4 as moderate disease. Lopinavir concentrations reported as below the limit of quantification (BLQ) were assigned a value of 0.08 mg/L (half the limit of quantification).

Pre-treatment characteristics were described with summary statistics (median, interquartile range (IQR) and proportions). Individual lopinavir concentrations during follow-up were presented by means of time-series plots and summary statistics. To account for missing data, 10 multiple imputations were conducted using the Amelia II software package[15] in R. We imputed 10 datasets for pre-treatment WAZ, pre-treatment HAZ, pre-treatment CD4%, WHO stage, $\log_{10}$ pre-treatment VL, adherence and lopinavir concentrations. The imputation model included all pre-treatment (WAZ, HAZ, CD4%, WHO stage, VL) and follow-up (adherence and lopinavir concentration) variables, as well as time (weeks on treatment) and viral load ($\leq$400, or >400 copies/mL). All results of our multivariate analysis are based on the imputed datasets and combined using Rubin's rules[16].

Cox proportional hazard regression modeling for multiple failure events was used to estimate the crude and adjusted hazard ratios of VL >400 copies/mL for the following pre-determined pre-treatment and follow-up variables: age at starting ART, pre-treatment WAZ and HAZ respectively, pre-treatment $\log_{10}$ VL, pre-treatment CD4%, pre-treatment WHO stage and lopinavir concentrations. Hazard ratios (HR) are reported together with the 95% confidence intervals (CI). In addition to the crude and adjusted hazard ratios, we also present the hazard ratios obtained for a model, with variables selected by Akaike's information criterion (AIC). We assumed the model to include $\log_{10}$ pre-treatment VL and adjusted the AIC with inverse probability weights (AICw) due to missing data[17].

We modeled the effect of lopinavir concentration on the hazard of VL >400 copies/mL in the adjusted models as a dichotomous variable based on cut-offs of 1 mg/L and 4 mg/L. Additionally, we modeled the effect of lopinavir concentrations on VL >400 copies/mL non-linearly via penalized splines, representing this in a figure. Finally we compared two adjusted models by means of AICw: the first model included all pre-treatment variables and lopinavir concentrations at each visit whereas and the second model also included all pre-treatment variables and percentage adherence at each visit. Data was analyzed using the statistical software package R[18].

3.4 **Results**

3.4.1 **Study Population**

A total of 322 children exposed to nevirapine for PMTCT who met clinical and immunological criteria were enrolled into the study. All participants were initiated on LPV/r-based regimen. Thirty-eight (12%) children died and 40 (12%) were lost to follow-up before samples were collected for lopinavir concentration measurement. Four (2%) children on TB treatment and 3 (1%) on full-dose ritonavir (previously used instead of LPV/r for treatment of children less than six months of age) were excluded from this analysis. The sample size for analysis was thus reduced to 237 children. **Table 1** shows the pre-treatment characteristics of the study population.

3.4.2 **Plasma lopinavir Concentrations**

A total of 487 plasma samples from 237 children with a median number of 2 samples per child were analyzed to determine plasma concentrations of lopinavir. The median (IQR) sampling time was 3.50 (2.67-4.25) h after the dosing time reported by the caregiver, and 12% of the samples were BLQ**. Figure 1** presents plasma lopinavir concentrations of all children from 10 to 80 weeks of the study. We determined the population median lopinavir concentrations at each scheduled visit and found it to be similar for all visits. Sampling times after the dose were similar for samples <1.0 mg/L vs. >1.0 mg/L (median 3.37 [IQR: 2.60-4.42] h vs. 3.50 [2.67-4.25] h) and for samples <4.0 mg/L vs. >4.0 mg/L (median 3.33 [2.58-4.17] h vs. 3.50 [2.75-4.25] h). The percentage of samples below 1mg/L and 4mg/L at each clinic visit are shown in **Figure 1** and **Table 2**.

3.4.3 **Predictors of Viral Load >400 copies/mL**

We performed Cox proportional hazards regression analysis to evaluate the risks for VL >400 copies/mL. The results showed reduced risk of VL >400 copies/mL for increased lopinavir concentrations (HR=0.96 [95%Cl: 0.93-0.98] for each 1 mg/L, p=0.002), and increased risk for pre-treatment HAZ (HR=2.24 [95%Cl: 1.17-4.28] for moderate stunting, p=0.015; and HR=2.92 [95%Cl: 1.67-5.03] for severe stunting, p=0.0001 relative to those with normal HAZ). After adjustment for other covariates both lopinavir concentrations and pre-treatment HAZ remained significant (**Table 3**). Utilizing model selection with AICw yields a model with similar estimated hazard ratios for low lopinavir concentrations (HR= 0.96 [95%CI: 0.93-0.99], p=0.005) as well as moderate (HR=2.19 [95%Cl: 1.19-4.05]) and severe stunting (HR= 0.45

[95%CI: 0.24-0.84], p=0.009), confirming the stability of the adjusted model. A high $\log_{10}$ pre-treatment VL was associated with hazard ratios >1 although these did not reach significance in either the crude (HR= 1.62 [95%CI: 0.77-3.34], p=0.205) or adjusted (HR= 1.56 [95%CI: 0.91-3.54], p=0.269) models. Due to the high percentage (38%) of missing data, adherence was not included in the primary analysis. However, in a sensitivity analysis we compared two adjusted models using AICw, where the first model included lopinavir concentrations and the second model included recorded adherence. The results revealed that the AICw favors the model including lopinavir concentrations (AICw= 1220.7) compared with the model including adcherence (AICw= 1228.4).

We also fitted separate models where lopinavir concentrations were dichotomized based on the cut-offs of 1.0 mg/L (**Table 4**) and 4.0 mg/L (**Table 5**), respectively. The results showed that children with lopinavir concentrations of less than 1.0 mg/L or 4.0 mg/L (crude HR=2.3[95% CI: 1.63-3.26]; adjusted HR=1.74[95% CI: 1.36-2.23]) have an increased hazard of VL >400 copies/mL in both crude and adjusted models. Similarly we showed that moderate and severe stunting were significantly associated with increased hazards of VL>400 copies/mL in both crude and adjusted models. We compared the two models by means of AICw and showed that the model with 1.0 mg/L cut-off (AICw=1326.34) described the data better than the model with 4.0 mg/L cut-off (AICw=1331.03).

3.4.4 **Non-linear Effect of Lopinavir Concentrations on the Risk of Viremia**

We modeled the non-linear effect of lopinavir concentrations on the hazard of VL >400 copies/mL and failed to show any distinct threshold. Nonetheless, we showed that increasing lopinavir concentrations were associated with reduced hazard of VL >400 copies/mL across the full range of LPV concentrations studied (**Figure 2**).

3.5 **Discussion**

We used Cox regression models to describe the association of lopinavir concentrations during the first year of treatment and pre-treatment characteristics with the hazard of viraemia (VL >400 copies/mL) in a cohort of young, nevirapine-exposed South African children initiated on a PI-based regimen. Our data suggests that with increasing lopinavir concentrations the hazard of VL > 400 copies/mL is reduced. We also found a significant association with

moderate (HAZ -2 to -3) and severe (HAZ <-3) pre-treatment stunting with a greater chance of VL >400 copies/mL, while no association was found for the other pre-treatment characteristics, including WAZ.

Using the Cox regression models, we found that children with lopinavir concentrations below the cut-offs of 1.0 mg/L or 4.0 mg/L have an increased hazard of virologic failure, but the effect was stronger at the lower threshold. Moreover, we determined the non-linear effect of lopinavir concentrations on the hazard of VL >400 copies/mL (**Figure 2**) and showed that decreased concentrations correlated with increased risk of virological failure across the full range of lopinavir concentrations studied. This suggests that in addition to adherence related changes in drug exposure, individual variability in lopinavir concentrations may be important for therapeutic outcomes. However, high lopinavir concentrations would likely increase the risk of toxicity. Low lopinavir concentrations, especially below 1.0 mg/L, are likely to reflect poor adherence and could provide an objective measure of non-adherence. ART adherence is difficult to assess in paediatric patients as there is considerable social pressure for caregivers to report complete adherence and measuring returned medication is difficult, when compared to pill counts which can be done for adults. An objective adherence measure would be useful in children failing a LPV/r-based regimen as PI mutations are rarely found, provided that there was no prior exposure to other protease inhibitors[9]. Antiretroviral resistance testing, which is expensive, could be limited to children with lopinavir concentrations that are above 1.0 mg/L, as has been suggested in pilot study in adults[10].

HIV infection adversely affects growth. Prior to ART, studies demonstrated that perinatally acquired HIV is associated with poor growth outcome marked by high mortality, stunting and wasting. In our data, we found a significant association with pre-treatment HAZ, but not with WAZ. This suggests that children who are stunted have a higher hazard of virologic failure. Our data is consistent with other reports in the literature with regard to the effect of stunting on virologic failure[19].

Our study has several limitations that are worth highlighting. Firstly, in our study, there was missing data, which we dealt with by multiple imputation. This approach has been shown to be superior to complete case analysis in which only subjects who do not have missing values are analyzed[16]. If data are missing at random and thus the probability for value to be missing depends only on observed quantities, then no bias is introduced. We found the missing at

random assumption to be reasonable in our study given that the missing data related mainly to data not being measured, or due to insufficient sample volume or a lost sample. Secondly, we did not observe the time of morning dose prior to sampling for lopinavir concentration. Hence our analysis did not include adjustment of lopinavir concentrations for the time after the dose. Nevertheless, we have shown that the lopinavir concentrations in samples taken 0.42-9.00 hours after the last dose predict VL >400 copies/mL, which would allow laboratories to do lopinavir assays to decide whether to proceed to the much more expensive genotypic resistance tests. To exclude potential bias due to inclusion of 1) early viral load data which (if >400 copies/mL) may indicate failure to suppress at that time point, rather than virological failure, and, 2) children followed up to more than 52 weeks, we conducted two sensitivity analyses (not shown), the first excluding visits before 24 weeks, and the second excluding visits after the planned follow up period. Our findings were not substantially altered in either analysis.

The use of TDM is complicated by insufficient knowledge of the target plasma concentrations particularly in children on ART in whom the optimal drug concentrations have not been clearly defined[20]. In this study, we used reference values for plasma lopinavir concentrations derived largely from adult studies. The recommended minimum lopinavir trough concentrations are 1.0 mg/L in treatment naïve patients and 4.0 mg/L in treatment experienced patients[21]. We found that lopinavir concentrations 0.42-9.00 hours after the last dose (analogous to the time after dose for samples collected at a typical clinic visit when the child has taken his/her ART in the morning) predicted the risk of viraemia. The lopinavir concentrations taken during the clinic visits were in keeping with those described in other studies amongst children of a similar age[22,23].

Strengths of the study include a relatively large sample size and the cohort design, which provides a higher level of evidence for the relationship between explanatory and outcome variables compared to studies with a cross-sectional or case-control design. Another strength of the study was repeated plasma drug concentration measurement, at each follow-up visit, which made it possible to assess each child's lopinavir concentration profile and its correlation with treatment success.

In conclusion lopinavir concentrations were associated with the hazard of VL >400 copies/mL. Low lopinavir concentrations could be used as a proxy for treatment non-adherence to guide determination of eligibility for resistance testing. Furthermore, our findings provide preliminary data to support developing optimal target concentrations of lopinavir required for viral suppression in children, which could be used as part of therapeutic drug monitoring to optimize the efficacy of ART regimens in children. Moderate and severe stunting were also associated with virological response to LPV/r-based ART suggesting that the reasons for poor responses in stunted children should be investigated further and that this group may be targeted for appropriate interventions.

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3.7 **References**

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Table 1: Characteristics of the 237 HIV-infected children initiating LPV/r-based antiretroviral therapy (ART) and included in this analysis.

Variable	Median	IQR	Missing Data (%)
Age Start ART (Months)	10	5-14	0
Pre-treatment VL(copies/mL)			13
<100 000	20(8%)		
100 000-750 000	61(26%)		
>750 000	125(53%)		
Pre-treatment WAZ	-2.17	-3.35 to -1.21	11
Pre-treatment HAZ	-3.34	-4.57 to -3.41	12
Pre-treatment CD4%	18.80	12.70-25.35	5
Sex			
Male	109(46%)		0
Female	128(54%)		
WHO Stage			
Early	42(22%)		18
Moderate	147(78%)		

Figure 1: Lopinavir concentrations at scheduled visits of all children in the study. The individual lines connect each child

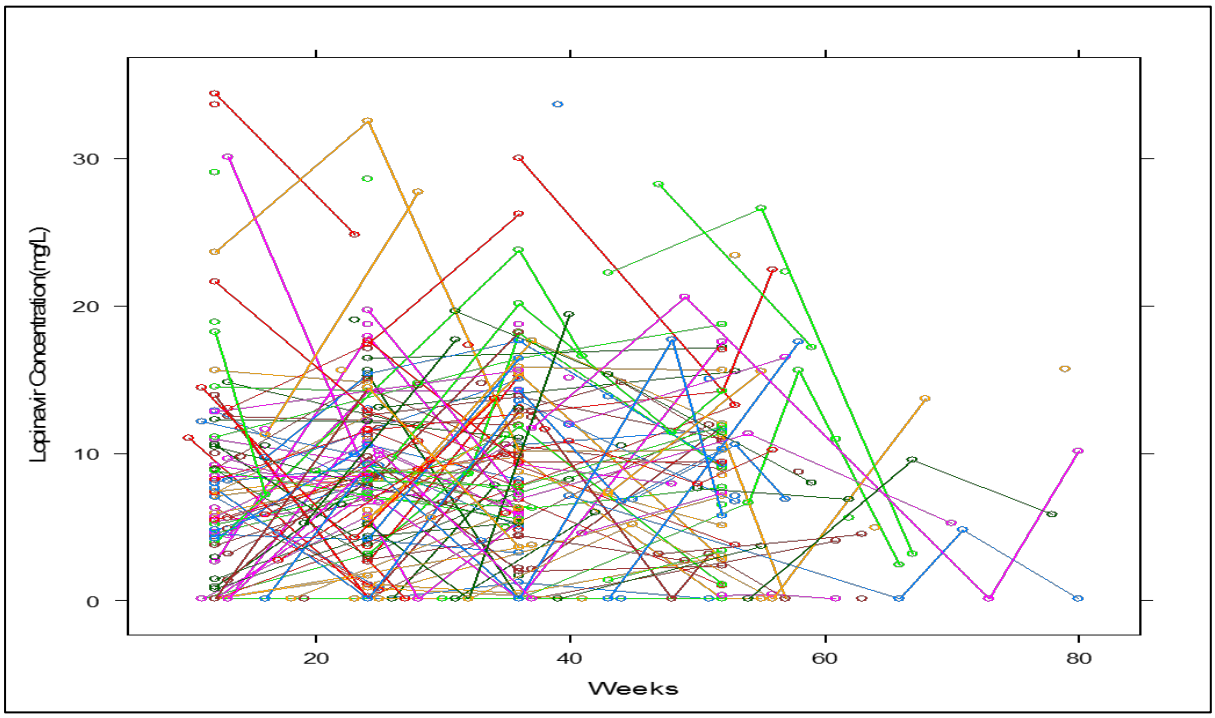

Table 2: Lopinavir concentrations, time of sampling, body weight and lopinavir dose, by study week

Weeks	12(n=74)	24(n=132)	36(n=128)	52(n=100)	52+(n=53)
LPV, mg/L	7.03(3.36-10.95;0.08-34.40)	8.21(4.18-12.20;0.08-32.50)	8.57(4.97-13.00;0.08-30.00)	8.22(3.12-12.00;0.08-33.60)	6.76(2.59-13.20; 0.08-26.60)
%Samples<1mg/L, n(%)	13 (18)	16 (12)	18 (14)	16 (16)	16 (30)
%Samples<4mg/L, n(%)	17 (23%)	16 (17)	23(18)	19(19)	16(30)
Time after dose, h	3.58(3.00-4.17; 1.25-9.00)	3.50(2.50-4.17;0.50-6.41)	3.41(2.58-4.25;1.25-6.91)	3.50(2.75-4.42;0.42-6.08)	3.33(2.37-4.23; 0.58-6.67)
Total Body Weight, kg	8.38(7.49–9.50;4.20–12.50)	9.25(8.04–10.25;4.03–15.44)	9.80(8.62–10.80;5.85–16.40)	10.10(8.90–11.00; 5.52–16.40)	10.60(9.75–11.93; 6.30–15.55)
Dose [mg/m²]	225.24(220.17-232.75; 135.48–287.89)	221.83(213.20–229.26; 174.41–442.48)	224.04(216.72–231.26; 195.95–355.40)	224.72(216.67–231.73; 192.02–321.08)	224.89(221.84–230.51;204.46–302.30)

Data are in Median (IQR; Range), unless otherwise indicated. LPV, Lopinavir

Table3: Cox Proportional Hazards regression analysis for failure to achieve virological suppression for crude and the adjusted models after multiple imputation of all covariates.

Characteristic	**Crude Model**			**Adjusted Model**			**AICw**		
	HR	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**
lopinavir (for each 1.0 mg/L lopinavir)	0.96	0.93-0.98	0.005	0.96	0.94-0.99	0.019	0.96	0.93-0.99	0.005
Age (>9 months.)	**Reference**			**Reference**					
Age (<9 months)	1.21	0.54-2.73	0.64	1.24	0.56-2.74	0.59			
Pre-treatment WAZ(normal)	**Reference**			**Reference**					
Pre-treatment WAZ(Moderate)	1.06	0.75-1.48	0.72	0.91	0.61-1.33	0.63			
WAZ(severe)	1.87	0.61-1.25	0.45	1.15	0.77-1.72	0.49			
Pre-treatment HAZ(normal)	**Reference**			**Reference**			**Reference**		
Pre-treatment HAZ(Moderate**)**	2.24	1.17-4.28	0.015	2.20	1.18-4.09	0.012	2.19	1.19-4.05	0.011
Pre-treatment HAZ(Severe)	2.92	1.69-5.03	0.0001	2.83	1.66-4.82	0.0001	2.83	1.67-4.78	0.0001
Pre-treatment Log_{10} VL <5	**Reference**			**Reference**			**Reference**		
Pre-treatment Log_{10} VL >5	1.62	0.77-3.44	0.21	1.56	0.91-3.54	0.27	1.58	0.72-3.44	0.252
Pre-treatment CD4%≥25	**Reference**			**Reference**					
Pre-treatment CD4%<25	1.02	0.68-1.53	0.91	1.09	0.73-1.65	0.65			
WHO Stage(Early)	**Reference**			**Reference**					
WHO Stage(Moderate)	1.22	0.70-2.13	0.47	1.21	0.69-2.01	0.49			

Table 4: Cox Proportional Hazards regression analysis for failure to achieve virological suppression for the crude model and the adjusted model after multiple imputation of all covariates using lopinavir with a cut-off of 1mg/L

Characteristic	Crude Model			Adjusted Model			AICw		
	HR	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**
lopinavir >1.0 mg/L	**Reference**			**Reference**			**Reference**		
lopinavir <1.0 mg/L	2.3	1.63-3.26	0.0001	2.11	1.62-2.75	0.001	2.14	1.52-3.02	0.0001
Age (>9 months.)	**Reference**			**Reference**					
Age (<9 months.)	1.21	0.53-2.76	0.76	1.23	0.60-2.73	0.50			
Pre-treatment WAZ(normal)	**Reference**			**Reference**					
Pre-treatment WAZ(Moderate)	1.06	0.75-1.48	0.72	0.91	0.61-1.33	0.63			
Pre-treatment WAZ(severe)	1.87	0.61-1.25	0.45	1.15	0.77-1.72	0.49			
Pre-treatment HAZ(normal)	**Reference**			**Reference**			**Reference**		
Pre-treatment HAZ(Moderate**)**	2.24	1.17-4.28	0.015	2.20	1.18-4.09	0.012	2.32	1.24-4.37	0.009
Pre-treatment HAZ(severe)	2.92	1.69-5.03	0.0001	2.83	1.66-4.82	0.0001	2.90	1.67-5.05	0.0001
Pre-treatment Log_{10} VL <5	**Reference**			**Reference**			**Reference**		
Pre-treatment Log_{10} VL >5	1.67	0.79-3.50	0.17	1.69	0.81-3.53	0.16	1.58	0.72-3.44	0.252
Pre-treatment CD4%≥25	**Reference**			**Reference**					
Pre-treatment CD4%<25	1.09	0.69-1.72	0.71	1.15	0.74-1.77	0.53			
WHO Stage(Early)	**Reference**			**Reference**					
WHO Stage(Moderate)	1.26	0.75-2.11	0.38	1.25	0.73-2.13	0.49			

Table 5: Cox Proportional Hazards regression analysis for failure to achieve virological suppression for the crude model and the adjusted model after multiple imputation of all0 covariates using lopinavir with a cut-off of 4mg/L

Characteristic	Crude Model			Adjusted Model			AICw		
	HR	**95%CI**	**P Value**	**HR**	**95%CI**	**P Value**	**HR**	**95%CI**	**P Value**
lopinavir >4.0 mg/L	**Reference**			**Reference**			**Reference**		
lopinavir <4.0 mg/L	2.3	1.63-3.26	0.0001	1.74	1.36-2.23	0.019	1.77	1.29-2.43	0.0001
Age (>9months.)	**Reference**			**Reference**					
Age (<9months.)	1.21	0.53-2.76	0.76	1.23	0.60-2.73	0.50			
Pre-treatment WAZ(normal)	**Reference**			**Reference**					
Pre-treatment WAZ(Moderate)	1.06	0.75-1.48	0.72	0.91	0.61-1.33	0.63			
Pre-treatment WAZ(Severe)	1.87	0.61-1.25	0.45	1.15	0.77-1.72	0.49			
Pre-treatment HAZ(normal)	**Reference**			**Reference**			**Reference**		
Pre-treatment HAZ(Moderate**)**	2.24	1.17-4.28	0.015	2.20	1.18-4.09	0.012	2.19	1.19-4.21	0.012
Pre-treatment HAZ(Severe)	2.92	1.69-5.03	0.0001	2.83	1.66-4.82	0.0001	2.83	1.64-4.87	0.0001
Pre-treatment Log_{10} VL <5	**Reference**			**Reference**			**Reference**		
Pre-treatment Log_{10} VL >5	1.67	0.79-3.50	0.17	1.79	0.88-3.63	0.21	1.58	0.72-3.44	0.252
Pre-treatment CD4%≥25	**Reference**			**Reference**					
Pre-treatment CD4%<25	1.02	0.68-1.53	0.906	1.14	0.73-1.78	0.46			
WHO Stage(Early)	**Reference**			**Reference**					
WHO Stage(Moderate)	1.26	0.75-2.11	0.38	1.22	0.72-2.07	0.48			

Author Contributions

Title "Effect of Lopinavir and Nevirapine Concentrations on Viral Outcomes in Protease Inhibitor-experienced HIV-infected Children".

Moholisa RR, Schomaker M, Kuhn L, Castel S, Wiesner L, Coovadia A, Strehlau R, Patel F, Pinillos F, Abrams EJ, Maartens G, McIlleron H. Pediatr Infect Dis J. 2016;**35**:e378-e383."

RR Moholisa: Responsible for the data handling, processing and analysis under; Wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures

M Schomaker: Guided the analysis strategy and provided guidance from the intital draft of the manuscript especially the statistical analysis section and the discussion

A Coovadia, R Strehlau, F Patel, F Pinillos and EJ Abrams: Responsible for the overall study design and collecting all the data.

S Castel and L Weisner: performed the laboratory analysis of measuring lopinavir and nevirapine concentrations

G Maartens and H McIlleron: Oversaw overall analysis strategy planning and mentoring, guided the writing of the manuscript from beginning to end

Chapter 4

Title: Effect of Lopinavir and Nevirapine Concentrations on Viral Outcomes in Protease Inhibitor-Experienced HIV-Infected Children

Authors: [1]Retsilisitsoe R. Moholisa, [2]Michael Schomaker, [3]Louise Kuhn, [1]Sandra Castel, [1]Lubbe Wiesner, [4]Ashraf Coovadia, [4]Renate Strehlau, [4]Faeezah Patel, [4]Francoise Pinillos, [5]Elaine J. Abrams, [1,6]Gary Maartens, [1,6]Helen McIlleron.

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town,[2]Centre for Infectious Diseases Epidemiology and Research, School of Public Health and Family Medicine, University of Cape Town,[3]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York,[4]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand ,[5]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[6] Institute of Infectious Diseases and Molecular Medicine, University of Cape Town

4.1 **Abstract**

4.1.1 **Background:** Adequate exposure to antiretroviral drugs is necessary to achieve and sustain viral suppression. However, the target antiretroviral concentrations associated with long term viral suppression have not been adequately defined in children.

4.1.2 **Aim:** We assessed the relationship between plasma lopinavir or nevirapine concentrations and the risk of subsequent viremia in children initially suppressed on antiretroviral therapy.

4.1.3 **Methods:** After an induction phase of antiretroviral treatment, 195 children with viral suppression (viral load ≤400 copies/mL) were randomized to remain on a lopinavir/ritonavir-based regimen or to switch to a nevirapine-based regimen (together with lamivudine and stavudine). Viral load and lopinavir or nevirapine concentrations were measured at clinic visits

4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post-randomization. Cox multiple failure event models were used to estimate the effects of drug concentrations on the hazard of viremia (viral load >50 copies/mL)

4.1.4 **Results:** At randomization, the median (IQR) age, $CD4^+$ T-Lymphocyte percentage, weight-for-age and weight-for-height z-scores were 19 (16-24) months, 29 (23-37) %, -0.6(-1.3 to 0.2) and -3.2 (-4.1 to -2.1) respectively. The proportion of children with viral load 51-400 copies/mL at randomization was 43%. The hazard of subsequent viremia during follow-up was increased for lopinavir concentrations <1.0 mg/L vs ≥1.0 mg/L (adjusted hazard ratio 0.62 [95% CI, 0.40-0.94]) and for children with viral loads 51-400 copies/mL at randomization. Nevirapine concentrations were not significantly associated with subsequent viremia.

4.1.5 **Conclusion:** Plasma lopinavir concentrations predicted viral outcomes in children receiving lopinavir-based antiretroviral therapy. Our findings support a minimum target concentration of ≥1.0 mg/L of lopinavir to ensure sustained viral suppression.

4.2 **Introduction**

Combination antiretroviral therapy (ART) has significantly improved survival and quality of life of HIV infected children worldwide[1]. The maintenance of adequate drug exposures is necessary to prevent viral resistance and ART failure, and high levels of adherence are critical for maintaining viral suppression[2,3].

Current ART treatment guidelines for HIV-infected children recommend combination therapy of dual nucleoside analogue reverse transcriptase inhibitor (NRTI) combined with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a boosted protease inhibitor (PI).

Nevirapine (NVP) has a low barrier to develop viral resistance. Suboptimal NVP concentrations have been shown to select for the development of drug resistance mutations[4]. An ART regimen including the co-formulated PI lopinavir/ritonavir (LPV/r) has been shown to be superior to a NVP-based regimen for treating infants exposed to NVP perinatally[5]. LPV/r has a high barrier for resistance, however, the oral suspension of LPV/r has poor palatability which may result in poor treatment adherence[6,7].

Based largely on studies in adults, minimum trough concentrations of 1.0 mg/L and 3.0 mg/L are recommended for LPV and NVP, respectively[8,9]. Therapeutic drug monitoring is recommended by some guidelines for children on LPV or NVP[10], as the plasma concentrations of both drugs are highly variable even after observed doses. However few data exist on the relationship between plasma drug concentrations of LPV or NVP, and viral response in children.

We measured serial LPV and NVP concentrations from stored plasma in children enrolled in a clinical trial[7,10]. Once they had achieved viral suppression (<400 copies/mL), children were randomized to continue LPV/r or to switch to NVP. The purpose of our analysis is to evaluate the plasma LPV and NVP concentrations associated with maintenance of viral suppression.

4.3 **Methods**

4.3.1 **Study Participants**

Plasma LPV and NVP concentrations were retrospectively analyzed in samples collected from participants of the Neverest2 trial at clinic visits during the post-randomization period[7,11]. The Neverest2 trial was a randomized open-label clinical trial investigating treatment options for NVP exposed children who initiated PI-based ART when less than 24 months of age. HIV infected children attending the Rahima Moosa Mother and Child Hospital, Johannesburg,

South Africa, who achieved a viral load (VL) ≤400 copies/mL for at least 2 consecutive visits on LPV-based ART were eligible for randomization. Once criteria for randomization were met, the children were randomized 1:1 to continue their LPV/r regimen or switch LPV/r to NVP. NVP (Viramune® oral solution, Boehringer Ingelheim) was introduced at 120 mg/m^2 once daily for the first 2 weeks and thereafter at 200mg/m^2 12 hourly. Children randomized to continue LPV/r (Kaletra® oral solution, Abbott Laboratories, USA), received doses of 230 mg/m^2 12 hourly. Lamivudine and stavudine were used as the other two drugs. Doses were adjusted according to the growth of the children at each visit. Both NVP and LPV groups received additional adherence counselling, including specific instructions concerning the lead-in schedule and possible adverse effects for children switching to NVP.

Data collected at randomization included age, sex, VL and CD4$^+$ T lymphocyte percentage (CD4%). Weight-for-age z-score (WAZ) and height-for-age z-score (HAZ) at randomization were calculated using WHO software[7]. In both groups, blood samples were collected at randomization and at 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post-randomisation and at unscheduled clinic visits. Blood samples collected at each visit post-randomization were used to measure VL (post-randomization viremia was defined as VL >50 copies/mL), and LPV or NVP concentrations. The time of blood sample collection was documented, as was the time of the morning dose of antiretrovirals, as reported by the caregiver. Caregivers were requested to return medication bottles at each visit. The bottles were weighed and the contents reconciled with the expected usage of each medication to determine the degree of adherence. Adherence was defined as returning less than 20% of the expected volume of any of the three drugs, whereas returning more than 20% was defined as non-adherence. In children who were diagnosed with TB after randomization, concomitant TB treatment was

recorded at each visit. After 76 weeks, all children were enrolled in an extended follow-up period during which clinical care was provided and monitored.

4.3.2 **Laboratory Methods**

Plasma LPV and NVP concentrations were assayed using validated liquid chromatography tandem mass spectrometry methods developed in the Division of Clinical Pharmacology, Cape Town, South Africa. An AB Sciex 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode. The validated concentration range for the LPV assay was 0.16 mg/L to 20 mg/L and that for NVP was 0.1 mg/L to 15 mg/L. Inter- and intra-day coefficients of variation were below 10% for all quality control concentrations. The laboratory, at which the concentrations were assayed, participates in the International Inter-laboratory Pharmacology Quality Control Program, the AIDS Clinical Trial Group (ACTG).

4.3.3 **Statistical Analysis**

Children with a WAZ > -2 SD below the norm, were categorized as underweight. HAZ < -2 was regarded as indicating stunting. Immunity at randomization was categorized as low (CD4% less than 25%) or high (CD4% greater than or equal to 25%) whilst VL was categorized as low level viremia (VL 51-400 copies/mL) or suppressed (VL ≤50 copies/mL). TB treatment was a dichotomous variable (present or absent at each post-randomization visit). LPV and NVP concentrations below the limit of quantification (BLQ) were assigned values of 0.08 and 0.05 mg/L respectively (half the limit of quantification). Characteristics at randomization were described with summary statistics (median, interquartile range (IQR) and proportions).

Cox proportional hazard regression for multiple failure events was used to estimate the crude and adjusted hazard ratios for viremia (VL >50 copies/mL) associated with the following pre-determined variables: CD4%, age, WAZ, HAZ and VL at randomization, TB treatment post-

randomization, and LPV or NVP concentration at the current visit. In secondary analyses, we determined the crude and adjusted hazards of viremia associated with LPV or NVP concentration at the previous visit, and the crude and adjusted hazards of viremia associated with the average of two drug concentrations, derived from the current and prior visits, respectively. To account for missing CD4% and adherence data, as well as LPV and NVP concentrations, 10 multiple imputations were conducted using the Amelia II software package in R[13]. The imputation model included variables for WAZ, HAZ, VL and CD4% at the time of randomization as well as repeated measures of adherence, TB treatment, and NVP and LPV concentrations during follow-up, along with the time (weeks on treatment). All results in our crude and adjusted analyses are based on the imputed datasets and combined using Rubin's rules[14]. Hazard ratios (HR) are reported together with the 95% confidence intervals (CI). Akaike information criterion (AIC) for each imputed dataset was used to compare all the adjusted models.

We modelled the effect of LPV and NVP on the hazard not only linearly but also using binary cut-offs for drug concentrations. We determined Mixed effects logistic regression models were used to describe the hazard of viremia (VL >50 copies/mL) for concentrations below each cut-off value respectively compared to higher concentrations. Multivariate models were used to adjust for the time post-randomization (in weeks), and clustering by individual was used. We compared LPV cut-offs [0.5, 1.0, 2.0, 3.0, 4.0, 5.0 & 6.0 mg/L] and NVP cut-offs [2.0, 3.0, 4.0, 5.0, 6.0, 7.0 & 9.0 mg/L] by means of generalized cross validation (GCV)[15].

To graphically display the non-linear effects of LPV and NVP concentrations on the hazard of viremia we used penalized splines[16].

Finally, we compared two adjusted models by means of AIC in each imputed dataset; the first model included all variables at randomization and LPV or NVP concentrations at each visit,

whereas and the second model included all variables at randomization and percentage adherence at each visit in both the LPV and the NVP groups. Data was analysed using the statistical software package R[17].

4.4 **Results**

4.4.1 **Study Population**

A total of 195 children from the initial 322 children were enrolled in the post-randomization phase of the NEVEREST2 study. Of the 195 children, 96 were switched to NVP whilst 99 remained on a LPV regimen. **Table 1** shows the characteristics of children in both groups in the study at randomization, and indicates missing data. The characteristics in the two groups were similar.

4.4.2 **Plasma Lopinavir and Nevirapine Concentrations**

For the LPV group, a total of 1134 plasma samples from 99 children with a median of 8 samples per child were collected from 3 weeks to 209 weeks post-randomization (**Supplementary Figure 1A**). The blood was sampled a median 3.00 (IQR 2.00-3.91) hours after the reported dose of antiretrovirals, and 7% of the samples were BLQ with 6% missing. The median population LPV concentrations determined at 24, 50, 76, and 100 and 150 weeks, respectively, and were similar across all visits (**Table 2**).

For the NVP group, a total of 764 samples plasma samples from 96 children, with a median of 6 samples per child, were collected from 3 to 196 weeks post-randomization. For the NVP group, a total of 764 samples plasma samples from 96 children, with a median of 6 samples per child, were collected from 3 to 196 weeks post-randomization. The median time of sampling was 3.00 (IQR 2.17-3.92) hours after the reported dose, and 1% of samples were BLQ and 9% were missing. As with the LPV concentrations, the median NVP concentrations

were similar across all visits (**Table 2**). Five children had exceptionally high NVP concentrations i.e. NVP concentrations consistently above 40 mg/L for an average of 2 visits (**Supplementary Figure 1B**). The data for these five children were excluded in subsequent analyses. Two of these children had TB post-randomization and were switched to another ART regimen. One child who experienced toxicity and one child with viral failure were withdrawn, and one child was lost to follow-up.

4.4.3 **Predictors of Viremia (Viral load >50 copies/mL) in the LPV Group**

As shown in **Table 3**, the risk of viremia (VL >50 copies/mL) was estimated to be reduced by 5% for each 1.0 mg/L increment in the current visit LPV concentration (HR: 0.95 [95% CI 0.92, 0.98]; P<0.01). Children with low level viremia (VL 51-400 copies/mL) at the time of randomization had a 2.62-fold increased risk of viremia (HR: 2.62 [95% CI 1.62, 4.24]; P<0.01) (**Table 3)** compared to children with VL <50 copies/mL. After adjusting for other covariates both LPV concentrations (HR: 0.96 [95% CI 0.94, 0.99]; P=0.01) and VL at randomization (HR: 2.66 [95% CI 1.68, 4.22]; P <0.01) remained significant predictors of post-randomization viremia. We found the average of two LPV concentrations (at the current visit and previous visit, respectively) were predictive of viremia in the crude (HR: 0.94 [95% CI 0.91, 0.98]; P<0.01) and adjusted (HR: 0.96 [95% CI 0.92, 1.00; P=0.05) models (**Supplementary Table 1**), whereas LPV concentrations at the previous visit was less predictive of viremia in both crude (HR: 0.98 [95% CI 0.96, 1.01]; P=0.15) and adjusted (HR: 0.99[95% CI 0.96, 1.02]; P=0.36) models (**Supplementary Table 1**). The effect of low level viremia at randomization remained significant in all models. When we compared the three models by means of AIC in each imputed dataset, we showed that the models which included current visit LPV concentrations or the average of LPV concentrations at two visits described the data better than the model with previous visit LPV concentrations. Due to high percentage of missing data (24%),

adherence was not included in the primary analysis. However, in a secondary analysis we compared two models using AIC in each imputed dataset, where the first model included current LPV concentrations and the second model included recorded adherence (**Supplementary Table 2**). In each imputed dataset the model including LPV (low AIC values) was more predictive of viremia compared to model with adherence.

We used predictive modelling to compare logistic regression models (using GCV values) and thereby evaluating the effects of various cut-off concentrations, we showed that a cut-off concentration of 1mg/L best predicted viremia (**Figure 1**). A separate Cox regression model, in which LPV concentrations were dichotomized with a cut-off of 1.0 mg/L (**Table 3**), a 41% reduction in the risk of viremia in children with LPV concentrations ≥1.0 mg/L compared to children with LPV concentrations <1.0 mg/L was shown. These associations were preserved in the adjusted models in which low level viremia at randomization was also significantly associated with increased hazard of viremia.

4.4.4 **Predictors of Viremia (Viral load>50 copies/ml) in the NVP Group**

We assessed the risk of viremia (VL>50 copies/mL) in the NVP group using Cox proportional hazards models. Neither current visit concentrations **(Table 4**), previous visit (**supplementary Table 3**) NVP concentrations nor average of two NVP (**supplementary Table 3)** concentrations taken at the current and previous visits respectively, were associated with the risk of viremia in crude or adjusted models. We compared the three models by means of AIC in each imputed dataset and showed that the model with current visit and average of two visit NVP concentrations described the data similarly but better compared with the model with previous visit NVP concentration. Consistently high NVP concentrations were measured in 5 children, these outlying observations were excluded in the primary analysis. However in

the sensitivity analysis we included the 5 children and they were influential, biasing results to significance (**data not shown**). Due to high percentage of missing data (29%), adherence was not included in the primary analysis. As for the LPV arm, we showed that current visit NVP concentrations described the data better than recorded adherence in a sensitivity analysis (**supplementary Table 4**).

Based on GCV values for the logistic regression evaluating the effect of NVP concentration thresholds, an NVP concentration cut-off values of 5.0 mg/L best predicted viremia in the respective arm (**Figure 1**). A separate Cox regression model was performed where NVP was dichotomized to evaluate the effects of NVP concentrations ≥5.0 mg/L vs. <5.0 mg/L. While not statistically significant, there was a trend to a reduction in the risk of viremia in children with NVP concentrations ≥5.0 mg/L (crude HR: 0.64[95% CI 0.33, 1.27]; P=0.20) (**Table 4**)

4.5 **Discussion**

We evaluated the risk of viremia (VL >50 copies/mL) in treatment experienced children achieving viral suppression (VL <400 copies/mL) after switching to NVP or remaining on LPV/r. In keeping with our analysis of the pre-randomization phase of the same study[18], higher LPV concentrations are associated with sustained viral suppression. Our data suggests that children with LPV concentrations ≥1.0 mg/L have a reduction in the risk of viremia of about 40%, compared to children with LPV <1.0 mg/L. In children established on ART a LPV concentration of 1.0 mg/L (taken 2-4 hours after the claimed morning dose time) may therefore be used as a threshold for therapeutic drug monitoring (TDM).

We found 1.0 mg/L and 5.0 mg/L to be the most predictive threshold values of LPV and NVP, respectively, for the risk of viremia. However, the association between NVP concentration

<5.0 mg/L and viremia was not significant in the regression model. This finding is consistent with other reports that NVP concentrations do not predict viral response[9]. Pre-existing drug resistance most likely accounts for the viremia in the NVP group as all children enrolled in this trial had past exposure to single-dose nevirapine used for PMTCT[7,11].

Low level viremia at randomization was associated with increased risk of ongoing viremia. This finding was more marked in children on the LPV/r-based regimen, and was independent of other effects captured by the multivariate models. This suggests that the risk of future viremia, conferred by low level viremia at randomization was not modified by LPV exposure post-randomization, however our study was not designed to evaluate whether interventions to increase LPV exposure in those children with low level viremia would lead to suppression.

In contrast to NVP, a high proportion (55-100%) of LPV concentrations below the threshold of 1.0 mg/L were below the quantifiable limit of the LPV assay (0.16 mg/L) across all visits (**Table 2**). This suggests that poor adherence accounts for most LPV concentrations <1.0 mg/L, which were associated with viremia, and supports efforts to develop LPV/r formulations with improved palatability.

This study has several limitations that are worth highlighting. Firstly, antiretroviral drug dosing was not directly observed therefore, our only measure of adherence was caregiver-reported adherence, which likely contributed to intra-individual variability in LPV and NVP concentrations. Secondly, the time of sampling in relation to the dose is a key determinant of drug concentration. In this study we did not observe the time of dosing and this was not included in our analysis. Despite this limitation, we have shown that LPV concentrations taken after 3.0 (2.0-3.9) hours after the last dose predict viremia, suggesting that a sample taken at a routine morning clinic can be used for LPV concentration monitoring. Thirdly, there was

missing data, which was dealt with by multiple imputation. Previous studies have shown multiple imputation to be superior to complete case analysis in which only patients with complete data across all variables are analyzed[19]. If data are missing at random and thus the probability for value to be missing randomly depends only on observed quantities, then no bias is introduced. We assumed data to be missing at random and found it to be a reasonable assumption given that the missing data related mainly to insufficient sample volumes or a lost samples. Lastly, we acknowledge that there was some model uncertainty in the choice of the best cut-off. Nonetheless, we used generalized cross validation to find a model that minimizes the expected prediction error, however, it may well be that other models with other cut-offs have good predictive ability too.

Strengths of the study include a relatively large sample size with viral load and plasma drug concentration measurements at repeated clinic visits, which made it possible to assess the relationship between each child's LPV or NVP concentration and their VL at successive intervals.

Measuring drug concentrations can be used as an effective tool in ensuring that therapeutic targets of ART are met[20]. However, TDM is not routinely used in any low and middle income country programs to our knowledge. There is also currently insufficient knowledge of target plasma concentrations in children. Moreover, although minimum trough concentrations for LPV and NVP of 1.0 mg/L (in treatment naïve children) and 3.0 mg/L, respectively, have been recommended[7,20],it is challenging to obtain a sample 12 hours post dose in clinical practice. Our findings suggest that a single sample taken 2-4 hours after the dose is a useful predictor of viremia, at least for LPV, and can be used for TDM.

In conclusion, LPV concentrations were associated with the hazard of viremia. Our analysis suggests that LPV plasma concentration monitoring at a routine clinic visit may be a useful tool in identifying sub-therapeutic antiretroviral concentrations in children, and thereby assist with adherence support.

4.6 **Acknowledgements**

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Table 1: Characteristics at randomization of the 195 HIV-infected children remaining on a lopinavir/ritonavir-based regimen or switched to nevirapine-based antiretroviral therapy

	Characteristics of children in the LPV group (n=99)			**Characteristics of children in the NVP group (n=96)**		
Variable	**Median**	**IQR**	**Missing Data**	**Median**	**IQR**	**Missing Data**
Age(months)	20	16-25	0	18	15-22.25	0
VL ≤50 copies/ml **VL51-400 copies/ml**	55(56%) 44(44%)		0	53(55%) 43(45%)		0
CD4%	28.05	21.65-35.20	4	29.55	22.95-36.70	3
WAZ	-0.60	-1.26 to 0.07	0	-0.60	-1.17 to-0.13	0
HAZ	-3.18	-3.97 to -1.97	0	-2.80	-2.10 to-4.05	0
TB Treatment **No** **Yes**	 86(86%) 13(14%)		 0	 91(94%) 6(6%)		 0

Data are in Median (IQR) or n(%).Age, Age at randomization; VL, viral load at randomization; CD4%, $CD4^+$ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization.

Table2: Lopinavir and nevirpine concentrations, time of sampling, body weight and dose by study week

Characteristics	LPV Group						NVP Group					
Weeks	**24(n=99)**	**50(n=94)**	**76(n=99)**	**100(n=98)**	**124(n=78)**	**150+(n=64)**	**24(n=92)**	**50(n=75)**	**76(n=71)**	**100(n=61)**	**124(n=44)**	**150+(n=34)**
Median[Drug]mg/L	8.92 (5.34-12.9)	9.19 (5.51-13.9	10.20 (6.68-13.9)	9.69 (6.28-13.9)	11.60 (7.90-17.2)	11.90 (8.81-14.7)	9.1 (8.4-15.2)	9.51 (7.2-11.4)	11.2 (8.3-14.2)	11.85 (9.2-16.1)	12.4 (9.4-14.7)	11.85 (8.04- 16)
% Samples between LLQ and 1 mg/L for LPV, between LLQ and 3 mg/L for NVP	8	12	10	9	9	7	3	6	2	4	3	7
% BLQ	6	12	9	5	9	6	0	2	1	3	1	1
Time after Dose(h)	3 (2.50-4.25)	3.00 (2.18-4.00)	3.08 (2.33-4.00)	3.33 (2.25-4.25	3.00 (2.25-3.75)	2.83 (2.00-3.67)	3.08 (2.25-3.92)	3.17 (2.58-4.17)	3.33 (2.50-4.17)	3.08 (2.25-4.17)	3.25 (2.58-3.88)	3.00 (2.33-4.00)
Total Body Weight(kg)	11 (10-12)	12 (11-13)	13 (12-14)	13 (12-16)	16 (15-18)	17 (16-19)	11 (10-13)	12 (10-13)	13(12-14)	14 (12-15)	15 (13-16)	17 (15- 18)
Dose(mg/m^2)	228 (222-232)	225 (219-231)	226 (221-231)	226 (223- 232)	227 (223-231)	227 (223-231)	196 (193-200)	196 (192-200)	195 (192-198)	197 (193-199)	197 (192-199)	197 (195-200)

Data are in Median (IQR), unless otherwise stated; LLQ, lower limit of quantification; BLQ, below the assay limit of quantification; LPV, lopinavir; NVP, nevirapine

Table 3: Cox proportional hazards regression analysis describing the risk of viremia (VL >50 copies/mL) post-randomization in 99 children randomized to LPV/r-based treatment.

