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

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MSc Medical Biochemistry

Thesis Submitted For the Fulfilment of Doctor of Philosophy in Clinical Pharmacology

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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 pretreatment 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 \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{SLCO1B1}. \textit{CYP2B6} 516G→T and \textit{CYP2B6} 983T→C were associated with NVP CL/F. \textit{CYP2B6} 983T→C was associated with NVP C_{min} and AUC. Additionally, polymorphisms in the \textit{ABCB1} and \textit{CYP3A5} were independently associated with NVP CL/F, C_{min} and AUC. Polymorphisms in the \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{SLCO1B1} and lopinavir pharmacokinetics. Polymorphisms in the \textit{ABCB1}, \textit{CYP2B6 CYP3A4} and \textit{CYP3A5} predicted nevirapine pharmacokinetics.

**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 \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{SLCO1B1} and lopinavir pharmacokinetics. Polymorphisms in the \textit{ABCB1}, \textit{CYP2B6 CYP3A4} and \textit{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 (Cmin), 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
SUMMARY

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, C_{min} and AUC and genetic polymorphisms in \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{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
SUMMARY

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**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>95%CI</td>
<td>95 Percent Confidence Interval</td>
</tr>
<tr>
<td>3TC</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-Binding Cassette</td>
</tr>
<tr>
<td>ALAG1</td>
<td>Absorption Lag Time</td>
</tr>
<tr>
<td>ACTG</td>
<td>AIDS Clinical Trail Group</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
</tr>
<tr>
<td>AICw</td>
<td>Weighted-Akaike Information Criterion</td>
</tr>
<tr>
<td>APO</td>
<td>Apolipoproteins</td>
</tr>
<tr>
<td>ATV</td>
<td>Atazanavir</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral Therapy</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretrovirals</td>
</tr>
<tr>
<td>AZT</td>
<td>Zidovudine</td>
</tr>
<tr>
<td>BID</td>
<td>Bis in Die(Twice Daily)</td>
</tr>
<tr>
<td>BLQ</td>
<td>Below The Limit of Quantification</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the Concentration Time Curve</td>
</tr>
<tr>
<td>CD4+</td>
<td>CD4+ T Lymphocytes</td>
</tr>
<tr>
<td>CL/F</td>
<td>Clearance</td>
</tr>
<tr>
<td>CYPs</td>
<td>Cytochrome P450 Enzymes</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CV</td>
<td>Co-efficient of Variation</td>
</tr>
<tr>
<td>C_{max}</td>
<td>Maximum Concentration</td>
</tr>
<tr>
<td>C_{min}</td>
<td>Minimum Concentration</td>
</tr>
<tr>
<td>EBEs</td>
<td>Empirical Bayes Estimates</td>
</tr>
<tr>
<td>EC_{50}</td>
<td>Half Maximal Effective Concentration</td>
</tr>
<tr>
<td>EFV</td>
<td>Efavirenz</td>
</tr>
<tr>
<td>EMB</td>
<td>Expectation Maximum Likelihood Bootstrap</td>
</tr>
<tr>
<td>FM</td>
<td>Fast Metabolizers</td>
</tr>
<tr>
<td>FO</td>
<td>First Order</td>
</tr>
<tr>
<td>FOCE</td>
<td>First Order Conditional Estimation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>FOCEI</td>
<td>First Conditional Estimation with Interaction</td>
</tr>
<tr>
<td>GCV</td>
<td>Generalized Cross Validation</td>
</tr>
<tr>
<td>GIQ</td>
<td>Growth Inhibitory Quotient</td>
</tr>
<tr>
<td>GOFs</td>
<td>Goodness of Fit Plots</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome Wide-Association Studies</td>
</tr>
<tr>
<td>HAZ</td>
<td>Height for Age Z-Scores</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune-Deficiency Virus</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
</tr>
<tr>
<td>hr</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>HSR</td>
<td>Hypersensitivity Reaction</td>
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<tr>
<td>IIV</td>
<td>Inter-individual Variability</td>
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<tr>
<td>IM</td>
<td>Intermediate Metabolizers</td>
</tr>
<tr>
<td>IOV</td>
<td>Inter-occasion Variability</td>
</tr>
<tr>
<td>IPREDs</td>
<td>Individual Predications</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>IWRES</td>
<td>Individual Weighted Residuals</td>
</tr>
<tr>
<td>KA</td>
<td>Absorption Rate Constant</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>KTR</td>
<td>Transit Time Rate Constant</td>
</tr>
<tr>
<td>LAM</td>
<td>Lopinavir Associated Mutations</td>
</tr>
<tr>
<td>L/kg</td>
<td>Litres per Kilogram</td>
</tr>
<tr>
<td>LMS</td>
<td>Lopinavir Associated Scores</td>
</tr>
<tr>
<td>LPV</td>
<td>Lopinavir</td>
</tr>
<tr>
<td>LPV/r</td>
<td>Ritonavir-Boosted Lopinavir</td>
</tr>
<tr>
<td>PAM</td>
<td>Protease Inhibitor Associated Mutations</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>Pg-p</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PI</td>
<td>Protease Inhibitor</td>
</tr>
</tbody>
</table>
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/m²       Milligram Per Metres Squared
mL          Millilitre
mL/hr/kg    Millilitre Per Hour Per Kilogram
MTT         Mean Transit Time
NPDE        Normalized Prediction 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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TDM</td>
<td>Therapeutic Drug Monitoring</td>
</tr>
<tr>
<td>UGTs</td>
<td>Uridine 5' Diphospho-Glucuronosyltransferases</td>
</tr>
<tr>
<td>USM</td>
<td>Ultra Slow Metabolizer</td>
</tr>
<tr>
<td>V/F</td>
<td>Volume of Distribution</td>
</tr>
<tr>
<td>VL</td>
<td>Viral Load</td>
</tr>
<tr>
<td>VPC</td>
<td>Visual Predictive Check</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WAZ</td>
<td>Weight for Age Z-scores</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The research presented in this thesis materialised through the help of a number of people that I owe huge amount of gratitude.

I am very grateful to my main supervisor Professor Helen McIlreron, Division of Clinical Pharmacology University of Cape Town, I’m hugely indebted to you for guidance, mentorship and patience so that I can achieve this feat.

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My daughter Liakae Moholisa, you are the shining light of my life

To Bakoena BaMolibeli for giving birth to me and Ramasedi for the gift of the sun and enlightenment.

Lastly to my funders; National Research Foundation, Carnegie Foundation, HWSETA and University of Cape Town.
This Thesis is based on the following Manuscripts


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”.


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”.


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 McIIlerson and Dr Michael Schomaker.
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
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................................................... i
SUMMARY ............................................................................................................................................... iii
LIST OF ABBREVIATIONS ...................................................................................................................... vii
ACKNOWLEDGEMENTS ......................................................................................................................... xi
PUBLICATIONS ...................................................................................................................................... xii
DECLARATION OF WORK ......................................................................................................................... xiii
PERMISSION FROM CO-AUTHORS ........................................................................................................ xiv

Chapter 1
Introduction.................................................................................................................................................. 1
1.1 Antiretroviral Therapy in Children .................................................................................................... 1
1.1.1 Classes of Antiretroviral Drugs Used for Antiretroviral Therapy .............................................. 2
1.1.2 Current Recommendations for Antiretroviral Therapy in Children ......................................... 4
1.2 Drugs of Interest in This Thesis ...................................................................................................... 4
1.2.1 Mechanism of Action of Lopinavir ............................................................................................. 5
1.2.2 Mechanism of Action of Nevirapine ......................................................................................... 6
1.3 Pharmacodynamics of Antiretroviral Drugs ................................................................................... 6
1.3.1 Pharmacodynamics of Lopinavir in Children ......................................................................... 7
1.3.2 Pharmacodynamics of Nevirapine in Children ....................................................................... 9
1.4 Pharmacokinetics of Lopinavir in Children .................................................................................. 10
1.5 Pharmacokinetics of Nevirapine in Children ................................................................................ 11
1.5.1 Therapeutic Drug Monitoring of Lopinavir ............................................................................. 14
1.5.2 Therapeutic Drug Monitoring of Nevirapine ......................................................................... 15
1.6 Therapeutic Drug Monitoring in Children ...................................................................................... 12
1.6.1 Therapeutic Drug Monitoring of Lopinavir ............................................................................ 14
1.6.2 Therapeutic Drug Monitoring of Nevirapine ......................................................................... 15
1.6 Adherence Monitoring ..................................................................................................................... 16
1.7 Pharmacogenetics ............................................................................................................................ 17
1.7.1 Pharmacogenetics of Protease Inhibitors ................................................................................. 19
1.7.1.1 Pharmacogenetics of Lopinavir ....................................................................................... 20
1.7.2 Pharmacogenetics of Non-Nucleoside Reverse Transcriptase Inhibitors .............................. 20
1.7.2.1 Pharmacogenetics of Nevirapine .................................................................................... 23
1.8 Pharmacometrics ............................................................................................................................. 24
1.8.1 Population Pharmacokinetic Modelling .................................................................................... 25
1.8.2 Model Estimation ....................................................................................................................... 27
TABLE OF CONTENTS

1.8.3 Model Validation ............................................................................................................. 28
1.9 Thesis Rationale and Aims ............................................................................................... 30
References .............................................................................................................................. 31

Chapter 2

2.1 Study Population ............................................................................................................. 55
2.1.1 Study Design ................................................................................................................... 55
2.1.2 Ethical Consideration ...................................................................................................... 57
2.1.3 Laboratory Methods ....................................................................................................... 57
2.1.3.1 Pharmacokinetic Assays ............................................................................................... 57
2.1.3.2 DNA Extraction ............................................................................................................. 57
2.3 Statistical Modelling .......................................................................................................... 59
2.3.1 Multiple Imputation in R Package Amelia II (Paper 1 and Paper 2) ......................... 59
2.3.1.1 How Amelia Works ...................................................................................................... 59
2.3.1.2 Assumptions in Amelia ................................................................................................. 60
2.3.1.3 The Amelia Algorithm .................................................................................................. 61
2.3.2 Cox Proportional Hazards Model for Multiple Failure Events (Paper 1 and Paper 2) .... 62
2.4 Pharmacometric Analyses ................................................................................................. 64
2.4.1 Population Pharmacokinetic Modelling (Paper 3 and Paper 4) ..................................... 64
References ............................................................................................................................... 67

Chapter 3

3.1 Abstract .............................................................................................................................. 69
3.1.1 Background: .................................................................................................................... 69
3.1.2 Methods: .......................................................................................................................... 69
3.1.3 Results: ............................................................................................................................ 69
3.1.4 Conclusions: .................................................................................................................... 70
3.2 Introduction ........................................................................................................................ 70
3.3 Methods ............................................................................................................................. 71
3.3.1 Study Participants ........................................................................................................... 71
3.3.2 Laboratory Methods ....................................................................................................... 72
3.3.3 Statistical Analysis ........................................................................................................... 72
3.4 Results ................................................................................................................................. 74
3.4.1 Study Population ............................................................................................................. 74
3.4.3 Predictors of Viral Load >400 copies/mL ........................................................................ 74
# TABLE OF CONTENTS

3.4.4 Non-linear Effect of Lopinavir Concentrations on the Risk of Viremia ........................................ 75

3.5 **Discussion** .................................................................................................................................. 75

3.6 **Acknowledgements:** .................................................................................................................. 78

3.7 **References** .................................................................................................................................. 78

Chapter 4

4.1 **Abstract** .................................................................................................................................... 86

4.1.1 Background: ................................................................................................................................. 86

4.1.2 Aim: ............................................................................................................................................... 86

4.1.3 Methods: ....................................................................................................................................... 86

4.1.4 Results: ......................................................................................................................................... 87

4.1.5 Conclusion: .................................................................................................................................. 87

4.2 **Introduction** ................................................................................................................................. 87

4.3 **Methods** ..................................................................................................................................... 88

4.3.1 Study Participants ......................................................................................................................... 88

4.3.2 Laboratory Methods ..................................................................................................................... 90

4.3.3 Statistical Analysis ....................................................................................................................... 90

4.4 **Results** ....................................................................................................................................... 92

4.4.1 Study Population .......................................................................................................................... 92

4.4.2 Plasma Lopinavir and Nevirapine Concentrations ........................................................................ 92

4.4.3 Predictors of Viremia (Viral load >50 copies/mL) in the LPV Group ........................................ 93

4.4.4 Predictors of Viremia (Viral load>50 copies/ml) in the NVP Group ........................................... 94

4.5 **Discussion** .................................................................................................................................. 95

4.6 **Acknowledgements:** .................................................................................................................. 98

4.7 **References** .................................................................................................................................. 98

Chapter 5

5.1 **Abstract** .................................................................................................................................... 112

5.1.1 Aims: ............................................................................................................................................. 112

5.1.2 Methods: ....................................................................................................................................... 112

5.1.3 Results: ......................................................................................................................................... 113

5.1.4 Conclusions: ................................................................................................................................. 113

5.2 **Introduction** .................................................................................................................................. 113

5.3 **Methods** ..................................................................................................................................... 114

5.3.1 Study Population .......................................................................................................................... 114
TABLE OF CONTENTS

5.3.2 Laboratory Analysis ....................................................................................................... 116
5.3.4 Genotyping .................................................................................................................... 116
5.3.5 LPV Model Development .............................................................................................. 117
5.3.6 Covariate Model ............................................................................................................ 118
5.3.7 Statistical Analysis ......................................................................................................... 118
5.4 Results .................................................................................................................................. 119
5.4.1 Study Population ........................................................................................................... 119
5.4.2 Genotyping .................................................................................................................... 119
5.4.3 LPV Model Description .................................................................................................. 120
5.4.4 Model Evaluation .......................................................................................................... 120
5.4.5 Association between Genetic Polymorphisms and Model Derived CL/F, AUC0-12 and Cmin ....................................................................................................................................... 121
5.5 Discussion ......................................................................................................................... 122
5.6 Acknowledgements: .......................................................................................................... 124
5.7 References ......................................................................................................................... 124

Chapter 6
6.1 Abstract ............................................................................................................................ 138
6.1.1 Aims: ............................................................................................................................. 138
6.1.2 Methods: ....................................................................................................................... 138
6.1.3 Results: .......................................................................................................................... 139
6.1.4 Conclusions: .................................................................................................................. 139
6.2 Introduction ...................................................................................................................... 139
6.3 Methods ............................................................................................................................ 141
6.3.1 Study Participants ......................................................................................................... 141
6.3.2 Quantification of Nevirapine in Plasma ........................................................................ 142
6.3.3 Genotyping .................................................................................................................... 142
6.3.4 Population Pharmacokinetic Analysis ........................................................................... 143
6.3.5 Structural Model ........................................................................................................... 144
6.3.6 Covariate Model ........................................................................................................... 144
6.3.7 Statistical Analysis ......................................................................................................... 144
6.4 Results .................................................................................................................................. 145
6.4.1 Study Participants ......................................................................................................... 145
6.4.2 Genetic Polymorphisms ................................................................................................. 145
6.4.3 Nevirapine Population Pharmacokinetic Model ......................................................... 146
### TABLE OF CONTENTS

6.4.4 Effects of ABCB1, CYP3A4, CYP3A5 and CYP2B6 Genetic Polymorphisms on Model-Derived Nevirapine Indices ................................................................. 147

6.5 **Discussion** ........................................................................................................ 149

6.6 **Acknowledgements:** ..................................................................................... 152

6.7 **References** ...................................................................................................... 152

**Chapter 7**

7.1 **Discussion** ...................................................................................................... 168

7.2 **Limitations** ..................................................................................................... 173

7.3 **Conclusions** .................................................................................................... 175

7.4 **Implications For Clinical Care and Practice** ..................................................... 176

7.5 **References** ..................................................................................................... 177

**Appendix I** ........................................................................................................... 212

**Appendix II** .......................................................................................................... 212

**Appendix III** ......................................................................................................... 258

**Appendix IV** .......................................................................................................... 302

**Appendix V** ............................................................................................................ 312
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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{5,6}. ART requires maintenance of adequate drug exposure and, high levels of adherence in order to prevent viral resistance and ART failure\textsuperscript{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\textsuperscript{2}. Currently available antiretroviral (ARVs) drugs either block replication within the infected cell or prevent entry into the cell\textsuperscript{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\textsuperscript{+} T lymphocyte count and/or percentage\textsuperscript{2,3}. These defined laboratory measurements are assessed at baseline and repeated after durations ranging mostly from 24 or 48 weeks post initiation ART\textsuperscript{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\textsuperscript{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\textsuperscript{2}. 
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 coreceptors (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 cell7. 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 nucleus7.

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. 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.