Characteristic	Crude			Adjusted			Characteristic	Crude			Adjusted		
	HR	95%CI	P Value	HR	95%CI	P Value		HR	95%CI	P Value	HR	95%CI	P Value
LPV (mg/L)	0.95	0.92-0.98	<0.01	0.96	0.94-99	0.01	LPV <1 mg/L	Reference			Reference		
Age ≥20 months	Reference			Reference			LPV ≥1 mg/L)	0.59	0.40-0.94	0.03	0.62	0.40-0.95	0.03
Age <20 months	1.48	0.91-2.39	0.11	1.47	0.92-2.34	0.11	Age ≥20 months	Reference			Reference		
Normal WAZ	Reference			Reference			Age <20 months	1.48	0.91-2.39	0.34	1.47	0.92-2.34	0.09
Underweight	2.36	0.77-7.25	0.13	2.62	0.94-7.24	0.06	Normal WAZ	Reference			Reference		
Normal HAZ	Reference			Reference			Underweight	2.36	0.77-7.25	0.13	2.90	1.04-8.42	0.05
Stunted	0.64	0.36-1.12	0.12	0.78	0.43-1.39	0.40	Normal HAZ	Reference			Reference		
VL ≤50	Reference			Reference			Stunted	0.64	0.36-1.12	0.16	0.76	0.43-1.34	0.34
VL 51-400	2.62	1.62-4.24	<0.01	2.66	1.68-4.22	<0.01	VL ≤50	Reference			Reference		
CD4% ≥25	Reference			Reference			VL 51-400	2.62	1.62-4.24	<0.01	2.73	1.72-4.33	<0.01
CD4% <25	1.25	0.75-2.06	0.38	1.05	0.66-1.66	0.85	CD4% ≥25	Reference			Reference		
TB Treatment (No)	Reference			Reference			CD4% <25	1.25	0.75-2.06	0.28	1.05	0.66-1.66	0.85
TB Treatment (Yes)	0.36	0.13-1.05	0.07	0.41	0.15-1.15	0.09	TB Treatment (No)	Reference			Reference		
							TB Treatment (Yes)	0.36	0.13-1.05	0.07	0.41	0.15-1.15	0.09

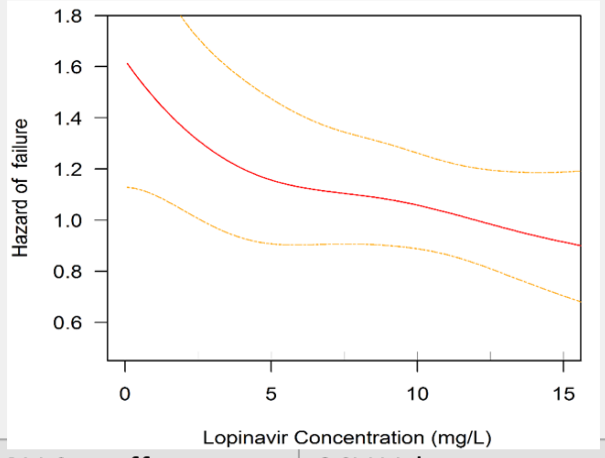
HR, hazard ratio; LPV, lopinavir concentration at each visit; Age, age at randomization; VL, viral load at randomization; CD4%, $CD4^+$ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization.

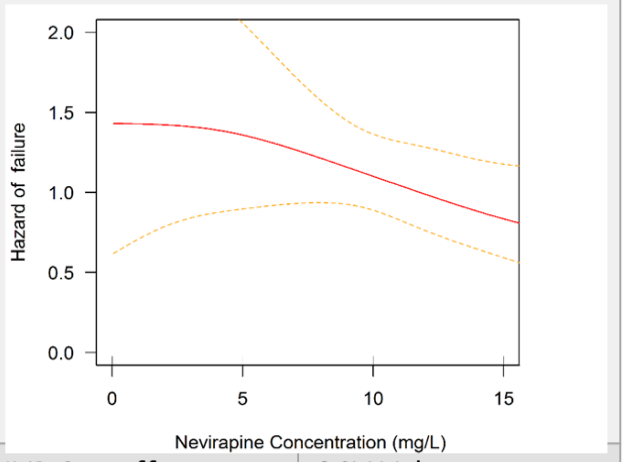
Table 4: Cox proportional hazards regression model describing the risk of viremia (VL >50 copies/mL) in children (n=96) associated with current visit plasma nevirapine concentrations.

Characteristic	Crude			Adjusted			Characteristic	Crude			Adjusted		
	HR	95%Cl	P Value	HR	95%Cl	P Value		HR	95%Cl	P Value	HR	95%Cl	P Value
NVP(mg/L)	0.95	0.90-1.01	0.11	0.96	0.91-1.01	0.13	NVP ≥5mg/L	Reference			Reference		
Age ≥18 months	Reference			Reference			NVP <5mg/L	0.64	0.33-1.27	0.20	0.69	0.33-1.41	0.31
Age <18 months	1.31	0.69-2.47	0.42	1.48	0.77-2.84	0.26	Age ≥18 months	Reference			Reference		
Normal WAZ	Reference			Reference			Age <18 months	1.31	0.69-2.47	0.42	1.48	0.77-2.82	0.24
Underweight	1.28	0.37-4.44	0.69	1.39	0.34-5.62	0.64	WAZ(normal)	Reference			Reference		
Normal HAZ	Reference			Reference			WAZ(advanced)	1.28	0.37-4.44	0.69	1.43	0.36-5.64	0.61
Stunted	0.87	0.46-1.66	0.67	0.88	0.47-1.65	0.67	HAZ(normal)	Reference			Reference		
VL ≤50	Reference			Reference			HAZ(advanced)	0.87	0.46-1.66	0.67	0.90	0.47-1.73	0.76
VL >50	1.69	0.91-3.16	0. 09	1.75	0.92-3.35	0.09	VL ≤50	Reference			Reference		
CD4% ≥25	Reference			Reference			VL >50	1.69	0.91-3.16	0. 09	1.79	0.93-3.44	0.08
CD4% <25	1.21	0.61-2.49	0.59	1.23	0.63-2.39	0.64	CD4% ≥25	Reference			Reference		
TB Treatment (No)	Reference			Reference			CD4% <25	1.21	0.61-2.49	0.59	1.19	0.61-2.31	0.61
TB Treatment(Yes)	1.12	0.34-3.64	0.86	1.19	0.28-5.33	0.82	TB Treatment (No)	Reference			Reference		
							TB Treatment (Yes)	1.12	0.34-3.64	0.86	1.15	0.26-5.11	0.85

HR, hazard ratio; NVP, nevirapine concentration at each visit; Age, Age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

FIGURE 1. Nonlinear effect of lopinavir and nevirapine concentrations on the hazard of viremia with determination of cutoffs using generalized cross validation. Left panel demonstrates lopinavir and the right panel presents nevirapine. GCV indicates generalized cross validation values; LPV cutoff, lopinavir concentrations cutoffs; NVP cutoff, nevirapine concentrations cutoffs





LPV Cut-off	GCV Value	NVP Cut-off	GCV Value
0.5 mg/L	0.8363767	2 mg/L	0.6384146
1 mg/L	0.8362492	3 mg/L	0.6384466
2 mg/L	0.8382971	4 mg/L	0.6396787
3 mg/L	0.8376631	5 mg/L	0.6355073
4 mg/L	0.8407137	6 mg/L	0.6374518
5 mg/L	0.8393880	7 mg/L	0.6377969
6 mg/L	0.8448085	9 mg/L	0.6385802

Chapter 4

Supplementary Tables

Table 1: Cox Proportional Hazards regression analysis for the risk of viremia (VL>50/copies/mL) using previous visit lopinavir concentrations (n=99)

Characteristic	Crude Model			Adjusted Model		
	HR	**95%CI**	**P Value**	**HR**	**95%CI**	**P Value**
LPV(mg/L)	0.98	0.96-1.01	0.15	0.99	0.96-1.02	0.36
Age ≥20 months	**Reference**			**Reference**		
Age <20 months	1.48	0.91-2.39	0.34	1.43	0.90-2.27	0.13
Normal WAZ	**Reference**			**Reference**		
Underweight	2.36	0.77-7.25	0.13	2.83	0.99-8.07	0.05
Normal HAZ	**Reference**			**Reference**		
Stunted	0.64	0.36-1.12	0.16	0.77	0.43-1.38	0.39
VL ≤50	**Reference**			**Reference**		
VL 51-400	2.62	1.62-4.24	<0.01	2.71	1.70-4.31	<0.01
CD4% ≥25	**Reference**			**Reference**		
CD4% <25	1.25	0.75-2.06	0.28	1.06	0.66-1.69	0.82
TB Treatment (No)	**Reference**			**Reference**		
TB Treatment(Yes)	0.36	0.13-1.05	0.07	0.40	0.15-1.10	0.07

HR, hazard ratio; LPV, previous visit lopinavir concentration; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Table 2: Cox proportional hazards regression model describing the risk of viremia (VL >50 copies/mL) associated with the average of two lopinavir concentrations taken at the current and previous visits respectively.

Characteristic	Crude Model			Adjusted Model		
	HR	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**
LPV(mg/L)	0.94	0.91-0.98	<0.01	0.96	0.92-1.00	0.05
Age ≥20 months	**Reference**			**Reference**		
Age <20 months	1.48	0.91-2.39	0.34	1.47	0.91-2.34	0.09
Normal WAZ	**Reference**			**Reference**		
Underweight	2.36	0.77-7.25	0.13	2.58	0.92-7.22	0.07
Normal HAZ	**Reference**			**Reference**		
Stunted	0.64	0.36-1.12	0.16	078	0.44-1.42	0.42
VL ≤50	**Reference**			**Reference**		
VL 51-400	2.62	1.62-4.24	<0.01	2.64	1.66-4.19	<0.01
CD4% ≥25	**Reference**			**Reference**		
CD4% <25	1.25	0.75-2.06	0.28	1.02	0.66-1.69	0.81
TB Treatment (No)	**Reference**			**Reference**		
TB Treatment(Yes)	0.36	0.13-1.05	0.07	0.39	0.14-1.09	0.07

HR, hazard ratio; LPV, average of previous and current visit lopinavir concentration; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Table 3: Cox Proportional Hazards regression evaluating the risk of viremia (VL >50 copies/mL) associated with adherence (volume of lopinavir/ritonavir oral solution returned, divided by the volume dispensed at the previous visit, expressed as a percentage), in the lopinavir group (n=99).

Characteristic	Crude Model			Adjusted Model		
	HR	95%CI	P Value	HR	95%CI	P Value
Adherence (%)	0.99	0.96-1.03	0.62	0.99	0.96-1.02	0.43
Age ≥20 months	**Reference**			**Reference**		
Age <20 months	1.48	0.91-2.39	0.34	1.44	0.91-2.28	0.12
Normal WAZ	**Reference**			**Reference**		
Underweight	2.36	0.77-7.25	0.13	2.91	1.02-8.30	0.05
Normal HAZ	**Reference**			**Reference**		
Stunted	0.64	0.36-1.12	0.16	0.77	0.43-1.40	0.39
VL ≤50	**Reference**			**Reference**		
VL 51-400	2.62	1.62-4.24	<0.001	2.78	1.76-4.40	<0.001
CD4% ≥25	**Reference**			**Reference**		
CD4% <25	1.25	0.75-2.06	0.28	1.08	0.68-1.73	0.74
TB Treatment (No)	**Reference**			**Reference**		
TB Treatment(Yes)	0.36	0.13-1.05	0.07	0.39	0.14-1.06	0.07

HR, hazard ratio; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Table 4: Cox Proportional Hazards regression analysis describing the risk of viremia (VL >50/copies/mL) in 96 children, associated with the plasma nevirapine concentration at the previous visit.

Characteristic	Crude Model			Adjusted Model		
	HR	**95%Cl**	**P Value**	**HR**	**95%Cl**	**P Value**
NVP(mg/L)	0.96	0.91-1.01	0.13	0.96	0.91-1.02	0.17
Age ≥18 months	**Reference**			**Reference**		
Age <18 months	1.31	0.69-2.47	0.42	1.46	0.76-2.80	0.26
Normal WAZ	**Reference**			**Reference**		
Underweight	1.28	0.37-4.44	0.69	1.35	0.34-5.35	0.67
Normal HAZ	**Reference**			**Reference**		
Stunted	0.87	0.46-1.66	0.67	0.91	0.47-1.75	0.79
VL ≤50	**Reference**			**Reference**		
VL 51-400	1.69	0.91-3.16	0. 09	1.79	0.94-3.42	0.07
CD4% ≥25	**Reference**			**Reference**		
CD4% <25	1.21	0.61-2.49	0.59	1.20	0.61-2.34	0.60
TB Treatment (No)	**Reference**			**Reference**		
TB Treatment(Yes)	1.12	0.34-3.64	0.86	1.19	0.27-5.40	0.81

HR, hazard ratio; NVP, previous visit nevirapine concentration; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Table 5: Cox proportional hazards regression analysis for the risk of viremia (VL >50 copies/mL) associated with the average of two nevirapine concentrations taken at the current and previous visits, respectively, in 96 children.

Characteristic	Crude Model			Adjusted Model		
	HR	95%CI	P Value	HR	95%CI	P Value
NVP (mg/L)	0.94	0.88-1.01	0.07	0.95	0.88-1.01	0.09
Age ≥18 months	Reference			Reference		
Age <18 months	1.31	0.69-2.47	0.42	1.47	0.76-2.84	0.26
Normal WAZ	Reference			Reference		
Underweight	1.28	0.37-4.44	0.69	1.35	0.34-5.34	0.68
Normal HAZ	Reference			Reference		
Stunted	0.87	0.46-1.66	0.67	0.89	0.47-1.67	0.71
VL ≤50	Reference			Reference		
VL 51-400	1.69	0.91-3.16	0. 09	1.74	0.91-3.32	0.09
CD4% ≥25	Reference			Reference		
CD4% <25	1.21	0.61-2.49	0.59	1.22	0.62-2.39	0.56
TB Treatment (No)	Reference			Reference		
TB Treatment(Yes)	1.12	0.34-3.64	0.86	1.19	0.27-5.40	0.81

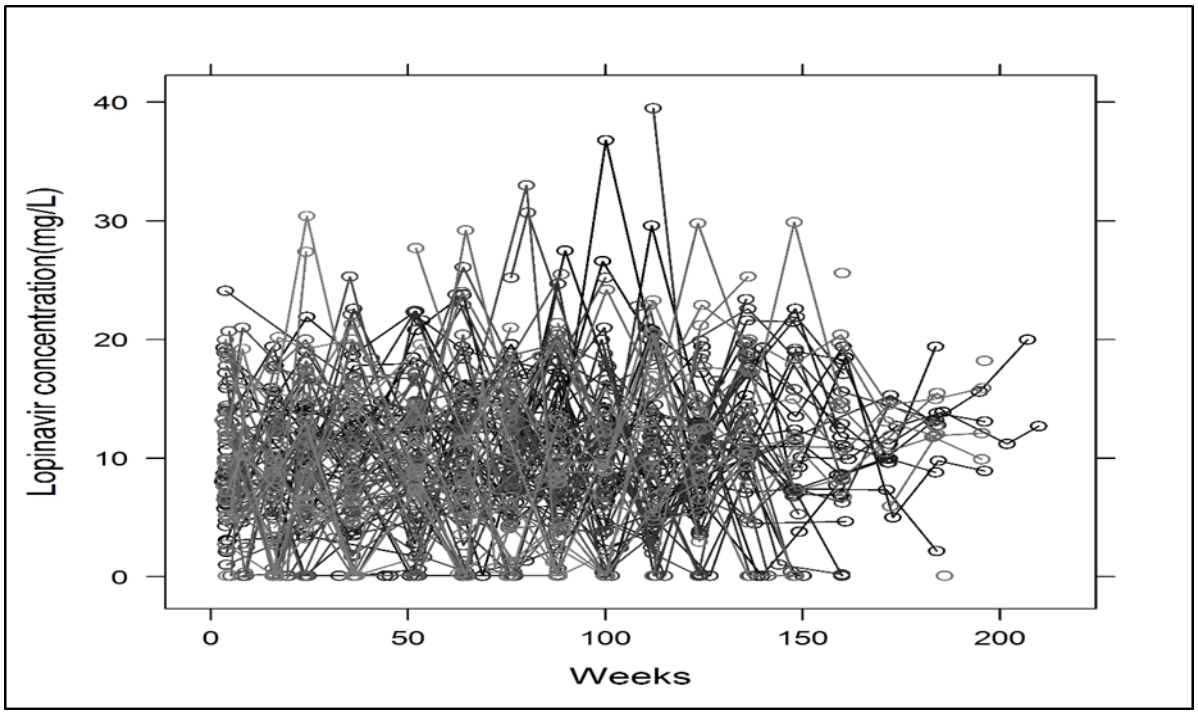
HR, hazard ratio; NVP, average of previous and current visit nevirapine concentration; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Table 6: Cox Proportional Hazards regression analysis evaluating the risk viremia (V L>50/copies/mL) associated with adherence (the amount of nevirapine returned, divided by the amount dispensed at the previous visit, expressed as a percentage) in the NVP group (n=96).

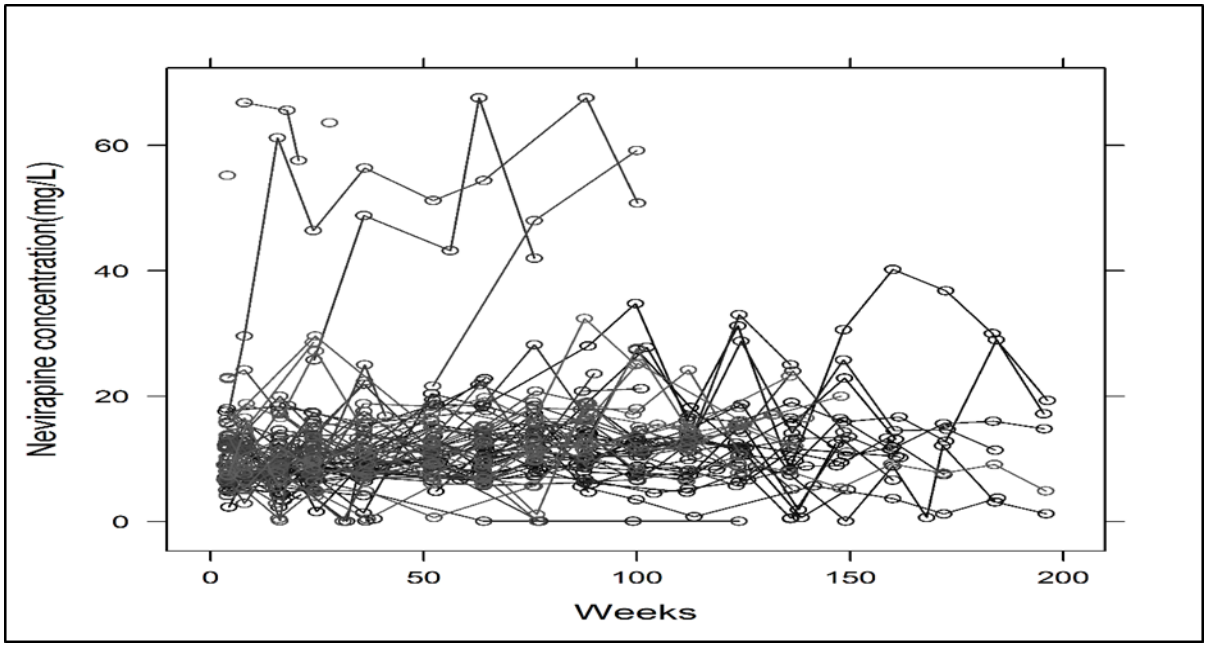
Characteristic	Crude Model			Adjusted Model		
	HR	**95%CI**	**P Value**	**HR**	**95%CI**	**P Value**
Adherence (%)	0.98	0.93-1.06	0.64	0.99	0.93-1.04	0.65
Age ≥18 months	**Reference**			**Reference**		
Age <18 months	1.31	0.69-2.47	0.42	1.66	0.89-3.10	0.11
Normal WAZ	**Reference**			**Reference**		
Underweight	1.28	0.37-4.44	0.69	1.04	0.32-3.32	0.94
Normal HAZ	**Reference**			**Reference**		
Stunted	0.87	0.46-1.66	0.67	0.92	0.47-1.81	0.82
VL ≤50	**Reference**			**Reference**		
VL 51-400	1.69	0.91-3.16	0. 09	2.12	1.12-3.99	0.02
CD4% ≥25	**Reference**			**Reference**		
CD4% <25	1.21	0.61-2.49	0.59	1.06	0.56-2.01	0.85
TB Treatment (No)	**Reference**			**Reference**		
TB Treatment(Yes)	1.12	0.34-3.64	0.86	1.00	0.24-4.17	0.99

HR, hazard ratio; Age, age at randomization; VL, viral load at randomization; CD4%, CD4+ T lymphocyte percentage at randomization; WAZ, weight for age z-scores at randomization; HAZ, height for age z-score at randomization; TB Treatment, concomitant tuberculosis treatment post-randomization

Supplementary Figure 1A: Plasma lopinavir concentrations measured in samples taken at clinic visits. Lines connect the individual concentrations taken at serial visits (indicated in weeks after randomization) in each child in the lopinavir group.



Supplementary Figure 1B: Plasma nevirapine concentrations plotted over time (weeks after randomization). The lines connect the individual concentrations of each child in the nevirapine group, which were measured in samples taken at serial clinic visits.



Author Contributions

Title "Associations between Lopinavir Pharmacokinetics and Genetic Variants in ABCB1, CYP3A4, CYP3A5 and SLCO1B1 in a Cohort of South-African Children**"**

[1]Retsilisitsoe R. Moholisa, [2,3,4]Tim R. Cressey, [1]Phumla Sinxadi , [5]Louise Kuhn, [6,8]Emile R. Chimusa ,[1]Sandra Meredith, [1]Lubbe Weisner, [7]Ashraaf Coovadia, [8]Renate Strehlua, [9]Elaine J. Abrams, [1,10]Gary Maartens, [1,10]Helen McIlleron, [10,11]David Haas

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, [2]PHPT/IRD 174, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; [3]Department of Immunology & Infectious Diseases, Boston, Harvard T.H Chan School of Public Health, MA, USA, [4]Department of Molecular & Clinical Pharmacology, University of Liverpool, UK,[5]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, Division of Human Genetics, Department of Pathology, University of Cape Town, [6]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [7]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[8]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, [9]Vanderbilt Medical Center and [10]School of Medicine, Department of Medicine, Nashville,Tannessee

RR Moholisa: Responsible for the data handling, processing and analysis under; Wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures

Tim R Cressey: Guided the analysis strategy and provided guidance from the initial draft of the manuscript especially the population pharmacokinetic modelling analysis section and the discussion.

Phumla P Sinxadi and David Haas: Guided the statistical analysis strategy and provided guidance from the initial draft of the manuscript especially the genotyping, statistical analysis section and the discussion.

Emile R Chimusa: Provided technical assistance with regards to plotting linkage disequilibrium plots and providing input for the write up of the manuscript

Luis Kuhn, Ashraaf Coovadia, Renate Strehlau, and Elaine J Abrams: Responsible for planning the study and collecting all the data.

Sandra Castel and Lubbe Weisner: performed the laboratory analysis of measuring lopinavir and nevirapine concentrations

G Maartens and H McIlleron: Oversaw overall analysis strategy planning and mentoring, guided the writing of the manuscript from beginning to end

Chapter 5

Title: Associations between Lopinavir Pharmacokinetics and Genetic Variants in ABCB1, CYP3A4, CYP3A5 and SLCO1B1 in a Cohort of South-African Children.

[1]Retsilisitsoe R. Moholisa, [2,3,4]Tim R. Cressey, [1]Phumla Sinxadi , [5]Louise Kuhn, [6,8]Emile R. Chimusa ,[1]Sandra Meredith, [1]Lubbe Weisner, [7]Ashraaf Coovadia, [8]Renate Strehlua, [9]Elaine J. Abrams, [1,10]Gary Maartens, [1,10]Helen McIlleron, [10,11]David Haas.

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, [2]PHPT/IRD 174, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; [3]Department of Immunology & Infectious Diseases, Boston, Harvard T.H Chan School of Public Health, MA, USA, [4]Department of Molecular & Clinical Pharmacology, University of Liverpool, UK,[5]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, Division of Human Genetics, Department of Pathology, University of Cape Town, [6]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [7]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[8]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, [9]Vanderbilt Medical Center and [10]School of Medicine, Department of Medicine, Nashville,Tannessee

5.1 **Abstract**

5.1.1 **Aims:** To quantify demographic and host genotypic effects on lopinavir (LPV) disposition in HIV-positive children.

5.1.2 **Methods:** Steady-state LPV pharmacokinetics were predicted using a population nonlinear mixed effects model. Using the final model we estimated individual clearance (CL/F), area under concentration time curve (AUC_{0-12}) and minimum concentrations (C_{min}). We explored associations between pharmacokinetic parameters and genotypes in selected genes relevant to LPV absorption and disposition.

5.1.3 **Results:** A one compartment model with absorption lag time best described 1683 LPV plasma concentrations in 237 children. There was an age-driven effect on relative bioavailability of LPV. After correcting for multiple testing, there was no statistically significant associations between LPV CL/F, C_{min} or AUC_{0-12} and polymorphisms in *ABCB1*, *CYP3A4, CYP3A5* or *SLCO1B1*.

5.1.4 **Conclusions:** Relative bioavailability of lopinavir was driven by age in South African children. Genetic polymorphisms in candidate genes were not significantly associated with LPV pharmacokinetics.

5.2 **Introduction**

Lopinavir (LPV) co-formulated with ritonavir is recommended as first-line antiretroviral treatment (ART) in children infected with HIV in South Africa. LPV has low oral bioavailability due its high first pass metabolism mediated by cytochrome P450 (CYP) 3A4, and is a substrate of efflux transporter P-glycoprotein (P-gp)[1,2]. Co-formulation with low-dose ritonavir leads to increased LPV exposure via inhibition of intestinal and hepatic CYP3A and P-gp, respectively[3]. LPV is characterized by large pharmacokinetic inter-individual variability, which in part can be explained by body weight, age, sex, orsomucoid plasma levels, drug-drug interactions, liver disease, pregnancy and host genetics[4–7].

Genetic polymorphisms in the CYP3A4 and A5 have been reported to affect inter-individual variability in the absorption and disposition of several HIV protease inhibitors including LPV[8–11]. Moreover, polymorphisms in the *ABCB1* (which encodes Pg-p) have been reported to be associated with variability in absorption, disposition, drug response and toxicity of other PIs in adult studies[7]. In one paediatric study, *ABCB1* 3435C→T(rs1045642) polymorphism was

associated with higher plasma concentrations and virological response to nelfinavir whereas there was no association LPV pharmacokinetics(PK) [12,13]. The *SLCO1B1* 521T→C(rs4149056) polymorphism has been associated with increased LPV trough concentrations in adult male patients and increased LPV area under concentration time curve (AUC) in children[13,14]. However the clinical importance of this polymorphism for LPV PK is uncertain.

Studies of LPV to date have been performed largely in adults of European descent. The aim of the present study was to investigate whether genetic polymorphisms in *ABCB1, CYP3A4*, *CYP3A5* and *SCLO1B1* affect the steady-state PK of LPV in a cohort of South African children.

5.3 **Methods**

5.3.1 **Study Population**

Plasma LPV concentrations were retrospectively analyzed in stored samples collected at clinic visits during the pre-randomization and post-randomization periods from participants of the NEVEREST2 trial[15,16]. Treatment responses during both phases have been previously described[15,16]. The study population included HIV-positive children attending the Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa. At baseline, treatment eligibility criteria included WHO stage III or IV disease, $CD4^+$ lymphocyte percentage (CD4%) of less than 25% if younger than 12 months or less than 20% if older than 12 months, or recurrent (more than twice yearly) or prolonged (>4 weeks) hospitalization for HIV related complications. All children received 230/57.5 mg/m^2 of ritonavir-boosted LPV (Kaletra® oral solution, Abbott laboratories, USA), 1 mg/kg of stavudine and 4 mg/kg of lamivudine as oral solutions every 12 hours. At each visit, drug doses were adjusted according to growth. The caregivers of the children were provided with comprehensive counseling about treatment adherence.

Chapter 5

The children randomized into the NEVEREST2 study were accrued from a cohort of 323 nevirapine-exposed children less than 24 months of age, who met clinical and immunologic criteria for treatment, and who initiated a LPV-based regimen as their first treatment regimen. Data from children in the NEVEREST2 study population were included in prior publications about immune reconstitution inflammatory syndrome[17] and initial response to LPV-based antiretroviral therapy[18]. Data collected prior to starting LPV/r therapy included, age, sex, pre-treatment HIV plasma viral load (VL), pre-treatment CD4% and WHO stage. Weight-for-age z-score (WAZ) and height-for-age z-score (HAZ) collected pre-treatment and post-treatment initiation were calculated using World Health Organization (WHO) software. Blood samples for LPV concentration determination were collected during the pre-randomization phase at clinic visits 12, 24, 36 and 52 weeks after starting treatment, and at unscheduled clinic visits. After randomization additional samples were collected at 0, 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post-randomisation and at unscheduled clinic visits. The time of blood sample collection was documented, as was the time of the morning dose of antiretrovirals, as reported by the caregiver. Caregivers were requested to return medication bottles at each visit. Bottles were weighed and the contents reconciled with the expected usage of each medication to determine the degree of adherence. Adherence was defined as returning less than ≤20% of the expected volume of any of the three drugs, whereas non-adherence was defined as returning >20%. In children with tuberculosis (TB), concomitant TB treatment was recorded at each visit. Children diagnosed with TB received a double dose of LPV/r.

5.3.2 **Laboratory Analysis**

Plasma LPV concentrations were measured using validated liquid chromatography tandem mass spectrometry methods developed in the Division of Clinical Pharmacology, University of Cape Town, South Africa. An AB Sciex 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode. The assay was validated over the concentration range of 0.16-20 mg/L. Inter- and intra-day coefficients of variation were below 10% for all quality control concentrations. The assay laboratory, participates in the International Inter-laboratory Pharmacology Quality Control Program, the AIDS Clinical Trial Group.

5.3.4 **Genotyping**

Human DNA was extracted from buffy coats using the QIAsymphony DNA midi kit which utilises magnetic-particle technology to isolate and purify DNA. Targeted genotyping of *CYP2A6* -48T→G (rs28399433) was done by TaqMan™ (Applied Biosystems, Foster City, CA). Genotyping of *SLCO1B1* 521T→C (rs4149056) and *SLCO1B1* rs4149032 was done as part of a custom designed MassARRAY® iPLEX Gold (Sequenom Inc., San Diego, California, USA). Genotypes were confirmed by visual inspection of plots, and all samples were genotyped in duplicate. Additional genotyping was done by Illumina HumanCore Exome assay (Illumina, San Diego, CA).Each HumanCore Exome plate included a HapMap trio, as well as duplicates scattered across each plate for QC purposes. The average genotype call rate for each sample was 98.7%, and call rates for 93% of samples exceeded 98%. Genotyping was done at the Vanderbilt Technologies for Advanced Genomics (VANTAGE), by laboratory personnel with no knowledge of clinical data.

5.3.5 **LPV Model Development**

The population means and variances of LPV pharmacokinetic parameters at steady state were estimated using non-linear mixed-effects regression. NONMEM software version7.3 (ICON Development Solutions, Ellicott City, MD, USA) was used to fit the LPV concentration-time data using two-step estimation method : (i) first-order conditional estimation method with interaction was used to generate population typical parameters and support points from the empirical Bayes estimates (EBE); (ii) the nonparametric estimation was used to estimate the population probability of each support point[19]. LPV concentrations below 0.08 mg/L, were treated as values below the limit of quantification (BLQ) samples using the M5 method, where all BQL observations are replaced by BQL/2 as suggested by Beal *et al* [20]. PsN 4.6.8, Pirana and Xpose were used to facilitate the model building process and for diagnosing the model. The stepwise model building process was guided by differences in the objective function value (OFV; proportional to -2 log likelihood), inspection of goodness of fit plots and visual predictive checks, biological plausibility and clinical relevance. The differences of >3.84 drop in OFV between two nested models after adding one parameter to the model was considered significant. Nonparametric bootstrap (n=200) was used to evaluate the stability and robustness of final parameters estimates of the model. Both LPV minimum concentrations (C_{min}) and AUC were calculated using model derived EBE for individual parameters for each sampling occasion and patient.

One or two compartment disposition models with first order absorption and elimination were tested, as well as delayed absorption using previously published models[21,22]. The inter-individual (IIV) and inter-occasion (IOV) variability of LPV pharmacokinetic parameters were assumed to be log-normally distributed and was approximately interpreted as a deviation

proportional to the typical value, and is reported as %CV. Correlation between of pharmacokinetic parameters were also investigated especially at the IIV level. Residual unexplained variability (RUV) was tested using the combined proportional (PROP) and additive (ADD) structure. Implausible outliers were identified using visual inspections and excluded based on normalised prediction distribution errors (NPDE >2.5).

5.3.6 **Covariate Model**

Clearance (CL/F) and volume (V/F) parameters were scaled allometrically at early stage as previously suggested[23]. Maturation on CL/F was tested using exponential or sigmoidal function with or without the Hill coefficient models. Other covariates tested include sex and concomitant TB treatment.

5.3.7 **Statistical Analysis**

Genetic associations were tested for significance against model derived PK parameters CL, AUC and C_{min}. Geometric means were calculated for each individual for all PK parameters and were used in the subsequent analysis. Bonferroni correction was used to account for multiple testing. Hardy-Weinberg equilibrium was assessed using exact tests for all genotypes. Data was analysed using Plink version 1.90(http://pgnu.mgh.harvard.edu/~purcell/plink).

Haplotypic blocks were defined using the D' confidence intervals method in Haploview[24] and haplotype phases were inferred using the standard E-M algorithm in PLINK[25]. Linkage disequilibrium (LD) plots and values were generated with Haploview (www.broad.mit.edu/mpg/haploview/).

5.4 **Results**

5.4.1 **Study Population**

Pharmacokinetic data was available in 237 children with a median of 2 samples per child, and a total of 487 plasma samples during the pre-randomization phase. Post-randomization, 1134 plasma samples from 99 children with a median of 8 samples per child were available for pharmacokinetic analysis. Of the 237 children, 176 were successfully genotyped and were analysed further. **Table 1** presents the characteristics of the study population.

5.4.2 **Genotyping**

Among the 237 study participants, 100 polymorphisms (27 in *ABCB1*; 6 in *CYP3A4;* 10 in *CYP3A5*; 59 in *SLCO1B1*) were genotyped in 174 patients, of which 21 were monomorphic. The remaining 79 polymorphisms were in Hardy-Weinberg equilibrium (HWE) based on the Bonferroni adjusted P value threshold of 0.0005; eight had unadjusted P values of <0.05 (*SCLO1B1 rs1084178*, P=0.003; *SLCO1B1 rs7967354*, P=0.01; *SLCO1B1 rs4149008*, P=0.02; *SLCO1B1 rs4140389*, P=0.02; *ABCB1 rs6465118*, P=0.03; *SLCO1B1 rs4149009*, P=0.03; *CYP3A4 rs28451617*, P=0.04; ABCB1 rs10225473, P=0.05). **Supplementary Table 1** presents minor allele frequencies, genotype frequencies and HWE P-values of the 100 polymorphisms in 176 patients. We did not observe any strong LD association (r^2>0.80) with *ABCB1* 3435C→T (rs1045642) and 4036A→G (rs3842) and other polymorphisms (**Supplementary Figure 1**). Furthermore, no strong LD association with polymorphisms in the *CYP3A4* were observed (**Supplementary Figure 2**). In *CYP3A5*, there was a strong LD association between rs1859690, rs15524 and rs10211 or rs15524 and rs10211. There was a strong LD between rs10256106 and rs10264272 (**Supplementary Figure 3**). There was no strong LD association with between *SLCO1B1* 521T→C (rs4149056), rs4149032, 388→G (rs2306283) and 463C→A (rs11045819)

and other genotypes. *SLCO1B1* 463C→A (rs11045819) was in LD (r^2 >0.6) with rs11045852, rs11045854, rs74064211, rs74064213 and rs11045863 (**Supplementary Figure 4**).

5.4.3 **LPV Model Description**

LPV pharmacokinetics were best described using a one compartment model with first order absorption and elimination. Final model estimates of the PK parameters are presented in **Table 2**. Allometric scaling based on body weight on both CL/F and V/F improved the model (**Equations 1.1** and **1.2**). Inclusion of sex did not improve the model. Our model did not find a maturation effect on CL/F, but there was an age-driven effect on bioavailability (**Figure 1A),** which was described using a sigmoidal model (**Equation 2**). Bioavailability was 60% after 3 months and reached 90% after 56 months. Concomitant TB treatment increased CL of LPV by 59.5% (**Figure 1B**). The correlation between IIV CL and V was not supported by the data.

The absorption parameters were not well estimated in our model and therefore were fixed to the values reported in the literature[21,22] and this improved the stability of the model. We did not have intravenous data so we could not estimate bioavailability and so we fixed to it 1 and IIV and IOV were estimated. We derived individual estimates for CL/F, C_{min} and AUC from our final model and used in the subsequent analysis.

5.4.4 **Model Evaluation**

Visual predictive checks (500 simulations) for the final LPV model is shown in **Figure 2**. The 5th, 50th and 95th percentiles of the data are in agreement with the 95% confidence interval of each percentile of the simulated data, supporting adequacy of the model. Bootstrap results (**Table 2**) confirmed robustness of the final parameter estimates.

5.4.5 Association between Genetic Polymorphisms and Model Derived CL/F, AUC_{0-12} and C_{min}

A total of 131 individuals with genotype and phenotype were included in the analysis. **Table 3** presents a summary of data for LPV PK and genotype. The pharmacokinetic data (CL/F, C_{min}, and AUC) were not normally distributed and therefore individual geometric means were calculated and used for subsequent analysis. The median C_{min} was 4.27(3.42-5.29) mg/L and the median AUC_{0-12} was 113.31(94.32-134.28) mg.h/L. There was no significant association between any polymorphism and LPV CL, C_{min} or AUC, after adjusting for multiple comparisons (Bonferroni P-value of 0.0005) (**Table 3**). Without adjusting for multiple comparisons, there were nominally significant associations with LPV CL/F and *ABCB1* rs10267099 [β:-0.10(-0.17 to -0.02, P= 1.71 x 10^{-02})], *CYP3A4* rs473706 [β:-0.05(-0.10 to -0.004, P= 3.54 x 10^{-02})], and 3 in *SLCO1B1* rs73250843 [β:0.12 (0.04-0.19, , P= 4.04 x 10^{-03})], rs11045819 [β:-0.08(-0.15 to -0.01 P= 2.85 x 10^{-02}], and rs4149032 [β:-0.05(-0.10 to -0.003, P= 3.91 x 10^{-02})]).

We found nominally significant associations between LPV C_{min} and *SLCO1B1* rs112403792 [β:0.73(0.30-1.16, P=1.12 x 10^{-03})], rs11045819 [β:0.78(0.25-1.30, P=4.35 x 10^{-03})], rs4149057[β:0.72(0.22-1.21, P=5.06 x 10^{-03})], rs7975594 [β:0.70(0.07-1.32, P= 3.02 x 10^{-02})], rs2306283[β:0.52(0.05-0.99, P= 3.13 x 10^{-02})], and rs1000691 [β:-0.40(-0.78 to -0.02, P= 4.02 x 10^{-02})], rs112108376 [β:0.61(0.02-1.19, P= 4.02 x 10^{-02})], without adjustment for multiple comparisons.

Lastly we found nominal associations with LPV AUC and seven *SLCO1B1* polymorphisms in the gene (rs112403792[β:16.60(7.17-26.04, P=7.58 x 10^{-04})], rs11045819 [β:16.86(5.29-28.43, P=4.99 x 10^{-02})], rs4149057 [β:15.69(4.80-26.58, P=5.49 x 10^{-02})], rs2306283 [β:12.11(0.72-22.50, P=2.39 x 10^{-02})], rs7975594 [β:15.26(1.47-29.05, P=3.19 x 10^{-02})], rs112108376 [β:14.31(1.37-27.24, P=3.20 x 10^{-02})], and rs1000691 [β:-8.99(-17.35 to -0.64, P=3.66 x 10^{-02})].

5.5 **Discussion**

This study evaluated associations between of *CYP3A4*, *CYP3A5*, *ABCB1* and *SLCO1B1* polymorphisms on and LPV PK in a cohort of South African children. A model based approach was used to satisfactorily describe LPV PK. A one compartment with first order absorption, delay and linear elimination, including body weight effects on CL/F and V/F described LPV PK. We also found an age-driven effect on relative bioavailability and an effect of concomitant TB therapy effect on CL/F, similar to previous reports[21,26]. Our model estimates for both CL/F and V/F were consistent with values in previous studies[21,22,27]. We also found results of model derived C_{min} and AUC_{0-12} similar to those in the literature[28]. Our dataset was sparse (1 sample per occasion) and we had limited data in the absorption phase, hence we could not estimate both absorption rate constant (KA) and absorption lag time (ALAG). Nonetheless, both inter-individual and inter-occasion variability for both parameters were well estimated in our model.

We used candidate gene approach to evaluate associations between polymorphisms in *ABCB1*, *CYP3A4*, *CYP3A5* and *SLCO1B1* genes and LPV PK. LPV is a substrate of *SLCO1B1*, and at least one genetic variant is known to affect LPV PK. Indeed reports have shown that 521T→C (rs4149056) is associated with increased plasma LPV concentrations[4,14,29,30]. In our study, we found no significant association between 56 polymorphisms in *SLCO1B1* and LPV CL/F, C_{min} and AUC_{0-12} after adjusting for multiple comparisons. Specifically, we found no association between *SLCO1B1* 521T→C (rs4149056) and LPV PK (p-values 0.47, 0.67 and 0.70 for CL, C_{min} and AUC_{0-12}, respectively. This is mostly likely due to the low frequency of the 521CC allele. Only 1 patient had heterozygous *SLCO1B1* 521CT genotype and the rest had wild type alleles.

We found nominally significant negative association between *SLCO1B1* rs4149032 and LPV CL/F, but no associations were found between this polymorphism and LPV C_{min} or AUC_{0-12}. These data are in contrast with a previous report, which showed an association between *SLCO1B1* rs4149032 and LPV PK[4]. This polymorphism was common in our population (MAF 21%). Therefore, the lack of an association cannot be attributed to low frequency. We also found a nominal negative association between *SLCO1B1* 463C→A (rs11045819) and LPV CL/F, with positive association with LPV C_{min} and AUC_{0-12}. These data are in contrast with a previous report, which showed an association between *SLCO1B1* rs4149032 or 463C→A (rs11045819) and increased LPV CL/F[4].

We found nominal association between *SLCO1B1* 388A→G (rs2306283) and increased C_{min} and AUC_{0-12}. This is in contracts to previous reports which found no association between this polymorphism and LPV concentrations in children[13] or adults[14]. We found trends between *SLCO1B1* rs112403792 and rs4149057 with increased LPV C_{min} and AUC_{0-12}. There are no previous reports regarding these trends and these polymorphisms.

There was no significant association between polymorphisms in *ABCB1* and LPV CL/F, C_{min} and AUC_{0-12} after adjusting for multiple comparisons, similar to previous reports in children[13] or adult studies[31]. We found nominally significant association of rs10267099 with LPV CL/F. This association has not been reported.

In our study neither polymorphisms in the *CYP3A4* and/or *CYP3A5* genes were associated with LPV CL, C_{min} and AUC_{0-12} after adjusting for multiple comparisons. Our results were consistent with previously published data on the lack of effect of polymorphisms in both genes on LPV PK in children and adult studies of African descent[13,32].

In summary, this is the first study to quantify effects of *ABCB1, CYP3A4*, *CYP3A5* and *SLCO1B1* polymorphisms on LPV PK. These results increase our understanding of factors that influence LPV PK variability. Effects of interaction between these genes remain to be elucidated.