Table 1: Different classes and types of antiretroviral drugs

<table>
<thead>
<tr>
<th>Antiretroviral Class</th>
<th>Site of Action</th>
<th>Representative Antiretroviral Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside Reverse Transcriptase Inhibitors (NRTIs)</td>
<td>Interrupt the HIV replication cycle via competitive inhibition of HIV reverse transcriptase and termination of the DNA chain</td>
<td>Lamivudine (3TC), Zidovudine (AZT), Didanosine (DDI), Stavudine (4DT), Abacavir (ABC), Tenofovir (TDF), Entricitabine</td>
</tr>
<tr>
<td>Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)</td>
<td>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</td>
<td>Nevirapine (NVP), Efavirenz (EFV), Etravirine, Rilpivirine</td>
</tr>
<tr>
<td>Protease Inhibitors (PIs)</td>
<td>Function as competitive inhibitors that directly bind to HIV protease and prevent subsequent cleavage of polypeptide</td>
<td>Lopinavir (LPV), Atazanavir (ATV) Fosamprenavir, Ritonavir (RTV) Tipranavir, Saquinavir (SQV), Indinavir (IDV)</td>
</tr>
<tr>
<td>Fusion or Entry Inhibitors</td>
<td>Act extracellularly to prevent the fusion or entry of HIV to the CD4 or other target cell</td>
<td>Enfuvirtide Maraviroc</td>
</tr>
<tr>
<td>Integrase Inhibitors</td>
<td>Competitively inhibit the strand transfer reaction by binding metallic ions in the active site of the enzyme</td>
<td>Elvitegravir Dolutegravir Raltegravir</td>
</tr>
</tbody>
</table>
Chapter 1
Literature Review

1.1.2 Current Recommendations for Antiretroviral Therapy in Children

The current South African guidelines\textsuperscript{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\textsuperscript{6} similarly recommend that for children <3 years 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\textsubscript{50}=6.5nM) is a second generation PI used both in drug naïve and drug experienced patients. LPV (\textbf{Figure 2} was developed by Abbot Laboratories USA and is marketed co-formulated with ritonavir (LPV/r) under the name Kaletra\textsuperscript{®} or Aluvia\textsuperscript{®} since 2000. LPV inhibits HIV-1 protease with an EC\textsubscript{50} of 17nM\textsuperscript{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.

\textbf{Figure 2:} Chemical Structure of Lopinavir
Chapter 1
Literature Review

NVP (MW=266.30 g/mol, EC\textsubscript{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\textsuperscript{12} (Figure 3). NVP is slightly soluble in water at neutral pH and is insoluble in nonpolar solvents\textsuperscript{13}. Currently NVP is marketed as Viramune\textsuperscript{®} (Boehringer Ingelheim, Germany) and it is available as a 200mg tablet and a 10 mg/ml oral suspension\textsuperscript{14}.

![Figure 3: Structure of Nevirapine](image)

1.2.1 Mechanism of Action of Lopinavir

LPV is a highly potent and specific inhibitor of HIV-1 protease\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{15}. LPV mean EC\textsubscript{50} ranged from 4-11nmol/L in the absence of serum against several isolates of HIV-1 subtype B\textsuperscript{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)\textsuperscript{22}. Moreover, 0.5nmol/L LPV inhibited 93% of the wild-type virus protease activity and displayed a $\geq10^{5}$-fold specificity to HIV protease more than mammalian proteases renin and cathepsin D and E in vitro\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{12}. NVP penetrates cell free virions and thus inactivates virion-associated reverse transcriptase \textit{in situ}\textsuperscript{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\textsuperscript{12}. NVP has been shown to inhibit many HIV-1 strains in vitro\textsuperscript{27}. Human T-lymphocyte cultures display 10 µg/L 50% inhibitory concentration (IC\textsubscript{50})\textsuperscript{27}. NVP has also been shown to be active against strains that are resistant to NRTIs\textsuperscript{28}. NVP has been shown to have synergistic antiviral activity when combined with PIs and/or NRTIs\textsuperscript{28}. NVP resistant HIV-1 isolates have been shown to have 100-250 fold decreased susceptibility to NVP in vitro\textsuperscript{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\textsuperscript{30,31}. Thus, resistant strains develop, when NVP is as monotherapy, within weeks\textsuperscript{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\textsuperscript{14,33}.

1.3 Pharmacodynamics of Antiretroviral Drugs

Pharmacodynamics (PD) is broadly defined as “what the drug does to the body” \textsuperscript{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\textsuperscript{35}. It thus becomes very important to incorporate the knowledge of ARV PD to optimise their use.
Chapter 1

Literature Review

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. 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. Immunological surrogates are usually tied to prediction of opportunistic infections and survival, whereas virological markers are used to predict treatment success or failure. Typically, both these markers are measured only after 12, 24 or 48 weeks post initiation therapy. Consequently, numerous strategies have been proposed to predict ART outcomes even before the first dose, including therapeutic target concentrations and inhibitory quotients (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 [Cmax], area under the concentration time curve [AUC], and trough plasma concentrations [Ctrough/min]) similar to those found in adults. 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.

1.3.1 Pharmacodynamics of Lopinavir in Children

The target LPV Cmin 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. Studies in children, have shown that low LPV Cmin (<1.0 mg/L) is associated with increased risk of virologic failure. Similar to adult data, minimum target plasma Cmin of LPV and other PIs are recommended for treatment-naïve children, and higher target trough concentrations are required in PI-experienced paediatric patients. A LPV population PK model of treatment experienced children aged 4-18 years found a median Cmin of 5.9 mg/L lower than that found reported in adults, even though the children received 20% higher doses recommended in
Chapter 1

Literature Review

children. 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. 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. 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. Furthermore, low-dose LPV/r is inappropriate for PI-experienced children. 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 Cmin to IC50 (concentration of drug required for 50% inhibition of viral replication in vitro). The virtual GIQ uses the fold-change in virtual IC50 (derived from the genotype), multiplied by a reference wild-type protein-adjusted IC50 and the normalized GIQ is the patient-specific GIQ divided by a reference GIQ calculated as the ratio of typical Cmin for a given dose and wild-type viral IC50, 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). 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. 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. 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. Except for poor palatability and gastrointestinal intolerance, LPV is well tolerated by children. 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. 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.

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. Maintaining NVP Cmin above 3.0 mg/L has been associated with long term virological suppression. Recently, as study in 322 African children showed that children with a Cmin <3.0 mg/L had increased hazard of virological non-suppression. Moreover, some children and adult data showed that a higher NVP Cmin of >4.3 mg/L reduced emergence of resistance mutations compared with lower concentrations (3-4.3 mg/L). 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. Sub-therapeutic nevirapine concentration were more pronounced in children <8 years of age; supporting the evidence that younger children metabolized NVP more rapidly than older children. However, there was no clinically relevant effect of NVP concentration on virological outcome (viral load≥200 copies/mL). 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. The most common side effect associated with NVP concentrations is skin rash, whereas NVP-associated hepatotoxicity is not associated with NVP plasma concentrations.

1.4 Pharmacokinetics of Lopinavir in Children

Several studies have been completed that assessed the PK of LPV/r in a capsule formulation. The corrected protein binding steady-state concentrations of LPV required to inhibit HIV replication by 50% (EC50) were shown to be 0.07 µg/mL in two studies with different dosing regimens. 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. Both the soft-gelatin capsule and the tablet formulations comparatively displayed serum PK Cmin values of 5.17 µg/mL and 5.64 µg/mL respectively as well as respective Cmax values of 6.97 µg/mL and 10.26 µg/mL. However, this differences were not statistically significant.

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. 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. 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 α1 acid glycoprotein. The mean plasma Cmax of LPV is 9.8±3.7 µg/mL, occurring approximately 4 hours post dose. The mean trough concentration is 5.2 µg/mL with the mean elimination half-life (T1/2) 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. Examining the penetration of drugs such as LPV is important due to the fact that HIV replication occurs at intracellular level. 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. A study found that following 400/100mg LPV/r capsules, the plasma and peripheral
Chapter 1

Literature Review

blood mononuclear cells, EC$_{50}$ was reached with a $C_{\text{max}}$ 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 $C_{\text{max}}$ 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%$^{12,84}$.
Chapter 1
Literature Review

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. 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. 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. 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. 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 $C_{\text{max}}$ compared to males. Moreover, NVP PK is not significantly affected by age (range 18-68 years). However, NVP PK is significantly associated with race, with black people having 39% higher concentrations that Caucasians.

In newborn infants, NVP washout elimination is extensive and highly variable with median $T_{1/2}$ of 64.9 hours in two studies. 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). In new-borns, absorption was variable and extensive with $T_{\text{max}}$ of 8.2 hours (range 2-26.1 hours). In older infants, NVP clearance is rapid, averaging around 120 ml/h/kg, during the first 2 years of life. Thereafter, NVP clearance decreases gradually to an average of 60ml/h/kg by 8-10 years of age.

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. 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.
Chapter 1
Literature Review

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\textsuperscript{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\textsuperscript{2} LPV/r regimen BID plus SQV 50mg/kg. In 19 children, the PK showed a median C\textsubscript{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\textsubscript{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\textsuperscript{40}.