5.6 **Acknowledgements:**

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Table 1: Summary of the All the Data of Participants Pre-and-Post Randomization

Characteristic	Median(IQR)	Range
Age(Months)	32(21-44)	4-74
BW(Kg)	12.5(10.5-14.65)	4.03-27.65
Dose(mg/m^2)	226	222-232
CD4$^+$(%)	29(20.7-34.8)	7.87-64
VL(Copies/mL)	50(48.9-98)	25-5x10^6

Table 2: Final Model Parameter Estimates

Parameter	Typical Values (Of Typical Child Weighting 13 Kg)	Bootstrap Values[Median(97.5%CI)]
CL/F[L/hr]	1.05	1.04(0.68-1.61)
V/F[L]	8.03	8.03(6.51-10.88)
Ka[hr^{-1}]	0.74 FIX	--------
Alag[hr]	0.37 FIX	--------
HILL	1 FIX	
PMA50[months]	26.7	26.51(9.68-31.75)
RIF Effect on CL/F[%]	59.5%	59.49%(50.95%-61.77%)
Variability		
IIV CL	44.1%	42.15%(40.22%-46.30%)
IIV V	28.8%	28.24%(20.08%-31.55%)
IIV KA	-------	
IIV ALAG	26.9%	26.47%(20.06%-26.95%)
IIV F	42.9%	41.15%(41.11%-41.29%)
IOV CL	------	
IOV KA	32.3%	31.48%(31.39%-31.47%)
IOV ALAG	46.7%	44.38%(43.68%-44.49%)
IOV F	37.9%	36.89%(36.57%-42.49%)
RUV		
Prop[%]	11.7%	11.68%(10.07%-14.13%)
Add[mg/L]	0.10	0.104(0.103%-3.51%)
Cmin[Median,(IQR)]	4.27(3.42-5.29)	
AUC_{0-12}[Median, (IQR)]	113.31(94.32-134.28)	

Alag, absorption lag time; Add, additive error; CL/F, clearance; F, bioavailability; IIV, inter-individual variability; IOV, inter-occasion variability; KA, absorption rate constant; PMA50, post menstrual age; Prop, proportional error; RUV, residual unexplained variability; V/F, volume

Table 3: Genetic associations with lopinavir CL, C_{min} and AUC

LPV CL/F			LPV C_{min}			AUC		
CHR	**SNP(Gene)**	**β(95%CI,P Value)**	**CHR**	**SNP(Gene)**	**β(95%CI,P Value)**	**CHR**	**SNP(Gene)**	**β(95%CI,P Value)**
12	rs73250843 (SLCO1B1)	0.12(0.04-0.19,P=0.004)	**12**	rs112403792 (SLCO1B1)	0.73(0.30-1.16, P=0.001)	**12**	rs112403792 (SLCO1B1)	16.60(7.17-26.04, P=0.0007)
7	rs10267099 (ABCB1)	-0.10(-0.17 to -0.02, P= 0.02)	**12**	rs11045819 (SLCO1B1)	0.78(0.25-1.30, P=0.004)	**12**	rs11045819 (SLCO1B1)	16.86(5.29-28.43, P=0.005)
12	rs11045819 (SLCO1B1)	-0.08(-0.15 to -0.01,P=0.03)	**12**	rs4149057 (SLCO1B1)	0.72(0.22-1.21, P=0.005)	**12**	rs4149057 (SLCO1B1)	15.69(4.80-26.58, P=0.006)
7	rs473706 (CYP3A4)	-0.05(-0.10 to -0.004,P=0.03)	**12**	rs7975594 (SLCO1B1)	0.70(0.07 -1.32, P=0.03)	**12**	rs2306283 (SLCO1B1)	12.11(1.72-22.50, P=0.02)
12	rs4149032 (SLCO1B1)	-0.05(-0.10 to -0.003,P=0.04)	**12**	rs2306283 (SLCO1B1)	0.52(0.05-0.99, P=0.03)	**12**	rs7975594 (SLCO1B1)	15.26(1.47-29.05, P=0.03)
12	rs11045863 (SLCO1B1)	-0.08(-0.17 to 0.002,P=0.06)	**12**	rs1000691 (SLCO1B1)	-0.40(-0.78 to -0.02, P=0.04)	**12**	rs112108376 (SLCO1B1)	14.31(1.37-27.24, P=0.03)
7	rs1002204 (ABCB1)	0.06(-0.002 to 0.13,P=0.06)	**12**	rs112108376 (SLCO1B1)	0.61(0.02-1.19, P=0.05)	**12**	rs1000691 (SLCO1B1)	-8.99(-17.35 to -0.64, P=0.04)
12	rs112403792 (SLCO1B1)	-0.06(-0.12 to 0.002,P=0.06)	**12**	rs6487213 (SLCO1B1)	0.30(-0.03 to 0.63, P=0.07)	**12**	rs6487213 (SLCO1B1)	7.32(0.03-14.61, P=0.05)
7	rs10276036 (ABCB1)	0.05(-0.003 to 0.11, P=0.06)	**7**	rs113539362 (CYP3A5)	0.52(-0.06 to 1.10, P=0.08)	**7**	rs473706 (CYP3A4)	7.54(-0.004 to 15.08, P=0.05)
12	rs11045852 (SLCO1B1)	-0.08(-0.17 to 0.006,P=0.07)	**7**	rs1002204 (ABCB1)	-0.39(-0.87 to 0.09, P=0.11)	**7**	rs10267099 (ABCB1)	11.82(-1.17 to 24.81, P=0.08)

Figure 1: Covariate associated relationships with LPV PK Parameters, **A** age related changes on LPV bioavailability, **B** effect of concomitant tuberculosis rifampicin therapy of LPV clearance

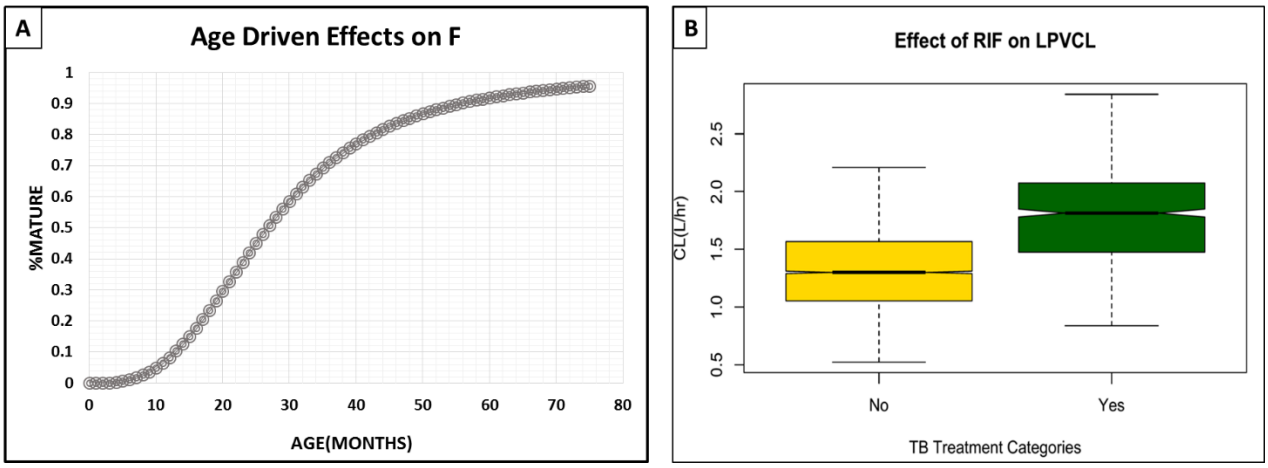
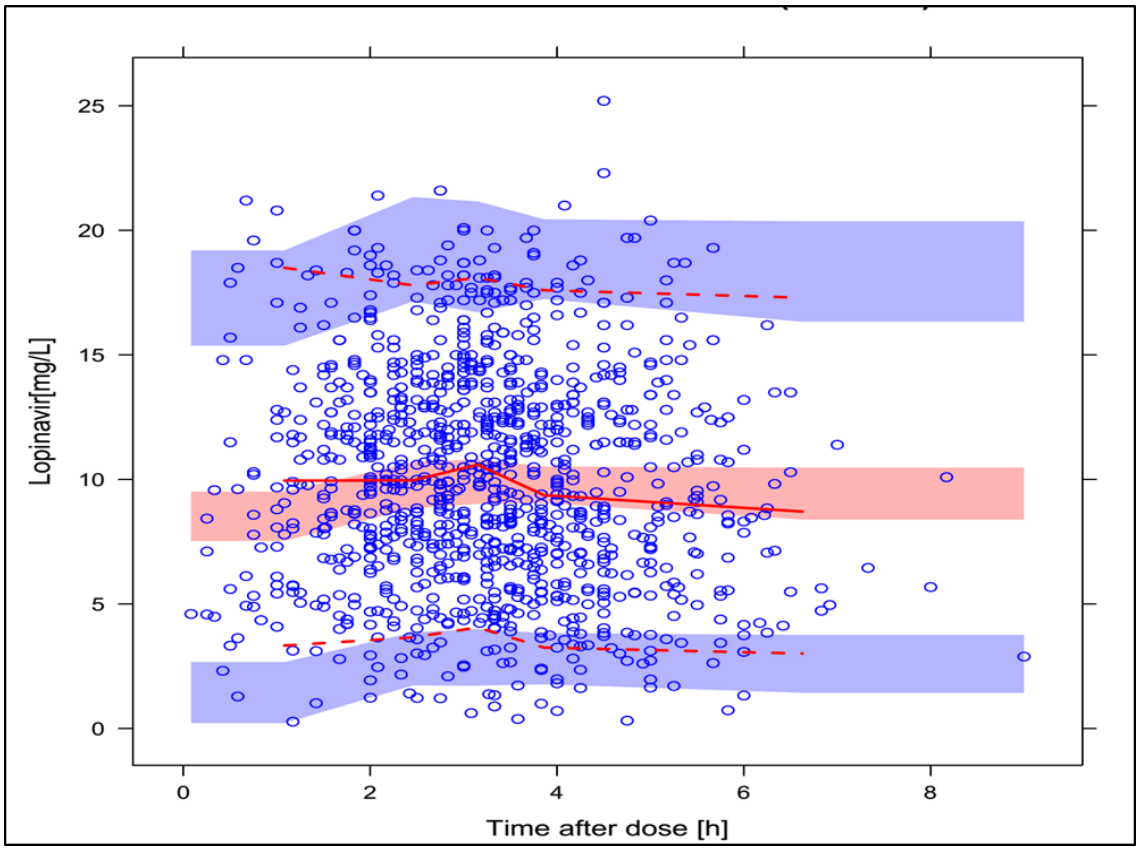


Figure 2: Visual Predicative Check of the Final Model



Supplemental Table S1: Minor allele frequencies of the 100 polymorphisms in 174 South African Children

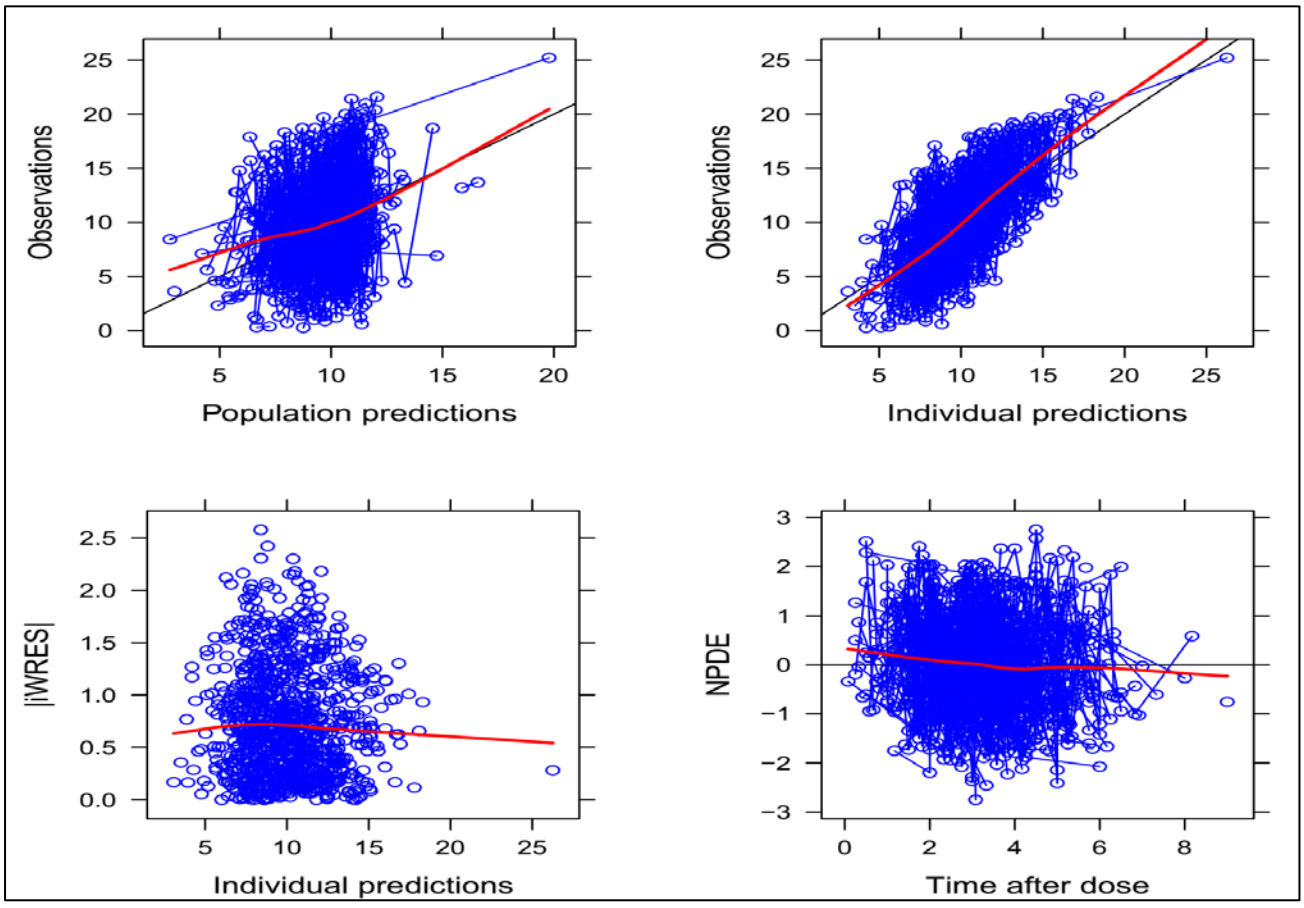
Gene	SNP	CHR	Minor Allele	Major Allele	Minor Allele Frequency	Genotype Frequency	P-value
ABCB1	rs1002204	7	T	G	0.12	1/40/135	0.47
ABCB1	rs10225473	7	G	A	0.14	7/36/133	0.05
ABCB1	rs10248420	7	A	G	0.34	19/83/74	0.62
ABCB1	rs10260862	7	C	G	0.33	21/74/79	0.61
ABCB1	rs10267099	7	G	A	0.09	0/30/146	0.62
ABCB1	rs10276036	7	C	T	0.15	4/46/126	1
ABCB1	rs1045642	7	T	C	0.09	3/26/147	0.15
ABCB1	rs11760837	7	C	T	0.20	9/52/115	0.34
ABCB1	rs17064	7	T	A	0.16	4/50/122	1
ABCB1	rs17209837	7	C	T	0.27	11/73/92	0.57
ABCB1	rs1858923	7	C	T	0.07	0/23/153	1
ABCB1	rs1882478	7	C	T	0.31	22/66/88	0.11
ABCB1	rs1922240	7	C	T	0.24	7/69/100	0.30
ABCB1	rs1989830	7	T	C	0.27	12/71/93	0.85
ABCB1	rs2229107	7	T	A	0.12	0/42/134	0.14
ABCB1	rs2235023	7	A	G	0.31	18/73/85	0.72
ABCB1	rs2235033	7	T	C	0.46	35/93/48	0.45
ABCB1	rs28364275	7	C	T	0.10	1/32/143	1
ABCB1	rs28364277	7	T	C	0.12	2/39/135	1
ABCB1	rs28364278	7	I	D	0.13	2/41/133	0.74
ABCB1	rs3747806	7	C	T	0.27	17/62/97	0.13
ABCB1	rs3789243	7	A	G	0.35	16/88/69	0.13
ABCB1	rs3842	7	C	T	0.18	4/57/115	0.45
ABCB1	rs4148751	7	G	A	0.26	10/70/96	0.69
ABCB1	rs6465118	7	A	G	0.23	4/73/99	0.03
ABCB1	rs6961419	7	C	T	0.48	35/98/43	0.17
ABCB1	rs8187789	7	A	G	0.05	0/19/157	1
CYP3A4	rs17161886	7	C	A	0.25	9/69/97	0.55
CYP3A4	rs28451617	7	T	C	0.15	8/38/130	0.04
CYP3A4	rs3735451	7	A	G	0.13	3/40/133	1
CYP3A4	rs473706	7	T	C	0.25	14/58/103	0.16
CYP3A4	rs651430	7	T	C	0.13	3/38/135	0.74
CYP3A4	rs667660	7	A	C	0.24	10/66/100	1
CYP3A4	rs7801671	7	A	C	0.22	7/62/107	0.82
CYP3A5	rs10211	7	A	G	0.32	21/70/85	0.30
CYP3A5	rs10256106	7	G	C	0.18	8/47/121	0.21
CYP3A5	rs10264272	7	T	C	0.20	9/51/116	0.34
CYP3A5	rs113539362	7	A	G	0.09	0/30/146	0.62
CYP3A5	rs15524	7	T	C	0.32	21/71/84	0.31

CYP3A5	rs1859690	7	A	G	0.32	20/72/84	0.49
CYP3A5	rs2687134	7	C	A	0.37	25/79/71	0.75
CYP3A5	rs6977165	7	C	T	0.16	3/51/122	0.58
CYP3A5	rs773049150	7	I	D	0.10	2/32/142	0.70
CYP3A5	rs8175345	7	T	C	0.10	1/33/142	1
SLCO1B1	rs1000691	12	A	G	0.18	9/44/123	0.07
SLCO1B1	rs10841763	12	C	T	0.13	3/41/132	1
SLCO1B1	rs10841778	12	G	T	0.13	0/31/145	0.37
SLCO1B1	rs10841781	12	G	A	0.28	6/87/83	0.003
SLCO1B1	rs10841782	12	T	C	0.18	3/59/114	0.21
SLCO1B1	rs11045819	12	A	C	0.08	1/27/148	1
SLCO1B1	rs11045852	12	G	A	0.06	0/21/155	1
SLCO1B1	rs11045854	12	A	G	0.06	0/21/155	1
SLCO1B1	rs11045863	12	T	C	0.06	0/22/154	1
SLCO1B1	rs11045917	12	A	G	0.05	0/17/158	1
SLCO1B1	rs11045919	12	T	G	0.49	45/83/48	0.45
SLCO1B1	rs11045920	12	A	C	0.08	1/25/150	1
SLCO1B1	rs112108376	12	A	G	0.08	1/25/150	1
SLCO1B1	rs112403792	12	T	C	0.14	3/44/129	1
SLCO1B1	rs113341884	12	T	G	0.05	0/17/157	1
SLCO1B1	rs11568557	12	C	G	0.08	0/27/149	0.60
SLCO1B1	rs11568565	12	A	G	0.08	1/27/148	1
SLCO1B1	rs11835045	12	C	T	0.27	11/73/89	0.57
SLCO1B1	rs12317843	12	T	C	0.46	40/83/53	0.54
SLCO1B1	rs12578392	12	C	T	0.23	8/64/104	0.83
SLCO1B1	rs2010668	12	A	C	0.24	11/63/102	0.84
SLCO1B1	rs2291075	12	C	T	0.44	37/81/58	0.36
SLCO1B1	rs2306283	12	A	G	0.13	3/39/134	1
SLCO1B1	rs34671512	12	C	A	0.11	3/34/139	0.47
SLCO1B1	rs4140389	12	C	A	0.49	49/71/53	0.02
SLCO1B1	rs4149006	12	T	G	0.15	0/53/123	0.02
SLCO1B1	rs4149008	12	G	A	0.39	34/68/74	0.02
SLCO1B1	rs4149009	12	C	T	0.31	23/63/90	0.03
SLCO1B1	rs4149014	12	G	T	0.05	0/16/160	1
SLCO1B1	rs4149032	12	C	T	0.21	10/55/110	0.37
SLCO1B1	rs4149050	12	T	C	0.43	37/76/63	0.12
SLCO1B1	rs4149056	12	C	T	0	0/1/175	1
SLCO1B1	rs4149057	12	C	T	0.1	1/34/141	1
SLCO1B1	rs4149063	12	T	G	0.25	12/65/99	0.84
SLCO1B1	rs4149087	12	G	T	0.23	8/64/104	0.83
SLCO1B1	rs4149088	12	G	A	0.23	8/64/104	0.83
SLCO1B1	rs57040246	12	T	C	0.04	1/12/163	0.24
SLCO1B1	rs6487213	12	C	T	0.3	17/71/88	0.59
SLCO1B1	rs71446763	12	A	G	0.08	0/27/149	0.60

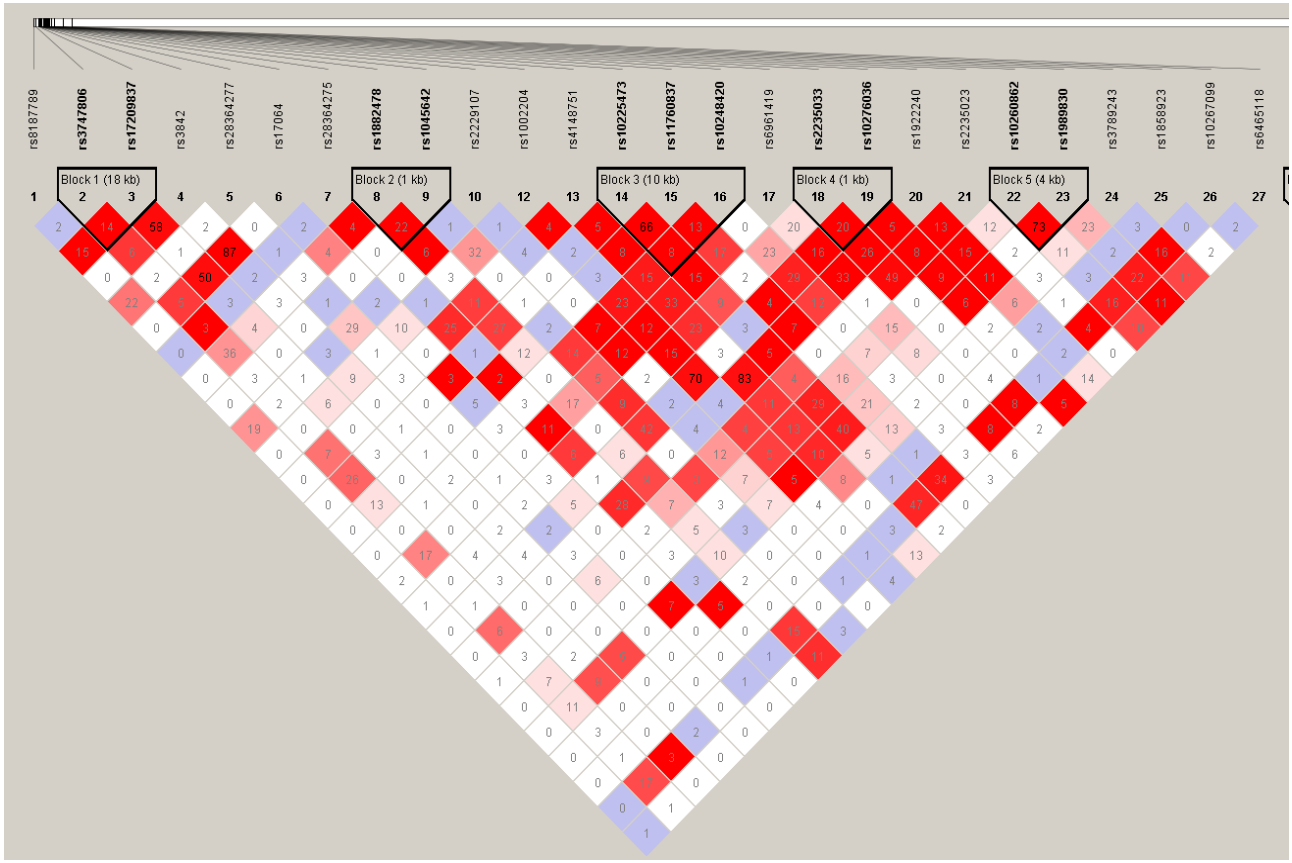
SLCO1B1	rs7302619	12	A	G	0.27	10/75/91	0.34
SLCO1B1	rs73241802	12	A	C	0.08	0/28/148	0.60
SLCO1B1	rs73250843	12	A	G	0.07	1/23/152	0.60
SLCO1B1	rs74064211	12	T	C	0.06	0/21/155	1
SLCO1B1	rs74064213	12	G	A	0.05	0/19/157	1
SLCO1B1	rs75967989	12	G	A	0.08	1/26/149	1
SLCO1B1	rs78801100	12	T	C	0.06	1/20/153	0.51
SLCO1B1	rs7966613	12	G	A	0.17	6/48/122	0.60
SLCO1B1	rs7967354	12	T	C	0.39	35/68/73	0.01
SLCO1B1	rs7975594	12	C	T	0.06	1/18/157	0.44
SLCO1B1	rs7976818	12	T	C	0.22	9/59/108	0.83
SLCO1B1	rs852550	12	G	A	0.28	15/70/91	0.85
SLCO1B1	rs981262	12	C	T	0.48	39/91/46	0.76
SLCO1B1	rs999278	12	A	C	0.04	0/13/163	1
SLCO1B7	rs111512821	12	C	T	0.08	0/28/148	0.60
SLCO1B8	rs11045927	12	G	T	0.22	35/91/50	0.65
SLCO1B9	rs11045906	12	G	A	0.46	7/63/106	0.66

CHR, chromosome; SNP, single nucleotide polymorphism

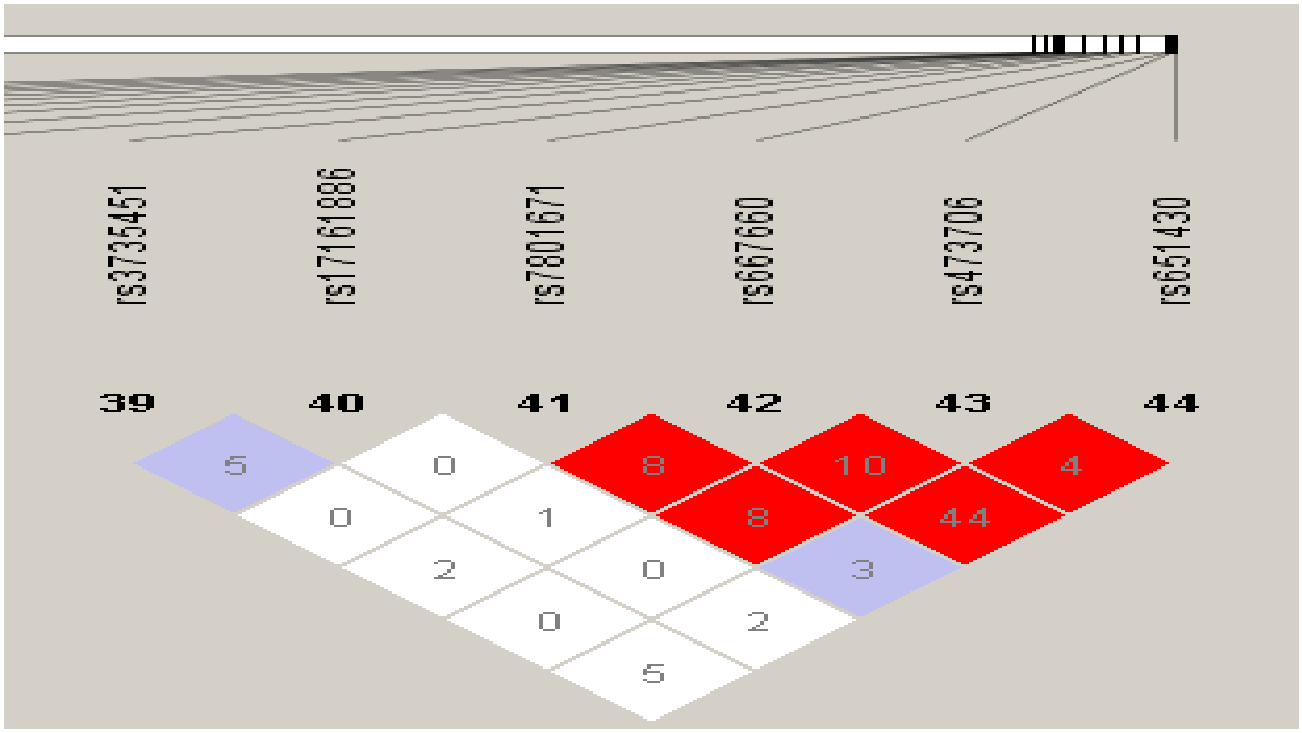
Supplementary Figure 1: Depicts the goodness of fit plots of the final model



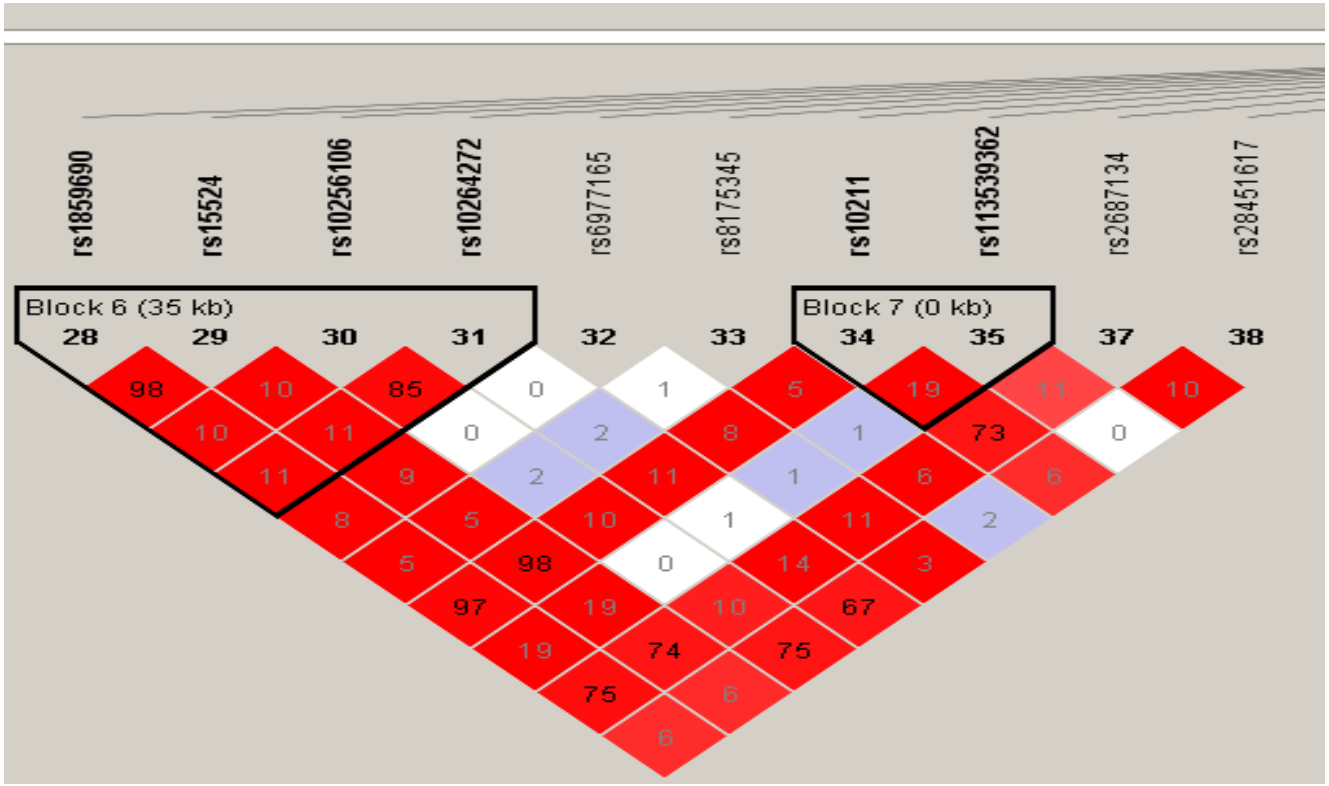
Supplementary Figure 2: Shows the linkage disequilibrium of ABCB1 gene



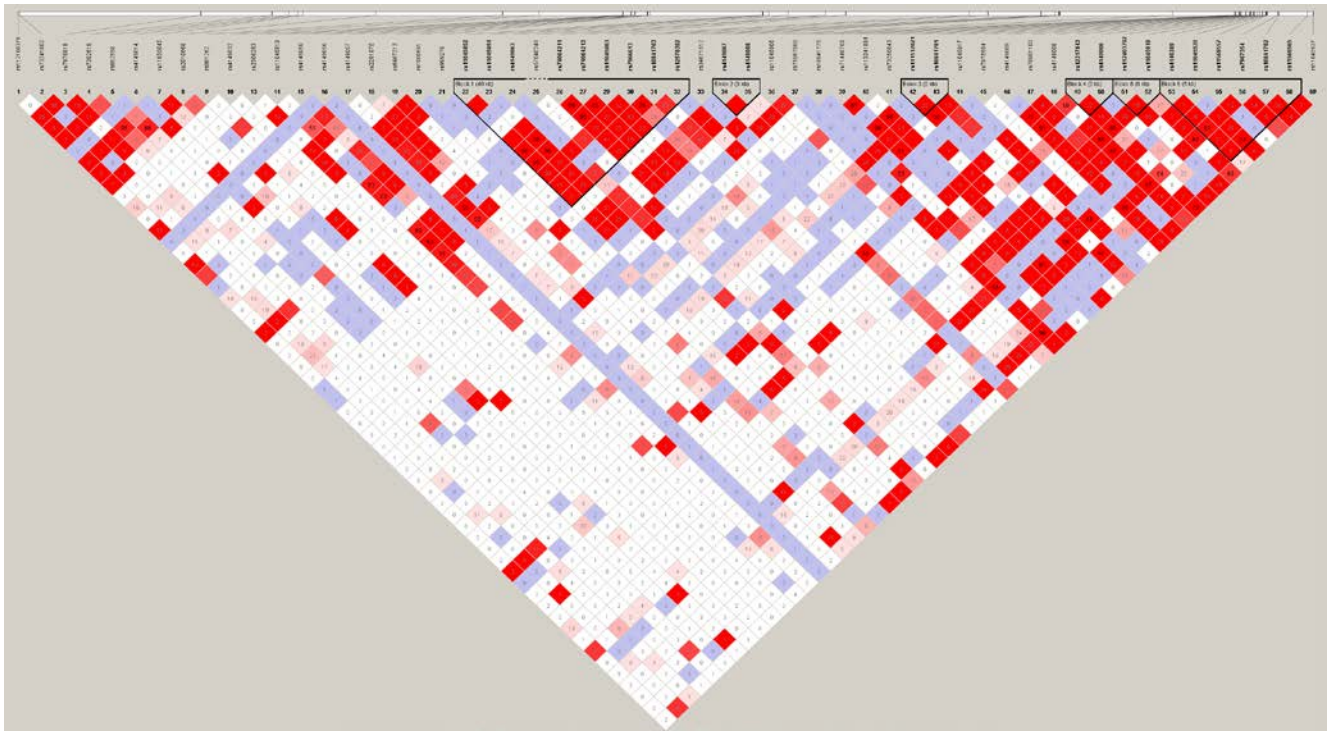
Supplementary Figure 3: Presents the linkage disequilibrium of CYP3A4 gene



Supplementary Figure 3: Demonstrates the linkage disequilibrium of CYP3A5 gene



Supplementary Figure 4: Illustrates the linkage disequilibrium of SLCO1B1 gene



Equation 1

$$CL/Fstd = CL/Fstd * (\frac{TWBT}{12.5})^{0.75} \quad \textbf{(1.1)}$$

$$V/Fstd = V/Fstd * (\frac{TWBT}{12.5})^{1} \quad \textbf{(1.2)}$$

Equation 2

PMA = AGE + (9/12)

TVPMA = MEDAGE + (9/12)

$$F = 1/\left(1 + \frac{PMA}{TVPMA+PMA50}\right) EXP^{-Hill}$$

Author Contributions

Title "Associations between CYP2B6, CY3A4, CYP3A5 and ABCB1 Genotypes and Nevirapine Pharmacokinetics in HIV-Positive South African Children**"**

[1]Retsilisitsoe R. Moholisa, [2,3,4]Tim R. Cressey, [1]Phumla Sinxadi , [5]Louise Kuhn, [6,8]Emile R. Chimusa ,[1]Sandra Meredith, [1]Lubbe Weisner, [7]Ashraaf Coovadia, [8]Renate Strehlua, [9]Elaine J. Abrams, [1,10]Gary Maartens, [1,10]Helen McIlleron, [10,11]David Haas

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, [2]PHPT/IRD 174, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; [3]Department of Immunology & Infectious Diseases, Boston, Harvard T.H Chan School of Public Health, MA, USA, [4]Department of Molecular & Clinical Pharmacology, University of Liverpool, UK,[5]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, Division of Human Genetics, Department of Pathology, University of Cape Town, [6]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [7]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[8]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, [9]Vanderbilt Medical Center and [10]School of Medicine, Department of Medicine, Nashville,Tannessee

RR Moholisa: Responsible for the data handling, processing and analysis under; Wrote the initial drafts of the manuscript till the final version that was submitted for publication including producing all tables and figures

Tim R Cressey: Guided the analysis strategy and provided guidance from the initial draft of the manuscript especially the population pharmacokinetic modelling analysis section and the discussion.

Phumla P Sinxadi and David Haas: Guided the analysis strategy and provided guidance from the initial draft of the manuscript especially the genotyping, statistical analysis section and the discussion.

Emile R Chimusa: Provided technical assistance with regards to plotting linkage disequilibrium plots and providing input for the write up of the manuscript

Luis Kuhn, Ashraaf Coovadia, Renate Strehlau, and Elaine J Abrams: Responsible for planning the study and collecting all the data.

Sandra Castel and Lubbe Weisner: performed the laboratory analysis of measuring lopinavir and nevirapine concentrations

G Maartens and H McIlleron: Oversaw overall analysis strategy planning and mentoring, guided the writing of the manuscript from beginning to end

Chapter 6

Title: Associations between CYP2B6, CY3A4, CYP3A5 and ABCB1 Genotypes and Nevirapine Pharmacokinetics in HIV-Positive South African Children.

[1]Retsilisitsoe R. Moholisa, [2,3,4]Tim R. Cressey, [1]Phumla Sinxadi , [5]Louise Kuhn, [6,9]Emile R. Chimusa ,[1]Sandra Meredith, [1]Lubbe Weisner, [7]Ashraaf Coovadia, [7]Renate Strehlua, [8]Elaine J. Abrams, [1,9]Gary Maartens, [1,9]Helen McIlleron, [10,11]David W. Haas.

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, [2]PHPT/IRD 174, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; [3]Department of Immunology & Infectious Diseases, Boston, Harvard T.H Chan School of Public Health, MA, USA, [4]Department of Molecular & Clinical Pharmacology, University of Liverpool, UK,[5]Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, [6]Division of Human Genetics, Department of Pathology, University of Cape Town, [7]Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, [8]ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York,[9]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, [10]Vanderbilt University Medical Center, Department of Medicine, Nashville,Tannessee, and [11]Meharry Medical College, Department of Internal Medicine, Nashville, Tennessee

6.1 **Abstract**

6.1.1 **Aims:** To assess whether clinical factors or host genotype are associated with nevirapine pharmacokinetics of nevirapine (NVP) at steady-state in HIV-positive South African children.

6.1.2 **Methods:** Steady-state population pharmacokinetic parameters for NVP were estimated using nonlinear mixed-effects modelling. The final model was used to derive individual oral clearances (CL/F), minimum concentrations (C_{min}) and area under the concentration time curves (AUC_{0-12}). We explored relationships with genotypes in selected genes relevant to NVP disposition and model-derived pharmacokinetic indices.

6.1.3 **Results:** A total of 95 children were included in the analysis. Nevirapine pharmacokinetics were best described by a one-compartment disposition model coupled with elimination through a well-stirred liver model accounting for first-pass effect and transit absorption. Among 60 children with genotype data, there were 16 *CYP2B6* extensive metabolizer, 40 *CYP2B6* intermediate metabolizer and 4 *CYP2B6* slow metabolizer genotypes, and based composite *CYP2B6* 15582/516/983 genotypes. By univariate analysis, several *CYP2B6* and genotypes were associated with NVP pharmacokinetics: *CYP2B6* 516G→T and 983T→C were associated with CL/F. *CYP2B6* 983T→C was associated with C_{min} and $AUC_{0\text{-}12}$ in a univariate analysis and after adjusting for *CYP2B6* 516G→T. *CYP2B6* 15582C→T was associated with and CL/F, C_{min} and $AUC_{0\text{-}12}$ after adjusting for *CYP2B6* 516G→T and 983T→C. Polymorphisms in *ABCB1* and *CYP3A5* were independently associated with CL/F, C_{min} and $AUC_{0\text{-}12}$

6.1.4 **Conclusions:** In HIV-positive Black South African children, *CYP2B6* genotype was associated with NVP pharmacokinetics

6.2 **Introduction**

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor used as part of combination antiretroviral therapy (ART) for HIV-1 infection in adults and children in resource-limited settings[1]. The success of NVP in African nations has been partly due its affordability and availability in fixed-dose combinations[2–4]. Though efficacious and safe, NVP has a low genetic barrier to resistance, and sub-therapeutic drug exposure can increase the risk of drug resistance and treatment failure[5,6]. Nonetheless, NVP still has several advantages over HIV protease inhibitors including its ability to be formulated as a heat-stable liquid and

fewer potential drug-drug interactions, particularly with anti-tuberculosis medications. Also, the bioavailability of NVP is not affected by food intake and does not cause significant central nervous system compared adverse events, unlike the non-nucleoside reverse transcriptase inhibitor efavirenz[7].

Antiretroviral agents, including NVP are characterised by considerable inter-individual variability in metabolism, some of which is due to genetic differences in drug-metabolising enzymes and efflux/influx transporters. NVP is metabolised predominantly by cytochrome (CYP) *2B6* and *CYP3A* isoforms[8]. Previous studies have shown an association between the *CYP2B6* 516G→T (rs3745274) polymorphism and NVP concentrations in children and adults[9–11]. Patients homozygous for *CYP2B6* 516TT genotype had lower oral clearance and higher trough concentrations[9,12]. A less frequent *CYP2B6* polymorphism, 983T→C (rs28399499), has been associated with increased steady-state exposure of NVP[13]. Both *CYP2B6* 516T and 983C are more frequent with African ancestry than with European ancestary[14,15]. A genome wide association study of White, Black and Hispanic adults in the United States found an independent association between a third polymorphism, *CYP2B6* 15582C→T (rs4803419) and efavirenz trough concentrations [16]. Subsequently, a study in South African adults and children showed that the composite genotype defined by *CYP2B6* 516G→T, 983T→C and 15582C→T was associated with increased plasma efavirenz exposure[17]. *CYP2B6* 15582C→T has been associated with slower clearance (CL/F) of NVP in Cambodian adults[18], and longer time for plasma concentrations to fall below the protein-adjusted IC_{50} in women of African ancestry[19]. The impact of *CYP3A4* and *CYP3A5* genetic polymorphisms on NVP pharmacokinetics is less studied.

Chapter 6

The aims of the present study were to characterise the steady-state population pharmacokinetics of NVP in South African children, and to quantify associations between polymorphisms in selected genes and NVP disposition in this patient population.

6.3 **Methods**

6.3.1 **Study Participants**

Pharmacokinetics of NVP were retrospectively analyzed in samples collected from participants of the NEVEREST2 trial at clinic visits during the post-randomization period[20,21]. The NEVEREST2 trial was a randomized, open-label clinical trial investigating treatment options for NVP exposed children who initiated protease inhibitor-based ART when less than 24 months of age. Children eligible for randomization were HIV-positive, attending the Rahima Moosa Mother and Child Hospital in, Johannesburg, South Africa, and who achieved plasma HIV-1 RNA ≤400 copies/mL for at least 2 consecutive visits on lopinavir/ritonavir (LPV/r) based ART. Once criteria for randomization were met, the children were randomized 1:1 to continue their LPV/r regimen or switch from LPV/r to NVP. NVP (Viramune® oral solution, Boehringer Ingelheim) was administered at 120 mg/m^2 once daily for the first 2 weeks and thereafter at 200mg/m^2 every 12 hours thereafter. Lamivudine and stavudine were used as concomitant nucleoside reverse transcriptase inhibitors. Doses were adjusted at each visit according to the growth of the children. Additional adherence counselling was offered, including specific instructions concerning the lead-in schedule and possible adverse effects of switching to NVP.

Data collected at randomization included age, sex, plasma HIV-1 RNA and CD4$^+$ T lymphocyte percentage (CD4%). Weight-for-age z-score and height-for-age z-score at randomization were

calculated using WHO software[20]. Blood samples for measuring NVP concentrations were collected at randomization at 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post-randomisation and at unscheduled clinic visits. The time of blood sample collection was documented, as was the time of the morning dose of antiretrovirals, as reported by the caregiver. In children who were diagnosed with tuberculosis, concomitant tuberculosis treatment was recorded at each visit.

6.3.2 **Quantification of Nevirapine in Plasma**

Plasma NVP concentrations were assayed using validated liquid chromatography tandem mass spectrometry methods developed in the Division of Clinical Pharmacology, University of Cape Town, South Africa. An AB Sciex 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode. The validated concentration range for NVP was 0.1 mg/L to 15 mg/L. Inter- and intra-day coefficients of variation were below 10% for all quality control concentrations. The laboratory, at which the concentrations were assayed, participates in the International Inter-laboratory Pharmacology Quality Control Program of, the AIDS Clinical Trial Group[22].

6.3.3 **Genotyping**

Human DNA was extracted from buffy coats using the QIAsymphony (QIAGEN, Hilden, Germany) DNA midi kit which utilises magnetic-particle technology to isolate and purify DNA. Targeted genotyping of *CYP2B6* 516G→T (rs3745274), *CYP2A6* -48T→G (rs28399433) and *CYP2B7* rs4124633 were done by TaqMan™ (Applied Biosystems, Foster City, CA). Genotyping of *CYP2B6* 983T→C (rs28399499), 15582C→T (rs4803419) was done as part of a custom designed MassARRAY® iPLEX Gold (Sequenom Inc., San Diego, California, USA). Genotypes were confirmed by visual inspection of plots, and all samples were genotyped in duplicate. Additional selected genotypes were extracted from Illumina HumanCore Exome assay data

(Illumina, San Diego, CA). All HumanCore Exome plates contained a HapMap trio, as well as duplicates scattered across each plate for QC purposes. Call rates for 93% of samples exceeded 98%, and the average call rate for the project was 98.7%. All genotyping was done at the Vanderbilt Technologies for Advanced Genomics (VANTAGE). Laboratory personnel with no knowledge of clinical data performed genotyping.

6.3.4 **Population Pharmacokinetic Analysis**

A population pharmacokinetic analysis of steady state NVP plasma concentration data was performed to estimate average population pharmacokinetic parameters and interpatient variability. Non-linear mixed effects modelling software NONMEM 7.3[23] was used and concentration-time data was fitted using a two-step estimation method (i) first-order conditional estimation method with interaction was used to generate support points from the empirical bayes estimates (EBES); (ii) the nonparametric estimation was used to estimate the population probability of each support point. PsN 4.6.8, Pirana 2.9.6 and Xpose 4.5.3 were used to facilitate the model-building process and for model assessment[24]. A stepwise model building process was guided by changes in the objective function value (OFV; equivalent to -2 log likelihood), inspection of goodness-of fit plots and visual predictive checks (VPC), biological plausibility and clinical relevance. A >3.84 point drop in OFV between two nested models after adding one parameter was considered statistically significant ($p \leq 0.05$, chi square distribution with one degree of freedom). Nonparametric bootstrap (n=200) was used to evaluate the stability and robustness of final parameter estimates of the model. Both NVP C_{min} and $AUC_{0\text{-}12}$ were calculated using model-derived empirical Bayes estimates (EBE) of individual subject parameters at each sampling occasion.

6.3.5 **Structural Model**

We used a model previously developed by Bienczak *et al*[11]. One or two compartment disposition models with first-order absorption and elimination were tested, as well as delayed and transit-compartment absorption[25]. Hepatic elimination of NVP was tested using a previously described semi-mechanistic well-stirred model[26]. The hepatic model assumed the following parameters: i) fraction unbound (fu) of NVP of 40%, hepatic plasma flow (QH) of 50 L/h[27] and a liver volume (VH) of 1L[26], allometrically scaled for a typical individual weighing 70 kg. Inter-individual (IIV) and inter-occasion variability (IOV) were tested on all pharmacokinetic parameters assuming a log-normal distribution. Residual unexplained variability (RUV) was tested using the combined proportional (PROP) and additive (ADD) model structures. Samples below the limit of quantification (BLQ) were handled using the M5 method[28] and implausible outliers were identified using visual inspections and excluded based on normalised prediction distribution errors (NPDE >2.5).

6.3.6 **Covariate Model**

Clearance (CL/F) and volume (V/F) parameters were scaled allometrically at an early stage as previously suggested[29]. Maturation of intrinsic clearance (CL_{int}) and pre-hepatic bioavailability (F_{PREH}) were tested using power, exponential and sigmoidal functions with or without fixing the Hill coefficient[29]. Other covariates tested include sex and concomitant tuberculosis treatment.

6.3.7 **Statistical Analysis**

The model derived pharmacokinetic parameters CL/F, AUC and C_{min} were used to test for genetic associations. Bonferroni correction was used to account for multiple testing. Hardy-Weinberg equilibrium was assessed using exact tests. Composite *CYP2B6* 516/983/15582 genotypes were assigned as follows: extensive metaboliser (15582CC-516-GG-983-TT or

15582CT-516-GG-983-TT), intermediate metaboliser (15582TT-516GG-983TT, 15582CC-516GT-983TT, 15582CC-516GG-983CT, 15582CT-516GT-983TT or 15582CT-516GG-983CT), slow metaboliser (15582CC-516TT-983TT, 15582CC-516GT-983CT or 15582CC-516GG-983CC). For exploratory analyses, an additional 61 polymorphisms (in *ABCB1*, *CYP2B6*, *CYP3A4*, and *CYP3A5*) were analyzed. All tests used a 5% two-sided significance level. Data was analysed using Plink version 1.90 (http://pgnu.mgh.harvard.edu/~purcell/plink).

Haplotypic blocks were defined using the r^2 method in Haploview[30] and haplotype phases were inferred using the standard E-M algorithm in PLINK[31]. Linkage disequilibrium (LD) plots and values were generated with Haploview (www.broad.mit.edu/mpg/haploview/).

6.4 **Results**

6.4.1 **Study Participants**

Pharmacokinetic data were available from 96 children, from which 764 plasma samples were collected, with a median of 6 samples per child over a follow-up period of 3 to 196 weeks. Of the 96 DNA samples, 60 were successfully genotyped and were included the subsequent analyses. **Table 1** presents characteristics of the study population.

6.4.2 **Genetic Polymorphisms**

Among 60 children with genotype data, 66 polymorphisms were genotyped (28 in *ABCB1*, 1in *CYP2A6*, 21 in *CYP2B6*, 1 in *CYP2B7*, 7 in CYP3A4, 11 in CYP3A5). All genotypes were in Hardy-Weinberg equilibrium (HWE) based on an adjusted Bonferroni P value threshold of 0.001, except *CYP2B6* rs707265 (P=0.0009); three had unadjusted P values ≤ 0.05 (*ABCB1* rs6465116, P=0.03; *ABCB1* rs1022547, P=0.05; *CYP3A4* rs2845161, P=0.04). Minor allele frequencies, genotype frequencies and HWE P values are presented in Supplementary **Table 1**. Based on

composite CYP2B6 15582/516/983 genotype, there were 16 extensive metabolizer, 40 intermediate metabolizer and 4 slow metabolizer genotypes. No polymorphisms were in strong LD with *CYP2B6* 516G→T (rs3745274), 983T→C (rs28399499) or 15582C→T (4803417), based on threshold of $r^2 \geq 0.80$ (**Supplementary Figure 1**). Furthermore, no strong LD association with ABCB1 3435C→T (rs1045642), 4036A→G (rs3842) and other polymorphisms were observed (**Supplementary Figure 2**). No strong LD association with polymorphisms in the *CYP3A4* were observed (**Supplementary Figure 3**)**.** However, in *CYP3A5*, there was strong LD between rs1859690, rs15524 and rs10211 or rs15524 and rs10211 as well as rs10256106 and rs10264272 (**Supplementary Figure 4**).

6.4.3 **Nevirapine Population Pharmacokinetic Model**

A one compartment disposition model best described NVP pharmacokinetics. A transit compartment was used to describe absorption, and elimination was described using a semi-physiological hepatic extraction. The absorption parameters could not be estimated in our model due to sparseness of the data (1 sample per occasion) and were therefore fixed previously published values[11]. **Table 2** presents final NVP population pharmacokinetic parameter estimates, their precision (obtained through non-parametric bootstrap) and inclusion of covariates and random effects based on statistical significance (drop in OFV value) and biological plausibility. Model adequacy was evaluated using GOF plots and VPC (**Figure 2A**).

The effect of body size was accounted for on all CL/F and V/F parameters and significantly improved the model fit (21 point drop in OFV). Implementing a well-stirred liver model resulted in a drop in OFV of 150 points, without adding extra parameters. CLint was used to parameterize the model and the model identified distinct F_{preH} and FH of bioavailability

because changes in the liver activity mechanistically also affected FH. F_{preH} was fixed to one, whereas BSV and BOV were estimated. Our model did not find an age driven effects on CLint. Age-driven differences were identified on F_{preH} using an exponential model (**Figure 2B**). F_{preH} was estimated to be 50.4% from older children, with a half-life of 0.84 years.

BSV was identified for all parameters except for MTT. Absorption parameters KA and MTT displayed the largest BOV of all parameters, 40.2% and 244.1%, respectively. The combined error model (additive and proportional error) best described the RUV.

6.4.4 **Effects of *ABCB1*, *CYP3A4*, *CYP3A5* and *CYP2B6* Genetic Polymorphisms on Model-Derived Nevirapine Indices**

Pharmacokinetic data were not normally distributed in 55 children with both phenotype and genotype, so we used geometric means for subsequent analysis. The median C_{min} and AUC_{0-12} were 4.67 mg/L (IQR 3.71- 5.77) and 48.03 mg.hr/L (IQR 38.66-57.91), respectively. Regarding NVP CL/F, in univariate (unadjusted) linear regression models, there were no significant associations with genotypes when using Bonferroni-adjusted P≤0.001. Nonetheless, we found nominal associations with *ABCB1* rs1002204 (β:0.32[0.05-0.59, P= 2.29 x 10^{-2}]), *CYP2B6* 983T→C (β:-0.26[(-0.50 to -0.02, P= 3.91 x 10^{-2}]), 516G→T (-0.16[-0.31 to -0.01, P= 4.15 x 10^{-2}]), and rs7250597 (β:0.17[0.01-0.33, P= 4.20 x 10^{-2}]) , *CYP3A5* rs185690 (β:-0.18[-0.34 to -0.01, P= 4.35 x 10^{-2}]) and rs15524 (β:-0.18[-0.34 to -0.01, P= 4.35 x 10^{-2}]), respectively. After adjusting for *CYP2B6* 516G→T, we found significant nominal associations between NVP CL/F and *ABCB1* rs1002204 (β:-0.14[-0.28 to 0.01, P= 4.93 x 10^{-2}]), *CYP2B6* 983 T→C (β:-0.19[-0.33 to -0.04, P= 1.38 x 10^{-2}]) and *CYP2B6* 15582C→T (β:-0.16[-0.31 to 0.01, P= 4.84 x 10^{-2}]). After adjusting for the *CYP2B6* 516/983 haplotype, we found a significant associations between NVP CL/F and all genotypes in the first model including *CYP2B6* 15582C→T (β:-0.21[-0.37 to -0.05, P= 1.33 x 10^{-2}]). Similarly, after adjusting for composite

CYP2B6 15582/516/983 haplotype, we found significant associations between NVP CL/F and above polymorphisms. **Table 3** presents associations between genetic polymorphisms and NVP CL/F

Regarding NVP C_{min} (**Table 4**), we found a Bonferroni-adjusted (P≤0.001) significant association by using a linear regression with *CYP2B6* 983 T→C (β:6.07(95%CI:3.14-9.01, P= 1.65 x 10^{-4}]), and nominal associations with CYP2A6 -48T→G (β:7.14[95%CI:1.92-12.37, P= 9.83 x 10^{-3}]), *CYP2B7* rs4124633 (β:2.98[95%CI:0.30-5.65, P= 3.38 x 10^{-2}]), *CYP3A5* rs10211 (β:2.74[95%CI:0.54-4.94, P= 1.80 x 10^{-2}]), rs1859690 (β:2.70[95%CI:0.49-4.91, P= 2.03 x 10^{-2}]), rs15524(2.70[95%CI:0.49-4.91,P= 2.03 x 10^{-2}]), and rs113539362 (β:4.44[0.51-8.36, P= 3.09 x 10^{-2}]). After adjusting for *CYP2B6* 516G→T, there was a strong association with NVP C_{min} and *CYP2B6* 983T→C (β:3.24[95%CI:1.53-4.94, P= 4.83 x 10^{-4}]) and CYP2A6 -48T→G (β:2.16[95%CI:0.27-4.05, P= 2.94 x 10^{-2}) but not with *CYP2B6* 15582C→T (β:1.71[95%CI:-0.34 to 3.77, P= 0.11]). Adjusting for composite *CYP2B6* 516/983 genotype was associated with increased NVP C_{min} across all genotypes in the initial model including *CYP2B6* 15582C→T (β: 3.31[95%CI:1.28-5.35,P= 2.36 x 10^{-3}]). Likewise, after adjusting for the composite *CYP2B6* 15582/516/983 haplotype, there were significant associations with the aforementioned polymorphisms.

Regarding NVP AUC_{0-12}, by univariate regression, (**Table 5**),there was a significant Bonferroni-adjusted association with *CYP2B6* 983T→C (β:59.3[95%CI:30.6-88.2, P= 1.71 x 10^{-4}]) and nominal associations with *CYP2A6* -48T→G (69.6[195%CI:8.3-120.8, P= 1.02 x 10^{-2}]), *CYP2B7* rs4124633 (β:29.3[95%CI:0.30-5.65, P= 3.30 x 10^{-2}]), *CYP3A5* rs10211 (β:26.9[95%CI:5.4-48.4, P= 1.77 x 10^{-2}]), *CYP3A5* rs1859690 (β:26.9[95%CI:5.4-48.4, P= 2.00 x 10^{-2}]), rs15524(β:26.5[95%CI:4.8-48.2, P= 2.00 x 10^{-2}]), and *CYP3A5* rs113539362(β:43.8[95%CI:5.4-

82.2, P= 2.97 x 10^{-2}]. After adjusting for *CYP2B6* 516G→T, there was a significant association with *CYP2B6* 983T→C (β:21.9[95%CI:9.6-34.2, P= 9.96 x 10^{-4}]), and nominal associations with *CYP2B6* rs8100458 β:15.7[95%CI:1.7-29.6, P= 3.23 x 10^{-2}) and *CYP2B6* 15582C→T (β:15.1[95%CI:1.1-29.2, P= 4.02 x 10^{-2}]), *CYP2A6* -48T→G (β:15.5[95%1.9-29, P= 2.99 x 10^{-2}]), and *CYP2B7* rs4124633 (β:13.9[95%CI: 0.1-27.8, P= 5.48 x 10^{-2}]). After adjusting for composite *CYP2B6* 516/983 genotype, there were significant associations with all polymorphisms in the initial model including *CYP2B6* 15582C→T (β:32.2[95%CI:11.9-52.3,P=2.93 x 10^{-3}]). Similarly, after adjusting for composite *CYP2B6* 15582/516/983 haplotype, there were significant associations with the above polymorphisms.

6.5 **Discussion**

NVP remains one of the most widely prescribed drugs for treating HIV in resource limited settings. The present study characterised relationships between NVP pharmacokinetics and genetic polymorphisms in South African children. Our population pharmacokinetic model estimated allometrically-scaled oral clearance to be 2.08L/h, which was lower reported in previous study by our group (than 3.8L/h[11])and lower than other studies in children and adults. V/F of 19.7L was comparable to previously published data[11]. Our model could not estimate the absorption parameters (absorption rate constant [KA], number of transits [NN] and mean transit time [MTT]) due to limited number of samples within the absorption phase. However, we found largest IOV in KA and MTT similar to previous results from our group[11].

We evaluated associations with genetic polymorphisms in *ABCB1*, *CYP2A6, CYP2B7, CYP2B6, CYP3A4* and *CYP3A5* using a candidate gene approach. Previous studies have shown associations between NVP pharmacokinetics and *CYP2B6* 516G→T[10,12] and 983T→C[30–33].

CYP2B6 15582C→T has also been associated with NVP PK in Cambodian and African adults[18,35]. Our study replicated this findings in black South African children. In the present study, minor allele frequencies of *CYP2B6* 516G→T, *CYP2B6* 983T→C, *CYP2B6* 15582C→T were 0.39, 0.11, 0.06, respectively, similar to a previous report from our group[17]. By univariate analysis, *ABCB1* rs1002204 and *CYP2B6* rs7250597 were nominally associated with increased NVP CL/F, whereas *CYP3A5* rs1859690, *CYP3A5* rs15524, *CYP2B6* 516G→T and *CYP2B6* 983T→C were nominally associated with decreased NVP CL/F. The effect of *CYP2B6* 516G→T and *CYP2B6* 983T→C on NVP CL/F are consistent with previous reports in African adults[36]. After adjusting for *CYP2B6 516G→T*, there were nominal associations with *ABCB1 rs1002204, CYP2B6 983T→C* and *CYP2B6 15582 C→T*. Adjusting for composite *CYP2B6* 516/983 or *CYP2B6* 15582/516/983 genotype, there were significant associations with the aforementioned genotypes.

In a univariate analysis, we found significant associations between NVP C_{min} and *CYP2B6 983T→C* and nominal associations with *CYP3A5* rs10211*, CYP3A5* rs1859690*, CYP3A5* rs15524, *CYP3A5* rs113539362, *CYP2B6* rs8100458, *CYP2A6* -48T→G*,* and *CYP2B7* rs4124633. After adjusting for *CYP2B6* 516G→T, both *CYP2B6* 983T→C and *CYP2A6* -48T→G remained significant. Furthermore, after adjusting for the composite CYP2B6 516/983 or *CYP2B6* 15582/516/983 genotypes, the above-mentioned polymorphisms were significantly associated with NVP C_{min}. These findings are consistent with previous reports from our group with regards the relationship between *CYP2B6* 983T→C and NVP C_{min}[11]. Similarly, in a univariate analysis, we found significant association with NVP AUC_{0-12} and *CYP2B6* 983T→C, and nominal associations with *CYP3A5* rs10211*, CYP3A5* rs1859690*, CYP3A5* rs15524, *CYP3A5* rs113539362, *CYP2A6* -48T→G*, CYP2B7* rs4124633 and *CYP2B6* rs8100458. After adjusting for *CYP2B6* 516G→T, only polymorphisms in CYP2B6 remained significant including 15582C→T.

Likewise, after adjusting for the composite *CYP2B6* 516/983 or *CYP2B6* 15582/516/983 genotype, all the aforementioned polymorphisms were significantly associated with NVP $AUC_{0\text{-}12}$.

We found little association between *CYP2B6* 516G→T and NVP pharmacokinetics in our study. This is consistent with a previous report in African American adults[9]. In contrast, *CYP2B6* 983T→C was consistently associated with NVP pharmacokinetics and suggesting fundamental differences between the two polymorphisms, consistent with previous reports[37,38] . This may be because C*YP2B6* 983T→C reduces hepatic *CYP2B6* expression and/or activity to a much greater extent than does *CYP2B6* 516G→T. This is reinforced by evidence that *CYP2B6* 983T→C has a greater effect on efavirenz PK than does *CYP2B6* 516G→T[16]. In a univariate analysis, lack of association between 15582C→T and NVP pharmacokinetics may reflect this polymorphism's weak effect on *CYP2B6* expression and/or activity. Interestingly, we found associations with *ABCB1* rs1002204 and NVP CL/F even after adjusting for *CYP2B6* genotypes. Furthermore, we found significant association with several *CYP3A5* polymorphisms and NVP PK after adjusting for composite *CYP2B6* 516/983 or composite *CYP2B6* 516/983/15582 genotype.

In summary, the present study extends our understanding of the influence of genetic polymorphisms on NVP PK. Improved knowledge of the impact of genetic variants on NVP pharmacokinetics may ultimately improve the clinical management of HIV infection in children.

Chapter 6

6.6 **Acknowledgements:**

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6.7 **References**

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Table 1: Summary of the data

Characteristic	Median(IQR)	Range
Age(Months)	30(24-42)	10-77
BW(Kg)	13(11-15)	6.6-20
Dose(mg/m^2)	105(90-120)	55-165
CD4%	32(24-39)	7.86-64
VL	50(25-73)	25-5.6 x 10^6

Table 2: Final Model Parameter Estimates

Parameter	Typical Values(For 13KG Child)	Bootstrap Values[Median(95%CI)]
CL_{int} [L/hr]	2.07	2.08(1.38-4.74)
V/F[L]	19.7	19.70(13.73-26.86)
Ka[hr^{-1}]	0.84 FIX	
MTT[hr]	0.56 FIX	
NN	3 FIX	
F at Birth[%]	50.4	50.35(44.81-55.08)
KBIO[Years]	0.84	0.84(0.50-0.95)
Variability		
IIV CL	20.9%	20.15%(15.57%-23.45%)
IIV V	22.3%	22.48%(18.41%-24.53%)
IIV KA	51.7%	48.44%(46.76%-54.19%)
IIV MTT	--------	
IIV F	15.7%	15.43%(13.53-17.04)
IOV CL	11.6%	
IOV KA	40.2%	
IOV MTT	244.1%	
IOV F	13.5%	
RUV		
Prop[%]	10.7	10.6(0.07-16.40)
Add[mg/L]	2.13	2.14(0.15-2.83)
Cmin[Median,(IQR)]	5.03(3.96-5.98)	
AUC[Median, (IQR)]	50.62(40.31-59.39)	

Add, additive error; CL/F, clearance; F, bioavailability; IIV, inter-individual variability; IOV, inter-occasion variability; KA, absorption rate constant; PMA50, post menstrual age; Prop, proportional error; RUV, residual unexplained variability; V/F, volume

Figure 1: **A** Visual Predicative Check of Nevirapine Final Model. **B** Age driven effects on Nevirapine bioavailability in Children.

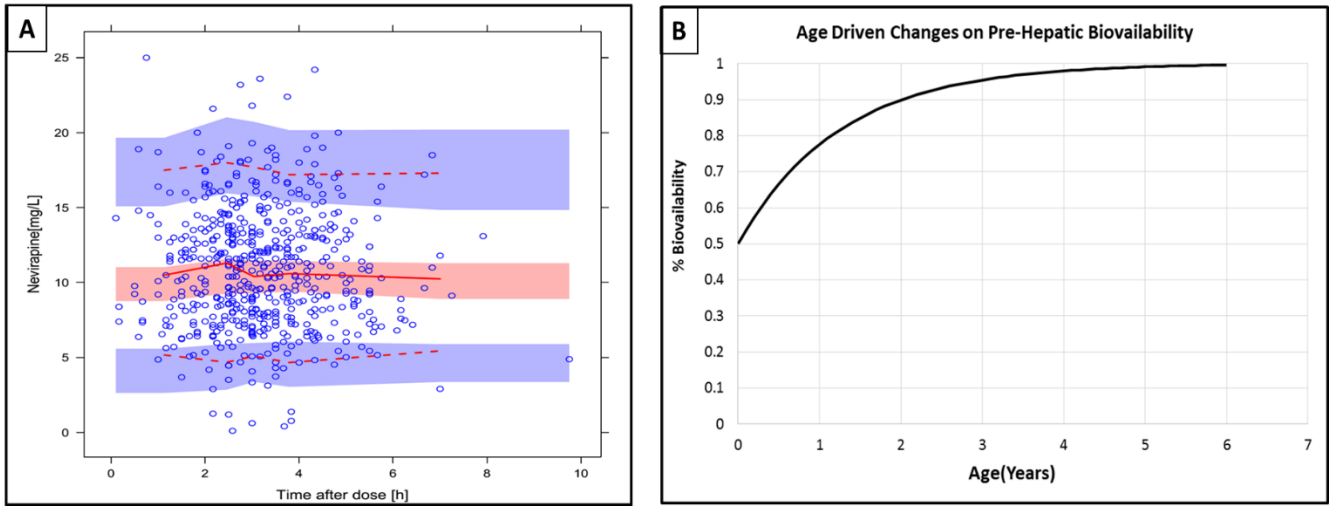
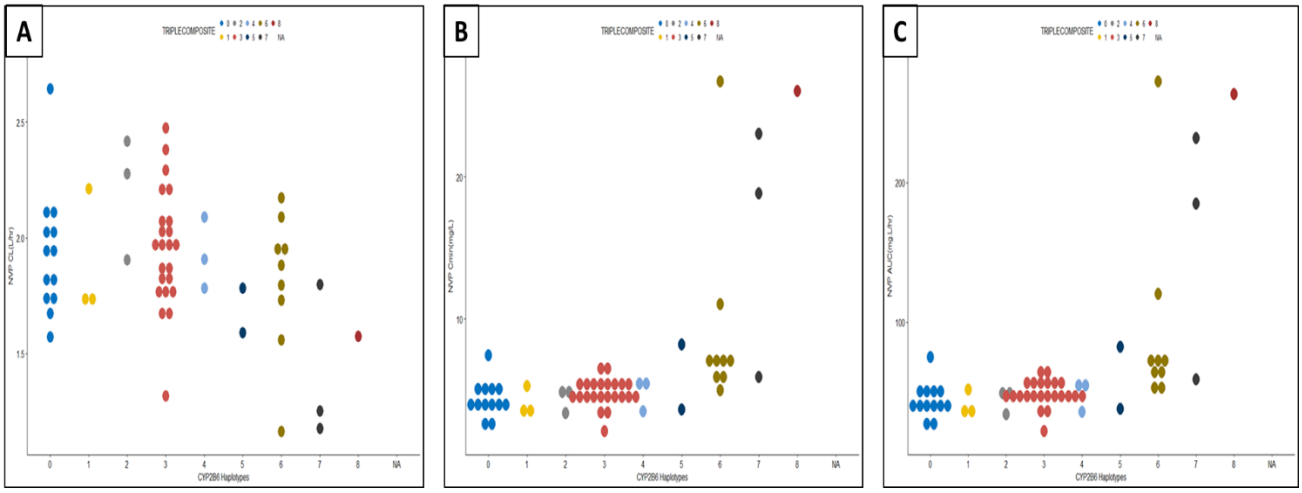


Figure 2: A composite 15582/516/983 haplotypes versus NVP CL/F. B composite 15582/516/983 haplotypes versus NVP C_{min}. C composite 15582/516/983 haplotypes versus NVP AUC.



0(15582CC-516GG-983TT); 1(15582CT-516GG-983TT); 2(15582TT-516GG-983TT); 3(15582CC-516GT-983TT); 4(15582CC-516GG-983CT); 5(15582CT-516GT-983TT); 6(15582CT-516GG-983CT); 7(15582CC-516TT-983TT), 8(15582CC-516GT-983CT)

Table 3: Relationships between Selected Genotypes and Nevirapine CL/F

CHR	SNP (Gene)	Unadjusted β(95%CI,P)	516G→ T Adjusted β(95%CI,P)	516G→T, 983T→C Adjusted β(95%CI,P)	516G→T, 983T→C and 15582C→>T Adjusted β(95%CI,P)
7	rs1002204 (ABCB1)	0.32(0.05-0.59,P=0.02)	-0.14(-0.28 to 0.01,P=0.05)	-0.21(-0.36 to -0.07,P=0.007)	-0.09(-0.13 to -0.05,P=9.3E-0.6)
19	983T→C (CYP2B6)	-0.26(-0.50 to -0.02,P=0.04)	-0.19(-0.33 to -0.04,P=0.01)	N/A	N/A
19	516G→T (CYP2B6)	-0.16(-0.31 to -0.01,P=0.04)	N/A	N/A	N/A
19	rs7250597 (CYP2B6)	0.17(0.01-0.33,P=0.04)	-0.11(-0.25 to 0.04,P=0.16)	-0.19(-0.34 to -0.03,P=0.02)	-0.08(-0.12 to -0.04,P=0.0001)
7	rs1859690 (CYP3A5)	-0.18(-0.34 to -0.01,P=0.05)	-0.13(-0.28 to 0.02, P=0.10)	-0.17(-0.34 to 0.01,P=0.04)	-0.09(-0.13 to -0.05,P=0.0002)
7	rs15524 (CYP3A5)	-0.18(-0.34 to -0.01,P=0.05)	-0.13(-0.28 to 0.02, P=0.10)	0.17(-0.34 to 0.01,P=0.04)	-0.09(-0.13 to -0.05,P=0.0002)
7	rs17064 (ABCB1)	0.18(0.01-0.37,P=0.06)	-0.13(-0.28 to 0.01, P=0.07)	-0.21(-0.36 to -0.06,P=0.009)	-0.09(-0.14 to -0.05,P=2.3E-0.5)
7	rs2687134 (CYP3A5)	-0.15(-0.31 to 0.01,P=0.06)	-0.13(-0.28 to 0.02, P=0.09)	-0.18(-0.34 to 0.01,P=0.04)	-0.09(-0.13 to -0.05,P=0.0001)
7	rs1922240 (ABCB1)	-0.19(-0.40 to 0.01,P=0.06)	-0.09(-0.24 to 0.06, P=0.23)	-0.18(-0.34 to -0.02,P=0.03)	-0.09(-0.13 to -0.05,P=0.0002)
7	rs10211 (CYP3A5)	-0.18(-0.33 to 0.01,P=0.07)	-0.13(-0.28 to 0.02, P=0.09)	-0.17(-0.34 to -0.03,P=0.03)	-0.09(-0.13 to -0.05,P=0.0002)
19	15582C→T (CYP2B6)	0.13(-0.05 to 0.31, P=0.16)	-0.16(-0.31 to 0.01,P=0.05)	-0.21(-0.37 to -0.05,P=0.01)	N/A

CHR, chromosome; SNP, single nucleotide polymorphism.

Table 4: Summary of Selected Genotypes Associated with Nevirapine C_{min}

CHR	SNP (Gene)	Unadjusted β(95%CI,P)	516G→ T Adjusted β(95%CI,P)	516G→T, 983T→C Adjusted β(95%CI,P)	516G→T, 983T→C and 15582C→>T Adjusted β(95%CI,P)
19	983T→C (CYP2B6)	6.07(3.14-9.01,P=0.0002)	3.24(1.53-4.94,P=0.005)	N/A	N/A
19	-48T→G (CYP2A6)	7.14(1.92-12.37,P=0.009)	2.16(0.27-4.05,P=0.03)	3.29(1.41-5.18,P=0.001)	1.30(0.79-1.82,P=7.0E-06)
7	rs10211 (CYP3A5)	2.74(0.54-4.94,P=0.02)	1.16(-0.90 to 3.22,P=0.27)	2.87(0.82-4.93,P=0.008	131(0.77-1.85,P=1.4E-0.5)
7	rs1859690 (CYP3A5)	2.70(0.49-4.91,P=0.02)	1.24(-0.80 to 3.28,P=0.24)	2.93(0.91-4.93,P=0.006)	1.31(0.79-1.84,P=9.4E-0.6)
7	rs15524 (CYP3A5)	2.70(0.49-4.91,P=0.02)	1.24(-0.80 to 3.28,P=0.24)	2.93(0.91-4.93,P=0.006)	1.31(0.79-1.84,P=9.4E-0.6)
7	rs113539362 (CYP3A5)	4.44(0.51-8.36,P=0.03)	1.71(-0.24 to 3.66,P=0.09)	3.13(1.16-5.10,P=0.003)	1.33(0.81-1.85,P=6.9E-0.6)
19	rs4124633 (CYP2B7)	2.98(0.30-5.65,P0.03)	1.51(-0.48 to 3.49, P=0.14)	3.12(1.15-5.10,P=0.003)	1.34(0.82-1.86,P=4.5E-0.6)
19	rs8100458 (CYP2B6)	3.36(-0.14 to 6.86, P=0.07)	1.56(-0.44 to 3.57,P=0.13)	3.05(0.98-5.12,P=0.006)	1.36(0.81-1.90,P=1.0E-0.5)
19	516G→T (CYP2B6)	1.81(-0.21 to 3.82, P=0.08)	N/A	N/A	N/A
19	15582C→T (CYP2B6)	-1.97(-3.46 to 1.41,P=0.41)	1.71(-0.34 to 3.77, P=0.11)	3.31(1.28-5.35,P=0.002)	N/A

CHR, chromosome; SNP, single nucleotide polymorphism.

Table 5: Summary of Genetic associations with Nevirapine AUC

CHR	SNP (Gene)	Unadjusted β(95%CI,P)	516G→ T Adjusted β(95%CI,P)	516G→T, 983T→C Adjusted β(95%CI,P)	516G→T, 983T→C and 15582C→>T Adjusted β(95%CI,P)
19	983T→C (CYP2B6)	59.3(30.6-88.2, P=0.0002)	21.9(9.6-34.2, P=0.001)	N/A	N/A
19	-48T→G (CYP2A6)	69.6(18.3-120.8, P=0.01)	15.5(1.9-29, P=0.03)	32.3(13.6-50.6,P=0.001)	12.7(7.7-17.7,P=8.4E-0.06)
7	rs10211 (CYP3A5)	26.9(5.4-48.4, P=0.02)	13.7(-0.70 to 28.10, P=0.07	27.9(7.8-48.1,P=0.009)	12.7(7.5-17.9,P=1.6E-0.5)
7	rs1859690 (CYP3A5)	26.5(4.8-48.2, P=0.02)	13.8(-0.50 to 28, P=0.06)	28.6(8.7-48.4,P=0.007)	12.8(7.6-17.9,P=1.1E-0.5)
7	rs15524 (CYP3A5)	26.5(4.8-48.2, P=0.02)	13.8(-0.50 to 28, P=0.06)	28.6(8.7-48.4,P=0.007)	12.8(7.6-17.9,P=1.1E-0.5)
7	rs113539362 (CYP3A5)	43.8(5.4-82.2, P=0.03)	13.6(-0.1 to 27.2, P=0.06)	30.5(11.2-49.7,P=0.003)	12.9(7.8-18.1,P=8.3E-0.6)
19	rs4124633 (CYP2B7)	29.3(0.30-5.65, P0.03)	13.9(0.1-27.8, P=0.05)	30.5(11.1-49.8, P=0.003)	13.6(8.0-18.1,P=5.4E-0.6)
19	rs8100458 (CYP2B6)	33.35(3.1 to 55.5, P=0.06)	15.7(1.7-29.6, P=0.03)	29.6(9.3-49.9, P=0.006)	13.5(7.8-18.5,P=1.3E-05)
19	516G→T (CYP2B6)	17.4 (-2.3 to 37.2, P=0.09)	N/A	N/A	N/A
19	15582C→T (CYP2B6)	-19.2 (-59.0 to 20.7, P=0.35)	15.1(1.1-29.2, P=0.04)	32.2(11.9-52.3,P=0.003)	N/A

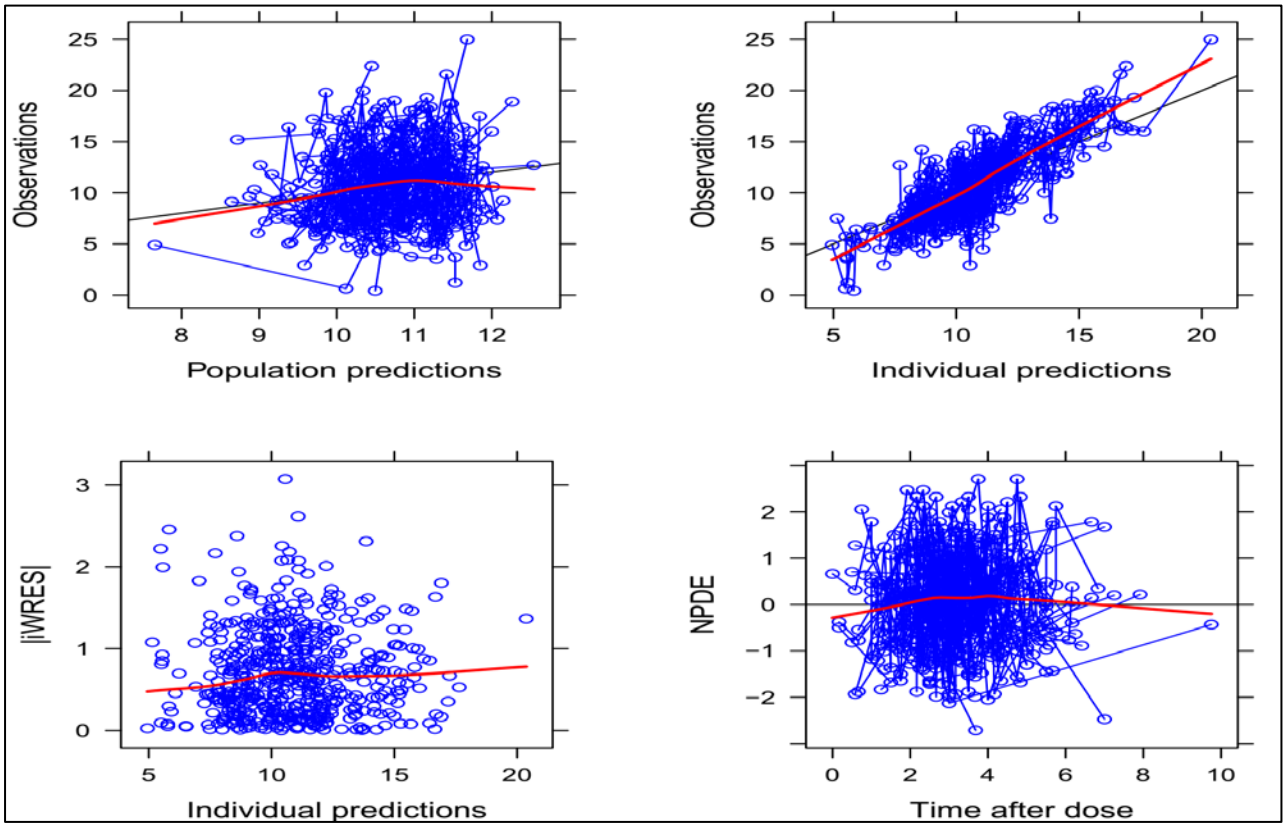
CHR, chromosome; SNP, single nucleotide polymorphism.

Supplemental Table S1: Minor allele frequencies, Genotype Frequencies, HWE P Values of the 66 polymorphisms in 60 South African Children

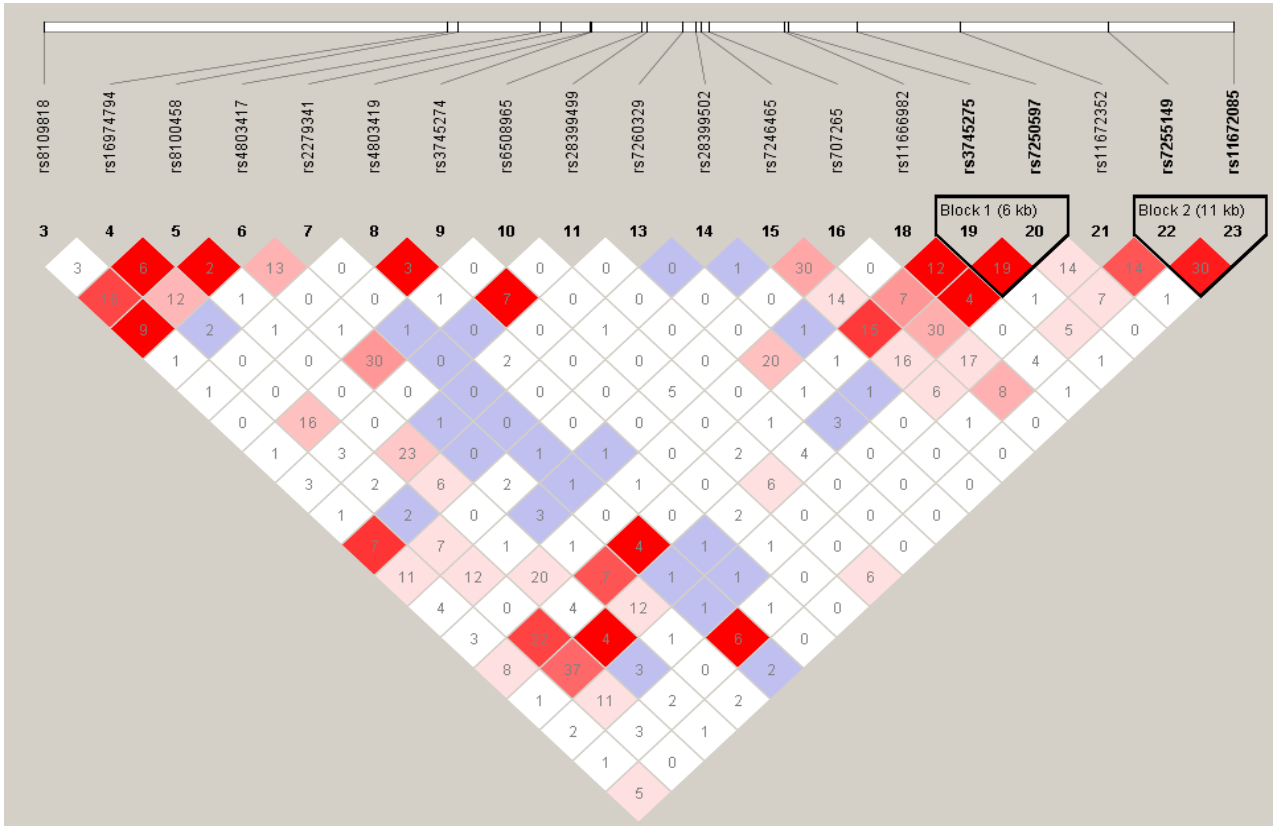
Gene	SNP	CHR	Minor Allele	Major Allele	Minor Allele Frequencies	Genotype Frequencies	P-value
ABCB1	rs1002204	7	T	G	0.12	1/40/135	0.4744
ABCB1	rs10225473	7	G	A	0.14	7/36/133	0.05431
ABCB1	rs10248420	7	A	G	0.34	19/83/74	0.6183
ABCB1	rs10260862	7	C	G	0.33	21/74/79	0.6089
ABCB1	rs10267099	7	G	A	0.09	0/30/146	0.6167
ABCB1	rs10276036	7	C	T	0.15	4/46/126	1
ABCB1	rs1045642	7	T	C	0.09	3/26/147	0.1544
ABCB1	rs11760837	7	C	T	0.20	9/52/115	0.3439
ABCB1	rs17064	7	T	A	0.16	4/50/122	1
ABCB1	rs17209837	7	C	T	0.27	11/73/92	0.5691
ABCB1	rs1858923	7	C	T	0.07	0/23/153	1
ABCB1	rs1882478	7	C	T	0.31	22/66/88	0.1129
ABCB1	rs1922240	7	C	T	0.24	7/69/100	0.2999
ABCB1	rs1989830	7	T	C	0.27	12/71/93	0.8496
ABCB1	rs2229107	7	T	A	0.12	0/42/134	0.1386
ABCB1	rs2235023	7	A	G	0.31	18/73/85	0.7246
ABCB1	rs2235033	7	T	C	0.46	35/93/48	0.4511
ABCB1	rs28364275	7	C	T	0.10	1/32/143	1
ABCB1	rs28364277	7	T	C	0.12	2/39/135	1
ABCB1	rs28364278	7	I	D	0.13	2/41/133	0.7434
ABCB1	rs3747806	7	C	T	0.27	17/62/97	0.1324
ABCB1	rs3789243	7	A	G	0.35	16/88/69	0.1319
ABCB1	rs3842	7	C	T	0.18	4/57/115	0.4527
ABCB1	rs4148751	7	G	A	0.26	10/70/96	0.6923
ABCB1	rs6465118	7	A	G	0.23	4/73/99	0.03157
ABCB1	rs651430	7	T	C	0.48	3/38/135	0.7366
ABCB1	rs6961419	7	C	T	0.05	35/98/43	0.1732
ABCB1	rs8187789	7	A	G	0.21	0/19/157	1
CYP2A6	rs28399433	19	C	A	0.11	2/23/151	0.2627
CYP2B7	rs4124633	19	G	A	0.13	4/40/132	0.5351
CYP2B6	rs1042389	19	C	T	0.15	6/62/108	0.5027
CYP2B6	rs11666982	19	T	G	0.27	7/39/130	0.07895
CYP2B6	rs11672085	19	T	C	0.17	11/73/92	0.5691
CYP2B6	rs11672352	19	G	A	0.30	8/43/125	0.1046
CYP2B6	rs16974794	19	G	A	0.05	18/71/87	0.5926
CYP2B6	rs2279341	19	C	G	0.08	0/19/157	1
CYP2B6	rs28399499	19	C	T	0.05	3/33/140	0.4482
CYP2B6	rs28399502	19	A	C	0.39	1/17/158	0.4013
CYP2B6	rs3745274	19	T	G	0.42	31/75/70	0.2037
CYP2B6	rs3745275	19	A	G	0.14	36/75/65	0.1204
CYP2B6	rs4803417	19	C	A	0.06	4/39/133	0.5182

CYP2B6	rs4803419	19	T	C	0.18	0/20/156	1
CYP2B6	rs6508965	19	T	C	0.18	6/52/118	1
CYP2B6	rs707265	19	A	G	0.22	0/65/111	0.000874
CYP2B6	rs7246465	19	T	C	0.22	12/55/109	0.1925
CYP2B6	rs7250597	19	T	C	0.48	10/56/110	0.3823
CYP2B6	rs7255149	19	A	C	0.10	38/93/45	0.5455
CYP2B6	rs7260329	19	A	G	0.13	0/36/140	0.2223
CYP2B6	rs8100458	19	C	T	0.39	1/43/132	0.3158
CYP2B6	rs8109818	19	G	A	0.25	27/82/66	0.874
CYP3A4	rs17161886	7	C	A	0.15	9/69/97	0.5476
CYP3A4	rs28451617	7	T	C	0.13	8/38/130	0.03657
CYP3A4	rs3735451	7	A	G	0.25	3/40/133	1
CYP3A4	rs473706	7	T	C	0.13	14/58/103	0.1571
CYP3A4	rs667660	7	A	C	0.24	10/66/100	1
CYP3A4	rs7801671	7	A	C	0.22	7/62/107	0.8232
CYP3A5	rs10211	7	A	G	0.32	21/70/85	0.2968
CYP3A5	rs10256106	7	G	C	0.18	8/47/121	0.2071
CYP3A5	rs10264272	7	T	C	0.20	9/51/116	0.3353
CYP3A5	rs113539362	7	A	G	0.09	0/30/146	0.6167
CYP3A5	rs15524	7	T	C	0.32	21/71/84	0.3051
CYP3A5	rs1859690	7	A	G	0.32	20/72/84	0.4869
CYP3A5	rs2687134	7	C	A	0.37	25/79/71	0.7452
CYP3A5	rs6977165	7	C	T	0.16	3/51/122	0.5768
CYP3A5	rs773049150	7	I	D	0.10	2/32/142	0.6961
CYP3A5	rs8175345	7	T	C	0.10	1/33/142	1

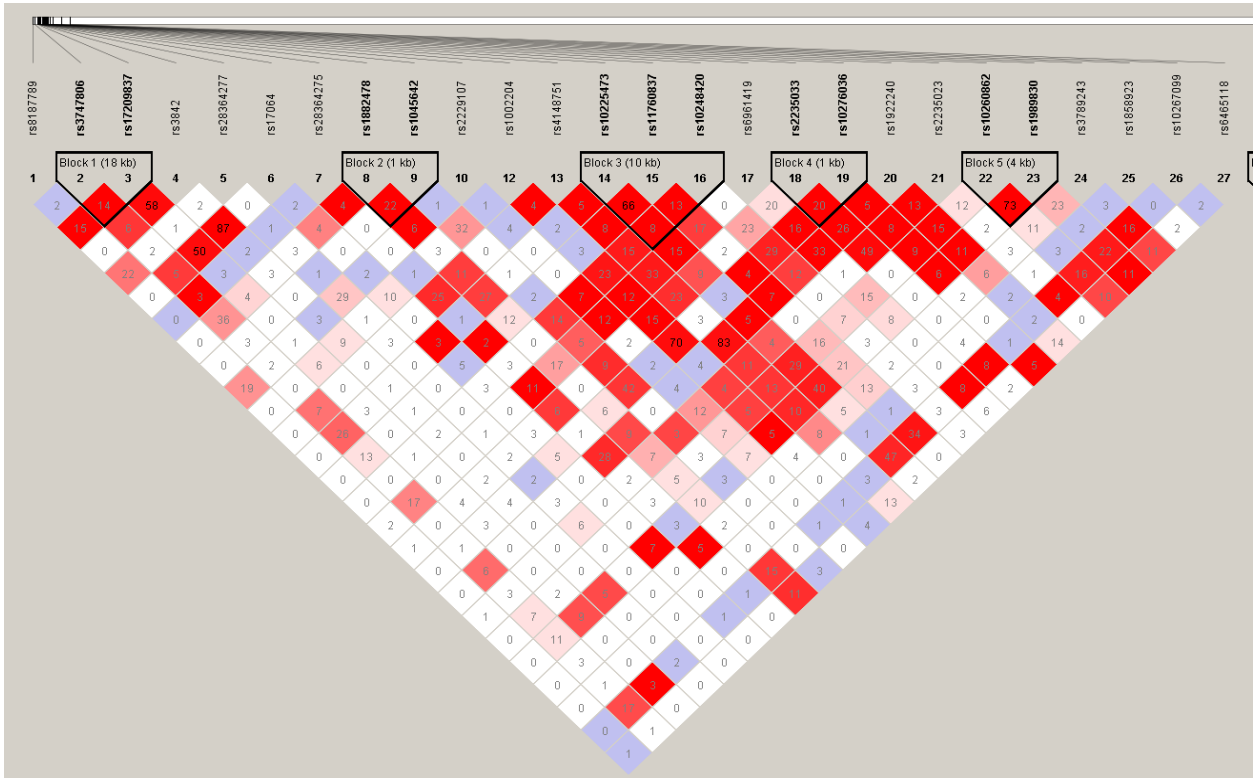
Supplementary Figure 1: Goodness of fit plots of the final model