Another prospective study of 126 PI-experienced patients was done to explore the utility of GIQ by predicting virological response to LPV therapy\textsuperscript{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\textsubscript{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\textsuperscript{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\textsuperscript{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\textsubscript{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\(_{\text{min}}\) <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\(_{\text{min}}\). Previous guidelines were based on the median C\(_{\text{min}}\) value found in the INCAS trail, however findings the studies by Vries-Sluijs \textit{et al}, Duong \textit{et al} and the 2NN trail suggest the C\(_{\text{min}}\) cut-off to be 3.0mg/L.\(^{40,50,53}\)
1.6 Adherence Monitoring

Adherence to ART directly correlates with clinical and virological outcomes.\textsuperscript{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\%\textsuperscript{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\textsuperscript{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\textsuperscript{122–125}. Moreover, in Sub-Saharan Africa conditions of extreme poverty and livelihood insecurities add a further dimension to adherence costs\textsuperscript{125}. Another factor that compounds to this problem include non-disclosure due to fear of stigmatization\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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
Chapter 1

Literature Review

resistance, prior treatment failure or poor absorption of drugs. In the literature, discordance has been shown between viral suppression and adherence in those contexts\textsuperscript{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\textsuperscript{130}. Single nucleotide polymorphisms (SNPs) are defined as sequence variations occurring in human DNA as a single nucleotide changes at allele frequencies greater 1%\textsuperscript{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\textsuperscript{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
Chapter 1
Literature Review

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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{135,136}. 
Table 2: Genetic polymorphisms and the clinical relevance of proteins involved in PI metabolism and disposition

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

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<sup>148–150</sup>.

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<sup>151–156</sup>. 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<sup>157</sup>. 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)<sup>149</sup>. Both NVP and EFV are extensively metabolised by highly polymorphic CYP2B6 enzyme<sup>158</sup>. 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\textsuperscript{149}. Amid various alleles, the CYP2B6*6 haplotype (516 G $\rightarrow$ T and 785 A $\rightarrow$ 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\textsuperscript{159–162}. Moreover, though the CYP2B6*16 (785 A $\rightarrow$ G, 983 T $\rightarrow$ C) or CYP2B6*18 (983 T $\rightarrow$ C) polymorphisms are common in black populations and leads to decreased protein expression, they do not affect its intrinsic catalytic activity\textsuperscript{163}.

NVP is predominantly metabolized by CYP3A4 and 2B6 with a minor contribution from CYP3A5 to its hydroxynevirapine metabolites\textsuperscript{164}. EFV is primarily CYP2B6 with a minor contribution from CYP3A4\textsuperscript{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\textsuperscript{134,166}. 
## Chapter 1

### Literature Review

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene(Protein)</th>
<th>Variant</th>
<th>PK Effect</th>
</tr>
</thead>
</table>
| NVP  | CYP3A4        | *1B or -392A→G | *1/*1B: Higher C<sub>min</sub><sup>146</sup>  
Not predictive of hepatoxicity<sup>167</sup> |
|      | CYP3A5        | *1 or 6986A→ | Higher AUC<sup>168</sup>  
No association with C<sub>min</sub><sup>167</sup>  
Not predictive of hepatoxicity<sup>167</sup> |
|      | CYP2B6        | 516G→T   | Increased plasma levels<sup>164,169</sup> |
|      |               | 983T→C   | Increased plasma levels<sup>170,171</sup> |
|      |               | 1459C→T  | Decreased plasma levels<sup>146</sup> |
|      | ABCB1(Pg-p)   | 3435TC→T | TT: Higher C<sub>min</sub><sup>172</sup>  
Decreased risk of hepatoxicity<sup>173,174</sup> |
|      | MRP7(ABCC10)  | c.28473T→C | CC: Lower plasma levels<sup>175</sup> |
| EFV  | CYP3A4        | *1B or -392A→G | GG: higher EFV AUC<sup>146</sup> |
|      | CYP3A5        | *1 or A6986 | No association with plasma levels<sup>160</sup> |
|      | CYP2B6        | 516C→T   | TT: High plasma levels<sup>136</sup>  
Increased risk of CNS adverse events<sup>176,177</sup> |
|      |               | 785A→G   | High plasma levels<sup>178–180</sup>  
Increased risk of CNS adverse events<sup>160</sup> |
|      |               | 983T→C   | High plasma levels<sup>163,181,182</sup>  
Increased risk of CNS adverse events<sup>176</sup> |
|      | ABCB1(Pg-p)   | 3435TC→T | Dispute in the literature regarding influence on plasma EFV levels<sup>183,184</sup>  
Decreased likelihood of virological failure and decrease emergence of resistant virus<sup>185</sup>  
TT: affect plasma levels<sup>146</sup> |
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\textsuperscript{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\textsuperscript{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 \textit{CYP2B6} metabolism, whereas hepatotoxicity is most likely mediated by MHC class II and unaffected by \textit{CYP2B6} polymorphism.\textsuperscript{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 \textit{HLA-DRB1*01:01} variant and CD4\(^+\) T lymphocyte count greater than 25\% prominently increases the risk of developing NVP associated HSR and hepatotoxicity\textsuperscript{190,191}. Similarly, other HLA class alleles such as \textit{HLA*B14:02}, \textit{HLA-Cw08} and \textit{HLA-B*35:05} have been associated with NVP associated drug reactions\textsuperscript{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\textsuperscript{195}. Interestingly, a GWAS study was conducted in Thai population whereby genetic variations in the \textit{CCHCR1} gene were strongly associated with NVP-induced rash\textsuperscript{196}.

NVP is metabolized into its major metabolites 2- and 3-hydroxynevirapine, respectively, mainly by CYPs 3\textit{A4} and 2\textit{B6}\textsuperscript{136,164}. In the literature, several studies have shown that the 516 \textit{G}→\textit{T} and 983\textit{T}→\textit{C} polymorphisms in the \textit{CYP2B6} gene are associated with NVP PK in ethnically diverse populations\textsuperscript{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 \textit{G}→\textit{T} polymorphism, 1.86 L/h compared to 2.95 L/h in patients with wild type allele\textsuperscript{198}. Additionally, Mahungu et al showed that the 516 \textit{G}→\textit{T} significantly predicted NVP trough concentrations\textsuperscript{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.\textsuperscript{154,203}

1.8.1 \textbf{Population Pharmacokinetic Modelling}

Population pharmacokinetic (PopPK) modelling involves using nonlinear mixed effects model(s) to simultaneously evaluate data from individuals in a population.\textsuperscript{204,205} (Table 4 presents advantages and disadvantages of PopPK). “Nonlinear” can be defined as dependent variable (e.g. drug concentration) being non-linearly 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:

\[ Y_{ijk} = f(X_{ijk}, P_{jk}) \] \hspace{1cm} \text{Equation (1)}

Where \( Y_{ijk} \) is the \( j \)th observation of the dependent variable (usually drug concentrations) at the \( k \)th 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.\textsuperscript{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.\textsuperscript{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
Chapter 1
Literature Review

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:

\[ P_{jk} = \theta_j * e^{\eta_j + k_j} \]

Equation(2)

where \( P_{jk} \) is the individual parameter at the k occasion, \( \theta_j \) is the typical parameter estimate and \( \eta_j \) and \( k_j \) 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 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:

\[ Y_{ijk} = f(X_{ijk}) + \varepsilon_{ijk} \]

Equation(3)
Chapter 1

Literature Review

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

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

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

1.8.2 Model Estimation

Estimating PopPK can be done in a number of software packages, all of which are based on hierarchical nonlinear mixed effects modelling\textsuperscript{208,209}. In this thesis, NONMEM\textsuperscript{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<0.05211–214. 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 nonlinearly215. Nonetheless, in NONMEM, this handled by using approximations of the nonlinear model and also involve linearization of the random effects216. 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 defined217,218. 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 NONP218.

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 model219. There is numerous diagnostic graphical approaches, some of which rely on simulation to evaluate
Chapter 1

Literature Review

different aspects of model adequateness\textsuperscript{220}. Moreover, each diagnostic approach has
assumptions, strengths and weaknesses\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 reused (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 1

Literature Review


Chapter 1
Literature Review


Chapter 1
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Literature Review


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Literature Review


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 pediatric 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 (>2×times/year) or prolonged (>4 weeks) 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 pediatric 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**

<table>
<thead>
<tr>
<th>Time (months) after treatment</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>9 (if needed)</th>
<th>12 (if needed)</th>
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<tbody>
<tr>
<td>Weeks →</td>
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<tr>
<td>Time –1</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Ye</td>
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<td>No</td>
<td>Ye</td>
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<td>2</td>
<td>No</td>
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<td>No</td>
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<tr>
<td>6</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>12</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

 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**

<table>
<thead>
<tr>
<th>Months after randomized treatment (^1) started →</th>
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<th>2</th>
<th>4</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
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</thead>
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<tr>
<td>Weeks →</td>
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<tr>
<td>Time 0-R</td>
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<td>Yes</td>
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<tr>
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<td>Yes</td>
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</tr>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>2</td>
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<td>Yes</td>
<td>Yes</td>
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<td>4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>6</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>Yes</td>
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<td>9</td>
<td>Yes</td>
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<tr>
<td>12</td>
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<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
</tr>
</tbody>
</table>

 ALT, alanine transferase; FBC diff**, differential full blood count; HIV RNA, human immunodeficiency virus ribonucleic acid
Chapter 2
General Methods

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
Chapter 2
General Methods

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°C before further analysis.

Figure 1: Flow chart of the DNA purification using the QIAsymphony Automated Technology
Chapter 2
General Methods

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 nature 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 \((n \times k)\) dataset are denoted as \( D \) (with observed part \( D^{obs} \) and unobserved part \( D^{mis} \)), then this assumption is:

\[
D \sim \mathcal{N}(\mu, \Sigma) \tag{1}
\]

Stating that \( D \) has a multivariate normal distribution with mean vector \( \mu \) and covariance matrix \( \Sigma \). 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\(^3\). 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 \( m_{ij} = 1 \) if \( dij \in D^{mis} \) and \( m_{ij} = 0 \) 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:

\[
\rho(M|D) = \rho(M|D^{obs}) \tag{2}
\]

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.
Chapter 2
General Methods

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, \( \theta = (\mu, \Sigma) \). 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 \( \rho(D^{obs}, M | \theta) \). Using the MAR assumption, this can be broken up as:

\[
\rho(D^{obs}, M | \theta) = \rho(M | D^{obs}) \rho(D^{obs} | \theta) \tag{3}
\]

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

\[
L(\theta | D^{obs}) \propto \rho(D^{obs} | \theta) \tag{4}
\]

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

\[
\rho(D^{obs} | \theta) = \int \rho(D | \theta) dD^{mis} \tag{5}
\]

With this likelihood and a flat prior on \( \theta \), then the posterior is

\[
\rho(\theta | D^{obs}) \propto \rho(D^{obs} | \theta) \tag{6}
\]

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 (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. 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 \( \theta \), which is a linear regression with parameters that can be calculated directly from \( \theta \).
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:

\[
\log(h_i(t)) = \alpha + \beta_1 X_{i1} + \beta_2 X_{i2} + \cdots + \beta_k X_{ik} \]

\text{Figure 2: Schematic of Multiple Imputation Approach with the EMB Algorithm, Adapted from Honaker et al 2011}
or equivalently,

\[ h_i(t) = \exp(\alpha + \beta_1 X_i + \beta_2 X_i^2 + \cdots + \beta_k X_i^k) \]

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, \( \alpha \) log-baseline hazard, since \( \log h_i(t) = \alpha \) [or \( h_i(t) = e^\alpha \)] when all of the covariates (\( x \)) are 0. In contrast, the Cox model leaves the baseline hazard function \( \alpha(t) = \log h_0(t) \) unspecified:

\[ \log h_i(t) = \alpha(t) + \beta_1 X_i + \beta_2 X_i^2 + \cdots + \beta_k X_i^k \]  
\[ \tag{8} \]

or equivalently,

\[ h_i(t) = h_0(t) \exp(\alpha + \beta_1 X_i + \beta_2 X_i^2 + \cdots + \beta_k X_i^k) \]  
\[ \tag{9} \]

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 \( i_0 \) that differ in their \( x \)-values, with the corresponding linear predictors \( \eta \) independent of time \( t \).

\[ \eta_i = \beta_1 X_i + \beta_2 X_i^2 + \cdots + \beta_k X_i^k \]  
\[ \tag{10} \]

And

\[ \eta_i' = \beta_1 X_i + \beta_2 X_i^2 + \cdots + \beta_k X_i^k \]  
\[ \tag{11} \]

The hazard ratio for these two observations,

\[ \frac{h_i(t)}{h_{i'}(t)} = \frac{h_0(t)e^{\eta_i}}{h_0(t)e^{\eta_i'}} \]  
\[ \tag{12} \]

\[ = \frac{e^{\eta_i}}{e^{\eta_i'}} \]

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
Chapter 2
General Methods

(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. 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 \texttt{coxph} function (located in the survival package).

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. 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\textsuperscript{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:

$$k_{tr} = \frac{n + 1}{MTT} \text{..........................................................(13)}$$

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.:

$$P_{ij} = TV(P_{ij}) \times EXP^{\eta_{IP}} \text{..........................................................(14)}$$

where $P_{ij}$ is the jth pharmacokinetic parameter for the ith individual; TV($P_{ij}$) is the typical value of the jth population parameter, and $\eta_{ij}$ represents a random variable for the ith individual in the jth parameter(P) distributed with a mean of zero and variance of $\omega_{ij}$. 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:

$$C_{ij} = C_{ij}' \times (1 + \epsilon_1) + \epsilon_2$$

where $C_{ij}$ and $C_{ij}'$ are the $j$th observed and predicted blood concentrations for the $i$th individual, respectively. $\epsilon_1$ and $\epsilon_2$ are random variables distributed with a mean of zero and variances of $\sigma_1$ and $\sigma_2$, respectively.

For concentrations below the limit of quantification (BLQ), the Beal M5 method was used\textsuperscript{13}. For this method, whenever a measurement was recorded as being BLQ in the dataset, the observation was replaced with $\text{BLQ}/2$ 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:

\[
AUC = \frac{AMT \times BIO}{CL}
\]
\[
C_{min} = \frac{(BIO \times AMT)^{1/\tau}}{(V - (\tau - k) \times (1/(1 - Exp(-K \times 12)) - (1 - Exp(-\tau \times 12)))}
\]

Where $AUC$ is the area under curve from 0-12 hours, $AMT$ is the dose, $CL$ is the clearance. $C_{min}$ is the minimum concentration, $V$ is the volume of the central compartment, $K$ is the elimination rate constant, $\tau$ is the inter-dosing interval

References


Chapter 2
General Methods


Author Contributions

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

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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 McIIlerson: Oversaw overall analysis strategy planning and mentoring, guided the writing of the manuscript from beginning to end
Title: Plasma Lopinavir Concentrations Predict Virological Failure in a Cohort of South African Children Initiating a Protease-Inhibitor-Based Regimen

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\textsuperscript{1-3}. Failure to achieve virological suppression may be due to the presence of HIV quasispecies resistant to antiretroviral drugs\textsuperscript{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\textsuperscript{5,6}. LPV/r has a high barrier for the development of resistance. However, the oral suspension of LPV/r has poor palatability\textsuperscript{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\textsuperscript{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\textsuperscript{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.
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\textsuperscript{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\textsuperscript{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\textsuperscript{+} 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\textsuperscript{2} LPV/r (Kaletra\textsuperscript{®} 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\textsuperscript{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).
Chapter 3

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].
Chapter 3

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%CI: 0.93-0.98] for each 1 mg/L, p=0.002), and increased risk for pre-treatment HAZ (HR=2.24 [95%CI: 1.17-4.28] for moderate stunting, p=0.015; and HR=2.92 [95%CI: 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%CI: 1.19-4.05]) and severe stunting (HR= 0.45...
Chapter 3

[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 AIC_w, where the first model included lopinavir concentrations and the second model included recorded adherence. The results revealed that the AIC_w favors the model including lopinavir concentrations (AIC_w= 1220.7) compared with the model including adherence (AIC_w= 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 AIC_w and showed that the model with 1.0 mg/L cut-off (AIC_w=1326.34) described the data better than the model with 4.0 mg/L cut-off (AIC_w=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
Chapter 3

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 inhibitors9. 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 adults10.

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 failure19.

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 analyzed16. 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. 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. 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.

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.