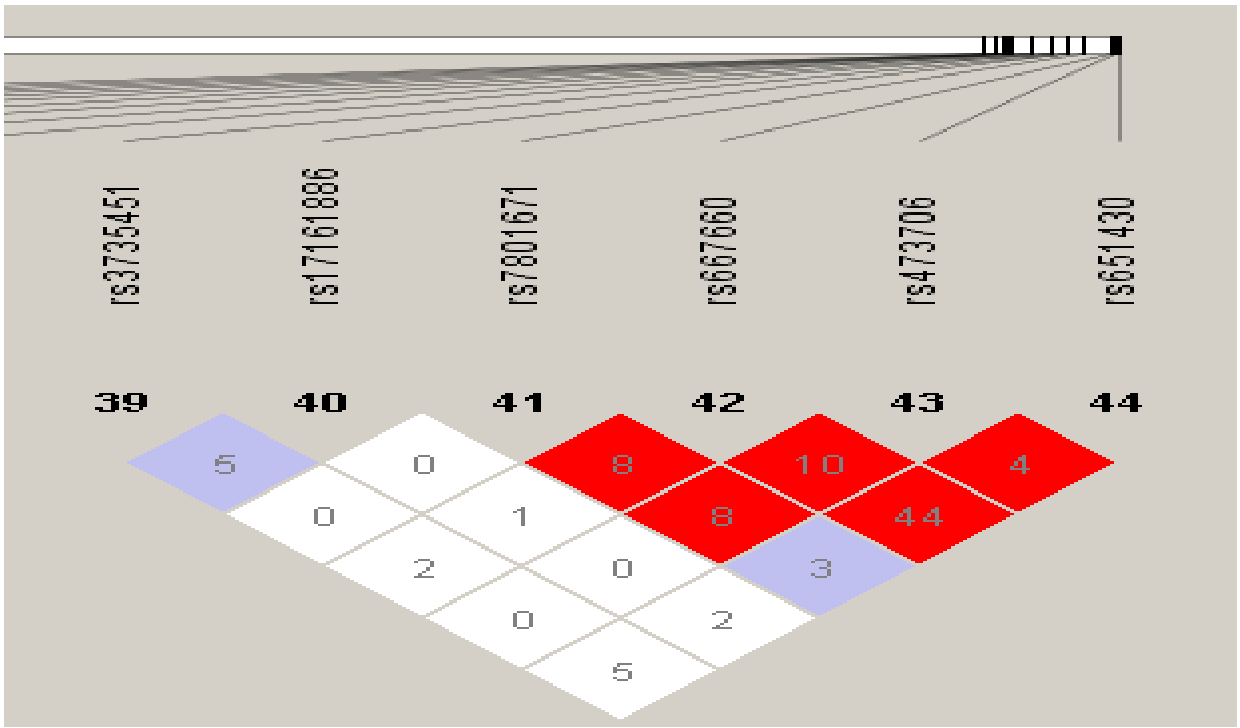
Supplementary Figure 2: Illustrates the linkage disequilibrium plot for CYP2B6



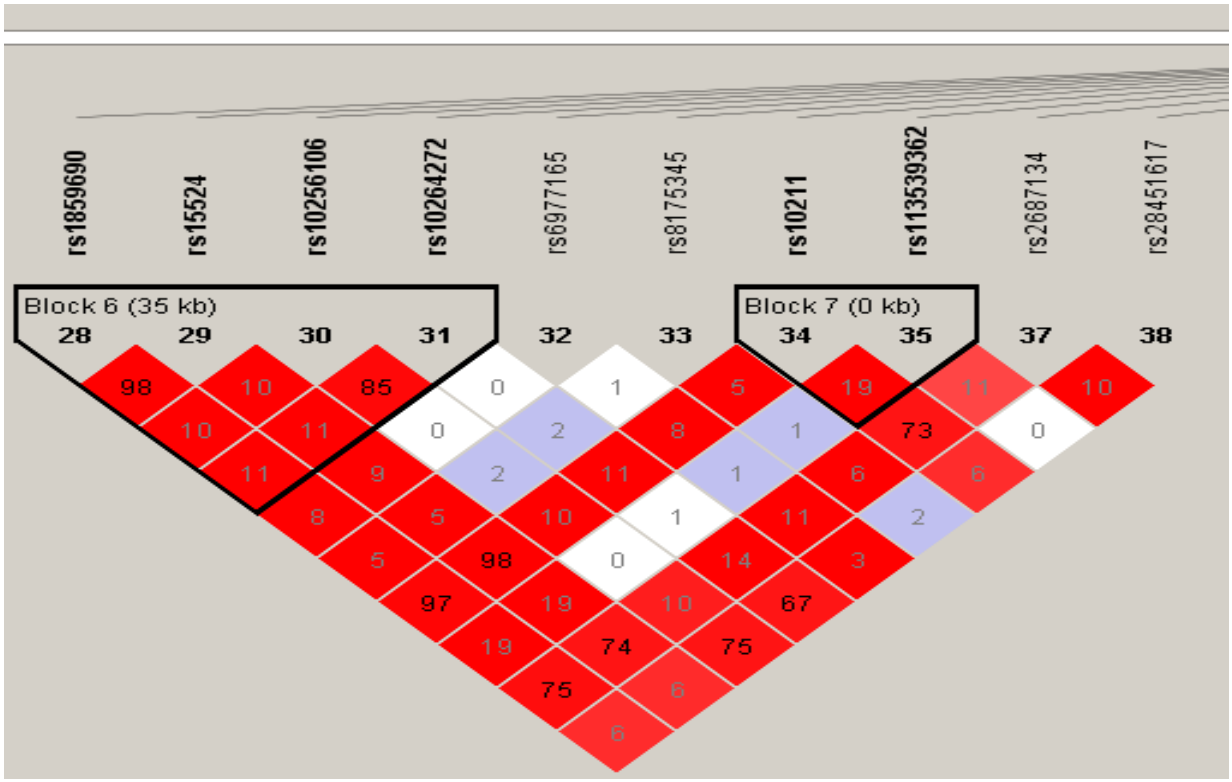
Supplementary Figure 3: Presents linkage disequilibrium plot for ABCB1



Supplementary Figure 4: Demonstrates the linkage disequilibrium plot for CYP3A4



Supplementary Figure 5: Shows the linkage disequilibrium plot for CYP3A5



Equation 1

$$CL/Fstd = CL/Fstd * (\frac{TWBT}{12.5})^{0.75} \quad \textbf{(1.1)}$$

$$V/Fstd = V/Fstd * (\frac{TWBT}{12.5})^{1} \quad \textbf{(1.2)}$$

Equation 2

$$FpreH = 1 - (1 - FpreH\_Birth)\, Exp^{-KpreH * AGE}$$

Chapter 7
Overall Discussions and Conclusions

7.1 **Discussion**

HIV in children continues to be a large health burden globally and in Sub-Saharan Africa, particularly in South Africa where high proportion of infections and deaths occur[1]. Majority of HIV infections in children result from transmission of the virus from the mother during pregnancy, during the birth process, or from breastfeeding[1,2]. Antiretroviral therapy greatly reduces HIV associated morbidity and mortality in children[3,4]. Therefore, greater understanding of the pharmacology of antiretroviral drugs in children is of paramount importance so as to optimize treatment in high burden environments, particularly in South African and Sub-Saharan Africa. Optimal use of currently available drugs is imperative considering that sub-therapeutic exposures can lead to resistance and treatment failure.

This thesis, adds to the body knowledge on the pharmacokinetics, pharmacogenetics and pharmacodynamics of antiretroviral drugs based on clinical data and retrospective measurement of antiretroviral drugs measurement (in single scheduled and unscheduled samples collected during clinic visits) as part a clinical trial conducted in a programmatic setting in South Africa. The patients received drug regimens and combinations based on standard regimens used in most parts of the world, hence the findings maybe applicable beyond South Africa. The work involved describing the relationship between serial clinic visit lopinavir concentrations and other pre-treatment clinical variables and virological outcomes in children during the induction phase of the NEVEREST2 trail. Another aim was to describe the relationship between maintenance of virological suppression and longitudinal drug concentrations in children on 2 different antiretroviral regimens (lopinavir or nevirapine) and other important clinical variables during the post randomization phase. For both aims, Cox proportional hazard multiple failure events were employed to describe a time to treatment

failure. Another aim pharmacokinetics. Before this could be done, population pharmacokinetic models were developed in order to describe antiretroviral drugs pharmacokinetics and from the final models three pharmacokinetic indices, namely; clearance (CL/F), minimum concentration ($C_{min)}$ and area under the concentration time curve (AUC_{0-12}), were obtained for each individual. These were then used to assess their associations with genetic polymorphisms in pre-selected genes.

In paper 1, plasma lopinavir concentrations in 237 children collected at the same time as viral load tests at serial clinic visits were used to determine relationship concentrations and virological response in children. Furthermore, other pre-determined relevant pre-treatment variables were used to predict virological response. Children with lopinavir concentrations <1.0 mg/L had a (HR 2.3[95% CI 1.63, 3.26]) fold risk of virological failure (viral load>400 copies/mL). A cut-off value of 4.0 mg/L (HR 1.74[95% CI 1.36, 2.23]) was also associated with an increased risk of virological failure. Furthermore, a non-linear effect of lopinavir was shown graphically on the hazard of viral load>400 copies/mL. It demonstrated that lower concentration predicted virological failure across a full range of lopinavir concentrations. This finding indicates that in addition to adherence-related changes in drug exposure, individual variability in lopinavir concentrations might play a vital role in therapeutic outcomes. This finding suggests that low lopinavir concentrations (especially <1.0 mg/L) measured at 0.42-9 hours post dose reflect poor medication adherence and this can be used an objective for adherence to antiretroviral therapy as well as therapeutic drug monitoring. This finding confirms that a 1.0 mg/L trough concentrations derived from adults[5] is also applicable to children in predicting virological response. The lopinavir concentrations described in this thesis confirms findings in our settings, similar to those found in children of comparable age

reported elsewhere[6,7]. There was also significant increase in the hazard of viral load>400 copies/mL with pre-treatment moderate (HAZ -2 to -3sd) and severe stunting (HAZ> -3 sd) but not with other pre-treatment characteristics. This finding is consistent with a report in another study in children[8]. This suggests appropriated interventions may be required in children who are stunted in order to improve their virological outcomes.

For paper 2, 195 children who achieved virological suppression (viral load<400 copies/mL) for 2 consecutive visits after induction phase of antiretroviral therapy were randomized, of which 99 to remained on a lopinavir-based regimen and 95 were switched to a nevirapine-based regimen for the post-randomization phase. Viral loads and lopinavir or nevirapine concentrations were measured at serial clinic visits. The hazard of viremia (viral load>50 copies/mL) were determined for lopinavir or nevirapine and clinical relevant variables at baseline. In the 99 children remaining on lopinavir, the hazard of viremia was increased by 40% in children with concentrations <1.0 mg/L versus >1.0 mg/L. Furthermore, several lopinavir concentrations cut-offs (0.5-6.0 mg/L) were compared using generalized cross validation and the method found the 1.0 mg/L the most predictive. This suggests that 1.0 mg/L could be used as a target concentration for lopinavir concentrations taken 2-4 post dose during a routine clinical visit and could be used as a measure for therapeutic drug monitoring in children established on antiretroviral therapy. There was also an increased hazard of viremia in children with low level viremia (viral load 51-400 copies/mL) at baseline suggesting the risk of future viremia was not modified by lopinavir exposure post-randomization. In the 95 children switched to nevirapine-based regimen, there was no association between nevirapine concentrations taken 2-3.9 hours post dose and the hazard of viremia. Nonetheless, a cut-off 5.0 mg/L was shown to reduce the hazard of viremia by 36% though

the association was not statistically significant. These findings were consistent with previous reports whereby it shown that nevirapine concentrations do not predict virological response in children established on antiretroviral treatment[9].

In paper 3, a population model was developed to describe the pharmacokinetics of lopinavir in children and a one compartment model best described the data. Allometric scaling using total body weight was applied on CL/F and volume and thus improved the model fit. Both lopinavir estimates of oral CL/F and volume were comparable to previous studies[10,11]. There was an age-driven effect on lopinavir relative bioavailability and an effect of concomitant tuberculosis therapy on lopinavir clearance, similar to other reports in the literature[10,12].

From the final model, individual CL/F, C_{min}, and AUC_{0-12} were obtained and this were used to examine for associations with genetic polymorphisms in the *ABCB1*, *CYP3A4*, *CYP3A5* and *SLCO1B1*. Lopinavir is a substrate for *SLCO1B1* and reports have shown that a genetic variant *SLCO1B1* 521T→C (rs4149056) is associated with lopinavir pharmacokinetics[13–16]. When adjusting for multiple comparisons, there were no significant associations between 56 *SLCO1B1* polymorphisms and lopinavir CL/F, C_{min} or AUC_{0-12}, specifically 521→C due low frequency of the 521CC allele. There was nominally significant association with rs4149032 (MAF 21%) and reduced lopinavir CL/F, contrasting previous reports[13]. There were significant nominal associations between *SLCO1B1* 463C→A (rs11045819) and decreased lopinavir CL/F or increased C_{min} and AUC_{0-12}, however the results are in contrast with previous reports[13]. Furthermore, there were nominally significant associations with *SLCO1B1* 388A→G (rs2306283) and increased lopinavir C_{min} or AUC_{0-12}, contrasting previous reports[14,17]. Additionally, there were trends of increased C_{min} and AUC_{0-12} with *SLCO1B1* rs112403792 and rs4149057, however this have not been reported previously.

Similarly, when adjusting for multiple comparisons, there were no significant associations between *ABCB1* polymorphisms and lopinavir CL/F, C_{min} or AUC_{0-12}. Nonetheless, there was a trend of reduced in lopinavir CL/F for rs10267099, which has not been reported previously. Neither polymorphisms in *CYP3A4* and *CYP3A5* were associated with lopinavir pharmacokinetics.

For paper 4, a one compartment population model with transit compartment and a well stirred liver model satisfactorily described nevirapine pharmacokinetics of 96 children. The application of allometric scaling for total body weight on oral CL/F and volume of distribution accounted for the effect of size. The estimate oral CL/F was lower than that from a previous study whereas volume was comparable[18]. There was no maturation effect on CL/F, however there was an age-driven effect on relative bioavailability, similar to previous reports[18]. From the final model, individual CL/F, C_{min} and AUC_{0-12} were obtained and were used to evaluate for associations with genetic polymorphisms in *ABCB1*, *CYP2A6, CYP2B7, CYP2B6, CYP3A4* and *CYP3A5*.

Nevirapine is substrate of *CYP2B6*, previous studies have shown associations between 516G→T[18–20] and 983T→C[21–23] variants and nevirapine pharmacokinetics. When adjusting for multiple comparisons in a univariate analysis, there were nominal associations between nevirapine CL/F and *ABCB1* rs1002204 and *CYP2B6* rs7250597*, CYP3A5* rs1859690, *CYP3A5* rs15524, *CYP2B6* 516G→T and 983T→C. Adjusting for *CYP2B6* 516G→T genotype resulted in nominal associations with *ABCB1* rs1002204, *CYP2B6* 983T→C and *CYP2B6* 15582 C→T. When adjusting for *CYP2B6* 516/983 haplotype and the composite *CYP2B6* 516/983/15582 haplotype, there were significant associations with the above-mentioned polymorphisms.

Chapter 7

Overall Discussions and Conclusions

Regarding nevirapine C_{min}, when adjusting for multiple comparison by univariate analysis, there was a significant association with *CYP2B6* 983T→C and nominal associations with *CYP3A5* rs10211*, CYP3A5* rs1859690*, CYP3A5* rs15524, *CYP3A5* rs113539362, *CYP2B6* rs8100458, *CYP2A6* -48T→G*,* and *CYP2B7* rs4124633. When adjusting *CYP2B6* 516G→T, there were nominal associations with only *CYP2B6* 983T→C and *CYP2A6* -48T→G. After adjusting for *CYP2B6* 516/983 haplotype there were nominal association between the aforementioned genotypes in the first model and nevirapine C_{min}, whereas adjusting composite *CYP2B6* 516/983/15582 haplotype, they were significantly associated with nevirapine C_{min}.

For nevirapine AUC_{0-12}, in a univariate analysis, when adjusting for multiple comparisons, there was a significant association between nevirapine AUC_{0-12} and *CYP2B6* 983T→C and associations nominally with *CYP3A5* rs10211*, CYP3A5* rs1859690*, CYP3A5* rs15524, *CYP3A5* rs113539362, *CYP2A6* -48T→G*, CYP2B7* rs4124633 and *CYP2B6* rs8100458. After adjusting for *CYP2B6* 516G→T, there were nominal associations between nevirapine AUC and *CYP2B6* 983T→C, rs8100458 and 15582C→T and/or *CYP2A6* -48T→G. After adjusting for *CYP2B6* 516/983 haplotype, there nominal associations with polymorphisms mentioned in the first model including 15582C→T. Adjusting for the composite 516/983/15582 haplotype resulted in significant associations with polymorphisms aforementioned in the first and third models, respectively.

7.2 **Limitations**

The findings from these studies need to be interpreted within the context of their limitations. For the studies investigating associations between drug concentration and virological response (paper 1 and 2) the limitations are as follows: First, there was missing data which

was accounted by multiple imputation, as this approach has be shown to be superior to complete case analysis in which subjects who do not have missing values are analysed[24]; Second, the timing of dosing of lopinavir or nevirapine was not directly observed by the study team and the analysis did not include adjustment for the time after dose. Nonetheless, to minimize recall bias, caregivers were requested to record the time of last dose on the morning before pharmacokinetic sampling; Third, adherence was self-reported and therefore, incomplete adherence cannot be excluded, which could have important effects on the observed concentrations; Fourth, lopinavir or nevirapine concentrations were used and not the area under the curve, the pharmacokinetic parameter that better describes the drug exposure.

Many children on ART for treating HIV experience undetectable levels of viral load (<50 copies/mL). However, some patients experience transient viremia[24,25]. Viral blips might result from release of drug-sensitive virions from the latent reservoir or might signal viral replication that occurs as a result of lack of adherence to drug treatment or increases in target cells secondary to infection[26]. The clinical significance of this phenomenon remains controversial in the literature. Nonetheless, in this thesis, the impact of viral blips on virological outcomes was not assessed and this could have led to biased in the relationship between clinic visit concentrations and the hazard of viremia especially that of lopinavir.

For paper 3, there were 176 out of a total of 237 patients with genotype data. Furthermore, 131 patients had both genotype and phenotype limiting the sample size. This could have resulted in decreased statistical power in investigating the associations between genotype and phenotype.

Similarly, in paper 4, there were 60 out of a total of 96 patients with genotype data. Furthermore, 55 patients had both genotype and phenotype further limiting the sample size. Sample size might have limited the ability to extensively identify novel genetic associations with nevirapine pharmacokinetics.

7.3 **Conclusions**

In conclusion all the aims of this work were achieved. Cox proportional hazard models were used to investigate the relationship between serial visits viral loads and lopinavir concentrations and other clinical relevant variables. The findings showed that children with lopinavir concentrations <1.0 mg/L taken 0.42-9 hours post dose had a higher hazard of viral load>400 copies/mL. This can be used as proxy for treatment non-adherence and can be used as target concentration for therapeutic drug monitoring in optimizing antiretroviral treatment in children initiating lopinavir-based regimen. This finding was subsequently confirmed children established on a lopinavir-based regimen when using a threshold of viral load>50 copies/mL. Furthermore, it was confirmed that nevirapine concentrations do not predict the hazard of viremia (viral load>50 copies) in children established on antiretroviral treatment.

Pharmacometric models were developed for lopinavir and nevirapine and associations between both drugs pharmacokinetics and genetic polymorphisms relevant to both drugs were explored. There were nominal associations between lopinavir pharmacokinetics and genetic polymorphisms in *SLCO1B1* and *ABCB1*. This thesis confirmed significant associations between nevirapine pharmacokinetics and *CYP2B6* polymorphisms.

Based on the work from this thesis, further larger studies are needed to confirm the proposed target concentrations for lopinavir and their usefulness for therapeutic drug monitoring.

Moreover, more pharmacogenetic studies are needed to confirm the influence of genetic polymorphisms in *SLCO1B1* and *ABCB1* in children especially those of African descent. Furthermore, analysis gene-gene interaction are crucial for better understanding of lopinavir pharmacokinetics. Regarding nevirapine, more studies are needed in order to elucidate clinical relevancy of the contribution of genetic variants to nevirapine pharmacokinetics. Datasets from more recent studies could be used to build models incorporating genetic effects which can then be used for clinical trial simulation to confirm the most efficient designs for optimizing therapy of nevirapine-based regimen.

7.4 **Implication for Clinical Care and Practice**

The findings in this thesis improves our understanding on relationship between longitudinal lopinavir or nevirapine concentrations and virological failure. Furthermore, this thesis also adds to the knowledge on the genetic determinants of lopinavir or nevirapine pharmacokinetics in African children, providing insights into the host factors associated with drug exposure. Some of the findings potential public health implications.

Currently therapeutic drug monitoring is not routinely used in low and middle-income countries. Moreover, it has been shown that measuring drug concentrations can be used as an effective tool in ensuring that therapeutic targets of ART targets are met. Interestingly, there is also insufficient knowledge on target concentrations in children. The findings in this study suggests that a single sample taken 0.42-9(in children initiating LPV/r regimen) or 2-4 hours (in children established on LPV/r regimen) after the dose is useful predictor of lopinavir concentrations and can used for therapeutic drug monitoring, and therefore assist in adherence support.

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The track record of randomized clinical trials that incorporate clinical phenotypes and genotypes in children on ART remains fraught. Furthermore, the use of pharmacogenetic features could be a useful tool in clinical practice. Moreover, the study of genetic profiles of patients could help in optimizations of therapeutic management of HIV-infected children through test introduced into clinical practice. LPV is recommended as first-line therapy in children and is likely to remain so due to its high barrier for developing drug resistance and tolerability. LPV is a substrate of *SLCO1B1* and the clinical utility of genotyping or sequencing of *SLCO1B1* still needs further work and cannot be recommended based on our findings. Nevirapine is prescribed as the 2nd preferred drug in children due to its affordability, availability in fixed dose combinations, and safety and efficacy profile. NVP is a substrate of *CYP2B6* and *CYP2B6* is highly polymorphic, especially in patients of African ancestry. Slow metabolizer genotypes are prevalent in Sub-Saharan African populations and therefore the clinical utility and cost-effectiveness of monitoring the effect *CYP2B6* slow genotypes on NVP pharmacokinetics could be useful and remains to be determined.

7.5 **References**

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APPENDIX I

Cox Proportional Hazards Mutliple failure Event Model of Lopinavir During The Pre-randomization Phase

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library(splines)

library(survival)

library(pspline)

LPVPRE<-read.csv("PRERANDATA6.csv",header=T,sep=",",stringsAsFactors=FALSE)

attach(LPVPRE)

###################

### Descriptives ####

###################

# percentage Missingness

round(apply(apply(cbind(lpv,adhstatus,baselinecd4pc,agestartarv,whostage2,baselinewfa,vlsupp,hfacat),c(1,2)
,is.na),2,mean),digits=3)

surv.vl1 <- Surv(X_t0,X_t,vlsupp)       # setting survival time from Stata

summary(surv.vl1)

##################

## Imputation   ####

##################

library(foreign)

library(Amelia)

library(norm)

set.seed(666)

M=10 #Set Number of Impuations to Be Done

impdat <-
LPVPRE[,c("id","X_t0","X_t","vlsupp","lpv","adhstatus","baselinecd4pc","logbaselinevl","agestartarv","whostag
e2","baselinewfa","baselinehfa")]

round(apply(apply(impdat,c(1,2),is.na),2,mean),digits=3)   # percentage of missing values

myimp <- amelia(impdat, m=M, p2s=1, noms=c("adhstatus","vlsupp","whostage2")
,cs=c("id"),ts=c("X_t0"),bounds=matrix(c(4,6,7,13,0,0,0,0,50,100,6,100),ncol=3,nrow=3),logs="lpv",lags="lpv",l
eads="lpv",polytime=3,splinetime=3,empri=1000)

ameliabind(myimp)

plot(myimp)

or

par(mfrow=c(2,2))

compare.density(myimp,var="baselinecd4pc")

compare.density(myimp,var="logbaselinevl")
```

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```
compare.density(myimp,var="baselinewfa")

compare.density(myimp,var="baselinehfa")

compare.density(myimp,var="whostage2")

dev.off()

par(mfrow=c(1,1))

suppressWarnings(disperse(myimp, dims=1, m=10))

suppressWarnings(disperse(myimp, dims=2, m=10))

par(mfrow=c(2,1))

suppressWarnings(tscsPlot(myimp,var="baselinecd4pc",cs=3011,draws=10))

suppressWarnings(tscsPlot(myimp,var="baselinewfa",cs=3011,draws=10))

par(mfrow=c(1,1))

overimpute(myimp, var = "baselinecd4pc")

overimpute(myimp, var = "whostage2")

overimpute(myimp, var = "baselinewfa")

overimpute(myimp, var = "baselinehfa")

overimpute(myimp, var = "lpv")

summary(myimp)


# Categorization of relevant variables

for(i in 1:10){

myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$logbaselinevl,breaks=c(-
1,5,1000000000)))

colnames(myimp$imputations[[i]])[13]<-c("logvlcat")

myimp$imputations[[i]] <-
cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$baselinecd4pc,breaks=c(-1,25,1000000000)))

colnames(myimp$imputations[[i]])[14]<-c("cd4pccat")

myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$agestartarv,breaks=c(-
1,9,1000000000)))

colnames(myimp$imputations[[i]])[15]<-c("agecat")

myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$baselinewfa,breaks=c(-
1000,-3,-2,10)))

colnames(myimp$imputations[[i]])[16]<-c("wfacat")

myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$baselinehfa,breaks=c(-
1000,-3,-2,10)))

colnames(myimp$imputations[[i]])[17]<-c("hfacat")
```

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```
myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$lpv,breaks=c(-
1,1,1000000000)))

colnames(myimp$imputations[[i]])[18]<-c("lpvcat1")

myimp$imputations[[i]] <- cbind(myimp$imputations[[i]],cut(myimp$imputations[[i]]$lpv,breaks=c(-
1,4,1000000000)))

colnames(myimp$imputations[[i]])[19]<-c("lpvcat4")

}

write.csv(myimp$imputations[[1]],"m1.csv")

head(myimp$imputations[[1]])


hfacat1=myimp$imputations[hfacat==1]=0

hfacat1


write.csv(myimp$imputations,file="LPV.csv")

lpvimp=read.csv("myimp.csv",header=T,sep=",",stringsAsFactors=FALSE)




################################

## Table 3: Cox Regression   #

## (crude, adj, selected)    #

################################


# Adjusted analysis

#Using Lopinavir as continous variable

m2_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]]$X_t,method="breslow")

m2_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")
```

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```
m2_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpv  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest <-
list(coef(m2_1),coef(m2_2),coef(m2_3),coef(m2_4),coef(m2_5),coef(m2_6),coef(m2_7),coef(m2_8),coef(m2_
9),coef(m2_10))

mystd <-
list(summary(m2_1)[[7]][,4],summary(m2_2)[[7]][,4],summary(m2_3)[[7]][,4],summary(m2_4)[[7]][,4],summar
y(m2_5)[[7]][,4],summary(m2_6)[[7]][,4],summary(m2_7)[[7]][,4],summary(m2_8)[[7]][,4],summary(m2_9)[[7]
][,4],summary(m2_10)[[7]][,4])

my2a <- mi.inference(myest, mystd, confidence=0.95)

my_2 <- round(cbind(exp(my2a$est),exp(my2a$lower),exp(my2a$upper),my2a$signif),digits=3)

my_2     # overall results with Hazard ratios, CI, and p-values


#Using Lopinavir with a cut-off of 1mg/L

m3_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m3_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m3_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m3_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")
```

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```
m3_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m3_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m3_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m3_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m3_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m3_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat1  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest <-
list(coef(m3_1),coef(m3_2),coef(m3_3),coef(m3_4),coef(m3_5),coef(m3_6),coef(m3_7),coef(m3_8),coef(m3_
9),coef(m3_10))

mystd <-
list(summary(m2_1)[[7]][,4],summary(m2_2)[[7]][,4],summary(m2_3)[[7]][,4],summary(m3_4)[[7]][,4],summar
y(m3_5)[[7]][,4],summary(m2_6)[[7]][,4],summary(m3_7)[[7]][,4],summary(m3_8)[[7]][,4],summary(m3_9)[[7]
][,4],summary(m3_10)[[7]][,4])

my3a <- mi.inference(myest, mystd, confidence=0.95)

my_3 <- round(cbind(exp(my3a$est),exp(my3a$lower),exp(my3a$upper),my3a$signif),digits=2)

my_3    # overall results with Hazard ratios, CI, and p-values


#Using Lopinavir with a cut-off of 4mg/L

m4_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")
```

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```
m4_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m4_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat4  + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest <-
list(coef(m4_1),coef(m4_2),coef(m4_3),coef(m4_4),coef(m4_5),coef(m4_6),coef(m4_7),coef(m4_8),coef(m4_
9),coef(m4_10))

mystd <-
list(summary(m2_1)[[7]][,4],summary(m2_2)[[7]][,4],summary(m2_3)[[7]][,4],summary(m4_4)[[7]][,4],summar
y(m4_5)[[7]][,4],summary(m2_6)[[7]][,4],summary(m4_7)[[7]][,4],summary(m4_8)[[7]][,4],summary(m4_9)[[7]
][,4],summary(m4_10)[[7]][,4])

my4a <- mi.inference(myest, mystd, confidence=0.95)

my_4 <- round(cbind(exp(my4a$est),exp(my4a$lower),exp(my4a$upper),my2a$signif),digits=3)

my_4     # overall results with Hazard ratios, CI, and p-values


# Crude analysis

m2_11<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_21<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_31<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_41<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_51<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_61<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_71<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_81<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_91<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[9]],method="breslow")
```

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```
m2_101<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[10]],method="breslow")

myest1 <-
list(coef(m2_11),coef(m2_21),coef(m2_31),coef(m2_41),coef(m2_51),coef(m2_61),coef(m2_71),coef(m2_81),
coef(m2_91),coef(m2_101))

mystd1 <-
list(summary(m2_11)[[7]][,4],summary(m2_21)[[7]][,4],summary(m2_31)[[7]][,4],summary(m2_41)[[7]][,4],su
mmary(m2_51)[[7]][,4],summary(m2_61)[[7]][,4],summary(m2_71)[[7]][,4],summary(m2_81)[[7]][,4],summar
y(m2_91)[[7]][,4],summary(m2_101)[[7]][,4])

my2a1 <- mi.inference(myest1, mystd1, confidence=0.95)

my_21 <- round(cbind(exp(my2a1$est),exp(my2a1$lower),exp(my2a1$upper),my2a1$signif),digits=3)

my_21


m2_13<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_23<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_33<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_43<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_53<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_63<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_73<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_83<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_93<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_103<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")


myest3 <-
list(coef(m2_13),coef(m2_23),coef(m2_33),coef(m2_43),coef(m2_53),coef(m2_63),coef(m2_73),coef(m2_83),
coef(m2_93),coef(m2_103))

mystd3 <-
list(summary(m2_13)[[7]][,4],summary(m2_23)[[7]][,4],summary(m2_33)[[7]][,4],summary(m2_43)[[7]][,4],su
mmary(m2_53)[[7]][,4],summary(m2_63)[[7]][,4],summary(m2_73)[[7]][,4],summary(m2_83)[[7]][,4],summar
y(m2_93)[[7]][,4],summary(m2_103)[[7]][,4])
```

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```
my2a3 <- mi.inference(myest3, mystd3, confidence=0.95)

my_23 <- round(cbind(exp(my2a3$est),exp(my2a3$lower),exp(my2a3$upper),my2a3$signif),digits=3)

my_23


m2_14<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_24<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_34<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_44<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_54<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_64<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_74<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_84<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_94<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_104<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(logvlcat) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest4 <-
list(coef(m2_14),coef(m2_24),coef(m2_34),coef(m2_44),coef(m2_54),coef(m2_64),coef(m2_74),coef(m2_84),
coef(m2_94),coef(m2_104))

mystd4 <-
list(summary(m2_14)[[7]][,4],summary(m2_24)[[7]][,4],summary(m2_34)[[7]][,4],summary(m2_44)[[7]][,4],su
mmary(m2_54)[[7]][,4],summary(m2_64)[[7]][,4],summary(m2_74)[[7]][,4],summary(m2_84)[[7]][,4],summar
y(m2_94)[[7]][,4],summary(m2_104)[[7]][,4])

my2a4 <- mi.inference(myest4, mystd4, confidence=0.95)

my_24 <- round(cbind(exp(my2a4$est),exp(my2a4$lower),exp(my2a4$upper),my2a4$signif),digits=3)

my_24


m2_15<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_25<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[2]],method="breslow")
```

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m2_35<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_45<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_55<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_65<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_75<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_85<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_95<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_105<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest5 <-
list(coef(m2_15),coef(m2_25),coef(m2_35),coef(m2_45),coef(m2_55),coef(m2_65),coef(m2_75),coef(m2_85),
coef(m2_95),coef(m2_105))

mystd5 <-
list(summary(m2_15)[[7]][,4],summary(m2_25)[[7]][,4],summary(m2_35)[[7]][,4],summary(m2_45)[[7]][,4],su
mmary(m2_55)[[7]][,4],summary(m2_65)[[7]][,4],summary(m2_75)[[7]][,4],summary(m2_85)[[7]][,4],summar
y(m2_95)[[7]][,4],summary(m2_105)[[7]][,4])

my2a5 <- mi.inference(myest5, mystd5, confidence=0.95)

my_25 <- round(cbind(exp(my2a5$est),exp(my2a5$lower),exp(my2a5$upper),my2a5$signif),digits=3)

my_25


m2_16<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_26<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_36<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_46<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_56<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_66<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[6]],method="breslow")
```

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m2_76<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_86<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_96<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_106<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(whostage2)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest6 <-
list(coef(m2_16),coef(m2_26),coef(m2_36),coef(m2_46),coef(m2_56),coef(m2_66),coef(m2_76),coef(m2_86),
coef(m2_96),coef(m2_106))

mystd6 <-
list(summary(m2_16)[[7]][,4],summary(m2_26)[[7]][,4],summary(m2_36)[[7]][,4],summary(m2_46)[[7]][,4],su
mmary(m2_56)[[7]][,4],summary(m2_66)[[7]][,4],summary(m2_76)[[7]][,4],summary(m2_86)[[7]][,4],summar
y(m2_96)[[7]][,4],summary(m2_106)[[7]][,4])

my2a6<- mi.inference(myest6, mystd6, confidence=0.95)

my_26 <- round(cbind(exp(my2a6$est),exp(my2a6$lower),exp(my2a6$upper),my2a6$signif),digits=3)

my_26


m2_17<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_27<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_37<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_47<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_57<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_67<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_77<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_87<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_97<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_107<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor(wfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")
```

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```
myest7 <-
list(coef(m2_17),coef(m2_27),coef(m2_37),coef(m2_47),coef(m2_57),coef(m2_67),coef(m2_77),coef(m2_87),
coef(m2_97),coef(m2_107))

mystd7 <-
list(summary(m2_17)[[7]][,4],summary(m2_27)[[7]][,4],summary(m2_37)[[7]][,4],summary(m2_47)[[7]][,4],su
mmary(m2_57)[[7]][,4],summary(m2_67)[[7]][,4],summary(m2_71)[[7]][,4],summary(m2_81)[[7]][,4],summar
y(m2_91)[[7]][,4],summary(m2_107)[[7]][,4])

my2a7 <- mi.inference(myest7, mystd7, confidence=0.95)

my_27 <- round(cbind(exp(my2a7$est),exp(my2a7$lower),exp(my2a7$upper),my2a7$signif),digits=3)

my_27


m2_18<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_28<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_38<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_48<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_58<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_68<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_78<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_88<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_98<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_108<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor(hfacat) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest8 <-
list(coef(m2_18),coef(m2_28),coef(m2_38),coef(m2_48),coef(m2_58),coef(m2_68),coef(m2_78),coef(m2_88),
coef(m2_98),coef(m2_108))

mystd8 <-
list(summary(m2_18)[[7]][,4],summary(m2_28)[[7]][,4],summary(m2_38)[[7]][,4],summary(m2_48)[[7]][,4],su
mmary(m2_58)[[7]][,4],summary(m2_68)[[7]][,4],summary(m2_78)[[7]][,4],summary(m2_88)[[7]][,4],summar
y(m2_98)[[7]][,4],summary(m2_108)[[7]][,4])

my2a8 <- mi.inference(myest8, mystd8, confidence=0.95)

my_28 <- round(cbind(exp(my2a8$est),exp(my2a8$lower),exp(my2a8$upper),my2a8$signif),digits=3)
```

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my_28

```
m2_19<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_29<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_39<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m2_49<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_59<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_69<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_79<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_89<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_99<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_109<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor(lpvcat1) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest9 <-
list(coef(m2_19),coef(m2_29),coef(m2_39),coef(m2_49),coef(m2_59),coef(m2_69),coef(m2_79),coef(m2_89),
coef(m2_99),coef(m2_109))

mystd9 <-
list(summary(m2_19)[[7]][,4],summary(m2_29)[[7]][,4],summary(m2_39)[[7]][,4],summary(m2_49)[[7]][,4],su
mmary(m2_59)[[7]][,4],summary(m2_69)[[7]][,4],summary(m2_79)[[7]][,4],summary(m2_89)[[7]][,4],summar
y(m2_99)[[7]][,4],summary(m2_109)[[7]][,4])

my2a9 <- mi.inference(myest9, mystd9, confidence=0.95)

my_29 <- round(cbind(exp(my2a9$est),exp(my2a9$lower),exp(my2a9$upper),my2a9$signif),digits=2)

my_29
```

```
m2_21<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m2_31<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m2_41<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[3]],method="breslow")
```

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```
m2_51<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m2_61<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m2_71<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m2_81<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m2_91<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m2_101<-coxph(Surv(X_t0,X_t, vlsupp)~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m2_111<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest11 <-
list(coef(m2_21),coef(m2_31),coef(m2_41),coef(m2_51),coef(m2_61),coef(m2_71),coef(m2_81),coef(m2_91),
coef(m2_101),coef(m2_111))

mystd11 <-
list(summary(m2_19)[[7]][,4],summary(m2_29)[[7]][,4],summary(m2_39)[[7]][,4],summary(m2_49)[[7]][,4],su
mmary(m2_59)[[7]][,4],summary(m2_69)[[7]][,4],summary(m2_79)[[7]][,4],summary(m2_89)[[7]][,4],summar
y(m2_99)[[7]][,4],summary(m2_109)[[7]][,4])

my2a11 <- mi.inference(myest9, mystd9, confidence=0.95)

my_31 <- round(cbind(exp(my2a11$est),exp(my2a11$lower),exp(my2a11$upper),my2a11$signif),digits=2)

my_31

# Model selection based on AICw

library(mgcv)


seldat <- impdat

seldat <- cbind(seldat,cut(seldat$logbaselinevl,breaks=c(-1,5,1000000000)))

colnames(seldat)[13]<-c("logvlcat")

seldat <- cbind(seldat,cut(seldat$baselinecd4pc,breaks=c(-1,25,1000000000)))

colnames(seldat)[14]<-c("cd4pccat")

seldat <- cbind(seldat,cut(seldat$agestartarv,breaks=c(-1,26,1000000000)))

colnames(seldat)[15]<-c("agecat")

seldat <- cbind(seldat,cut(seldat$baselinewfa,breaks=c(-1000,-3,-2,10)))

colnames(seldat)[16]<-c("wfacat")

seldat <- cbind(seldat,cut(seldat$baselinehfa,breaks=c(-1000,-3,-2,10)))
```

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```
colnames(seldat)[17]<-c("hfacat")

seldat <- cbind(seldat,cut(seldat$lpv,breaks=c(-1,1,1000000000)))

colnames(seldat)[18]<-c("lpvcat1")

seldat <- cbind(seldat,cut(seldat$lpv,breaks=c(-1,4,1000000000)))

colnames(seldat)[19]<-c("lpvcat4")

#Model Selection Using lopinar Concentration

mymissing<-as.numeric(apply(is.na(seldat),1,any))

probmod1 <-gam(mymissing~1 + vlsupp + whostage2 + s(baselinecd4pc) + s(agestartarv) + s(baselinewfa) +
s(baselinehfa) + s(lpv)  ,family=binomial,data=seldat)

summary(probmod1)

probmod <-gam(mymissing~1 + vlsupp + whostage2  + s(baselinewfa) + s(baselinehfa) + s(lpv)
,family=binomial,data=seldat)

summary(probmod)

myprob <- rep(1, dim(seldat)[1])

mymissing2<-
as.numeric(apply(is.na(seldat[,c("vlsupp","baselinewfa","baselinehfa","lpv","whostage2")]),1,any))

myprob[mymissing2==0] <-predict(probmod,type="response")

myweights <- 1/myprob

myweights[mymissing==1] <- 0

summary(myweights)

cbind(seldat,mymissing,myweights)[1:100,]


# First get an idea on what is happening

library(MASS)

stepAIC(m2_1,direction = c("both"))   # LPV, VL, stage, HFA

stepAIC(m2_2,direction = c("both"))   # LPV, VL,     , HFA

stepAIC(m2_3,direction = c("both"))   # LPV,   , stage, HFA

stepAIC(m2_4,direction = c("both"))   # LPV,   ,     , HFA

stepAIC(m2_5,direction = c("both"))   # LPV,         , HFA

stepAIC(m2_6,direction = c("both"))   # LPV,   , stage, HFA

stepAIC(m2_7,direction = c("both"))   # LPV, VL,     , HFA

stepAIC(m2_8,direction = c("both"))   # LPV, VL, stage, HFA

stepAIC(m2_9,direction = c("both"))   # LPV, VL, stage, HFA

stepAIC(m2_10,direction = c("both"))  # LPV, VL,
```

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```
# Candidate models: LPV for sure, VL forced (but explore anyway), stage and hfa to check


m5_1 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + as.factor(logvlcat) + as.factor(whostage2) + as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")

m5_2 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + as.factor(logvlcat)                  + as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")

m5_3 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + as.factor(logvlcat) + as.factor(whostage2)               +
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")

m5_4 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + as.factor(logvlcat) +
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")

m5_5 <- coxph(Surv(X_t0,X_t, vlsupp)~       + as.factor(logvlcat)                  + as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")


m5_6 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv +                                 as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")


m5_10 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m5_11 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv + cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")



extractAIC(m5_1)

extractAIC(m5_2)    # AICw selected model (when VL forced)

extractAIC(m5_3)

extractAIC(m5_4)

extractAIC(m5_5)


extractAIC(m5_6)    # without VL (not considered)


extractAIC(m5_10)    # Full model

extractAIC(m5_11)    # Null model


# Estimate selected model after MI
```

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```
# Adjusted analysis

m3_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m3_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m3_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m3_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m3_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m3_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m3_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m3_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m3_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m3_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpv  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")


myest <-
list(coef(m3_1),coef(m3_2),coef(m3_3),coef(m3_4),coef(m3_5),coef(m3_6),coef(m3_7),coef(m3_8),coef(m3_
9),coef(m3_10))

mystd <-
list(summary(m3_1)[[7]][,4],summary(m3_2)[[7]][,4],summary(m3_3)[[7]][,4],summary(m3_4)[[7]][,4],summar
y(m3_5)[[7]][,4],summary(m3_6)[[7]][,4],summary(m3_7)[[7]][,4],summary(m3_8)[[7]][,4],summary(m3_9)[[7]
][,4],summary(m3_10)[[7]][,4])

my3a <- mi.inference(myest, mystd, confidence=0.95)

my_3 <- round(cbind(exp(my3a$est),exp(my3a$lower),exp(my3a$upper),my3a$signif),digits=3)

my_3     # overall results with Hazard ratios, CI, and p-values


###############

#  Summary   #

###############

MIsummary <- matrix(rep(NA,12*9),nrow=9,ncol=12)

MIsummary[,1:4] <- my_2

MIsummary[1,5:8]     <- my_21
```

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```
MIsummary[2,5:8]    <- my_23

MIsummary[3,5:8]    <- my_24

MIsummary[4,5:8]    <- my_25

MIsummary[5,5:8]    <- my_26

MIsummary[6:7,5:8]   <- my_27

MIsummary[8:9,5:8]   <- my_28

MIsummary[c(1,3,8:9),9:12] <- my_3


rownames(MIsummary) <- c("LPV conc.","CD4% (high)","logVL (5+)","Age (>1/2 yr.)","Stage (adv.)","WFA (-2 to
-3sd.)","WFA (> -2sd.)","HFA (-2 to -3sd.)","HFA (> -2sd.)")

colnames(MIsummary) <-c("Adj.","","","","Crude","","","","AIC sel.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_MI1.csv")


#####################################

# Using LPV with a cut-off of 1mg/L#

#####################################

m4_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m4_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat1  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")
```

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```
myest <-
list(coef(m4_1),coef(m4_2),coef(m4_3),coef(m4_4),coef(m4_5),coef(m4_6),coef(m4_7),coef(m4_8),coef(m4_
9),coef(m4_10))

mystd <-
list(summary(m4_1)[[7]][,4],summary(m4_2)[[7]][,4],summary(m4_3)[[7]][,4],summary(m4_4)[[7]][,4],summar
y(m4_5)[[7]][,4],summary(m4_6)[[7]][,4],summary(m4_7)[[7]][,4],summary(m4_8)[[7]][,4],summary(m4_9)[[7]
][,4],summary(m4_10)[[7]][,4])

my4a <- mi.inference(myest, mystd, confidence=0.95)

my_4 <- round(cbind(exp(my4a$est),exp(my4a$lower),exp(my4a$upper),my4a$signif),digits=3)

my_4     # overall results with Hazard ratios, CI, and p-values


MIsummary <- matrix(rep(NA,12*9),nrow=9,ncol=12)

MIsummary[,1:4] <- my_2

MIsummary[1,5:8]     <- my_21

MIsummary[2,5:8]     <- my_23

MIsummary[3,5:8]     <- my_24

MIsummary[4,5:8]     <- my_25

MIsummary[5,5:8]     <- my_26

MIsummary[6:7,5:8]   <- my_27

MIsummary[8:9,5:8]   <- my_28

MIsummary[c(1,3,8:9),9:12] <- my_4


rownames(MIsummary) <- c("LPVCAT1","CD4% (high)","logVL (5+)","Age (>1/2 yr.)","Stage (adv.)","WFA (-2 to
-3sd.)","WFA (> -2sd.)","HFA (-2 to -3sd.)","HFA (> -2sd.)")

colnames(MIsummary) <-c("Adj.","","","","Crude","","","","AIC sel.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_MI4.csv")


####################################

# Using LPV with a cut-off of 4mg/L#

####################################

m5_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")
```

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```
m5_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat4  + as.factor(logvlcat)  + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")


myest <-
list(coef(m5_1),coef(m5_2),coef(m5_3),coef(m5_4),coef(m5_5),coef(m5_6),coef(m5_7),coef(m5_8),coef(m5_
9),coef(m5_10))

mystd <-
list(summary(m5_1)[[7]][,4],summary(m5_2)[[7]][,4],summary(m5_3)[[7]][,4],summary(m5_4)[[7]][,4],summar
y(m5_5)[[7]][,4],summary(m5_6)[[7]][,4],summary(m5_7)[[7]][,4],summary(m5_8)[[7]][,4],summary(m5_9)[[7]
][,4],summary(m5_10)[[7]][,4])

my5a <- mi.inference(myest, mystd, confidence=0.95)

my_5 <- round(cbind(exp(my5a$est),exp(my5a$lower),exp(my5a$upper),my5a$signif),digits=3)

my_5     # overall results with Hazard ratios, CI, and p-values


MIsummary <- matrix(rep(NA,12*9),nrow=9,ncol=12)

MIsummary[,1:4] <- my_2

MIsummary[1,5:8]     <- my_21

MIsummary[2,5:8]     <- my_23

MIsummary[3,5:8]     <- my_24

MIsummary[4,5:8]     <- my_25

MIsummary[5,5:8]     <- my_26

MIsummary[6:7,5:8]   <- my_27

MIsummary[8:9,5:8]   <- my_28

MIsummary[c(1,3,8:9),9:12] <- my_5
```

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```
rownames(MIsummary) <- c("LPVCAT4","CD4% (high)","logVL (5+)","Age (>1/2 yr.)","Stage (adv.)","WFA (-2 to -3sd.)","WFA (> -2sd.)","HFA (-2 to -3sd.)","HFA (> -2sd.)")

colnames(MIsummary) <-c("Adj.","","","","Crude","","","","AIC sel.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_MI3.csv")

#############

# Figure 1 #

#############


# Spline representation

# Imputation based approach

library(pspline)

Lpvm31 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[1]], robust=TRUE, method="breslow")

Lpvm32 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[2]], robust=TRUE, method="breslow")

Lpvm33 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[3]], robust=TRUE, method="breslow")

Lpvm34 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[4]], robust=TRUE, method="breslow")

Lpvm35 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[5]], robust=TRUE, method="breslow")

Lpvm36 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[6]], robust=TRUE, method="breslow")

Lpvm37 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[7]], robust=TRUE, method="breslow")

Lpvm38 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[8]], robust=TRUE, method="breslow")

Lpvm39 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+ as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id), data=myimp$imputations[[9]], robust=TRUE, method="breslow")
```

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```
Lpvm310 <- coxph(Surv(X_t0,X_t,vlsupp) ~ pspline(lpv, df=4)+ as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[10]], robust=TRUE, method="breslow")

Lpvm311 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[11]], robust=TRUE, method="breslow")

Lpvm312 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[12]], robust=TRUE, method="breslow")

Lpvm313 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[13]], robust=TRUE, method="breslow")

Lpvm314 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[14]], robust=TRUE, method="breslow")

Lpvm315 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[15]], robust=TRUE, method="breslow")

Lpvm316 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[16]], robust=TRUE, method="breslow")

Lpvm317 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[17]], robust=TRUE, method="breslow")

Lpvm318 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[18]], robust=TRUE, method="breslow")

Lpvm319 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[19]], robust=TRUE, method="breslow")

Lpvm320 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4)+ as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[20]], robust=TRUE, method="breslow")

Lpvm321 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[21]], robust=TRUE, method="breslow")

Lpvm322 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[22]], robust=TRUE, method="breslow")

Lpvm323 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[23]], robust=TRUE, method="breslow")
```

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Lpvm324 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[24]], robust=TRUE, method="breslow")

Lpvm325 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2)+
as.factor(cd4pccat)+ as.factor(hfacat) + as.factor(agecat)+ as.factor(wfacat)+ cluster(id),
data=myimp$imputations[[25]], robust=TRUE, method="breslow")


predicted31 <- predict(Lpvm31, type = "terms" , se.fit = TRUE , terms = 1)

predicted32 <- predict(Lpvm32, type = "terms" , se.fit = TRUE , terms = 1)

predicted33 <- predict(Lpvm33, type = "terms" , se.fit = TRUE , terms = 1)

predicted34 <- predict(Lpvm34, type = "terms" , se.fit = TRUE , terms = 1)

predicted35 <- predict(Lpvm35, type = "terms" , se.fit = TRUE , terms = 1)

predicted36 <- predict(Lpvm36, type = "terms" , se.fit = TRUE , terms = 1)

predicted37 <- predict(Lpvm37, type = "terms" , se.fit = TRUE , terms = 1)

predicted38 <- predict(Lpvm38, type = "terms" , se.fit = TRUE , terms = 1)

predicted39 <- predict(Lpvm39, type = "terms" , se.fit = TRUE , terms = 1)

predicted310 <- predict(Lpvm310, type = "terms" , se.fit = TRUE , terms = 1)

predicted311 <- predict(Lpvm311, type = "terms" , se.fit = TRUE , terms = 1)

predicted312 <- predict(Lpvm312, type = "terms" , se.fit = TRUE , terms = 1)

predicted313 <- predict(Lpvm313, type = "terms" , se.fit = TRUE , terms = 1)

predicted314 <- predict(Lpvm314, type = "terms" , se.fit = TRUE , terms = 1)

predicted315 <- predict(Lpvm315, type = "terms" , se.fit = TRUE , terms = 1)

predicted316 <- predict(Lpvm316, type = "terms" , se.fit = TRUE , terms = 1)

predicted317 <- predict(Lpvm317, type = "terms" , se.fit = TRUE , terms = 1)

predicted318 <- predict(Lpvm318, type = "terms" , se.fit = TRUE , terms = 1)

predicted319 <- predict(Lpvm319, type = "terms" , se.fit = TRUE , terms = 1)

predicted320 <- predict(Lpvm320, type = "terms" , se.fit = TRUE , terms = 1)

predicted321 <- predict(Lpvm321, type = "terms" , se.fit = TRUE , terms = 1)

predicted322 <- predict(Lpvm322, type = "terms" , se.fit = TRUE , terms = 1)

predicted323 <- predict(Lpvm323, type = "terms" , se.fit = TRUE , terms = 1)

predicted324 <- predict(Lpvm324, type = "terms" , se.fit = TRUE , terms = 1)

predicted325 <- predict(Lpvm325, type = "terms" , se.fit = TRUE , terms = 1)


par(mfrow=c(3,3))
```

```
termplot(Lpvm31,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm32,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm33,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm34,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm35,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm36,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm37,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm38,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm39,terms=1,ylim=c(-0.75,0.3))


lp <-
(1/25)*(predicted31$fit+predicted32$fit+predicted33$fit+predicted34$fit+predicted35$fit+predicted36$fit+predicted37$fit+predicted38$fit+predicted39$fit+predicted310$fit+predicted311$fit+predicted312$fit+predicted313$fit+predicted314$fit+predicted315$fit+predicted316$fit+predicted317$fit+predicted318$fit+predicted319$fit+predicted320$fit+predicted321$fit+predicted322$fit+predicted323$fit+predicted324$fit+predicted325$fit)[is.na(seldat$lpv)==F]

within <-
(1/25)*(predicted31$se^2+predicted32$se^2+predicted33$se^2+predicted34$se^2+predicted35$se^2+predicted36$se^2+predicted37$se^2+predicted38$se^2+predicted39$se^2+predicted310$se^2+predicted311$se^2+predicted312$se^2+predicted313$se^2+predicted314$se^2+predicted315$se^2+predicted316$se^2+predicted317$se^2+predicted318$se^2+predicted319$se^2+predicted320$se^2+predicted321$se^2+predicted322$se^2+predicted323$se^2+predicted324$se^2+predicted325$se^2)[is.na(seldat$lpv)==F]

mycoefflist <-
cbind(c(predicted31$fit),c(predicted32$fit),c(predicted33$fit),c(predicted34$fit),c(predicted35$fit),c(predicted36$fit),c(predicted37$fit),c(predicted38$fit),c(predicted39$fit),c(predicted310$fit),c(predicted311$fit),c(predicted312$fit),c(predicted313$fit),c(predicted314$fit),c(predicted315$fit),c(predicted316$fit),c(predicted317$fit),c(predicted318$fit),c(predicted319$fit),c(predicted320$fit),c(predicted321$fit),c(predicted322$fit),c(predicted323$fit),c(predicted324$fit),c(predicted325$fit))[is.na(seldat$lpv)==F,]

mycoeff <- apply(mycoefflist,1,mean)

coeffdiff<-(matrix(cbind(rep(mycoeff,M)),ncol=M,nrow=length(mycoeff))-mycoefflist)^2

between <-  apply(coeffdiff,1,sum)

variance <- within + ((M+1)/(M*(M-1)))*between

se <- round(sqrt(variance),digits=5)


par(mfrow=c(1,1))

pdf(file="C:/Users/01429265/Documents/Project_Ray/Figure1.pdf")

plot(0 , xlab=" Lopinavir concentration (mg/L)" , ylab = "Hazard of virological failure" , axes=T, main = "" , type = "n" , xlim=c(0,15) , las=1,ylim=c(0.5,1.75))

lines(sm.spline(myimp$imputations[[1]]$lpv[is.na(seldat$lpv)==F], exp(lp)), col = "red" , lwd = 0.8)
```

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```
lines(sm.spline(myimp$imputations[[1]]$lpv[is.na(seldat$lpv)==F], exp(lp + 1.96 * sqrt(variance))) , col =
"orange" , lty = 2 , lwd = 0.4)

lines(sm.spline(myimp$imputations[[1]]$lpv[is.na(seldat$lpv)==F], exp(lp - 1.96 * sqrt(variance))) , col =
"orange" , lty = 2 , lwd = 0.4)

axis(side = 1 , at = c(seq(0,17.5,2.5)), labels = F , tick = T , tcl = 0.4 , lwd.ticks = 0.1)

#axis(2,at=c(seq(0.5,1.75,0.25)),las=1)

#legend("top",col=c("red","blue","blue"),legend=c("Multiple Imputation (n=524)","Complete cases (adj.,
n=213)","Complete cases (crude, n=452)"), lty=c(1,1,2),lwd=1.5, cex=1.25, bty="n",ncol=1)

dev.off()



#####################################

# Model Selection ######################

#####################################


m4_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m4_0b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m4_0c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m4_0d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")

extractAIC(m4_0a)[2]

extractAIC(m4_0b)[2]

extractAIC(m4_0c)[2]

extractAIC(m4_0d)[2]


m4_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")
```

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m4_1c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_1d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

extractAIC(m4_1a)[2]

extractAIC(m4_1b)[2]

extractAIC(m4_1c)[2]

extractAIC(m4_1d)[2]


m4_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_2c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_2d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

extractAIC(m4_2a)[2]

extractAIC(m4_2b)[2]

extractAIC(m4_2c)[2]

extractAIC(m4_2d)[2]


m4_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_3c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_3d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")
```

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extractAIC(m4_3a)[2]

extractAIC(m4_3b)[2]

extractAIC(m4_3c)[2]

extractAIC(m4_3d)[2]


m4_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_4c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_4d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

extractAIC(m4_4a)[2]

extractAIC(m4_4b)[2]

extractAIC(m4_4c)[2]

extractAIC(m4_4d)[2]


m4_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_5c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_5d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

extractAIC(m4_5a)[2]

extractAIC(m4_5b)[2]

extractAIC(m4_5c)[2]

extractAIC(m4_5d)[2]
```

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m4_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_6c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_6d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

extractAIC(m4_6a)[2]

extractAIC(m4_6b)[2]

extractAIC(m4_6c)[2]

extractAIC(m4_6d)[2]


m4_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_7c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_7d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

extractAIC(m4_7a)[2]

extractAIC(m4_7b)[2]

extractAIC(m4_7c)[2]

extractAIC(m4_7d)[2]


m4_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")
```

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m4_8c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_8d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

extractAIC(m4_8a)[2]

extractAIC(m4_8b)[2]

extractAIC(m4_8c)[2]

extractAIC(m4_8d)[2]


m4_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),,data=myimp$imputations[[9]],method="breslow")

m4_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),,data=myimp$imputations[[9]],method="breslow")

m4_9c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),,data=myimp$imputations[[9]],method="breslow")

m4_9d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

extractAIC(m4_9a)[2]

extractAIC(m4_9b)[2]

extractAIC(m4_9c)[2]

extractAIC(m4_9d)[2]


m4_10a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

m4_10b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

m4_10c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")

m4_10d<-coxph(Surv(X_t0,X_t, vlsupp) ~pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")
```

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```
extractAIC(m4_10a)[2]

extractAIC(m4_10b)[2]

extractAIC(m4_10c)[2]

extractAIC(m4_10d)[2]


################################

# Adherence vs. Concentration #####

################################

m5_0 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpvcat1   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m5_1 <- coxph(Surv(X_t0,X_t, vlsupp)~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m5_2 <- coxph(Surv(X_t0,X_t, vlsupp)~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=seldat,weights=myweights+1e-
08,method="breslow")

m5_3 <- coxph(Surv(X_t0,X_t, vlsupp)~ pspline(lpv) + as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=seldat,weights=myweights+1e-08,method="breslow")


extractAIC(m5_0)

extractAIC(m5_1)

extractAIC(m5_2)

extractAIC(m5_3)


m5_31<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_32<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_33<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_34<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")
```

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m5_35<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_36<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_37<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_38<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_39<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_310<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpv   + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) +
as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")


m5_41<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_42<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_43<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_44<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_45<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_46<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_47<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_48<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[8]],method="breslow")
```

APPENDIX I

```
m5_49<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat)
+ as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_410<-coxph(Surv(X_t0,X_t, vlsupp) ~ adhstatus+ as.factor(cd4pccat) + as.factor(logvlcat) +
as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+
cluster(id),data=myimp$imputations[[10]],method="breslow")



0.1*(extractAIC(m5_31)[2]+extractAIC(m5_32)[2]+extractAIC(m5_33)[2]+extractAIC(m5_34)[2]+extractAIC(m5
_35)[2]+extractAIC(m5_36)[2]+extractAIC(m5_37)[2]+extractAIC(m5_38)[2]+extractAIC(m5_39)[2]+extractAIC(
m5_310)[2])

0.1*(extractAIC(m5_41)[2]+extractAIC(m5_42)[2]+extractAIC(m5_43)[2]+extractAIC(m5_44)[2]+extractAIC(m5
_45)[2]+extractAIC(m5_46)[2]+extractAIC(m5_47)[2]+extractAIC(m5_48)[2]+extractAIC(m5_49)[2]+extractAIC(
m5_410)[2])


# Everything is indicating that it is better to use Lopinavir compared to adherence
```

APPENDIX II

Cox Proportional Hazards Mutliple failure Event Model of Lopinavir or Nevirapine During The Post-randomization Phase

```
library(splines)

library(survival)

library(pspline)

LPV/NVP<-read.csv("LPV_27_10_2015.csv", header=T,sep=",",stringsAsFactors=FALSE)

attach(LPV)

hist(lpvconc/nvpconc)

head(LPV/NVP)

######################

### Descriptives ####

######################

# percentage Missingness

round(apply(apply(cbind(lpvcorr,adstatus,t0rwfa,ageatran,t0rhfa,vlsupp,vlsupp1,t0rvl,t0rcd4pc,postrantb),c(1,
2),is.na),2,mean),digits=3)


surv.vl1 <- Surv(X_t0,X_t,vlsupp)      # setting survival time from Stata

summary(surv.vl1)

#################

## Imputation   #

#################

library(foreign)

library(Rcpp)

library(norm)

library(Amelia)

set.seed(666)

M=10

Impdat=LPV[,c("id","lpvcorr","adstatus","t0rvl","t0rcd4pc","ageatran","t0rwfa","t0rhfa","postrantb","X_t0","X
_t","vlsupp","vlsupp1")]

round(apply(apply(impdat,c(1,2),is.na),2,mean),digits=3)   # percentage of missing values

# Michael 1: Made changes here

myimp <- amelia(impdat, m=M,
p2s=1,noms=c("postrantb","vlsupp","vlsupp1"),cs=c("id"),ts=c("X_t0"),bounds=matrix(c(2,0,70, 3,0,100,
4,0,400, 5,0,100,
6,0,50),ncol=3,nrow=5,byrow=T),logs=c("lpvconc/nvpconc","adstatus"),polytime=3,splinetime=3,empri=20,inc
heck=TRUE,tolerance=0.001)
```

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```
plot(myimp)

par(mfrow=c(3,3))

compare.density(myimp,var="lpvcorr")

compare.density(myimp,var="adstatus")

compare.density(myimp,var="t0rvl")

compare.density(myimp,var="t0rcd4pc")

compare.density(myimp,var="ageatran")

compare.density(myimp,var="t0rwfa")

compare.density(myimp,var="t0rhfa")

compare.density(myimp,var="X_t")

dev.off()

par(mfrow=c(1,1))

suppressWarnings(disperse(myimp, dims=1, m=10))

suppressWarnings(disperse(myimp, dims=2, m=10))

par(mfrow=c(2,1))

suppressWarnings(tscsPlot(myimp,var="t0rcd4pc",cs=3011,draws=10))

suppressWarnings(tscsPlot(myimp,var="t0rwfa",cs=3011,draws=10))

par(mfrow=c(1,1))

overimpute(myimp,var="lpvcorr")

overimpute(myimp,var="adstatus")

overimpute(myimp,var="t0rvl")

overimpute(myimp,var="t0rcd4pc")

overimpute(myimp,var="t0rwfa")

overimpute(myimp,var="ageatran")

overimpute(myimp,var="t0rhfa")

#dev.off()

for(m in 1:M){

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],vlcat=cut(myimp$imputations[[m]]$t0rvl,breaks=c(-1,51,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],cd4pccat=cut(myimp$imputations[[m]]$t0rcd4pc,breaks=c(-
1,25,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],agecat=cut(myimp$imputations[[m]]$ageatran,breaks=c(-
1,20,1000000000)))
```

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```
myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],wfacat=cut(myimp$imputations[[m]]$t0rwfa,breaks=c(-1000,-2,10)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],hfacat=cut(myimp$imputations[[m]]$t0rhfa,breaks=c(-1000,-2,10)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat1=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,1,1000000000)))/

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat2=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,2,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat3=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,3,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat3.5=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,3.5,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat4=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,4,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat5=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,5,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvcat6=cut(myimp$imputations[[m]]$lpvcorr,breaks=c(-
1,6,1000000000)))

}


head(myimp$imputations[[1]])

write.amelia(obj=myimp , file.stem = "lpv3imp")



# function to create lags

shift.1<-function(x,shift_by=-1){

   stopifnot(is.numeric(shift_by))

   stopifnot(is.numeric(x))

   if (length(shift_by)>1)

      return(sapply(shift_by,shift, x=x))

   out<-NULL

   abs_shift_by=abs(shift_by)
```

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```
  if (shift_by > 0 )
    out<-c(tail(x,-abs_shift_by),rep(NA,abs_shift_by))
  else if (shift_by < 0 )
    out<-c(rep(NA,abs_shift_by), head(x,-abs_shift_by))
  else
    out<-x
  out
}
# ...use this function for longitudinal data, means apply them by patient
shift.l <- function(x,splitby){
unsplit(lapply(split(x,splitby),shift.1),splitby)
}
for(m in 1:M){
myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],lpvre=shift.l(myimp$imputations[[m]]$lpvcorr,myimp$imputations[[m]]$id))
myimp$imputations[[m]]$lpvre <-
replace((myimp$imputations[[m]])$lpvre,is.na((myimp$imputations[[m]])$lpvre),0)
myimp$imputations[[m]]<-transform(myimp$imputations[[m]],lpave=((lpvcorr+lpvre)/2))
}
head(myimp$imputations[[1]])
write.amelia(obj=myimp , file.stem = "lpv20imp")



################################
## Table 3: Cox Regression   #
## (crude, adj, selected)    #
################################
# Crude analysis
m1_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")
m1_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
m1_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")
```

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```
m1_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1 <-
list(coef(m1_1),coef(m1_2),coef(m1_3),coef(m1_4),coef(m1_5),coef(m1_6),coef(m1_7),coef(m1_8),coef(m1_
9),coef(m1_10))

mystd1 <-
list(summary(m1_1)[[7]][,4],summary(m1_2)[[7]][,4],summary(m1_3)[[7]][,4],summary(m1_4)[[7]][,4],summar
y(m1_5)[[7]][,4],summary(m1_6)[[7]][,4],summary(m1_7)[[7]][,4],summary(m1_8)[[7]][,4],summary(m1_9)[[7]
][,4],summary(m1_10)[[7]][,4])

my1a <- mi.inference(myest1, mystd1, confidence=0.95)

my_1 <- round(cbind(exp(my1a$est),exp(my1a$lower),exp(my1a$upper),my1a$signif),digits=3)

my_1    # significant



m1b_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1b_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1b_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1b_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1b_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1b_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1b_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
```

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```
m1b_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1b_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1b_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcorr +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1b <-
list(coef(m1b_1),coef(m1b_2),coef(m1b_3),coef(m1b_4),coef(m1b_5),coef(m1b_6),coef(m1b_7),coef(m1b_8),
coef(m1b_9),coef(m1b_10))

mystd1b <-
list(summary(m1b_1)[[7]][,4],summary(m1b_2)[[7]][,4],summary(m1b_3)[[7]][,4],summary(m1b_4)[[7]][,4],su
mmary(m1b_5)[[7]][,4],summary(m1b_6)[[7]][,4],summary(m1b_7)[[7]][,4],summary(m1b_8)[[7]][,4],summar
y(m1b_9)[[7]][,4],summary(m1b_10)[[7]][,4])

my1b <- mi.inference(myest1b, mystd1b, confidence=0.95)

my_1b <- round(cbind(exp(my1b$est),exp(my1b$lower),exp(my1b$upper),my1b$signif),digits=3)

my_1b     # significant


m1c_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1c_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1c_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1c_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1c_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1c_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1c_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1c_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1c_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1c_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvre +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1c <-
list(coef(m1c_1),coef(m1c_2),coef(m1c_3),coef(m1c_4),coef(m1c_5),coef(m1c_6),coef(m1c_7),coef(m1c_8),c
oef(m1c_9),coef(m1c_10))
```

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```
mystd1c <-
list(summary(m1c_1)[[7]][,4],summary(m1c_2)[[7]][,4],summary(m1c_3)[[7]][,4],summary(m1c_4)[[7]][,4],su
mmary(m1c_5)[[7]][,4],summary(m1c_6)[[7]][,4],summary(m1c_7)[[7]][,4],summary(m1c_8)[[7]][,4],summary(
m1c_9)[[7]][,4],summary(m1c_10)[[7]][,4])

my1c <- mi.inference(myest1c, mystd1c, confidence=0.95)

my_1c <- round(cbind(exp(my1c$est),exp(my1c$lower),exp(my1c$upper),my1c$signif),digits=3)

my_1c     # significant


m1d_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1d_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1d_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1d_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1d_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1d_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1d_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1d_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1d_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1d_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpave +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1d <-
list(coef(m1d_1),coef(m1d_2),coef(m1d_3),coef(m1d_4),coef(m1d_5),coef(m1d_6),coef(m1d_7),coef(m1d_8),
coef(m1d_9),coef(m1d_10))

mystd1d <-
list(summary(m1d_1)[[7]][,4],summary(m1d_2)[[7]][,4],summary(m1d_3)[[7]][,4],summary(m1d_4)[[7]][,4],su
mmary(m1d_5)[[7]][,4],summary(m1d_6)[[7]][,4],summary(m1d_7)[[7]][,4],summary(m1d_8)[[7]][,4],summar
y(m1d_9)[[7]][,4],summary(m1d_10)[[7]][,4])

my1d <- mi.inference(myest1d, mystd1d, confidence=0.95)

my_1d <- round(cbind(exp(my1d$est),exp(my1d$lower),exp(my1d$upper),my1d$signif),digits=3)

my_1d     # significant
```

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```
m1e_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1e_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1e_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1e_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1e_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1e_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1e_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1e_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1e_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1e_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat1 +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1e <-
list(coef(m1e_1),coef(m1e_2),coef(m1e_3),coef(m1e_4),coef(m1e_5),coef(m1e_6),coef(m1e_7),coef(m1e_8),
coef(m1e_9),coef(m1e_10))

mystd1e <-
list(summary(m1e_1)[[7]][,4],summary(m1e_2)[[7]][,4],summary(m1e_3)[[7]][,4],summary(m1e_4)[[7]][,4],su
mmary(m1e_5)[[7]][,4],summary(m1e_6)[[7]][,4],summary(m1e_7)[[7]][,4],summary(m1e_8)[[7]][,4],summary
(m1e_9)[[7]][,4],summary(m1e_10)[[7]][,4])

my1e <- mi.inference(myest1e, mystd1e, confidence=0.95)

my_1e <- round(cbind(exp(my1e$est),exp(my1e$lower),exp(my1e$upper),my1e$signif),digits=3)

my_1e


m1f_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1f_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1f_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1f_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")
```

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m1f_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1f_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1f_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1f_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1f_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1f_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1f <-
list(coef(m1f_1),coef(m1f_2),coef(m1f_3),coef(m1f_4),coef(m1f_5),coef(m1f_6),coef(m1f_7),coef(m1f_8),coef
(m1f_9),coef(m1f_10))

mystd1f <-
list(summary(m1df1)[[7]][,4],summary(m1f_2)[[7]][,4],summary(m1f_3)[[7]][,4],summary(m1f_4)[[7]][,4],sum
mary(m1f_5)[[7]][,4],summary(m1f_6)[[7]][,4],summary(m1f_7)[[7]][,4],summary(m1f_8)[[7]][,4],summary(m
1f_9)[[7]][,4],summary(m1f_10)[[7]][,4])

my1f <- mi.inference(myest1f, mystd1f, confidence=0.95)

my_1f <- round(cbind(exp(my1f$est),exp(my1f$lower),exp(my1f$upper),my1f$signif),digits=3)

my_1f     # significant


m2_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m2_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m2_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m2_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m2_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m2_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m2_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m2_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")
```

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m2_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m2_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest2 <-
list(coef(m2_1),coef(m2_2),coef(m2_3),coef(m2_4),coef(m2_5),coef(m2_6),coef(m2_7),coef(m2_8),coef(m2_
9),coef(m2_10))

mystd2 <-
list(summary(m2_1)[[7]][,4],summary(m2_2)[[7]][,4],summary(m2_3)[[7]][,4],summary(m2_4)[[7]][,4],summar
y(m2_5)[[7]][,4],summary(m2_6)[[7]][,4],summary(m2_7)[[7]][,4],summary(m2_8)[[7]][,4],summary(m2_9)[[7]
][,4],summary(m2_10)[[7]][,4])

my2a <- mi.inference(myest2, mystd2, confidence=0.95)

my_2 <- round(cbind(exp(my2a$est),exp(my2a$lower),exp(my2a$upper),my2a$signif),digits=3)

my_2


m3_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m3_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m3_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m3_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m3_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m3_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m3_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m3_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m3_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m3_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest3 <-
list(coef(m3_1),coef(m3_3),coef(m3_3),coef(m3_4),coef(m3_5),coef(m3_6),coef(m3_7),coef(m3_8),coef(m3_
9),coef(m3_10))

mystd3 <-
list(summary(m3_1)[[7]][,4],summary(m3_2)[[7]][,4],summary(m3_3)[[7]][,4],summary(m3_4)[[7]][,4],summar
```

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y(m3_5)[[7]][,4],summary(m3_6)[[7]][,4],summary(m3_7)[[7]][,4],summary(m3_8)[[7]][,4],summary(m3_9)[[7]
][,4],summary(m3_10)[[7]][,4])

my3a <- mi.inference(myest3, mystd3, confidence=0.95)

my_3 <- round(cbind(exp(my3a$est),exp(my3a$lower),exp(my3a$upper),my3a$signif),digits=3)

my_3


m4_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m4_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m4_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m4_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m4_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m4_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m4_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m4_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m4_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m4_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest4 <-
list(coef(m4_1),coef(m4_2),coef(m4_3),coef(m4_4),coef(m4_5),coef(m4_6),coef(m4_7),coef(m4_8),coef(m4_
9),coef(m4_10))

mystd4 <-
list(summary(m4_1)[[7]][,4],summary(m4_2)[[7]][,4],summary(m4_3)[[7]][,4],summary(m4_4)[[7]][,4],summar
y(m4_5)[[7]][,4],summary(m4_6)[[7]][,4],summary(m4_7)[[7]][,4],summary(m4_8)[[7]][,4],summary(m4_9)[[7]
][,4],summary(m4_10)[[7]][,4])

my4a <- mi.inference(myest4, mystd4, confidence=0.95)

my_4 <- round(cbind(exp(my4a$est),exp(my4a$lower),exp(my4a$upper),my4a$signif),digits=3)

my_4
```

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m6_1<-coxph(Surv(X_t0,X_t, vlsupp) ~as.factor (wfacat)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m6_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m6_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m6_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m6_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m6_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m6_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m6_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m6_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m6_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor (wfacat)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest6 <-
list(coef(m6_1),coef(m6_2),coef(m6_3),coef(m6_4),coef(m6_5),coef(m6_6),coef(m6_7),coef(m6_8),coef(m6_
9),coef(m6_10))

mystd6 <-
list(summary(m6_1)[[7]][,4],summary(m6_2)[[7]][,4],summary(m6_3)[[7]][,4],summary(m6_4)[[7]][,4],summar
y(m6_5)[[7]][,4],summary(m6_6)[[7]][,4],summary(m6_7)[[7]][,4],summary(m6_8)[[7]][,4],summary(m6_9)[[7]
][,4],summary(m6_10)[[7]][,4])

my6a <- mi.inference(myest6, mystd6, confidence=0.95)

my_6 <- round(cbind(exp(my6a$est),exp(my6a$lower),exp(my6a$upper),my6a$signif),digits=3)

my_6


m7_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m7_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m7_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m7_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")
```

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m7_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m7_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m7_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m7_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m7_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m7_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest7 <-
list(coef(m7_1),coef(m7_7),coef(m7_3),coef(m7_4),coef(m7_5),coef(m7_6),coef(m7_7),coef(m7_8),coef(m7_
9),coef(m7_10))

mystd7 <-
list(summary(m7_1)[[7]][,4],summary(m7_7)[[7]][,4],summary(m7_3)[[7]][,4],summary(m7_4)[[7]][,4],summar
y(m7_5)[[7]][,4],summary(m7_6)[[7]][,4],summary(m7_7)[[7]][,4],summary(m7_8)[[7]][,4],summary(m7_9)[[7]
][,4],summary(m7_10)[[7]][,4])

my7a <- mi.inference(myest7, mystd7, confidence=0.95)

my_7 <- round(cbind(exp(my7a$est),exp(my7a$lower),exp(my7a$upper),my7a$signif),digits=3)

my_7
```

```
m9_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m9_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m9_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m9_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m9_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m9_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m9_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
```

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m9_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m9_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m9_10<-coxph(Surv(X_t0,X_t, vlsupp)~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest9 <-
list(coef(m9_1),coef(m9_2),coef(m9_3),coef(m9_4),coef(m9_5),coef(m9_6),coef(m9_7),coef(m9_8),coef(m9_
9),coef(m9_10))

mystd9 <-
list(summary(m9_1)[[7]][,4],summary(m9_2)[[7]][,4],summary(m9_3)[[7]][,4],summary(m9_4)[[7]][,4],summar
y(m9_5)[[7]][,4],summary(m9_6)[[7]][,4],summary(m9_7)[[7]][,4],summary(m9_8)[[7]][,4],summary(m9_9)[[7]
][,4],summary(m9_10)[[7]][,4])

my9a <- mi.inference(myest9, mystd9, confidence=0.95)

my_9 <- round(cbind(exp(my9a$est),exp(my9a$lower),exp(my9a$upper),my9a$signif),digits=3)

my_9


# Adjusted analysis

# Using Lopinavir as continous variable

m11_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m11_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m11_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m11_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m11_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m11_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m11_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
```

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m11_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m11_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m11_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m11_1),coef(m11_2),coef(m11_3),coef(m11_4),coef(m11_5),coef(m11_6),coef(m11_7),coef(m11_8),
coef(m11_9),coef(m11_10))

mystd <-
list(summary(m11_1)[[7]][,4],summary(m11_2)[[7]][,4],summary(m11_3)[[7]][,4],summary(m11_4)[[7]][,4],su
mmary(m11_5)[[7]][,4],summary(m11_6)[[7]][,4],summary(m11_7)[[7]][,4],summary(m11_8)[[7]][,4],summar
y(m11_9)[[7]][,4],summary(m11_10)[[7]][,4])

my11a <- mi.inference(myest, mystd, confidence=0.95)

my_11 <- round(cbind(exp(my11a$est),exp(my11a$lower),exp(my11a$upper),my11a$signif),digits=3)

my_11     # overall results with Hazard ratios, CI, and p-values


m14_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m14_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m14_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m14_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m14_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m14_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m14_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
```

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```
m14_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m14_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m14_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m14_1),coef(m14_2),coef(m14_3),coef(m14_4),coef(m14_5),coef(m14_6),coef(m14_7),coef(m14_8),
coef(m14_9),coef(m14_10))

mystd <-
list(summary(m14_1)[[7]][,4],summary(m14_2)[[7]][,4],summary(m14_3)[[7]][,4],summary(m14_4)[[7]][,4],su
mmary(m14_5)[[7]][,4],summary(m14_6)[[7]][,4],summary(m14_7)[[7]][,4],summary(m14_8)[[7]][,4],summar
y(m14_9)[[7]][,4],summary(m14_10)[[7]][,4])

my14a <- mi.inference(myest, mystd, confidence=0.95)

my_14 <- round(cbind(exp(my14a$est),exp(my14a$lower),exp(my14a$upper),my14a$signif),digits=3)

my_14     # overall results with Hazard ratios, CI, and p-values


m15_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m15_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m15_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m15_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m15_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m15_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m15_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
```

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```
m15_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m15_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m15_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m15_1),coef(m15_2),coef(m15_3),coef(m15_4),coef(m15_5),coef(m15_6),coef(m15_7),coef(m15_8),
coef(m15_9),coef(m15_10))

mystd <-
list(summary(m15_1)[[7]][,4],summary(m15_2)[[7]][,4],summary(m15_3)[[7]][,4],summary(m15_4)[[7]][,4],su
mmary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summar
y(m15_9)[[7]][,4],summary(m15_10)[[7]][,4])

my15a <- mi.inference(myest, mystd, confidence=0.95)

my_15 <- round(cbind(exp(my15a$est),exp(my15a$lower),exp(my15a$upper),my15a$signif),digits=3)

my_15     # overall results with Hazard ratios, CI, and p-values


#############################################################################################
#########

# Cut-off Selection Using Cox Regression Approach vs Mixed Addative Logistic Regression Approach  #

#############################################################################################
#########

LPV2 <- LPV[,c("X_t","vlsupp","lpvcorr","id")]


# Approach 1: Cox regression

m2_0 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,0.25,100))    + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_1 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,0.5,100))     + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_2 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,0.75,100))    + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_3 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,1,100))       + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_4 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,2,100))       + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_5 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,3,100))       + cluster(id), data=LPV,
robust=TRUE, method="breslow")
```

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m2_6 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,4,100))      + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_7 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,5,100))      + cluster(id), data=LPV,
robust=TRUE, method="breslow")

m2_8 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(lpvcorr, breaks=c(0,6,100))      + cluster(id), data=LPV,
robust=TRUE, method="breslow")


# judge with AIC

extractAIC(m2_0)

extractAIC(m2_1)

extractAIC(m2_2)

extractAIC(m2_3)

extractAIC(m2_4)

extractAIC(m2_5)

extractAIC(m2_6)

extractAIC(m2_7)

extractAIC(m2_8)


# Michael 6: add this library

library(gamm4)

# Approach 2: mixed additive logistic regression    (not working so well)

m0_1a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,0.25,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_2a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,0.5,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_3a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,0.75,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_4a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,1,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_5a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,2,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_6a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,3,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_7a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,4,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

m0_8a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,5,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)
```

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m0_9a <- gamm4(vlsupp ~ cut(lpvcorr,
breaks=c(0,6,100))+s(X_t),random=~(1|id),data=na.omit(LPV2),family=binomial)

summary(m0_1a$mer)$AIC

summary(m0_2a$mer)$AIC

summary(m0_3a$mer)$AIC

summary(m0_4a$mer)$AIC

summary(m0_5a$mer)$AIC

summary(m0_6a$mer)$AIC

summary(m0_7a$mer)$AIC

summary(m0_8a$mer)$AIC

summary(m0_9a$mer)$AIC


# Approach 3 just additive logistic

library(mgcv)

m0_1b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,0.25,100))+s(X_t),data=na.omit(LPV2),family=binomial,scale=-
1)

m0_2b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,0.5,100))+s(X_t) ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_3b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,0.75,100))+s(X_t),data=na.omit(LPV2),family=binomial,scale=-
1)

m0_4b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,1,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_5b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,2,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_6b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,3,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_7b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,4,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_8b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,5,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_9b <- gam(vlsupp ~ cut(lpvcorr, breaks=c(0,6,100))+s(X_t)   ,data=na.omit(LPV2),family=binomial,scale=-1)

m0_1b$gcv

m0_2b$gcv

m0_3b$gcv

m0_4b$gcv # here we go!! Lowest GCV!

m0_5b$gcv

m0_6b$gcv

m0_7b$gcv

m0_8b$gcv

m0_9b$gcv
```

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```
# Michael 2: Let's discuss if multivariate or not

# Note: I neglected longitudinal structure, but Helen wanted predictive criterion and results make sense, we likely have to be pragmatic here


# Also, for figure: What is better: average or current?


m_current1 <- gam(vlsupp ~ s(lpvcorr)+s(X_t),data=myimp$imputations[[1]],family=binomial)

m_average1 <- gam(vlsupp ~ s(lpave)+s(X_t),data=myimp$imputations[[1]],family=binomial)

m_current2 <- gam(vlsupp ~ s(lpvcorr)+s(X_t),data=myimp$imputations[[2]],family=binomial)

m_average2 <- gam(vlsupp ~ s(lpave)+s(X_t),data=myimp$imputations[[2]],family=binomial)

m_current3 <- gam(vlsupp ~ s(lpvcorr)+s(X_t),data=myimp$imputations[[3]],family=binomial)

m_average3 <- gam(vlsupp ~ s(lpave)+s(X_t),data=myimp$imputations[[3]],family=binomial)

m_current4 <- gam(vlsupp ~ s(lpvcorr)+s(X_t),data=myimp$imputations[[4]],family=binomial)

m_average4 <- gam(vlsupp ~ s(lpave)+s(X_t),data=myimp$imputations[[4]],family=binomial)

m_current5 <- gam(vlsupp ~ s(lpvcorr)+s(X_t),data=myimp$imputations[[5]],family=binomial)

m_average5 <- gam(vlsupp ~ s(lpave)+s(X_t),data=myimp$imputations[[5]],family=binomial)


m_current1$gcv+m_current2$gcv+m_current3$gcv+m_current4$gcv +m_current5$gcv    # Michael 3: to discuss

m_average1$gcv+m_average2$gcv+m_average3$gcv+m_average4$gcv+m_average5$gcv



############

# Figure  #

############


# Spline representation

# Imputation based approach Using Current Visit LPV Conc's

Lpvm31 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id), data=myimp$imputations[[1]], robust=TRUE, method="breslow")

Lpvm32 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id), data=myimp$imputations[[2]], robust=TRUE, method="breslow")
```

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Lpvm33 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[3]], robust=TRUE, method="breslow")

Lpvm34 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[4]], robust=TRUE, method="breslow")

Lpvm35 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[5]], robust=TRUE, method="breslow")

Lpvm36 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[6]], robust=TRUE, method="breslow")

Lpvm37 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[7]], robust=TRUE, method="breslow")

Lpvm38 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[8]], robust=TRUE, method="breslow")

Lpvm39 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[9]], robust=TRUE, method="breslow")

Lpvm40 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(lpvcorr, df=4) + as.factor(vlcat) + as.factor(cd4pccat) +
as.factor (hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[10]], robust=TRUE, method="breslow")
```

```
predicted31 <- predict(Lpvm31, type = "terms" , se.fit = TRUE , terms = 1)

predicted32 <- predict(Lpvm32, type = "terms" , se.fit = TRUE , terms = 1)

predicted33 <- predict(Lpvm33, type = "terms" , se.fit = TRUE , terms = 1)

predicted34 <- predict(Lpvm34, type = "terms" , se.fit = TRUE , terms = 1)

predicted35 <- predict(Lpvm35, type = "terms" , se.fit = TRUE , terms = 1)

predicted36 <- predict(Lpvm36, type = "terms" , se.fit = TRUE , terms = 1)

predicted37 <- predict(Lpvm37, type = "terms" , se.fit = TRUE , terms = 1)

predicted38 <- predict(Lpvm38, type = "terms" , se.fit = TRUE , terms = 1)

predicted39 <- predict(Lpvm39, type = "terms" , se.fit = TRUE , terms = 1)

predicted40 <- predict(Lpvm40, type = "terms" , se.fit = TRUE , terms = 1)
```

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```
par(mfrow=c(3,3))

termplot(Lpvm31,terms=1)

termplot(Lpvm32,terms=1)

termplot(Lpvm33,terms=1)

termplot(Lpvm34,terms=1)

termplot(Lpvm35,terms=1)

termplot(Lpvm36,terms=1)

termplot(Lpvm37,terms=1)

termplot(Lpvm38,terms=1)

termplot(Lpvm39,terms=1)


# Michael 9: have defined "seldat" now and adapted

seldat <- impdat

lp <-
(1/10)*(predicted31$fit+predicted32$fit+predicted33$fit+predicted34$fit+predicted35$fit+predicted36$fit+predicted37$fit+predicted38$fit+predicted39$fit+predicted40$fit)[is.na(seldat$lpvcorr)==F]

within <-
(1/10)*(predicted31$se^2+predicted32$se^2+predicted33$se^2+predicted34$se^2+predicted35$se^2+predicted36$se^2+predicted37$se^2+predicted38$se^2+predicted39$se^2+predicted40$se^2)[is.na(seldat$lpvcorr)==F]

mycoefflist <-
cbind(c(predicted31$fit),c(predicted32$fit),c(predicted33$fit),c(predicted34$fit),c(predicted35$fit),c(predicted36$fit),c(predicted37$fit),c(predicted38$fit),c(predicted39$fit),c(predicted40$fit))[is.na(seldat$lpvcorr)==F,]

mycoeff <- apply(mycoefflist,1,mean)

coeffdiff<-(matrix(cbind(rep(mycoeff,M)),ncol=M,nrow=length(mycoeff))-mycoefflist)^2

between <-  apply(coeffdiff,1,sum)

variance <- within + ((M+1)/(M*(M-1)))*between

se <- round(sqrt(variance),digits=5)


# Main Figure: Nice

# Michael 3: changed

dev.off()

par(mfrow=c(1,1))

plot(0 , xlab=" Lopinavir Concentration (mg/L)" , ylab = "Hazard of  failure" , axes=T  ,main = "Non-Linear Effect of Lopinavir Concentration" ,type = "n" , xlim=c(0,15) , las=1,ylim=c(0.5,1.75))

lines(sm.spline(myimp$imputations[[5]]$lpvcorr[is.na(seldat$lpvcorr)==F], exp(lp)), col = "red" , lwd = 1)
```

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```
lines(sm.spline(myimp$imputations[[5]]$lpvcorr[is.na(seldat$lpvcorr)==F], exp(lp + 1.96 * sqrt(variance))) , col = "orange" , lty = 6 , lwd = 0.8)

lines(sm.spline(myimp$imputations[[5]]$lpvcorr[is.na(seldat$lpvcorr)==F], exp(lp - 1.96 * sqrt(variance))) , col = "orange" , lty = 6 , lwd = 0.8)

axis(side = 1 , at = c(seq(0,17.5,2.5)), labels = F , tick = T , tcl = 0.4 , lwd.ticks = 0.1)


png(file="test.png",width=5,height=5,units="cm",res=300, pointsize=6)

plot(rnorm(1000),rnorm(1000),xlab="some text")


tiff("LPV.tif", res=600, compression = "lzw", height=5, width=5, units="in")


tiff("outfile.tif", compression = "lzw")


dev.print(tiff, "image.tiff2", res=600, height=4, width=4, units="p")



tiff(file = "temp.tiff", width =672, height = 672, units = "px", res = 800,type = c("windows", "cairo"),family = "", restoreConsole = TRUE,antialias="cleartype")


tiff(file = "temp.tiff", width = 3200, height = 3200, units = "px", res = 800)

plot(plot)

dev.off()




png(filename = "LPV15mg.png", width = 3200, height = 3200, units = "px", pointsize = 12,

    bg = "white", res = 400, family = "", restoreConsole = TRUE,type = c("windows", "cairo"), antialias="cleartype")



#Average LPV

# Michael 4: discuss: average Lopinavir
```

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```
#####################################