3.6 Acknowledgements

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3.7 References


Chapter 3


Chapter 3


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

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

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

Data are in Median (IQR; Range), unless otherwise indicated. LPV, Lopinavir
Table 3: Cox Proportional Hazards regression analysis for failure to achieve virological suppression for crude and the adjusted models after multiple imputation of all covariates.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
<th>AICw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95%CI</td>
<td>P Value</td>
<td>HR 95%CI</td>
</tr>
<tr>
<td>lopinavir &gt;1.0 mg/L</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>lopinavir &lt;1.0 mg/L</td>
<td>2.3 1.63-3.26</td>
<td>0.0001</td>
<td>2.11 1.62-2.75</td>
</tr>
<tr>
<td>Age (&gt;9 months.)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Age (&lt;9 months.)</td>
<td>1.21 0.53-2.76</td>
<td>0.76</td>
<td>1.23 0.60-2.73</td>
</tr>
<tr>
<td>Pre-treatment WAZ(normal)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment WAZ(Moderate)</td>
<td>1.06 0.75-1.48</td>
<td>0.72</td>
<td>0.91 0.61-1.33</td>
</tr>
<tr>
<td>Pre-treatment WAZ(severe)</td>
<td>1.87 0.61-1.25</td>
<td>0.45</td>
<td>1.15 0.77-1.72</td>
</tr>
<tr>
<td>Pre-treatment HAZ(normal)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment HAZ(Moderate)</td>
<td>2.24 1.17-4.28</td>
<td>0.015</td>
<td>2.20 1.18-4.09</td>
</tr>
<tr>
<td>Pre-treatment HAZ(severe)</td>
<td>2.92 1.69-5.03</td>
<td>0.0001</td>
<td>2.83 1.66-4.82</td>
</tr>
<tr>
<td>Pre-treatment Log&lt;10 VL &lt;5</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment Log&lt;10 VL &gt;5</td>
<td>1.67 0.79-3.50</td>
<td>0.17</td>
<td>1.69 0.81-3.53</td>
</tr>
<tr>
<td>Pre-treatment CD4%&lt;25</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment CD4%&lt;25</td>
<td>1.09 0.69-1.72</td>
<td>0.71</td>
<td>1.15 0.74-1.77</td>
</tr>
<tr>
<td>WHO Stage(Early)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>WHO Stage(Moderate)</td>
<td>1.26 0.75-2.11</td>
<td>0.38</td>
<td>1.25 0.73-2.13</td>
</tr>
</tbody>
</table>
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 all covariates using lopinavir with a cut-off of 4mg/L

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
<th>AICw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95%CI</td>
<td>HR 95%CI</td>
<td>HR 95%CI</td>
</tr>
<tr>
<td>lopinavir &gt;4.0 mg/L</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>lopinavir &lt;4.0 mg/L</td>
<td>2.3 1.63-3.26</td>
<td>1.74 1.36-2.23</td>
<td>1.77 1.29-2.43</td>
</tr>
<tr>
<td>Age (&gt;9months.)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age (&lt;9months.)</td>
<td>1.21 0.53-2.76</td>
<td>1.23 0.60-2.73</td>
<td>0.50</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>WAZ(normal)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>1.06 0.75-1.48</td>
<td>0.91 0.61-1.33</td>
<td>0.63</td>
</tr>
<tr>
<td>WAZ(Moderate)</td>
<td>1.87 0.61-1.25</td>
<td>1.15 0.77-1.72</td>
<td>0.49</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>HAZ(normal)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>2.24 1.17-4.28</td>
<td>2.20 1.18-4.09</td>
<td>2.19 1.19-4.21</td>
</tr>
<tr>
<td>HAZ(Moderate)</td>
<td>2.92 1.69-5.03</td>
<td>2.83 1.66-4.82</td>
<td>2.83 1.64-4.87</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Log₁₀ VL &lt;5</td>
<td>1.67 0.79-3.50</td>
<td>1.79 0.88-3.63</td>
<td>1.58 0.72-3.44</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>CD4%≥25</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>1.02 0.68-1.53</td>
<td>1.14 0.73-1.78</td>
<td>0.46</td>
</tr>
<tr>
<td>WHO Stage(Early)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>WHO Stage(Moderate)</td>
<td>1.26 0.75-2.11</td>
<td>1.22 0.72-2.07</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Author Contributions

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


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

1Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, 2Centre for Infectious Diseases Epidemiology and Research, School of Public Health and Family Medicine, University of Cape Town, 3Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, 4Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, 5ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York, 6Institute 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\textsuperscript{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\textsuperscript{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/m² once daily for the first 2 weeks and thereafter at 200mg/m² 12 hourly. Children randomized to continue LPV/r (Kaletra® oral solution, Abbott Laboratories, USA), received doses of 230 mg/m² 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. 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 predetermined 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
congcentration 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\textsuperscript{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\textsuperscript{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)\textsuperscript{15}.

To graphically display the non-linear effects of LPV and NVP concentrations on the hazard of
viremia we used penalized splines\textsuperscript{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\textsuperscript{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. \textbf{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 (\textbf{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
Chapter 4

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
Chapter 4

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 study18, 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. 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.

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. 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\textsuperscript{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\textsuperscript{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\textsuperscript{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.
Chapter 4

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

National Institutes of Child Health and Human Development (NICHD) HD 47177 and Secure the Future Foundation RES 219(LK). South African National Research Foundation (RM, and grant 90729 to HM), Health and Welfare Sector Education and Training Authority and Carnegie Corporation (both RM). NIH grant UM1 AI 106701 through the AIDS Clinical Trials Group. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1 AI068634, UM1 AI068636 and UM1 AI106701. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

4.7 References


Chapter 4


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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Characteristics of children in the LPV group (n=99)</th>
<th>Characteristics of children in the NVP group (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Age(months)</td>
<td>20 (16-25)</td>
<td>18 (15-22.25)</td>
</tr>
<tr>
<td>VL ≤50 copies/ml</td>
<td>55 (56%)</td>
<td>53 (55%)</td>
</tr>
<tr>
<td>VL 51-400 copies/ml</td>
<td>44 (44%)</td>
<td>43 (45%)</td>
</tr>
<tr>
<td>CD4%</td>
<td>28.05 (21.65-35.20)</td>
<td>29.55 (22.95-36.70)</td>
</tr>
<tr>
<td>WAZ</td>
<td>-0.60 (-1.26 to 0.07)</td>
<td>-0.60 (-1.17 to 0.13)</td>
</tr>
<tr>
<td>HAZ</td>
<td>-3.18 (-3.97 to -1.97)</td>
<td>-2.80 (-2.10 to -4.05)</td>
</tr>
<tr>
<td>TB Treatment</td>
<td>86 (86%)</td>
<td>91 (94%)</td>
</tr>
<tr>
<td>No</td>
<td>13 (14%)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.
### Chapter 4

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>LPV Group</th>
<th>NVP Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks</strong></td>
<td>24(n=99)</td>
<td>50(n=94)</td>
</tr>
<tr>
<td></td>
<td>76(n=99)</td>
<td>100(n=98)</td>
</tr>
<tr>
<td></td>
<td>124(n=78)</td>
<td>150+(n=64)</td>
</tr>
<tr>
<td><strong>Median[Drug]mg/L</strong></td>
<td>8.92 (5.34-12.9)</td>
<td>9.19 (5.51-13.9)</td>
</tr>
<tr>
<td></td>
<td>10.20 (6.68-13.9)</td>
<td>9.69 (6.28-13.9)</td>
</tr>
<tr>
<td></td>
<td>11.60 (7.90-17.2)</td>
<td>11.90 (8.81-14.7)</td>
</tr>
<tr>
<td></td>
<td>9.1 (8.4-15.2)</td>
<td>9.51 (7.2-11.4)</td>
</tr>
<tr>
<td></td>
<td>11.2 (8.3-14.2)</td>
<td>11.85 (9.2-16.1)</td>
</tr>
<tr>
<td></td>
<td>11.4 (9.4-14.7)</td>
<td>11.85 (8.04-16)</td>
</tr>
<tr>
<td><strong>% Samples between LLQ and 1 mg/L for LPV, between LLQ and 3 mg/L for NVP</strong></td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td><strong>% BLQ</strong></td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Time after Dose(h)</strong></td>
<td>3 (2.50-4.25)</td>
<td>3.00 (2.18-4.00)</td>
</tr>
<tr>
<td></td>
<td>3.08 (2.33-4.25)</td>
<td>3.33 (2.25-4.25)</td>
</tr>
<tr>
<td></td>
<td>3.00 (2.25-3.75)</td>
<td>2.83 (2.00-3.67)</td>
</tr>
<tr>
<td></td>
<td>3.08 (2.25-3.92)</td>
<td>3.17 (2.58-4.17)</td>
</tr>
<tr>
<td></td>
<td>3.33 (2.50-4.17)</td>
<td>3.08 (2.25-4.17)</td>
</tr>
<tr>
<td></td>
<td>3.25 (2.58-3.88)</td>
<td>3.00 (2.33-4.00)</td>
</tr>
<tr>
<td><strong>Total Body Weight(kg)</strong></td>
<td>11 (10-12)</td>
<td>12 (11-13)</td>
</tr>
<tr>
<td></td>
<td>13 (12-14)</td>
<td>13 (12-16)</td>
</tr>
<tr>
<td></td>
<td>16 (15-18)</td>
<td>17 (16-19)</td>
</tr>
<tr>
<td></td>
<td>11 (10-13)</td>
<td>12 (10-13)</td>
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<tr>
<td></td>
<td>13(12-14)</td>
<td>14 (12-15)</td>
</tr>
<tr>
<td></td>
<td>15 (13-16)</td>
<td>17 (15-18)</td>
</tr>
<tr>
<td><strong>Dose(mg/m²)</strong></td>
<td>228 (222-232)</td>
<td>225 (219-231)</td>
</tr>
<tr>
<td></td>
<td>226 (221-231)</td>
<td>226 (223-232)</td>
</tr>
<tr>
<td></td>
<td>227 (223-231)</td>
<td>227 (223-231)</td>
</tr>
<tr>
<td></td>
<td>196 (193-200)</td>
<td>196 (192-200)</td>
</tr>
<tr>
<td></td>
<td>195 (192-198)</td>
<td>197 (193-199)</td>
</tr>
<tr>
<td></td>
<td>197 (192-199)</td>
<td>197 (195-200)</td>
</tr>
</tbody>
</table>