# Adjusted analysis, other versions #

#####################################



# Adjusted analysis

# Using Lopinavir 1 mg/L Cut off

m12_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m12_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m12_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m12_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m12_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m12_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m12_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m12_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m12_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m12_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m12_1),coef(m12_2),coef(m12_3),coef(m12_4),coef(m12_5),coef(m12_6),coef(m12_7),coef(m12_8),
coef(m12_9),coef(m12_10))

mystd <-
list(summary(m12_1)[[7]][,4],summary(m12_2)[[7]][,4],summary(m12_3)[[7]][,4],summary(m12_4)[[7]][,4],su
```

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```
mmary(m12_5)[[7]][,4],summary(m12_6)[[7]][,4],summary(m12_7)[[7]][,4],summary(m12_8)[[7]][,4],summar
y(m12_9)[[7]][,4],summary(m12_10)[[7]][,4])

my12a <- mi.inference(myest, mystd, confidence=0.95)

my_12 <- round(cbind(exp(my12a$est),exp(my12a$lower),exp(my12a$upper),my12a$signif),digits=3)

my_12


# Adjusted analysis

#Using Lopinavir 4 mg/L Cut off

m13_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m13_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m13_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m13_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m13_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m13_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m13_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m13_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m13_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m13_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat4  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m13_1),coef(m13_2),coef(m13_3),coef(m13_4),coef(m13_5),coef(m13_6),coef(m13_7),coef(m13_8),
coef(m13_9),coef(m13_10))
```

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```
mystd <-
list(summary(m13_1)[[7]][,4],summary(m13_2)[[7]][,4],summary(m13_3)[[7]][,4],summary(m13_4)[[7]][,4],summary(m13_5)[[7]][,4],summary(m13_6)[[7]][,4],summary(m13_7)[[7]][,4],summary(m13_8)[[7]][,4],summary(m13_9)[[7]][,4],summary(m13_10)[[7]][,4])

my13a <- mi.inference(myest, mystd, confidence=0.95)

my_13 <- round(cbind(exp(my13a$est),exp(my13a$lower),exp(my13a$upper),my13a$signif),digits=3)

my_13    # overall results with Hazard ratios, CI, and p-values    # overall results with Hazard ratios, CI, and p-valuess



#Using Lopinavir 2 mg/L Cut off

m14_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m14_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m14_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m14_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m14_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m14_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m14_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m14_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m14_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m14_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat2  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")
```

APPENDIX II

```
myest <-
list(coef(m14_1),coef(m14_2),coef(m14_3),coef(m14_4),coef(m14_5),coef(m14_6),coef(m14_7),coef(m14_8),
coef(m14_9),coef(m14_10))

mystd <-
list(summary(m14_1)[[7]][,4],summary(m14_2)[[7]][,4],summary(m14_3)[[7]][,4],summary(m14_4)[[7]][,4],su
mmary(m14_5)[[7]][,4],summary(m14_6)[[7]][,4],summary(m14_7)[[7]][,4],summary(m14_8)[[7]][,4],summar
y(m14_9)[[7]][,4],summary(m14_10)[[7]][,4])

my14a <- mi.inference(myest, mystd, confidence=0.95)

my_14 <- round(cbind(exp(my14a$est),exp(my14a$lower),exp(my14a$upper),my14a$signif),digits=3)

my_14     # overall results with Hazard ratios, CI, and p-values    # overall results with Hazard ratios, CI, and p-
valuess


#Using Lopinavir 3 mg/L Cut off

m15_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m15_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m15_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m15_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m15_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m15_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m15_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m15_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m15_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m15_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")
```

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```
myest <-
list(coef(m15_1),coef(m15_2),coef(m15_3),coef(m15_4),coef(m15_5),coef(m15_6),coef(m15_7),coef(m15_8),
coef(m15_9),coef(m15_10))

mystd <-
list(summary(m15_1)[[7]][,4],summary(m15_2)[[7]][,4],summary(m15_3)[[7]][,4],summary(m15_4)[[7]][,4],su
mmary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summar
y(m15_9)[[7]][,4],summary(m15_10)[[7]][,4])

my15a <- mi.inference(myest, mystd, confidence=0.95)

my_15 <- round(cbind(exp(my15a$est),exp(my15a$lower),exp(my15a$upper),my15a$signif),digits=3)

my_15     # overall results with Hazard ratios, CI, and p-values    # overall results with Hazard ratios, CI, and p-
valuess



#Using Lopinavir 5 mg/L Cut off

m16_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m16_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m16_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m16_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m16_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m16_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m16_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m16_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m16_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")
```

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```
m16_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m16_1),coef(m16_2),coef(m16_3),coef(m16_4),coef(m16_5),coef(m16_6),coef(m16_7),coef(m16_8),
coef(m16_9),coef(m16_10))

mystd <-
list(summary(m16_1)[[7]][,4],summary(m16_2)[[7]][,4],summary(m16_3)[[7]][,4],summary(m16_4)[[7]][,4],su
mmary(m16_5)[[7]][,4],summary(m16_6)[[7]][,4],summary(m16_7)[[7]][,4],summary(m16_8)[[7]][,4],summar
y(m16_9)[[7]][,4],summary(m16_10)[[7]][,4])

my16a <- mi.inference(myest, mystd, confidence=0.95)

my_16 <- round(cbind(exp(my16a$est),exp(my16a$lower),exp(my16a$upper),my16a$signif),digits=3)

my_16     # overall results with Hazard ratios, CI, and p-values    # overall results with Hazard ratios, CI, and p-
valuess


#Using Lopinavir 5 mg/L Cut off

m17_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m17_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m17_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m17_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m17_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m17_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m17_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m17_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m17_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")
```

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```
m17_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat3.5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m17_1),coef(m17_2),coef(m17_3),coef(m17_4),coef(m17_5),coef(m17_6),coef(m17_7),coef(m17_8),
coef(m17_9),coef(m17_10))

mystd <-
list(summary(m17_1)[[7]][,4],summary(m17_2)[[7]][,4],summary(m17_3)[[7]][,4],summary(m17_4)[[7]][,4],su
mmary(m17_5)[[7]][,4],summary(m17_6)[[7]][,4],summary(m17_7)[[7]][,4],summary(m17_8)[[7]][,4],summar
y(m17_9)[[7]][,4],summary(m17_10)[[7]][,4])

my17a <- mi.inference(myest, mystd, confidence=0.95)

my_17 <- round(cbind(exp(my17a$est),exp(my17a$lower),exp(my17a$upper),my17a$signif),digits=3)

my_17     # overall results with Hazard ratios, CI, and p-values    # overall results with Hazard ratios, CI, and p-
valuess




my_12

my_14

my_15

my_17

my_13

my_16


# Michael 5: Let's discuss if below relevant or not



##############################################



###############

#  Summary   #

###############


# Michael 17: I chose LPV cat1


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)
```

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```
MIsummary[1,1:4] <- my_1

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

#MIsummary[9:8,1:4] <- my_12



rownames(MIsummary) <- c("LPV conc.","VL (>50)","CD4% (Low)", "Age (>1/2 yr.)" ,"WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_LPVCONT.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1b

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10


rownames(MIsummary) <- c(" LPVCAT1","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_LPVCAt1.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1c
```

```
MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12


rownames(MIsummary) <- c(" LPVPRE","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA
(Advanced)","Postrantab(Yes)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_LPVPRE.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1d

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

#MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12


rownames(MIsummary) <- c(" LPVAVE","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA
(Advanced)","Postrantab(Yes)","Resistance(0)","Resistance(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_LPVAVE.csv")
```

APPENDIX II

```
MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1e

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12


rownames(MIsummary) <- c(" LPVCAT4","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)","Resistance(0)","Resistance(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_LPVCAt4.csv")




########################################

# Analysis with "interaction of time"##

########################################

# Michael 18: is not interaction but rather conditioned on a certain time period


#Used stsplit in Stat to generate the variable time1 split at (12 24 36 48 60 72) as this were scheduled visits for the study




# First 6 months = 6*4 =24weeks

# Michael 19: I replaced "<-" with "<=". Works now. You can summarize as above.

hist(impdat$lpvcorr[impdat$time1<=24]) # not much data above 20/30

m4_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) + as.factor(hfacat)  +as.factor(postrantb)+
```

```
cluster(id),data=(myimp$imputations[[1]])[myimp$imputations[[1]]$time1<=24,],
robust=TRUE,method="breslow")

m4_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[2]])[myimp$imputations[[2]]$time1<=24,],
robust=TRUE,method="breslow")

m4_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[3]])[myimp$imputations[[3]]$time1<=24,],
robust=TRUE,method="breslow")

m4_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[4]])[myimp$imputations[[4]]$time1<=24,],
robust=TRUE,method="breslow")

m4_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[5]])[myimp$imputations[[5]]$time1<=24,],
robust=TRUE,method="breslow")

m4_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[6]])[myimp$imputations[[6]]$time1<=24,],
robust=TRUE,method="breslow")

m4_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[7]])[myimp$imputations[[7]]$time1<=24,],
robust=TRUE,method="breslow")

m4_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[8]])[myimp$imputations[[8]]$time1<=24,],
robust=TRUE,method="breslow")

m4_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[9]])[myimp$imputations[[9]]$time1<=24,],
robust=TRUE,method="breslow")

m4_10a<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[10]])[myimp$imputations[[10]]$time1<=24,],
robust=TRUE,method="breslow")


# 6 months - 12 months

hist(impdat$lpvcorr[impdat$X_t<24]) # not much data above 20/30

m4_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb)  +
cluster(id),data=(myimp$imputations[[1]])[myimp$imputations[[1]]$time1<-24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")
```

APPENDIX II

```
m4_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[2]])[myimp$imputations[[2]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[3]])[myimp$imputations[[3]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[4]])[myimp$imputations[[4]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[5]])[myimp$imputations[[5]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[6]])[myimp$imputations[[6]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[7]])[myimp$imputations[[7]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[8]])[myimp$imputations[[8]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb) +
cluster(id),data=(myimp$imputations[[9]])[myimp$imputations[[9]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")

m4_10b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)  +as.factor(postrantb)  +
cluster(id),data=(myimp$imputations[[10]])[myimp$imputations[[10]]$X_t>24 &
myimp$imputations[[1]]$X_t<53,], robust=TRUE,method="breslow")


# after 1 year

hist(impdat$lpvcorr[impdat$X_t>53]) # not much data above 30

m4_1c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[1]])[myimp$imputations[[1]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_2c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
```

```
cluster(id),data=(myimp$imputations[[2]])[myimp$imputations[[2]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_3c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[3]])[myimp$imputations[[3]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_4c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[4]])[myimp$imputations[[4]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_5c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[5]])[myimp$imputations[[5]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_6c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[6]])[myimp$imputations[[6]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_7c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[7]])[myimp$imputations[[7]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_8c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[8]])[myimp$imputations[[8]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_9c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[9]])[myimp$imputations[[9]]$X_t>=53,],
robust=TRUE,method="breslow")

m4_10c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2)  + as.factor(cd4pccat)    + as.factor(wfacat) +
as.factor(hfacat)   +as.factor(postrantb)+
cluster(id),data=(myimp$imputations[[10]])[myimp$imputations[[10]]$X_t>=53,],
robust=TRUE,method="breslow")
```

```
##################################################

#Model Selection Using AIC for Each Imputed Data #

##################################################
```

APPENDIX II

```
m5_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_0b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) +
as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_0c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) +
as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_0d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(vlcat)+ as.factor(cd4pccat) +
as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

extractAIC(m5_0a)[2]

extractAIC(m5_0b)[2]

extractAIC(m5_0c)[2]

extractAIC(m5_0d)[2]


m5_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) +
as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) +
as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(vlcat)+ as.factor(cd4pccat) +
as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

extractAIC(m5_1a)[2]

extractAIC(m5_1b)[2]

extractAIC(m5_1c)[2]

extractAIC(m5_1d)[2]


m5_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")
```

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```
m5_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(vlcat)+ as.factor(cd4pccat) +
as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

extractAIC(m5_2a)[2]

extractAIC(m5_2b)[2]

extractAIC(m5_2c)[2]

extractAIC(m5_2d)[2]


m5_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(vlcat)+ as.factor(cd4pccat) +
as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

extractAIC(m5_3a)[2]

extractAIC(m5_3b)[2]

extractAIC(m5_3c)[2]

extractAIC(m5_3d)[2]


m5_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_4c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")
```

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```
m5_4d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

extractAIC(m5_4a)[2]

extractAIC(m5_4b)[2]

extractAIC(m5_4c)[2]

extractAIC(m5_4d)[2]


m5_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre            + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_5c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave            + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_5d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

extractAIC(m5_5a)[2]

extractAIC(m5_5b)[2]

extractAIC(m5_5c)[2]

extractAIC(m5_5d)[2]


m5_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre            + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave            + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

extractAIC(m5_6a)[2]

extractAIC(m5_6b)[2]

extractAIC(m5_6c)[2]
```

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```
extractAIC(m5_6d)[2]


m5_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre           + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_7c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave           + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_7d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

extractAIC(m5_7a)[2]

extractAIC(m5_7b)[2]

extractAIC(m5_7c)[2]

extractAIC(m5_7d)[2]


m5_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre           + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave           + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr)  + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

extractAIC(m5_8a)[2]

extractAIC(m5_8b)[2]

extractAIC(m5_8c)[2]

extractAIC(m5_8d)[2]


m5_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")
```

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```
m5_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m5_9c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpave + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m5_9d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(vlcat)+ as.factor(cd4pccat) +
as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

extractAIC(m5_9a)[2]

extractAIC(m5_9b)[2]

extractAIC(m5_9c)[2]

extractAIC(m5_9d)[2]


0.1*extractAIC(m5_0a)[2]+extractAIC(m5_1a)[2]+extractAIC(m5_2a)[2]+extractAIC(m5_3a)[2]+extractAIC(m5_
4a)[2]+extractAIC(m5_5a)[2]+extractAIC(m5_6a)[2]+extractAIC(m5_7a)[2]extractAIC(m5_8a)[2]+extractAIC(m
5_9a)[2]

0.1*extractAIC(m5_0a)[2]+extractAIC(m5_1b)[2]+extractAIC(m5_2b)[2]+extractAIC(m5_3b)[2]+extractAIC(m5
_4b)[2]+extractAIC(m5_5b)[2]+extractAIC(m5_6b)[2]+extractAIC(m5_7b)[2]extractAIC(m5_8b)[2]+extractAIC(
m5_9b)[2]

0.1*extractAIC(m5_0a)[2]+extractAIC(m5_1c)[2]+extractAIC(m5_2c)[2]+extractAIC(m5_3c)[2]+extractAIC(m5_
4c)[2]+extractAIC(m5_5c)[2]+extractAIC(m5_6c)[2]+extractAIC(m5_7c)[2]extractAIC(m5_8c)[2]+extractAIC(m5
_9c)[2]


m4_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_0b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

extractAIC(m4_0a)[2]

extractAIC(m4_0b)[2]


m4_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")
```

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```
extractAIC(m4_1a)[2]

extractAIC(m4_1b)[2]


m4_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

extractAIC(m4_2a)[2]

extractAIC(m4_2b)[2]


m4_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

extractAIC(m4_3a)[2]

extractAIC(m4_3b)[2]


m4_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

extractAIC(m4_4a)[2]

extractAIC(m4_4b)[2]


m4_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

extractAIC(m4_5a)[2]

extractAIC(m4_5b)[2]
```

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```
m4_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

extractAIC(m4_6a)[2]

extractAIC(m4_6b)[2]


m4_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

extractAIC(m4_7a)[2]

extractAIC(m4_7b)[2]


m4_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m4_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

extractAIC(m4_8a)[2]

extractAIC(m4_8b)[2]


m4_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m4_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat4          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

extractAIC(m4_9a)[2]

extractAIC(m4_9b)[2]



################################

# Adherent vs. Concentration  #
```

APPENDIX II

```
################################

m5_31<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_32<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_33<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_34<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_35<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_36<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_37<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_38<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_39<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_310<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")



m5_41<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_42<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")
```

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```
m5_43<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_44<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_45<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_46<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_47<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_48<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_49<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_410<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus+ as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest <-
list(coef(m5_41),coef(m5_42),coef(m5_43),coef(m5_44),coef(m5_45),coef(m5_46),coef(m5_47),coef(m5_48),
coef(m5_49),coef(m5_410))

mystd <-
list(summary(m5_41)[[7]][,4],summary(m5_42)[[7]][,4],summary(m5_43)[[7]][,4],summary(m5_44)[[7]][,4],su
mmary(m5_45)[[7]][,4],summary(m5_46)[[7]][,4],summary(m5_47)[[7]][,4],summary(m5_48)[[7]][,4],summar
y(m5_49)[[7]][,4],summary(m5_410)[[7]][,4])

my541a <- mi.inference(myest, mystd, confidence=0.95)

my_542 <- round(cbind(exp(my541a$est),exp(my541a$lower),exp(my541a$upper),my541a$signif),digits=3)

my_542
```

Michael 22: ...maybe here summary of adherence model

APPENDIX II

0.1*(extractAIC(m5_31)[2]+extractAIC(m5_32)[2]+extractAIC(m5_33)[2]+extractAIC(m5_34)[2]+extractAIC(m5_35)[2]+extractAIC(m5_36)[2]+extractAIC(m5_37)[2]+extractAIC(m5_38)[2]+extractAIC(m5_39)[2]+extractAIC(m5_310)[2])

0.1*(extractAIC(m5_41)[2]+extractAIC(m5_42)[2]+extractAIC(m5_43)[2]+extractAIC(m5_44)[2]+extractAIC(m5_45)[2]+extractAIC(m5_46)[2]+extractAIC(m5_47)[2]+extractAIC(m5_48)[2]+extractAIC(m5_49)[2]+extractAIC(m5_410)[2])

everything is indicating that it is better to use Lopinavir compared to adherence

APPENDIX III

Cox Proportional Hazards Mutliple failure Event Model of Nevirapine During The Post-randomization Phase

```
library(splines)

library(survival)

library(pspline)

NVP<-read.csv("NVP_02_09_2014.csv",header=T,sep=",",stringsAsFactors=FALSE)

attach(NVP)


######################

### Descriptives #######

######################

# percentage Missingness

round(apply(apply(cbind(nvp,adstatus,t0rwfa,ageatran,t0rhfa,vlsupp,vlsupp1,t0rvl,t0rcd4pc,postrantb),c(1,2),is.na),2,mean),digits=3)


surv.vl1 <- Surv(X_t0,X_t,vlsupp)       # setting survival time from Stata

summary(surv.vl1)

hist(nvp)

##################

## Imputation   #

##################


library(foreign)

library(Amelia)

library(norm)

set.seed(666)

M=10

impdat <-
NVP[,c("id","nvp","adstatus","t0rvl","t0rcd4pc","ageatran","t0rwfa","t0rhfa","postrantb","X_t0","X_t","vlsupp","vlsupp1")]

round(apply(apply(impdat,c(1,2),is.na),2,mean),digits=3)   # percentage of missing values

myimp <- amelia(impdat, m=M, p2s=1,
noms=c("postrantb","vlsupp","vlsupp1"),cs=c("id"),ts=c("X_t0"),bounds=matrix(c(2,0,70, 3,0,100,  4,0,400,
5,0,100,
6,0,50),ncol=3,nrow=5,byrow=T),logs=c("nvp","adstatus","X_t"),polytime=3,splinetime=3,empri=5,incheck=TRUE,tolerance=0.001)

plot(myimp)
```

APPENDIX III

```
par(mfrow=c(3,3))
compare.density(myimp,var="nvp")
compare.density(myimp,var="adstatus")
compare.density(myimp,var="t0rvl")
compare.density(myimp,var="t0rcd4pc")
compare.density(myimp,var="ageatran")
compare.density(myimp,var="t0rwfa")
compare.density(myimp,var="t0rhfa")
#compare.density(myimp,var="whostage")
#compare.density(myimp,var="postrantb")
#compare.density(myimp,var="")
#dev.off()

par(mfrow=c(1,1))
suppressWarnings(disperse(myimp, dims=1, m=10))
suppressWarnings(disperse(myimp, dims=2, m=10))        # excellent imp diagnostics here
par(mfrow=c(2,1))
suppressWarnings(tscsPlot(myimp,var="t0rcd4pc",cs=3011,draws=10))
suppressWarnings(tscsPlot(myimp,var="t0rwfa",cs=3011,draws=10))
par(mfrow=c(1,1))
overimpute(myimp,var="nvp")        # Not perfect, but only small amount imputed
overimpute(myimp,var="adstatus")       # I think we should be careful regarding  adhstatus
overimpute(myimp,var="t0rvl")
overimpute(myimp,var="t0rcd4pc")
overimpute(myimp,var="t0rwfa")
overimpute(myimp,var="ageatran")
overimpute(myimp,var="t0rhfa")
#overimpute(myimp,var="whostage")
#overimpute(myimp,var="postrantb")
#overimpute(myimp,var="")
#summary(myimp)

for(m in 1:M){
```

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```
myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],vlcat=cut(myimp$imputations[[m]]$t0rvl,breaks=c(-1,51,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],cd4pccat=cut(myimp$imputations[[m]]$t0rcd4pc,breaks=c(-
1,25,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],agecat=cut(myimp$imputations[[m]]$ageatran,breaks=c(-
1,18,1000000000)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],wfacat=cut(myimp$imputations[[m]]$t0rwfa,breaks=c(-1000,-2,10)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],hfacat=cut(myimp$imputations[[m]]$t0rhfa,breaks=c(-1000,-2,10)))

myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],nvpcat5=cut(myimp$imputations[[m]]$nvp,breaks=c(-1,5,1000000000)))

}

head(myimp$imputations[[1]])

# function to create lags

shift.1<-function(x,shift_by=-1){

 stopifnot(is.numeric(shift_by))

 stopifnot(is.numeric(x))

 if (length(shift_by)>1)

   return(sapply(shift_by,shift, x=x))

 out<-NULL

 abs_shift_by=abs(shift_by)

 if (shift_by > 0 )

   out<-c(tail(x,-abs_shift_by),rep(NA,abs_shift_by))

 else if (shift_by < 0 )

   out<-c(rep(NA,abs_shift_by), head(x,-abs_shift_by))

 else

   out<-x

 out

}

# use this function for longitudinal data, means apply them by patient

shift.l <- function(x,splitby){

 unsplit(lapply(split(x,splitby),shift.1),splitby)

}
```

APPENDIX III

```
for(m in 1:M){

  myimp$imputations[[m]]<-
transform(myimp$imputations[[m]],nvpre=shift.l(myimp$imputations[[m]]$nvp,myimp$imputations[[m]]$id))

  myimp$imputations[[m]]$nvpre <-
replace((myimp$imputations[[m]])$nvpre,is.na((myimp$imputations[[m]])$nvpre),0)

  myimp$imputations[[m]]<-transform(myimp$imputations[[m]],nvpave=((nvp+nvpre)/2))

}

head(myimp$imputations[[1]])

##############################

## Table 3: Cox Regression   #

## (crude, adj, selected)    #

##############################

# Crude analysis

m1_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvp  +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1 <-
list(coef(m1_1),coef(m1_2),coef(m1_3),coef(m1_4),coef(m1_5),coef(m1_6),coef(m1_7),coef(m1_8),coef(m1_
9),coef(m1_10))

mystd1 <-
list(summary(m1_1)[[7]][,4],summary(m1_2)[[7]][,4],summary(m1_3)[[7]][,4],summary(m1_4)[[7]][,4],summar
```

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```
y(m1_5)[[7]][,4],summary(m1_6)[[7]][,4],summary(m1_7)[[7]][,4],summary(m1_8)[[7]][,4],summary(m1_9)[[7]
][,4],summary(m1_10)[[7]][,4])

my1a <- mi.inference(myest1, mystd1, confidence=0.95)

my_1 <- round(cbind(exp(my1a$est),exp(my1a$lower),exp(my1a$upper),my1a$signif),digits=3)

my_1    # significant


m1b_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1b_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1b_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1b_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1b_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1b_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1b_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1b_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1b_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1b_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat5 +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1b <-
list(coef(m1b_1),coef(m1b_2),coef(m1b_3),coef(m1b_4),coef(m1b_5),coef(m1b_6),coef(m1b_7),coef(m1b_8),
coef(m1b_9),coef(m1b_10))

mystd1b <-
list(summary(m1b_1)[[7]][,4],summary(m1b_2)[[7]][,4],summary(m1b_3)[[7]][,4],summary(m1b_4)[[7]][,4],su
mmary(m1b_5)[[7]][,4],summary(m1b_6)[[7]][,4],summary(m1b_7)[[7]][,4],summary(m1b_8)[[7]][,4],summar
y(m1b_9)[[7]][,4],summary(m1b_10)[[7]][,4])

my1b <- mi.inference(myest1b, mystd1b, confidence=0.95)

my_1b <- round(cbind(exp(my1b$est),exp(my1b$lower),exp(my1b$upper),my1b$signif),digits=3)

my_1b    # significant


m1c_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")
```

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```
m1c_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1c_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1c_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1c_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1c_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1c_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1c_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1c_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1c_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpre +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1c <-
list(coef(m1c_1),coef(m1c_2),coef(m1c_3),coef(m1c_4),coef(m1c_5),coef(m1c_6),coef(m1c_7),coef(m1c_8),c
oef(m1c_9),coef(m1c_10))

mystd1c <-
list(summary(m1c_1)[[7]][,4],summary(m1c_2)[[7]][,4],summary(m1c_3)[[7]][,4],summary(m1c_4)[[7]][,4],su
mmary(m1c_5)[[7]][,4],summary(m1c_6)[[7]][,4],summary(m1c_7)[[7]][,4],summary(m1c_8)[[7]][,4],summary(
m1c_9)[[7]][,4],summary(m1c_10)[[7]][,4])

my1c <- mi.inference(myest1c, mystd1c, confidence=0.95)

my_1c <- round(cbind(exp(my1c$est),exp(my1c$lower),exp(my1c$upper),my1c$signif),digits=3)

my_1c     # significant


m1d_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1d_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1d_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1d_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1d_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")
```

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```
m1d_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1d_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1d_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1d_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1d_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpave +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1d <-
list(coef(m1d_1),coef(m1d_2),coef(m1d_3),coef(m1d_4),coef(m1d_5),coef(m1d_6),coef(m1d_7),coef(m1d_8),
coef(m1d_9),coef(m1d_10))

mystd1d <-
list(summary(m1d_1)[[7]][,4],summary(m1d_2)[[7]][,4],summary(m1d_3)[[7]][,4],summary(m1d_4)[[7]][,4],su
mmary(m1d_5)[[7]][,4],summary(m1d_6)[[7]][,4],summary(m1d_7)[[7]][,4],summary(m1d_8)[[7]][,4],summar
y(m1d_9)[[7]][,4],summary(m1d_10)[[7]][,4])

my1d <- mi.inference(myest1d, mystd1d, confidence=0.95)

my_1d <- round(cbind(exp(my1d$est),exp(my1d$lower),exp(my1d$upper),my1d$signif),digits=3)

my_1d     # significant


m1e_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1e_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1e_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1e_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1e_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1e_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1e_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1e_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1e_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")
```

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```
m1e_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat10 +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1e <-
list(coef(m1e_1),coef(m1e_2),coef(m1e_3),coef(m1e_4),coef(m1e_5),coef(m1e_6),coef(m1e_7),coef(m1e_8),
coef(m1e_9),coef(m1e_10))

mystd1e <-
list(summary(m1e_1)[[7]][,4],summary(m1e_2)[[7]][,4],summary(m1e_3)[[7]][,4],summary(m1e_4)[[7]][,4],su
mmary(m1e_5)[[7]][,4],summary(m1e_6)[[7]][,4],summary(m1e_7)[[7]][,4],summary(m1e_8)[[7]][,4],summary
(m1e_9)[[7]][,4],summary(m1e_10)[[7]][,4])

my1e <- mi.inference(myest1e, mystd1e, confidence=0.95)

my_1e <- round(cbind(exp(my1e$est),exp(my1e$lower),exp(my1e$upper),my1e$signif),digits=3)

my_1e


m1f_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1f_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1f_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1f_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1f_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1f_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1f_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1f_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1f_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1f_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ ageatran +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest1e <-
list(coef(m1f_1),coef(m1f_2),coef(m1f_3),coef(m1f_4),coef(m1f_5),coef(m1f_6),coef(m1f_7),coef(m1f_8),coef
(m1f_9),coef(m1f_10))

mystd1e <-
list(summary(m1f_1)[[7]][,4],summary(m1f_2)[[7]][,4],summary(m1f_3)[[7]][,4],summary(m1f_4)[[7]][,4],sum
mary(m1f_5)[[7]][,4],summary(m1f_6)[[7]][,4],summary(m1f_7)[[7]][,4],summary(m1f_8)[[7]][,4],summary(m
1f_9)[[7]][,4],summary(m1f_10)[[7]][,4])

my1e <- mi.inference(myest1e, mystd1e, confidence=0.95)
```

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```
my_1e <- round(cbind(exp(my1e$est),exp(my1e$lower),exp(my1e$upper),my1e$signif),digits=3)

my_1e



m1g_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m1g_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m1g_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m1g_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m1g_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m1g_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m1g_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m1g_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m1g_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m1g_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myestf <-
list(coef(m1g_1),coef(m1g_2),coef(m1g_3),coef(m1g_4),coef(m1g_5),coef(m1g_6),coef(m1g_7),coef(m1g_8),c
oef(m1g_9),coef(m1g_10))

mystdf <-
list(summary(m1g_1)[[7]][,4],summary(m1g_2)[[7]][,4],summary(m1g_3)[[7]][,4],summary(m1g_4)[[7]][,4],su
mmary(m1g_5)[[7]][,4],summary(m1g_6)[[7]][,4],summary(m1g_7)[[7]][,4],summary(m1g_8)[[7]][,4],summary
(m1g_9)[[7]][,4],summary(m1g_10)[[7]][,4])

my1f <- mi.inference(myestf, mystdf, confidence=0.95)

my_1f <- round(cbind(exp(my1f$est),exp(my1f$lower),exp(my1f$upper),my1f$signif),digits=3)

my_1f     # significant



m2_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")
```

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```
m2_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m2_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m2_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m2_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m2_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m2_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m2_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m2_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m2_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(vlcat) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest2 <-
list(coef(m2_1),coef(m2_2),coef(m2_3),coef(m2_4),coef(m2_5),coef(m2_6),coef(m2_7),coef(m2_8),coef(m2_
9),coef(m2_10))

mystd2 <-
list(summary(m2_1)[[7]][,4],summary(m2_2)[[7]][,4],summary(m2_3)[[7]][,4],summary(m2_4)[[7]][,4],summar
y(m2_5)[[7]][,4],summary(m2_6)[[7]][,4],summary(m2_7)[[7]][,4],summary(m2_8)[[7]][,4],summary(m2_9)[[7]
][,4],summary(m2_10)[[7]][,4])

my2a <- mi.inference(myest2, mystd2, confidence=0.95)

my_2 <- round(cbind(exp(my2a$est),exp(my2a$lower),exp(my2a$upper),my2a$signif),digits=3)

my_2


m3_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m3_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m3_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m3_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m3_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")
```

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```
m3_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m3_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m3_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m3_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m3_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(cd4pccat)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest3 <-
list(coef(m3_1),coef(m3_3),coef(m3_3),coef(m3_4),coef(m3_5),coef(m3_6),coef(m3_7),coef(m3_8),coef(m3_
9),coef(m3_10))

mystd3 <-
list(summary(m3_1)[[7]][,4],summary(m3_2)[[7]][,4],summary(m3_3)[[7]][,4],summary(m3_4)[[7]][,4],summar
y(m3_5)[[7]][,4],summary(m3_6)[[7]][,4],summary(m3_7)[[7]][,4],summary(m3_8)[[7]][,4],summary(m3_9)[[7]
][,4],summary(m3_10)[[7]][,4])

my3a <- mi.inference(myest3, mystd3, confidence=0.95)

my_3 <- round(cbind(exp(my3a$est),exp(my3a$lower),exp(my3a$upper),my3a$signif),digits=3)

my_3


m4_1<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m4_2<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m4_3<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m4_4<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m4_5<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m4_6<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m4_7<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m4_8<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m4_9<-coxph(Surv(X_t0,X_t, vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")
```

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```
m4_10<-coxph(Surv(X_t0,X_t,vlsupp) ~  as.factor(agecat) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

myest4 <-
list(coef(m4_1),coef(m4_2),coef(m4_3),coef(m4_4),coef(m4_5),coef(m4_6),coef(m4_7),coef(m4_8),coef(m4_
9),coef(m4_10))

mystd4 <-
list(summary(m4_1)[[7]][,4],summary(m4_2)[[7]][,4],summary(m4_3)[[7]][,4],summary(m4_4)[[7]][,4],summar
y(m4_5)[[7]][,4],summary(m4_6)[[7]][,4],summary(m4_7)[[7]][,4],summary(m4_8)[[7]][,4],summary(m4_9)[[7]
][,4],summary(m4_10)[[7]][,4])

my4a <- mi.inference(myest4, mystd4, confidence=0.95)

my_4 <- round(cbind(exp(my4a$est),exp(my4a$lower),exp(my4a$upper),my4a$signif),digits=3)

my_4
```

```
m6_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m6_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m6_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m6_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m6_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m6_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m6_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m6_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m6_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m6_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor (wfacat) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest6 <-
list(coef(m6_1),coef(m6_7),coef(m6_3),coef(m6_4),coef(m6_5),coef(m6_6),coef(m6_7),coef(m6_8),coef(m6_
9),coef(m6_10))

mystd6 <-
list(summary(m6_1)[[7]][,4],summary(m6_7)[[7]][,4],summary(m6_3)[[7]][,4],summary(m6_4)[[7]][,4],summar
```

```
y(m6_5)[[7]][,4],summary(m6_6)[[7]][,4],summary(m6_7)[[7]][,4],summary(m6_8)[[7]][,4],summary(m6_9)[[7]
][,4],summary(m6_10)[[7]][,4])

my6a <- mi.inference(myest6, mystd6, confidence=0.95)

my_6 <- round(cbind(exp(my6a$est),exp(my6a$lower),exp(my6a$upper),my6a$signif),digits=3)

my_6


m7_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m7_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m7_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m7_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m7_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m7_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m7_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m7_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m7_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m7_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ as.factor (hfacat) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest7 <-
list(coef(m7_1),coef(m7_7),coef(m7_3),coef(m7_4),coef(m7_5),coef(m7_6),coef(m7_7),coef(m7_8),coef(m7_
9),coef(m7_10))

mystd7 <-
list(summary(m7_1)[[7]][,4],summary(m7_7)[[7]][,4],summary(m7_3)[[7]][,4],summary(m7_4)[[7]][,4],summar
y(m7_5)[[7]][,4],summary(m7_6)[[7]][,4],summary(m7_7)[[7]][,4],summary(m7_8)[[7]][,4],summary(m7_9)[[7]
][,4],summary(m7_10)[[7]][,4])

my7a <- mi.inference(myest7, mystd7, confidence=0.95)

my_7 <- round(cbind(exp(my7a$est),exp(my7a$lower),exp(my7a$upper),my7a$signif),digits=3)

my_7
```

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m9_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m9_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")

m9_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m9_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m9_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m9_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m9_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m9_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m9_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m9_10<-coxph(Surv(X_t0,X_t, vlsupp)~ as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest9 <-
list(coef(m9_1),coef(m9_2),coef(m9_3),coef(m9_4),coef(m9_5),coef(m9_6),coef(m9_7),coef(m9_8),coef(m9_
9),coef(m9_10))

mystd9 <-
list(summary(m9_1)[[7]][,4],summary(m9_2)[[7]][,4],summary(m9_3)[[7]][,4],summary(m9_4)[[7]][,4],summar
y(m9_5)[[7]][,4],summary(m9_6)[[7]][,4],summary(m9_7)[[7]][,4],summary(m9_8)[[7]][,4],summary(m9_9)[[7]
][,4],summary(m9_10)[[7]][,4])

my9a <- mi.inference(myest9, mystd9, confidence=0.95)

my_9 <- round(cbind(exp(my9a$est),exp(my9a$lower),exp(my9a$upper),my9a$signif),digits=3)

my_9


######################

# Adjusted analysis##

######################

m11_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m11_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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m11_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m11_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m11_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m11_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m11_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m11_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m11_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m11_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m11_1),coef(m11_2),coef(m11_3),coef(m11_4),coef(m11_5),coef(m11_6),coef(m11_7),coef(m11_8),
coef(m11_9),coef(m11_10))

mystd <-
list(summary(m11_1)[[7]][,4],summary(m11_2)[[7]][,4],summary(m11_3)[[7]][,4],summary(m11_4)[[7]][,4],su
mmary(m11_5)[[7]][,4],summary(m11_6)[[7]][,4],summary(m11_7)[[7]][,4],summary(m11_8)[[7]][,4],summar
y(m11_9)[[7]][,4],summary(m11_10)[[7]][,4])

my11a <- mi.inference(myest, mystd, confidence=0.95)

my_11 <- round(cbind(exp(my11a$est),exp(my11a$lower),exp(my11a$upper),my11a$signif),digits=3)

my_11     # overall results with Hazard ratios, CI, and p-values


m14_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m14_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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m14_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m14_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m14_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m14_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m14_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m14_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m14_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m14_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m14_1),coef(m14_2),coef(m14_3),coef(m14_4),coef(m14_5),coef(m14_6),coef(m14_7),coef(m14_8),
coef(m14_9),coef(m14_10))

mystd <-
list(summary(m14_1)[[7]][,4],summary(m14_2)[[7]][,4],summary(m14_3)[[7]][,4],summary(m14_4)[[7]][,4],su
mmary(m14_5)[[7]][,4],summary(m14_6)[[7]][,4],summary(m14_7)[[7]][,4],summary(m14_8)[[7]][,4],summar
y(m14_9)[[7]][,4],summary(m14_10)[[7]][,4])

my14a <- mi.inference(myest, mystd, confidence=0.95)

my_14 <- round(cbind(exp(my14a$est),exp(my14a$lower),exp(my14a$upper),my14a$signif),digits=3)

my_14     # overall results with Hazard ratios, CI, and p-values


m15_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m15_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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m15_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m15_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m15_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m15_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m15_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m15_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m15_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m15_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpave  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m15_1),coef(m15_2),coef(m15_3),coef(m15_4),coef(m15_5),coef(m15_6),coef(m15_7),coef(m15_8),
coef(m15_9),coef(m15_10))

mystd <-
list(summary(m15_1)[[7]][,4],summary(m15_2)[[7]][,4],summary(m15_3)[[7]][,4],summary(m15_4)[[7]][,4],su
mmary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summar
y(m15_9)[[7]][,4],summary(m15_10)[[7]][,4])

my15a <- mi.inference(myest, mystd, confidence=0.95)

my_15 <- round(cbind(exp(my15a$est),exp(my15a$lower),exp(my15a$upper),my15a$signif),digits=3)

my_15     # overall results with Hazard ratios, CI, and p-values


m16_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m16_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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m16_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m16_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m16_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m16_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m16_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m16_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m16_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m16_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat5  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m16_1),coef(m16_2),coef(m16_3),coef(m16_4),coef(m16_5),coef(m16_6),coef(m16_7),coef(m16_8),
coef(m16_9),coef(m16_10))

mystd <-
list(summary(m16_1)[[7]][,4],summary(m16_2)[[7]][,4],summary(m16_3)[[7]][,4],summary(m16_4)[[7]][,4],su
mmary(m16_5)[[7]][,4],summary(m16_6)[[7]][,4],summary(m16_7)[[7]][,4],summary(m16_8)[[7]][,4],summar
y(m16_9)[[7]][,4],summary(m16_10)[[7]][,4])

my16a <- mi.inference(myest, mystd, confidence=0.95)

my_16 <- round(cbind(exp(my16a$est),exp(my16a$lower),exp(my16a$upper),my16a$signif),digits=3)

my_16     # overall results with Hazard ratios, CI, and p-values


m17_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m17_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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```
m17_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m17_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m17_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m17_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m17_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m17_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m17_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m17_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m17_1),coef(m17_2),coef(m17_3),coef(m17_4),coef(m17_5),coef(m17_6),coef(m17_7),coef(m17_8),
coef(m17_9),coef(m17_10))

mystd <-
list(summary(m17_1)[[7]][,4],summary(m17_2)[[7]][,4],summary(m17_3)[[7]][,4],summary(m17_4)[[7]][,4],su
mmary(m17_5)[[7]][,4],summary(m17_6)[[7]][,4],summary(m17_7)[[7]][,4],summary(m17_8)[[7]][,4],summar
y(m17_9)[[7]][,4],summary(m17_10)[[7]][,4])

my17a <- mi.inference(myest, mystd, confidence=0.95)

my_17 <- round(cbind(exp(my17a$est),exp(my17a$lower),exp(my17a$upper),my17a$signif),digits=3)

my_17     # overall results with Hazard ratios, CI, and p-values


m18_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m18_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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```
m18_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m18_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m18_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m18_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m18_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m18_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m18_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m18_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat15  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m18_1),coef(m18_2),coef(m18_3),coef(m18_4),coef(m18_5),coef(m18_6),coef(m18_7),coef(m18_8),
coef(m18_9),coef(m18_10))

mystd <-
list(summary(m18_1)[[7]][,4],summary(m18_2)[[7]][,4],summary(m18_3)[[7]][,4],summary(m18_4)[[7]][,4],su
mmary(m18_5)[[7]][,4],summary(m18_6)[[7]][,4],summary(m18_7)[[7]][,4],summary(m18_8)[[7]][,4],summar
y(m18_9)[[7]][,4],summary(m18_10)[[7]][,4])

my18a <- mi.inference(myest, mystd, confidence=0.95)

my_18 <- round(cbind(exp(my18a$est),exp(my18a$lower),exp(my18a$upper),my18a$signif),digits=3)

my_18


m19_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m19_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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```
m19_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m19_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m19_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m19_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m19_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m19_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m19_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m19_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat20  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m19_1),coef(m19_2),coef(m19_3),coef(m19_4),coef(m19_5),coef(m19_6),coef(m19_7),coef(m19_8),
coef(m19_9),coef(m19_10))

mystd <-
list(summary(m19_1)[[7]][,4],summary(m19_2)[[7]][,4],summary(m19_3)[[7]][,4],summary(m19_4)[[7]][,4],su
mmary(m19_5)[[7]][,4],summary(m19_6)[[7]][,4],summary(m19_7)[[7]][,4],summary(m19_8)[[7]][,4],summar
y(m19_9)[[7]][,4],summary(m19_10)[[7]][,4])

my19a <- mi.inference(myest, mystd, confidence=0.95)

my_19 <- round(cbind(exp(my19a$est),exp(my19a$lower),exp(my19a$upper),my19a$signif),digits=3)

my_19


m20_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m20_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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m20_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m20_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m20_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m20_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m20_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m20_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m20_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m20_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ nvpcat25  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m20_1),coef(m20_2),coef(m20_3),coef(m20_4),coef(m20_5),coef(m20_6),coef(m20_7),coef(m20_8),
coef(m20_9),coef(m20_10))

mystd <-
list(summary(m20_1)[[7]][,4],summary(m20_2)[[7]][,4],summary(m20_3)[[7]][,4],summary(m20_4)[[7]][,4],su
mmary(m20_5)[[7]][,4],summary(m20_6)[[7]][,4],summary(m20_7)[[7]][,4],summary(m20_8)[[7]][,4],summar
y(m20_9)[[7]][,4],summary(m20_10)[[7]][,4])

my20a <- mi.inference(myest, mystd, confidence=0.95)

my_20 <- round(cbind(exp(my20a$est),exp(my20a$lower),exp(my20a$upper),my20a$signif),digits=3)

my_20


m21_1<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")

m21_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
```

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```
m21_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")

m21_4<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")

m21_5<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")

m21_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")

m21_7<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")

m21_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")

m21_9<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")

m21_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb)+
cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")

myest <-
list(coef(m21_1),coef(m21_2),coef(m21_3),coef(m21_4),coef(m21_5),coef(m21_6),coef(m21_7),coef(m21_8),
coef(m21_9),coef(m21_10))

mystd <-
list(summary(m21_1)[[7]][,4],summary(m21_2)[[7]][,4],summary(m21_3)[[7]][,4],summary(m21_4)[[7]][,4],su
mmary(m21_5)[[7]][,4],summary(m21_6)[[7]][,4],summary(m21_7)[[7]][,4],summary(m21_8)[[7]][,4],summar
y(m21_9)[[7]][,4],summary(m21_10)[[7]][,4])

my21a <- mi.inference(myest, mystd, confidence=0.95)

my_21 <- round(cbind(exp(my21a$est),exp(my21a$lower),exp(my21a$upper),my21a$signif),digits=3)

my_21


##############################################################################################

# Cut-off Selection Using Cox Regression Approach vs Mixed Addative Logistic Regression Approach  ########

##############################################################################################

NVP<-read.csv("NVP_22_07_2014.csv",header=T,sep=",",stringsAsFactors=FALSE)

NVP2 <- NVP[,c("X_t","vlsupp","nvp","id")]
```

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```
# As checked above: we have no negative NVP values anymore, as it should be, so we can comment this out!

#NVP$nvp[NVP$nvp<0]<-NA

#NVP2$nvp[NVP2$nvp<0]<-NA


# Table 1 (Cutoffs)

# Approach 1: Cox regression

m0 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,0.25,100))    + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m1 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,0.5,100))     + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m2 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,0.75,100))    + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m3 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,1,100))       + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m4 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,1.5,100))     + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m5 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,3,100))       + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m6 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,6,100))       + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m7 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,9,100))       + cluster(id), data=NVP, robust=TRUE,
method="breslow")

m8 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ cut(nvp, breaks=c(0,12,100))      + cluster(id), data=NVP, robust=TRUE,
method="breslow")


# judge with AIC

extractAIC(m0)

extractAIC(m1)

extractAIC(m2)

extractAIC(m3)

extractAIC(m4)

extractAIC(m5)

extractAIC(m6)

extractAIC(m7)

extractAIC(m8)
```

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```
# add this library

library(gamm4)

# Approach 2: mixed additive logistic regression

m0_1 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,0.25,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_2 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,0.5,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_3 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,0.75,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_4 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,1,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_5 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,2,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_6 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,3,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_7 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,6,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_8 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,9,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

m0_9 <- gamm4(vlsupp ~ cut(nvp,
breaks=c(0,12,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial)

summary(m0_1$mer)$AIC

summary(m0_2$mer)$AIC

summary(m0_3$mer)$AIC

summary(m0_4$mer)$AIC

summary(m0_5$mer)$AIC

summary(m0_6$mer)$AIC

summary(m0_7$mer)$AIC

summary(m0_8$mer)$AIC

summary(m0_9$mer)$AIC


#add library

library(mgcv)

# Approach 3: additive logistic regression

m0_11 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,2,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)
```

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```
m0_12 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,3,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_13 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,4,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_14 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,5,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_15 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,6,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_16 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,7,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_17 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,9,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_18 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,10,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_19 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,11,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)

m0_20 <- gam(vlsupp ~ cut(nvp,
breaks=c(0,12,100))+s(X_t),random=~(1|id),data=na.omit(NVP2),family=binomial,scale=-1)


m0_11$gcv

m0_12$gcv

m0_13$gcv

m0_14$gcv

m0_15$gcv

m0_16$gcv

m0_17$gcv

m0_18$gcv

m0_19$gcv

m0_20$gcv


# Approach 4: like approach 3, but with imputed data

mi1_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi1_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)
```

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```
mi1_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi1_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi1_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi1_1$gcv+mi1_2$gcv+mi1_3$gcv+mi1_4$gcv+mi1_5$gcv


mi2_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,10,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi2_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,10,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi2_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,10,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi2_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,10,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi2_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,10,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi2_1$gcv+mi2_2$gcv+mi2_3$gcv+mi2_4$gcv+mi2_5$gcv


mi3_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,12.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi3_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,12.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi3_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,12.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi3_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,12.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi3_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,12.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)
```

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```
mi3_1$gcv+mi3_2$gcv+mi3_3$gcv+mi3_4$gcv+mi3_5$gcv


mi4_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,15,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi4_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,15,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi4_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,15,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi4_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,15,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi4_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,15,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi4_1$gcv+mi4_2$gcv+mi4_3$gcv+mi4_4$gcv+mi4_5$gcv


mi5_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,17.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi5_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,17.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi5_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,17.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi5_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,17.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi5_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,17.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi5_1$gcv+mi5_2$gcv+mi5_3$gcv+mi5_4$gcv+mi5_5$gcv


mi6_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,20,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi6_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,20,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)
```

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```
mi6_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,20,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi6_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,20,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi6_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,20,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi6_1$gcv+mi6_2$gcv+mi6_3$gcv+mi6_4$gcv+mi6_5$gcv


mi7_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,22.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi7_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,22.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi7_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,22.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi7_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,22.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi7_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,22.5,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi7_1$gcv+mi7_2$gcv+mi7_3$gcv+mi7_4$gcv+mi7_5$gcv     # somehwhere around here


mi8_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi8_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi8_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi8_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi8_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)
```

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```
mi8_1$gcv+mi8_2$gcv+mi8_3$gcv+mi8_4$gcv+mi8_5$gcv


mi9_1 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)

mi9_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)

mi9_3 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[3]],random=~(1|id),family=binomial)

mi9_4 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[4]],random=~(1|id),family=binomial)

mi9_5 <- gam(vlsupp ~ cut(nvp, breaks=c(0,25,100))+s(X_t)+ as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  +
as.factor(postrantb),data=myimp$imputations[[5]],random=~(1|id),family=binomial)

mi9_1$gcv+mi9_2$gcv+mi9_3$gcv+mi9_4$gcv+mi9_5$gcv


# For figure: What is better: average or current?

m_current <- gam(vlsupp ~ s(nvp)+s(X_t),data=myimp$imputations[[1]],family=binomial)

m_average <- gam(vlsupp ~ s(nvpave)+s(X_t),data=myimp$imputations[[1]],family=binomial)

m_current$gcv   # Michael 5: current NVP better, but to discuss with Ray, because method limited

m_average$gcv


#############

# Figure  ####

#############

# Spline representation

# Imputation based approach

Lpvm31 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[1]], robust=TRUE, method="breslow")

Lpvm32 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[2]], robust=TRUE, method="breslow")

Lpvm33 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[3]], robust=TRUE, method="breslow")
```

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```
Lpvm34 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[4]], robust=TRUE, method="breslow")

Lpvm35 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[5]], robust=TRUE, method="breslow")

Lpvm36 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[6]], robust=TRUE, method="breslow")

Lpvm37 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[7]], robust=TRUE, method="breslow")

Lpvm38 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[8]], robust=TRUE, method="breslow")

Lpvm39 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[9]], robust=TRUE, method="breslow")

Lpvm40 <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor
(hfacat) + as.factor(agecat)+ as.factor (wfacat)  + as.factor(postrantb) + cluster(id),
data=myimp$imputations[[10]], robust=TRUE, method="breslow")


predicted31 <- predict(Lpvm31, type = "terms" , se.fit = TRUE , terms = 1)

predicted32 <- predict(Lpvm32, type = "terms" , se.fit = TRUE , terms = 1)

predicted33 <- predict(Lpvm33, type = "terms" , se.fit = TRUE , terms = 1)

predicted34 <- predict(Lpvm34, type = "terms" , se.fit = TRUE , terms = 1)

predicted35 <- predict(Lpvm35, type = "terms" , se.fit = TRUE , terms = 1)

predicted36 <- predict(Lpvm36, type = "terms" , se.fit = TRUE , terms = 1)

predicted37 <- predict(Lpvm37, type = "terms" , se.fit = TRUE , terms = 1)

predicted38 <- predict(Lpvm38, type = "terms" , se.fit = TRUE , terms = 1)

predicted39 <- predict(Lpvm39, type = "terms" , se.fit = TRUE , terms = 1)

predicted40 <- predict(Lpvm40, type = "terms" , se.fit = TRUE , terms = 1)


par(mfrow=c(3,3))

termplot(Lpvm31,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm32,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm33,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm34,terms=1,ylim=c(-0.75,0.3))
```

```
termplot(Lpvm35,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm36,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm37,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm38,terms=1,ylim=c(-0.75,0.3))

termplot(Lpvm39,terms=1,ylim=c(-0.75,0.3))


# defined "seldat"

seldat <- impdat

lp <-
(1/10)*(predicted31$fit+predicted32$fit+predicted33$fit+predicted34$fit+predicted35$fit+predicted36$fit+predicted37$fit+predicted38$fit+predicted39$fit+predicted40$fit)[is.na(seldat$nvp)==F]

within <-
(1/10)*(predicted31$se^2+predicted32$se^2+predicted33$se^2+predicted34$se^2+predicted35$se^2+predicted36$se^2+predicted37$se^2+predicted38$se^2+predicted39$se^2+predicted40$se^2)[is.na(seldat$nvp)==F]

mycoefflist <-
cbind(c(predicted31$fit),c(predicted32$fit),c(predicted33$fit),c(predicted34$fit),c(predicted35$fit),c(predicted36$fit),c(predicted37$fit),c(predicted38$fit),c(predicted39$fit),c(predicted40$fit))[is.na(seldat$nvp)==F,]

mycoeff <- apply(mycoefflist,1,mean)

coeffdiff<-(matrix(cbind(rep(mycoeff,M)),ncol=M,nrow=length(mycoeff))-mycoefflist)^2

between <-  apply(coeffdiff,1,sum)

variance <- within + ((M+1)/(M*(M-1)))*between

se <- round(sqrt(variance),digits=5)

# Main Figure:

par(mfrow=c(1,1))

plot(0 , xlab=" Nevirapine Concentration (mg/L)" , ylab = "Hazard of  failure" , axes=T, main = "Non-Linear Effect of Nevirapine Concentration" ,type = "n" , xlim=c(0,15) , las=1,ylim=c(0,2))

lines(sm.spline(myimp$imputations[[1]]$nvp[is.na(impdat$nvp)==F], exp(lp)), col = "red" , lwd = 1)

lines(sm.spline(myimp$imputations[[1]]$nvp[is.na(impdat$nvp)==F], exp(lp + 1.96 * sqrt(variance))) , col = "orange" , lty = 6 , lwd = 0.8)

lines(sm.spline(myimp$imputations[[1]]$nvp[is.na(impdat$nvp)==F], exp(lp - 1.96 * sqrt(variance))) , col = "orange" , lty = 6 , lwd = 0.8)

axis(side = 1, at = c(seq(0,20,5)), labels = F , tick = T , tcl = 0.4 , lwd.ticks = 0.1)


tiff("NVP3.tif", res=600, compression = "lzw", height=5, width=5, units="in")

dev.off()
```

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```
ccm <- coxph(Surv(X_t0,X_t,vlsupp)  ~ pspline(nvp, df=4)+ cluster(id), data=impdat, robust=TRUE,
method="breslow" )

termplot(ccm)


#[is.na(impdat$nvp)==F]

#tiff(file = "temp.tiff", width = 672, height = 672 units = "px", res = 800,type = c("windows", "cairo")

#     family = "", restoreConsole = TRUE,antialias="cleartype")

#tiff(filename = "Rplot%03d.tiff, width = 3200, height = 3200, units = "px", pointsize = 12,

#     bg = "white", res = 400, family = "", restoreConsole = TRUE,type = c("windows", "cairo"), antialias=


###############

#  Summary   #

###############

MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

rownames(MIsummary) <- c("NVP conc.","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA
(Advanced)","Postrantab(Yes)","(0)","(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

write.csv(round(MIsummary, digits=3),file="Cox_LPVCONT.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1b

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7
```

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```
MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

 rownames(MIsummary) <- c(" NVP<3mg/L","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)","(0)","(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

write.csv(round(MIsummary, digits=3),file="Cox_nvpcat1.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1c

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12

rownames(MIsummary) <- c(" NVPRE","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)","(0)","(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

write.csv(round(MIsummary, digits=3),file="Cox_NVPRE.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1d

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12
```

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```
rownames(MIsummary) <- c(" NVPAVE","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)","(0)","(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

MIsummary

write.csv(round(MIsummary, digits=3),file="Cox_NVPAVE.csv")


MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)

MIsummary[1,1:4] <- my_1e

MIsummary[2,1:4] <- my_2

MIsummary[3,1:4] <- my_3

MIsummary[4,1:4] <- my_4

MIsummary[5,1:4] <- my_5

MIsummary[6,1:4] <- my_6

MIsummary[7,1:4] <- my_7

MIsummary[8,1:4] <- my_9

MIsummary[9:10,1:4] <- my_10

MIsummary[,5:8]    <- my_12

rownames(MIsummary) <- c(" NVP<10mg/L","VL (>50)","CD4% (Low)","Age (>1/2 yr.)","WFA (Advanced)","HFA (Advanced)","Postrantab(Yes)","(0)","(1)")

colnames(MIsummary) <-c("Crude","","","","Adj.","","","")

write.csv(round(MIsummary, digits=3),file="Cox_nvpcat4.csv")


##############################################

#Model Selection Using AIC for Each Imputed Data ##

##############################################

m5_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_0b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_0c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) + cluster(id),data=myimp$imputations[[1]],method="breslow")
```

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```
m5_0d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

extractAIC(m5_0a)[2]

extractAIC(m5_0b)[2]

extractAIC(m5_0c)[2]

extractAIC(m5_0d)[2]


m5_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave          + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_1d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

extractAIC(m5_1a)[2]

extractAIC(m5_1b)[2]

extractAIC(m5_1c)[2]

extractAIC(m5_1d)[2]


m5_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

extractAIC(m5_2a)[2]

extractAIC(m5_2b)[2]

extractAIC(m5_2c)[2]
```

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```
extractAIC(m5_2d)[2]


m5_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_3d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

extractAIC(m5_3a)[2]

extractAIC(m5_3b)[2]

extractAIC(m5_3c)[2]

extractAIC(m5_3d)[2]


m5_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_4c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_4d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

extractAIC(m5_4a)[2]

extractAIC(m5_4b)[2]

extractAIC(m5_4c)[2]

extractAIC(m5_4d)[2]


m5_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")
```

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```
m5_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_5c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_5d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

extractAIC(m5_5a)[2]

extractAIC(m5_5b)[2]

extractAIC(m5_5c)[2]

extractAIC(m5_5d)[2]


m5_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_6d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

extractAIC(m5_6a)[2]

extractAIC(m5_6b)[2]

extractAIC(m5_6c)[2]

extractAIC(m5_6d)[2]


m5_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_7c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")
```

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```
m5_7d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

extractAIC(m5_7a)[2]

extractAIC(m5_7b)[2]

extractAIC(m5_7c)[2]

extractAIC(m5_7d)[2]


m5_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_8d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

extractAIC(m5_8a)[2]

extractAIC(m5_8b)[2]

extractAIC(m5_8c)[2]

extractAIC(m5_8d)[2]


m5_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m5_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m5_9c<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m5_9d<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(nvp)  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)
+ as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

extractAIC(m5_9a)[2]

extractAIC(m5_9b)[2]

extractAIC(m5_9c)[2]
```

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```
extractAIC(m5_9d)[2]


m4_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m4_0b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

extractAIC(m4_0a)[2]

extractAIC(m4_0b)[2]


m4_1a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3          + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m4_1b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10          + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

extractAIC(m4_1a)[2]

extractAIC(m4_1b)[2]


m4_2a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3          + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m4_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10          + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

extractAIC(m4_2a)[2]

extractAIC(m4_2b)[2]


m4_3a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m4_3b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10          + as.factor(vlcat)+ as.factor(cd4pccat)  +
as.factor(agecat)  + as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

extractAIC(m4_3a)[2]

extractAIC(m4_3b)[2]
```

APPENDIX III

```
m4_4a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m4_4b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

extractAIC(m4_4a)[2]

extractAIC(m4_4b)[2]


m4_5a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m4_5b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

extractAIC(m4_5a)[2]

extractAIC(m4_5b)[2]


m4_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m4_6b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

extractAIC(m4_6a)[2]

extractAIC(m4_6b)[2]


m4_7a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m4_7b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

extractAIC(m4_7a)[2]

extractAIC(m4_7b)[2]


m4_8a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")
```

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```
m4_8b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

extractAIC(m4_8a)[2]

extractAIC(m4_8b)[2]


m4_9a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

m4_9b<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")

extractAIC(m4_9a)[2]

extractAIC(m4_9b)[2]

################################

# Adherence vs. Concentration #####

################################

 m5_31<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_32<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_33<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_34<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_35<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_36<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_37<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_38<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")
```

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```
m5_39<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp   + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_310<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp  + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")


m5_41<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[1]],method="breslow")

m5_42<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[2]],method="breslow")

m5_43<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_44<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[4]],method="breslow")

m5_45<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[5]],method="breslow")

m5_46<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[6]],method="breslow")

m5_47<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[7]],method="breslow")

m5_48<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[8]],method="breslow")

m5_49<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus + as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[9]],method="breslow")

m5_410<-coxph(Surv(X_t0,X_t, vlsupp) ~ adstatus+ as.factor(vlcat)+ as.factor(cd4pccat)  + as.factor(agecat)  +
as.factor (wfacat) + as.factor (hfacat)  + as.factor(postrantb) +
cluster(id),data=myimp$imputations[[10]],method="breslow")


0.1*(extractAIC(m5_31)[2]+extractAIC(m5_32)[2]+extractAIC(m5_33)[2]+extractAIC(m5_34)[2]+extractAIC(m5
_35)[2]+extractAIC(m5_36)[2]+extractAIC(m5_37)[2]+extractAIC(m5_38)[2]+extractAIC(m5_39)[2]+extractAIC(
m5_310)[2])
```

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0.1*(extractAIC(m5_41)[2]+extractAIC(m5_42)[2]+extractAIC(m5_43)[2]+extractAIC(m5_44)[2]+extractAIC(m5_45)[2]+extractAIC(m5_46)[2]+extractAIC(m5_47)[2]+extractAIC(m5_48)[2]+extractAIC(m5_49)[2]+extractAIC(m5_410)[2])

Everything is indicating that it is better to use Nevirapine compared to adherence

APPENDIX IV

Population Pharmacokinetic Model of Lopinavir In Children

```
;Model Desc: LPVRTV| CL vs K & V|BSV CL,V,BIO,KA,LAG| BOV KA,BIO,CL,LAG|EFFECT OF AGE on BIO|1COMP LPV + EFFECT OF TB TREAT|ADVAN2 TRANS1

$SIZES      MAXIDS=300 NO=120 LTH=20 LVR=100 MAXFCN=100000000

$PROBLEM    Effect of Clinic Visit LPVRTV in Children

$INPUT  ID WEEKS OCC ID_OCC=DROP TIME ABLAG=DROP WHAT=DROP AMT DV OLD_DV2=DROP DV1=DROP DV2=DROP MDV DVID II=DROP SS=DROP EVID BLQ TBT DRUG=DROP PREPOST AGE SEX=DROP MISKAL WT HT HDCIRMM BSA BMI WFA HFA WFH BMIFA CD4CNT CD4PC VL VLSUPP=DROP VLSUPP1=DROP HBCNT=DROP PLT=DROP ALT=DROP AST=DROP BILI=DROP NEUT=DROP MONO=DROP LMPHCNT=DROP EOSIN=DROP BASO=DROP HDL=DROP LDL=DROP TRGLCRDS=DROP PROB COMMENT=DROP

$DATA LPVRTV_09_06_2017.csv IGNORE=#

        IGNORE(PROB.GT.1)IGNORE(DVID.GT.1) IGNORE(BLQ.GT.0)

$ABBREVIATED DERIV2=NO

$SUBROUTINE ADVAN2 TRANS1

;-------------------------------------------------------------Initial Estimates from Chao------------

$THETA  (0,1.04989) ; 1 CL [L/h]

$THETA  (0,8.03299) ; 2 V [L]

$THETA  (0,0.74) FIX ; 3 KA [1/h]

$THETA  (0.004,0.103454,5) ; 4 ADD [mg/L]

$THETA  (0,0.11682) ; 5 PROP[%]

$THETA  (0,26.6646) ; 6 PMA50 [Months]

$THETA  (0,0.594982,1) ; 7 RIFCL

$THETA  (0,0.37) FIX ; 9 TVLAG

$OMEGA  BLOCK(1)

 0.177698  ;   1 IIV_CL

$OMEGA  BLOCK(1)

 0.07973  ;    3 IIV_V

$OMEGA  BLOCK(1)

 0.0991334  ;  3  IOV KA

$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) SAME
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.169358  ; 22  IIV BIO
$OMEGA  BLOCK(1)
 0.133771  ; 23 IOV BIO
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) FIX
 0  ; 42  IOV CL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) FIX
 0  ; 61  IIV_KA
$OMEGA  BLOCK(1)
 0.0700647  ; 62  IIV_LAG
$OMEGA  BLOCK(1)
 0.197  ; 63  IOV LAG
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$PK
;-----------------------------------------------------------------------Variability----------------------------------------
BSV_CL  = ETA(1)
BSV_V   = ETA(2)
BOV_KA = ETA(3)
IF (OCC.EQ.2)  BOV_KA  = ETA(4)
IF (OCC.EQ.3)  BOV_KA  = ETA(5)
IF (OCC.EQ.4)  BOV_KA  = ETA(6)
IF (OCC.EQ.5)  BOV_KA  = ETA(7)
IF (OCC.EQ.6)  BOV_KA  = ETA(8)
IF (OCC.EQ.7)  BOV_KA  = ETA(9)
IF (OCC.EQ.8)  BOV_KA  = ETA(10)
IF (OCC.EQ.9)  BOV_KA  = ETA(11)
IF (OCC.EQ.10) BOV_KA  = ETA(12)
IF (OCC.EQ.11) BOV_KA  = ETA(13)
IF (OCC.EQ.12) BOV_KA  = ETA(14)
IF (OCC.EQ.13) BOV_KA  = ETA(15)
IF (OCC.EQ.14) BOV_KA  = ETA(16)
IF (OCC.EQ.15) BOV_KA  = ETA(17)
IF (OCC.EQ.16) BOV_KA  = ETA(18)
IF (OCC.EQ.17) BOV_KA  = ETA(19)
```

```
IF (OCC.EQ.18) BOV_KA  = ETA(20)
IF (OCC.EQ.19) BOV_KA  = ETA(21)

BSV_BIO = ETA(22)
BOV_BIO = ETA(23)
IF (OCC.EQ.2)  BOV_BIO = ETA(24)
IF (OCC.EQ.3)  BOV_BIO = ETA(25)
IF (OCC.EQ.4)  BOV_BIO = ETA(26)
IF (OCC.EQ.5)  BOV_BIO = ETA(27)
IF (OCC.EQ.6)  BOV_BIO = ETA(28)
IF (OCC.EQ.7)  BOV_BIO = ETA(29)
IF (OCC.EQ.8)  BOV_BIO = ETA(30)
IF (OCC.EQ.9)  BOV_BIO = ETA(31)
IF (OCC.EQ.10) BOV_BIO = ETA(32)
IF (OCC.EQ.11) BOV_BIO = ETA(33)
IF (OCC.EQ.12) BOV_BIO = ETA(34)
IF (OCC.EQ.13) BOV_BIO = ETA(35)
IF (OCC.EQ.14) BOV_BIO = ETA(36)
IF (OCC.EQ.15) BOV_BIO = ETA(37)
IF (OCC.EQ.16) BOV_BIO = ETA(38)
IF (OCC.EQ.17) BOV_BIO = ETA(39)
IF (OCC.EQ.18) BOV_BIO = ETA(40)
IF (OCC.EQ.19) BOV_BIO = ETA(41)

BOV_CL = ETA(42)
IF (OCC.EQ.2)  BOV_CL = ETA(43)
IF (OCC.EQ.3)  BOV_CL = ETA(44)
IF (OCC.EQ.4)  BOV_CL = ETA(45)
IF (OCC.EQ.5)  BOV_CL = ETA(46)
IF (OCC.EQ.6)  BOV_CL = ETA(47)
IF (OCC.EQ.7)  BOV_CL = ETA(48)
IF (OCC.EQ.8)  BOV_CL = ETA(49)
```

```
IF (OCC.EQ.9)  BOV_CL = ETA(50)
IF (OCC.EQ.10) BOV_CL = ETA(51)
IF (OCC.EQ.11) BOV_CL = ETA(52)
IF (OCC.EQ.12) BOV_CL = ETA(53)
IF (OCC.EQ.13) BOV_CL = ETA(54)
IF (OCC.EQ.14) BOV_CL = ETA(55)
IF (OCC.EQ.15) BOV_CL = ETA(56)
IF (OCC.EQ.16) BOV_CL = ETA(57)
IF (OCC.EQ.17) BOV_CL = ETA(58)
IF (OCC.EQ.18) BOV_CL = ETA(59)
IF (OCC.EQ.19) BOV_CL = ETA(60)
BSV_KA  = ETA(61)
BSV_LAG  = ETA(62)

BOV_LAG = ETA(63)
IF (OCC.EQ.2)  BOV_LAG = ETA(64)
IF (OCC.EQ.3)  BOV_LAG = ETA(65)
IF (OCC.EQ.4)  BOV_LAG = ETA(66)
IF (OCC.EQ.5)  BOV_LAG = ETA(67)
IF (OCC.EQ.6)  BOV_LAG = ETA(68)
IF (OCC.EQ.7)  BOV_LAG = ETA(69)
IF (OCC.EQ.8)  BOV_LAG = ETA(70)
IF (OCC.EQ.9)  BOV_LAG = ETA(71)
IF (OCC.EQ.10) BOV_LAG = ETA(72)
IF (OCC.EQ.11) BOV_LAG = ETA(73)
IF (OCC.EQ.12) BOV_LAG = ETA(74)
IF (OCC.EQ.13) BOV_LAG = ETA(75)
IF (OCC.EQ.14) BOV_LAG = ETA(76)
IF (OCC.EQ.15) BOV_LAG = ETA(77)
IF (OCC.EQ.16) BOV_LAG = ETA(78)
IF (OCC.EQ.17) BOV_LAG = ETA(79)
IF (OCC.EQ.18) BOV_LAG = ETA(80)
IF (OCC.EQ.19) BOV_LAG = ETA(81)
```

APPENDIX IV

```
;----------------------------------------------------------------------------Allometric Scaling
SCL = (WT/10)**0.75
SV  = WT/10
;----------------------------------------------------------------------------Maturation function
MEDAGE=31
HILL   = 1
PMA50   = THETA(6)
PMA    = AGE + (9/12)
TVPMA   = MEDAGE +  (9/12)
FMAT = 1/(1+(PMA/TVPMA+PMA50)**(-HILL))
 ; ----------------------------------------------------------------------------LPV MODEL----------------------------------------------
;Effect of Concomittant TB Therapy
RIFCL = 0
IF (TBT.EQ.1) RIFCL = THETA(7) ;Baseline
TVCL  = THETA(1)*SCL*(1+RIFCL)
TVV   = THETA(2)*SV
TVKA  = THETA(3)
TVBIO = 1
TVLAG = THETA(8)
CL    = TVCL*EXP(BSV_CL + BOV_CL)
V     = TVV*EXP(BSV_V)
KA    = TVKA*EXP(BOV_KA + BSV_KA)
LAG =  TVLAG*EXP(BSV_LAG + BOV_LAG)
BIO = TVBIO*EXP(BSV_BIO + BOV_BIO)*FMAT

F1    = BIO
K     = CL/V
ALAG1 = LAG
S2    = V
;Dosing Compartment Initialization
 TAU_EQ = ALAG1+1/KA
 KA_EQ  = 1/TAU_EQ
```

APPENDIX IV

```
; DOSING IS EVERY 12H, HERE USE HF THE TOTAL DOSE
BASELINE = (((BIO*AMT) * KA_EQ) / (KA_EQ - K))  * ( (1 / (1- EXP(-K * 12))) - (1 /( 1- EXP(-KA_EQ*12))) )
 A_0 (1) = 0.0001
 A_0 (2) = BASELINE
 ;Calculating Cmin
CMIN= (((BIO*AMT/2) * KA_EQ) / (V*(KA_EQ - K)))*((1 / (1- EXP(-K * 12)))-(1 / (1- EXP(-KA_EQ*12))))


$ERROR
 ;(OBSERVATIONS ONLY)
IPRED = A(2)/V
ADD = THETA(4)   ;Addative Error
PROP= THETA(5)*IPRED ;Proportional Error
W = SQRT(ADD**2+PROP**2)
IF (W.LE.0.000001) W=0.000001

IRES=DV-IPRED
IWRES = IRES/W
Y= (IPRED)+W*EPS(1)

AA1 = A(1)
AA2 = A(2)
IF(AMT.EQ.0) THEN
        TDOS = 48
        PD   = AMT
        TAD  = TIME - TDOS
ENDIF
;---------------------------------- Handling LLOQ
LLOQ=0.08
IMPUTED_BLQ=LLOQ/2
; BLQ==1 are the first BLQ samples in a series
IF (ICALL/=4.AND.BLQ==1) THEN
PROP=0
```

APPENDIX IV

```
ADD = IMPUTED_BLQ*10000000000 ; A separate error, only for the BLQ data. It could be fixed to
IMPUTED_BLQ, which is normally LLOQ/2

ENDIF

; For simulation, like in case of VPC

IF (ICALL==4.AND.Y<=LLOQ) THEN

        Y=IMPUTED_BLQ ; All BLQ values in simulation get imputed to LLOQ/2. This also prevents negative
values

ENDIF

;Calculating AUC

AUC = BIO*AMT/CL

;Calculating Combined Variability

VAR_CL  = BSV_CL  + BOV_CL

VAR_BIO = BSV_BIO + BOV_BIO

VAR_AUC = VAR_CL  - VAR_BIO


$SIGMA  1  FIX

;-------------------------------------------------------------------------------------------------------------------------------

$ESTIMATION MAXEVAL=0 SIGL=10 ATOL=9 SIGDIG=3 PRINT=5 METHOD=COND

        INTER MSFO=msfo427 MCETA=100 NONINFETA=1 ETASTYPE=1

        RANMETHOD=4P NOABORT

$ESTIMATION MAXEVAL=9999 SIGL=10 ATOL=9 SIGDIG=3 PRINT=5 METHOD=COND

        INTER MSFO=msfo427 MCETA=5 NONINFETA=1 ETASTYPE=1

        RANMETHOD=4P NOABORT

$NONPARAMETRIC MARGINALS MSFO=msfo427 UNCONDITIONAL NPSUPP=300

;$COVARIANCE PRINT=E  MATRIX=S

$TABLE      FILE=sdtab427.csv ID WEEKS OCC TIME TAD AMT DV IPRED IRES

        IWRES PRED RES WRES CWRES NPDE OBJI ESAMPLE=1000 NOPRINT

        NOAPPEND ONEHEADER FORMAT=,

$TABLE      FILE=patab427.csv ID DVID CL V KA K ALAG1 F1 PMA50 PMA

        FMAT PROP ADD CMIN AUC TAU_EQ KA_EQ AA1 AA2 BSV_CL BSV_V

        BSV_KA BSV_LAG BSV_BIO BOV_KA BOV_BIO BOV_CL BOV_LAG RIFCL

        NOPRINT NOAPPEND ONEHEADER FORMAT=,

$TABLE      FILE=cotab427.csv AGE MISKAL WT HT HDCIRMM BSA BMI WFA HFA

        WFH BMIFA CD4CNT CD4PC VL NOPRINT NOAPPEND ONEHEADER FORMAT=,
```

APPENDIX IV

```
$TABLE      FILE=catab427.csv ID BLQ TBT PREPOST NOPRINT NOAPPEND
        ONEHEADER FORMAT=,
;$SIMULATION (123) ONLYSIM

;$TABLE  ID TIME Y DV CLL VL KAL CLR KAR NOPRINT NOAPPEND ONEHEADER FILE=sim.tab214
$TABLE      FILE=mytab427.csv ID OCC TIME TAD AMT DV IPRED IRES IWRES
        PRED RES WRES CWRES NPDE CL V KA K ALAG1 F1 PMA50 PMA FMAT
        PROP ADD CMIN AUC TAU_EQ KA_EQ AA1 AA2 BSV_CL BSV_V BSV_KA
        BSV_LAG BSV_BIO BOV_KA BOV_BIO BOV_CL BOV_LAG RIFCL VAR_CL
        VAR_BIO VAR_AUC AGE MISKAL WT HT HDCIRMM BSA BMI WFA HFA
        WFH BMIFA CD4CNT CD4PC VL BLQ TBT PREPOST NOPRINT NOAPPEND
        ONEHEADER FORMAT=,
```

APPENDIX V

Population Pharmacokinetic Model of Nevirapine In Children

```
;;Model Desc: NEVIRAPINE| CL vs K & V|BSV CL, V,KA,BIO|BOV CL,KA,MTT,BIO
BIO,MTT|1COMP+ALLOMETRY+TRANSIT+HEPATIC EXTRACTION+EXP ON BIO|ADVAN6 TRANS1

$SIZES  MAXIDS=100 LVR=90 LTH=20

$PROBLEM STEADY STATE NVP PK IN CHILDREN

$ABBREVIATED DERIV2=NO COMRES=2

$INPUT ID WEEKS OCC ID_OCC=DROP WHAT=DROP AMT TIME DV MDV EVID II=DROP SS=DROP BLQ
SEX=DROP BASEAGE=DROP VISAGE RANAGE TB RESISTANCE WT HT HDCIRMM BSA BMI WFA HFA WFH BMIFA
CD4CNT CD4PC VIRALOAD PROB COMMENT=DROP

$DATA NVP_20_06_17.csv IGNORE=# IGNORE=(PROB.GT.1)

$SUBROUTINE ADVAN=6 TRANS1 TOL=6

$MODEL NCOMPARTMENTS=3 COMP=(ABS DEFDOSE) COMP=(LIVER)

COMP=(CENTRAL DEFOBSERVATION)

$PK

;-------------------------------------------------------------------------------Allometric Scaling

SCL = (WT/13)**0.75

SV  = WT/13

;-------------------------------------------------------------------------------Variability

BSV_CL = ETA(1)

BSV_V  = ETA(2)

BOV_CL = ETA(3)

IF (OCC.EQ.2)  BOV_CL = ETA(4)

IF (OCC.EQ.3)  BOV_CL = ETA(5)

IF (OCC.EQ.4)  BOV_CL = ETA(6)

IF (OCC.EQ.5)  BOV_CL = ETA(7)

IF (OCC.EQ.6)  BOV_CL = ETA(8)

IF (OCC.EQ.7)  BOV_CL = ETA(9)

IF (OCC.EQ.8)  BOV_CL = ETA(10)

IF (OCC.EQ.9)  BOV_CL = ETA(11)

IF (OCC.EQ.10) BOV_CL = ETA(12)

IF (OCC.EQ.11) BOV_CL = ETA(13)

IF (OCC.EQ.12) BOV_CL = ETA(14)

IF (OCC.EQ.13) BOV_CL = ETA(15)

IF (OCC.EQ.14) BOV_CL = ETA(16)
```

```
IF (OCC.EQ.15) BOV_CL = ETA(17)
IF (OCC.EQ.16) BOV_CL = ETA(18)
IF (OCC.EQ.17) BOV_CL = ETA(19)
IF (OCC.EQ.18) BOV_CL = ETA(20)

BSV_KA = ETA(21)
BOV_KA = ETA(22)
IF (OCC.EQ.2)  BOV_KA = ETA(23)
IF (OCC.EQ.3)  BOV_KA = ETA(24)
IF (OCC.EQ.4)  BOV_KA = ETA(25)
IF (OCC.EQ.5)  BOV_KA = ETA(26)
IF (OCC.EQ.6)  BOV_KA = ETA(27)
IF (OCC.EQ.7)  BOV_KA = ETA(28)
IF (OCC.EQ.8)  BOV_KA = ETA(29)
IF (OCC.EQ.9)  BOV_KA = ETA(30)
IF (OCC.EQ.10) BOV_KA = ETA(31)
IF (OCC.EQ.11) BOV_KA = ETA(32)
IF (OCC.EQ.12) BOV_KA = ETA(33)
IF (OCC.EQ.13) BOV_KA = ETA(34)
IF (OCC.EQ.14) BOV_KA = ETA(35)
IF (OCC.EQ.15) BOV_KA = ETA(36)
IF (OCC.EQ.16) BOV_KA = ETA(37)
IF (OCC.EQ.17) BOV_KA = ETA(38)
IF (OCC.EQ.18) BOV_KA = ETA(39)

BSV_BIO = ETA(40)
BOV_BIO = ETA(41)
IF (OCC.EQ.2)  BOV_BIO = ETA(42)
IF (OCC.EQ.3)  BOV_BIO = ETA(43)
IF (OCC.EQ.4)  BOV_BIO = ETA(44)
IF (OCC.EQ.5)  BOV_BIO = ETA(45)
IF (OCC.EQ.6)  BOV_BIO = ETA(46)
IF (OCC.EQ.7)  BOV_BIO = ETA(47)
```

```
IF (OCC.EQ.8)  BOV_BIO = ETA(48)
IF (OCC.EQ.9)  BOV_BIO = ETA(49)
IF (OCC.EQ.10) BOV_BIO = ETA(50)
IF (OCC.EQ.11) BOV_BIO = ETA(51)
IF (OCC.EQ.12) BOV_BIO = ETA(52)
IF (OCC.EQ.13) BOV_BIO = ETA(53)
IF (OCC.EQ.14) BOV_BIO = ETA(54)
IF (OCC.EQ.15) BOV_BIO = ETA(55)
IF (OCC.EQ.16) BOV_BIO = ETA(56)
IF (OCC.EQ.17) BOV_BIO = ETA(57)
IF (OCC.EQ.18) BOV_BIO = ETA(58)

BSV_MTT = ETA(59)
BOV_MTT = ETA(60)
IF (OCC.EQ.2)  BOV_MTT = ETA(61)
IF (OCC.EQ.3)  BOV_MTT = ETA(62)
IF (OCC.EQ.4)  BOV_MTT = ETA(63)
IF (OCC.EQ.5)  BOV_MTT = ETA(64)
IF (OCC.EQ.6)  BOV_MTT = ETA(65)
IF (OCC.EQ.7)  BOV_MTT = ETA(66)
IF (OCC.EQ.8)  BOV_MTT = ETA(67)
IF (OCC.EQ.9)  BOV_MTT = ETA(68)
IF (OCC.EQ.10) BOV_MTT = ETA(69)
IF (OCC.EQ.11) BOV_MTT = ETA(70)
IF (OCC.EQ.12) BOV_MTT = ETA(71)
IF (OCC.EQ.13) BOV_MTT = ETA(72)
IF (OCC.EQ.14) BOV_MTT = ETA(73)
IF (OCC.EQ.15) BOV_MTT = ETA(74)
IF (OCC.EQ.16) BOV_MTT = ETA(75)
IF (OCC.EQ.17) BOV_MTT = ETA(76)
IF (OCC.EQ.18) BOV_MTT = ETA(77)
```

APPENDIX V

```
;-------------------------------------------------------------------------------Typical Parameters
TVCL  = THETA(1)
TVV   = THETA(2)
TVKA  = THETA(3)
TVNN  = THETA(6)
TVMTT = THETA(7)

CL   = TVCL*EXP (BSV_CL + BOV_CL)*SCL ;*MATCL
V3   = TVV*EXP(BSV_V)*SV
KA   = TVKA*EXP(BSV_KA + BOV_KA)
NN          = TVNN
MTT  = TVMTT*EXP(BSV_MTT + BOV_MTT)

; HEPATIC EXTRACTION
CLINT = CL
FU    = 0.4 ; fraction unbound in plasma
QH    = 50 *(WT/70)**0.75 ; hepatic plasma flow - adult = 50L/h
EH    = (FU * CLINT) / (QH +(FU *CLINT))  ; hepatic extraction ratio
FH    = 1 - EH ; bioavailibility after first pass metabolism
VH = 1 *(WT/70)
CLH= QH *EH      ; part metabolised
K   =(QH*EH)/VH  ; Metabolic rate constant from liver - ELIMINATION
K23 =(QH*FH)/VH  ; part that goes back to cent CMT -
;------------------ TRANFER RATE CONSTANTS ---------------------------------------------
K32 = QH/V3 ; FROM CENTRAL BACK TO LIVER
                                    ; THE ONES FROM LIVER TO CENTRAL AND EXTRACTION RATE COST DEFINED IN
$DES

 ; AGE EFFECT ON BIO
TVBIO     = FH ; bioavailibility after first pass
BIO_BIRTH = THETA(8)  ; BIO AT BIRTH
```

APPENDIX V

```
KBIO     = THETA(9)  ; AGE EFFECT CONSTANT

AGEBIO    = 1 - ((1-BIO_BIRTH)*(EXP(-(VISAGE*KBIO)))) ; AGE EFF ON F1 AS INVERSE EXP WITH INTERCEPT; AGE IN YEARS FROM BIRTH

BIO      = TVBIO *EXP(BSV_BIO + BOV_BIO)*AGEBIO  ;  PRE-HEPATIC BIO


S3 = V3

;----------------------------------------------------------------------------Transit Compartment

F1  = 0

KTR =(NN+1)/MTT


IF (NEWIND/=2.OR.EVID>=3) THEN ; new individual, or reset event

   ; The values read here will be stored in TDOS and PD in this very PK call.

         TNXD = TIME ; Time of the dose

         PNXD = AMT ; Amount. If it's zero, the DE is deactivated.

ENDIF


TDOS = TNXD ; This will either save here the temporary values if it's a new individual...

PD   = PNXD ; ...or the values which were read one record ahead during the execution of the previous record.


IF(AMT.GT.0) THEN ; This reads one record ahead and stores the data to be used when running the following record

; IF(AMT.GT.0.AND.ALAG1.EQ.0) THEN ; Use this instead if there is ALAG, as it will also checks if the ALAG is not 0

         TNXD = TIME

         PNXD = AMT

ENDIF


LNGAM = NN*LOG(NN)-NN+LOG(NN*(1+4*NN*(1+2*NN)))/6+0.572364942 ; approximation of log of gamma(n), 0.572364942 is LOG(PI)/2

; To speed up the computation, I calculate here all the non-time-varying quantities used in $DES

PIZZA = LOG(BIO*PD*KTR+0.00001)-LNGAM ; without +0.00001, it won't work with ETAs in bioavailability


;Dosing Compartment Initialization

 TAU_EQ = MTT+1/KA
```

APPENDIX V

```
 KA_EQ  = 1/TAU_EQ


; DOSING IS EVERY 12H, HERE USE HF THE TOTAL DOSE
BASELINE = (((BIO*AMT) * KA_EQ) / (KA_EQ - K))  * ( (1 / (1- EXP(-K * 12))) - (1 /( 1- EXP(-KA_EQ*12))) )
 A_0(1)= 0.0001
 A_0(2)= BASELINE *VH
 A_0(3)= BASELINE *V3


;Calculating Cmin
CMIN= (((BIO*PNXD/2) * KA_EQ) / (V3*(KA_EQ - K)))*((1 / (1- EXP(-K * 12)))-(1 / (1- EXP(-KA_EQ*12))))


IF (NEWIND.NE.2.OR.EVID.GE.3) THEN ; Each time I have a new subject, or a reset
        COM(1)=0
        COM(2)=0
        TDOS = 0
ENDIF


$DES
TEMPO=T-TDOS ; this is time after dose, it should always be >= 0
KTT=0
CP = A(3)/V3
IF (CP.GE.COM(1)) THEN
        COM(1) = CP          ; CMAX
        COM(2) = T - TDOS       ; TIME OF CMAX
ENDIF


DADT(1)=0
IF(PD.GT.0.AND.TEMPO.GT.0) THEN ; This happens only id PD>0, so only if a dose has been detected
        KTT=KTR*(TEMPO)
        DADT(1)=EXP(PIZZA+NN*LOG(KTT)-KTT)-KA*A(1)
ENDIF


DADT(2)= KA*A(1)- K*A(2)- K23*A(2)+K32*A(3)
```

```
DADT(3)= K23*A(2)-K32*A(3)


;-------------------------------------------------------------------------------Error
$ERROR
 ;(ONLY OBSERVATIONS)
IPRED = A(3)/V3
IRES  = DV - IPRED
PROP=THETA(4)*IPRED              ;proportional error
ADD=THETA(5) ;+ THETA(11)        ;additive error
W = SQRT(ADD**2 + PROP**2)
IF (W<0.0001) W = 0.0001
IWRES = IRES/W
Y = IPRED + W*ERR(1)
AA1 = A(1)
AA2 = A(2)
IF(AMT.EQ.0) THEN
          TDOS = 48
          PD   = AMT
          TAD  = TIME - TDOS
ENDIF
;Handling BLQ
LLOQ=0.05
IMPUTED_BLQ=LLOQ/2
 ;BLQ==1 are the first BLQ samples in a series
IF (ICALL/=4.AND.BLQ==1) THEN
PROP=0
ADD_BLQ = IMPUTED_BLQ*10000000000 ; A separate error, only for the BLQ data. It could be fixed to
IMPUTED_BLQ, which is normally LLOQ/2
ENDIF


 AA1=A(1) ; abs comp
 AA2=A(2) ; LIVER
 AA3=A(3) ; central comp
```

APPENDIX V

```
; For simulation, like in case of VPC
IF (ICALL==4.AND.Y<=LLOQ) THEN
        Y=IMPUTED_BLQ ; All BLQ values in simulation get imputed to LLOQ/2. This also prevents negative values
ENDIF
CMAX = COM(1) ; CMAX
TMAX = COM(2) ; TIME OF CMAX
;Calculate AUC
AUC = AMT*BIO / CL
; Reset CMAX code when a new dose is given
        COM(1)=0
        COM(2)=0
IF (ICALL==4.AND.Y.LE.0.1) Y=0.05 ; prevents negative simulated values
;----------Parameter Estimates

$THETA  (0,2.8833,10) ; 1 CL [L/h]
$THETA  (0,21.7866,40) ; 2 V [L]
$THETA  (0,0.84,10) FIX ; 3 KA [1/h]
$THETA  (0,0.115378,1) ; 4 PROP [%]
$THETA  (0.01,1.18234,10) ; 5 ADD [mg/L]
$THETA  3 FIX ; 6 NN []
$THETA  (0,0.56,8) FIX ; 7 MTT[h]
$THETA  (0,0.503515,1) ; 8 BIO_BIRTH[%]
$THETA  (0.05,0.843688,10) ; 9 KBIO[Years]
$OMEGA  BLOCK(1)
 0.253145  ;   1 BSV_CL
$OMEGA  BLOCK(1)
 0.220806  ;    2 BSV_V
$OMEGA  BLOCK(1)
 0.113794  ;          ;   3 BOV_CL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.23842  ;  21 BSV_KA
$OMEGA  BLOCK(1)
 0.173174  ;  22 BOV_KA
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.0285655  ; 40 BSV_BIO
$OMEGA  BLOCK(1)
 0.1995301  ; 41 BOV_BIO
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) FIX
 0  ; 59 BSV_MTT
$OMEGA  BLOCK(1)
 1.16439  ; 60 B0V_MTT
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
```

APPENDIX V

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

$SIGMA 1 FIX

$ESTIMATION MAXEVAL=0 SIGDIG=3 PRINT=1 NOABORT METHOD=COND INTER MSFO=msfo190 MCETA=100 RANMETHOD=4P ATOL=9

$ESTIMATION MAXEVAL=9999 SIGDIG=3 PRINT=1 NOABORT METHOD=COND INTER MSFO=msfo190 MCETA=5 ETASTYPE=1 RANMETHOD=4P ATOL=9

$NONPARAMETRIC MARGINALS MSFO=msfo190 UNCONDITIONAL NPSUPP=100

$TABLE FILE=sdtab190.csv ID WEEKS OCC TIME TAD DV IPRED IRES WRES PRED RES WRES CWRES NPDE OBJI ESAMPLE=300 WRESCHOL NOPRINT NOAPPEND ONEHEADER FORMAT=,

$TABLE FILE=patab190.csv ID WEEKS OCC TIME CL KA V3 K K23 K32 N MTT QH EH FH CLH VH BSV_V BIO_BIRTH KBIO AGEBIO BSV_CL BSV_KA BSV_BIO BSV_MTT BOV_CL BOV_KA BOV_BIO BOV_MTT NOPRINT NOAPPEND ONEHEADER FORMAT=,

$TABLE FILE=cotab190.csv ID RANAGE VISAGE WT HT HDCIRMM BSA BMI WFA HFA WFH BMIFA CD4CNT CD4PC VIRALOAD NOPRINT NOAPPEND ONEHEADER FORMAT=,

$TABLE FILE=catab190.csv ID TB RESISTANCE NOPRINT NOAPPEND ONEHEADER FORMAT=,

$TABLE FILE=mytab190.csv ID WEEKS OCC TIME TAD AMT DV AA1 AA2 IPRED IRES IWRES PRED RES WRES CWRES NPDE CL KA V3 K K23 K32 BIO_BIRTH KBIO AGEBIO CMIN AUC NN MTT TAU_EQ KA_EQ BASELINE QH EH FH CLH VH BSV_V BSV_CL BSV_KA BSV_MT BSV_BIO BOV_CL BOV_KA BOV_BIO BOV_MTT RANAGE VISAGE WT HT HDCIRMM BSA BMI WFA HFA WFH BMIFA CD4CNT CD4PC VIRALOAD TB RESISTANCE NOPRINT NOAPPEND ONEHEADER FORMAT=,