Data are in Median (IQR), unless otherwise stated; LLQ, lower limit of quantification; BLQ, below the assay limit of quantification; LPV, lopinavir; NVP, nevirapine.
Chapter 4

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude</th>
<th>95%CI</th>
<th>P Value</th>
<th>Adjusted</th>
<th>95%CI</th>
<th>P Value</th>
<th>Characteristic</th>
<th>Crude</th>
<th>95%CI</th>
<th>P Value</th>
<th>Adjusted</th>
<th>95%CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPV (mg/L)</td>
<td>0.95</td>
<td>0.92-0.98</td>
<td>&lt;0.01</td>
<td>0.96</td>
<td>0.94-0.99</td>
<td>0.01</td>
<td>LPV &lt;1 mg/L</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≥20 months</td>
<td>Reference</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td>LPV ≥1 mg/L</td>
<td>0.59</td>
<td>0.40-0.94</td>
<td>0.03</td>
<td>0.62</td>
<td>0.40-0.95</td>
<td>0.03</td>
</tr>
<tr>
<td>Age &lt;20 months</td>
<td>1.48</td>
<td>0.91-2.39</td>
<td>0.11</td>
<td>1.47</td>
<td>0.92-2.34</td>
<td>0.11</td>
<td>Age ≥20 months</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal WAZ</td>
<td>Reference</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td>Age &lt;20 months</td>
<td>1.48</td>
<td>0.91-2.39</td>
<td>0.34</td>
<td>1.47</td>
<td>0.92-2.34</td>
<td>0.09</td>
</tr>
<tr>
<td>Underweight</td>
<td>2.36</td>
<td>0.77-7.25</td>
<td>0.13</td>
<td>2.62</td>
<td>0.94-7.24</td>
<td>0.06</td>
<td>Normal WAZ</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal HAZ</td>
<td>Reference</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td>Underweight</td>
<td>2.36</td>
<td>0.77-7.25</td>
<td>0.13</td>
<td>2.90</td>
<td>1.04-8.42</td>
<td>0.05</td>
</tr>
<tr>
<td>Stunted</td>
<td>0.64</td>
<td>0.36-1.12</td>
<td>0.12</td>
<td>0.78</td>
<td>0.43-1.39</td>
<td>0.40</td>
<td>Normal HAZ</td>
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<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL ≤50</td>
<td>Reference</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td>Stunted</td>
<td>0.64</td>
<td>0.36-1.12</td>
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<td>0.76</td>
<td>0.43-1.34</td>
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</tr>
<tr>
<td>VL 51-400</td>
<td>2.62</td>
<td>1.62-4.24</td>
<td>&lt;0.01</td>
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<td>1.68-4.22</td>
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<td>VL ≤50</td>
<td>Reference</td>
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<tr>
<td>CD4% ≥25</td>
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<td></td>
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<td>Reference</td>
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<td>VL 51-400</td>
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<td>1.62-4.24</td>
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<td>CD4% &lt;25</td>
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<td>1.05</td>
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<td>CD4% ≥25</td>
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<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td>CD4% &lt;25</td>
<td>1.25</td>
<td>0.75-2.06</td>
<td>0.28</td>
<td>1.05</td>
<td>0.66-1.66</td>
<td>0.85</td>
</tr>
<tr>
<td>TB Treatment (Yes)</td>
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<td>0.13-1.05</td>
<td>0.07</td>
<td>0.41</td>
<td>0.15-1.15</td>
<td>0.09</td>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<td></td>
</tr>
<tr>
<td>TB Treatment (Yes)</td>
<td>0.36</td>
<td>0.13-1.05</td>
<td>0.07</td>
<td>0.41</td>
<td>0.15-1.15</td>
<td>0.09</td>
<td>TB Treatment (Yes)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude HR</th>
<th>95%CI</th>
<th>P Value</th>
<th>Adjusted HR</th>
<th>95%CI</th>
<th>P Value</th>
<th>Characteristic</th>
<th>Crude HR</th>
<th>95%CI</th>
<th>P Value</th>
<th>Adjusted HR</th>
<th>95%CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP (mg/L)</td>
<td>0.95</td>
<td>0.90-1.01</td>
<td>0.11</td>
<td>0.96</td>
<td>0.91-1.01</td>
<td>0.13</td>
<td>NVP ≥5mg/L</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<td>Age ≥18 months</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>NVP &lt;5mg/L</td>
<td>0.64</td>
<td>0.33-1.27</td>
<td>0.20</td>
<td>Age ≥18 months</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;18 months</td>
<td>1.31</td>
<td>0.69-2.47</td>
<td>0.42</td>
<td>1.48</td>
<td>0.77-2.84</td>
<td>0.26</td>
<td>Age &lt;18 months</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Normal WAZ</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>WAZ(normal)</td>
<td>1.28</td>
<td>0.37-4.44</td>
<td>0.69</td>
<td>WAZ(advanced)</td>
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<td>Reference</td>
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<td>Reference</td>
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</tr>
<tr>
<td>Underweight</td>
<td>1.28</td>
<td>0.37-4.44</td>
<td>0.69</td>
<td>1.39</td>
<td>0.34-5.62</td>
<td>0.64</td>
<td>Stunted</td>
<td>0.87</td>
<td>0.46-1.66</td>
<td>0.67</td>
<td>0.88</td>
<td>0.47-1.65</td>
<td>0.67</td>
</tr>
<tr>
<td>VL ≤50</td>
<td>Reference</td>
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<td>Reference</td>
<td>HAZ(normal)</td>
<td>Reference</td>
<td>Reference</td>
<td>VL &gt;50</td>
<td>1.69</td>
<td>0.91-3.16</td>
<td>0.09</td>
<td>1.75</td>
<td>0.92-3.35</td>
<td>0.09</td>
</tr>
<tr>
<td>CD4% ≥25</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>VL &gt;50</td>
<td>1.69</td>
<td>0.91-3.16</td>
<td>0.09</td>
<td>CD4% ≥25</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>CD4% ≥25</td>
<td>Reference</td>
</tr>
<tr>
<td>CD4% &lt;25</td>
<td>1.21</td>
<td>0.61-2.49</td>
<td>0.59</td>
<td>1.23</td>
<td>0.63-2.39</td>
<td>0.64</td>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
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<td>0.86</td>
<td>1.19</td>
<td>0.28-5.33</td>
<td>0.82</td>
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<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>TB Treatment (Yes)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>LPV Cut-off (mg/L)</th>
<th>GCV Value</th>
<th>NVP Cut-off (mg/L)</th>
<th>GCV Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.8363767</td>
<td>2</td>
<td>0.6384146</td>
</tr>
<tr>
<td>1</td>
<td>0.8362492</td>
<td>3</td>
<td>0.6384466</td>
</tr>
<tr>
<td>2</td>
<td>0.8382971</td>
<td>4</td>
<td>0.6396787</td>
</tr>
<tr>
<td>3</td>
<td>0.8376631</td>
<td>5</td>
<td>0.6355073</td>
</tr>
<tr>
<td>4</td>
<td>0.8407137</td>
<td>6</td>
<td>0.6374518</td>
</tr>
<tr>
<td>5</td>
<td>0.8393880</td>
<td>7</td>
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<tr>
<td>6</td>
<td>0.8448085</td>
<td>9</td>
<td>0.6385802</td>
</tr>
</tbody>
</table>
### Table 1: Cox Proportional Hazards regression analysis for the risk of viremia (VL>50/copies/mL) using previous visit lopinavir concentrations (n=99)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>LPV(mg/L)</td>
<td>0.98</td>
<td>0.96-1.01</td>
</tr>
<tr>
<td>Age ≥20 months</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;20 months</td>
<td>1.48</td>
<td>0.91-2.39</td>
</tr>
<tr>
<td>Normal WAZ</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Underweight</td>
<td>2.36</td>
<td>0.77-7.25</td>
</tr>
<tr>
<td>Normal HAZ</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Stunted</td>
<td>0.64</td>
<td>0.36-1.12</td>
</tr>
<tr>
<td>VL ≤50</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>VL 51-400</td>
<td>2.62</td>
<td>1.62-4.24</td>
</tr>
<tr>
<td>CD4% ≥25</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>CD4% &lt;25</td>
<td>1.25</td>
<td>0.75-2.06</td>
</tr>
<tr>
<td>TB Treatment (No)</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>TB Treatment (Yes)</td>
<td>0.36</td>
<td>0.13-1.05</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>LPV(mg/L)</td>
<td>0.94</td>
<td>0.91-0.98</td>
</tr>
<tr>
<td>Age ≥20 months</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;20 months</td>
<td>1.48</td>
<td>0.91-2.39</td>
</tr>
<tr>
<td>Normal WAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Underweight</td>
<td>2.36</td>
<td>0.77-7.25</td>
</tr>
<tr>
<td>Normal HAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Stunted</td>
<td>0.64</td>
<td>0.36-1.12</td>
</tr>
<tr>
<td>VL ≤50</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>VL 51-400</td>
<td>2.62</td>
<td>1.62-4.24</td>
</tr>
<tr>
<td>CD4% ≥25</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>CD4% &lt;25</td>
<td>1.25</td>
<td>0.75-2.06</td>
</tr>
<tr>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>TB Treatment(Yes)</td>
<td>0.36</td>
<td>0.13-1.05</td>
</tr>
</tbody>
</table>

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).

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

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>NVP(mg/L)</td>
<td>0.96</td>
<td>0.91-1.01</td>
</tr>
<tr>
<td>Age ≥18 months</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;18 months</td>
<td>1.31</td>
<td>0.69-2.47</td>
</tr>
<tr>
<td>Normal WAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Underweight</td>
<td>1.28</td>
<td>0.37-4.44</td>
</tr>
<tr>
<td>Normal HAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Stunted</td>
<td>0.87</td>
<td>0.46-1.66</td>
</tr>
<tr>
<td>VL ≤50</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>VL 51-400</td>
<td>1.69</td>
<td>0.91-3.16</td>
</tr>
<tr>
<td>CD4% ≥25</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>CD4% &lt;25</td>
<td>1.21</td>
<td>0.61-2.49</td>
</tr>
<tr>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>TB Treatment(Yes)</td>
<td>1.12</td>
<td>0.34-3.64</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>NVP (mg/L)</td>
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<td>0.88-1.01</td>
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<tr>
<td>Age ≥18 months</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;18 months</td>
<td>1.31</td>
<td>0.69-2.47</td>
</tr>
<tr>
<td>Normal WAZ</td>
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<tr>
<td>Underweight</td>
<td>1.28</td>
<td>0.37-4.44</td>
</tr>
<tr>
<td>Normal HAZ</td>
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<td>Reference</td>
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<tr>
<td>Stunted</td>
<td>0.87</td>
<td>0.46-1.66</td>
</tr>
<tr>
<td>VL ≤50</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>VL 51-400</td>
<td>1.69</td>
<td>0.91-3.16</td>
</tr>
<tr>
<td>CD4% ≥25</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>CD4% &lt;25</td>
<td>1.21</td>
<td>0.61-2.49</td>
</tr>
<tr>
<td>TB Treatment (No)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>TB Treatment (Yes)</td>
<td>1.12</td>
<td>0.34-3.64</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>Adherence (%)</td>
<td>0.98</td>
<td>0.93-1.06</td>
</tr>
<tr>
<td>Age ≥18 months</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age &lt;18 months</td>
<td>1.31</td>
<td>0.69-2.47</td>
</tr>
<tr>
<td>Normal WAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Underweight</td>
<td>1.28</td>
<td>0.37-4.44</td>
</tr>
<tr>
<td>Normal HAZ</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Stunted</td>
<td>0.87</td>
<td>0.46-1.66</td>
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<tr>
<td>VL ≤50</td>
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<td>Reference</td>
</tr>
<tr>
<td>VL 51-400</td>
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<td>0.91-3.16</td>
</tr>
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<tr>
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<tr>
<td>TB Treatment (Yes)</td>
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</table>

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
Chapter 4

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”

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

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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 McIlerson: 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.

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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\(_{\text{min}}\)). We explored associations between pharmacokinetic parameters and genotypes in selected genes relevant to LPV absorption and disposition.
Chapter 5

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, $C_{\text{min}}$ or $\text{AUC}_{0-12}$ 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 to 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, orsumucoid 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 P-gp) 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)\textsuperscript{12,13}. The \textit{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\textsuperscript{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 \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{SLCO1B1} 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\textsuperscript{15,16}. Treatment responses during both phases have been previously described\textsuperscript{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\textsuperscript{+} 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\textsuperscript{2} of ritonavir-boosted LPV (Kaletra\textregistered 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.
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 and initial response to LPV-based antiretroviral therapy. 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.
Chapter 5

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.
Chapter 5

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 version 7.3 (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. 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. 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 ($C_{\text{min}}$) 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. 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.
Chapter 5

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. 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 and haplotype phases were inferred using the standard E-M algorithm in PLINK. Linkage disequilibrium (LD) plots and values were generated with Haplovie (www.broad.mit.edu/mpg/haplovie/).
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\(\rightarrow\)T (rs1045642) and 4036A\(\rightarrow\)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\(\rightarrow\)C (rs4149056), rs4149032, 388\(\rightarrow\)G (rs2306283) and 463C\(\rightarrow\)A (rs11045819)
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\textsuperscript{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\textsubscript{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 5\textsuperscript{th}, 50\textsuperscript{th} and 95\textsuperscript{th} 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.
Association between Genetic Polymorphisms and Model Derived CL/F, AUC\textsubscript{0-12} and $C_{\text{min}}$

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, $C_{\text{min}}$, and AUC) were not normally distributed and therefore individual geometric means were calculated and used for subsequent analysis. The median $C_{\text{min}}$ was $4.27(3.42-5.29)$ mg/L and the median AUC\textsubscript{0-12} was $113.31(94.32-134.28)$ mg.h/L. There was no significant association between any polymorphism and LPV CL, $C_{\text{min}}$ 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 [$\beta$: -0.10 (-0.17 to -0.02, $P= 1.71 \times 10^{-02}$)], $CYP3A4$ rs473706 [$\beta$: -0.05 (-0.10 to -0.004, $P= 3.54 \times 10^{-02}$)], and 3 in $SLCO1B1$ rs73250843 [$\beta$: 0.12 (0.04-0.19, $P= 4.04 \times 10^{-03}$)], rs11045819 [$\beta$: -0.08 (-0.15 to -0.01 $P= 2.85 \times 10^{-02}$], and rs4149032 [$\beta$: -0.05 (-0.10 to -0.003, $P= 3.91 \times 10^{-02}$]).

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

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

This study evaluated associations between of \textit{CYP3A4}, \textit{CYP3A5}, \textit{ABCB1} and \textit{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\cite{21,26}. Our model estimates for both CL/F and V/F were consistent with values in previous studies\cite{21,22,27}. We also found results of model derived C\textsubscript{min} and AUC\textsubscript{0-12} similar to those in the literature\cite{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 \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{SLCO1B1} genes and LPV PK. LPV is a substrate of \textit{SLCO1B1}, and at least one genetic variant is known to affect LPV PK. Indeed reports have shown that 521T\textrarr;C (rs4149056) is associated with increased plasma LPV concentrations\cite{4,14,29,30}. In our study, we found no significant association between 56 polymorphisms in \textit{SLCO1B1} and LPV CL/F, C\textsubscript{min} and AUC\textsubscript{0-12} after adjusting for multiple comparisons. Specifically, we found no association between \textit{SLCO1B1} 521T\textrarr;C (rs4149056) and LPV PK (p-values 0.47, 0.67 and 0.70 for CL, C\textsubscript{min} and AUC\textsubscript{0-12}, respectively. This is mostly likely due to the low frequency of the 521CC allele. Only 1 patient had heterozygous \textit{SLCO1B1} 521CT genotype and the rest had wild type alleles.
We found nominally significant negative association between \textit{SLCO1B1} rs4149032 and LPV CL/F, but no associations were found between this polymorphism and LPV C\textsubscript{min} or AUC\textsubscript{0-12}. These data are in contrast with a previous report, which showed an association between \textit{SLCO1B1} rs4149032 and LPV PK\textsuperscript{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 \textit{SLCO1B1} 463C→A (rs11045819) and LPV CL/F, with positive association with LPV C\textsubscript{min} and AUC\textsubscript{0-12}. These data are in contrast with a previous report, which showed an association between \textit{SLCO1B1} rs4149032 or 463C→A (rs11045819) and increased LPV CL/F\textsuperscript{4}.

We found nominal association between \textit{SLCO1B1} 388A→G (rs2306283) and increased C\textsubscript{min} and AUC\textsubscript{0-12}. This is in contrast to previous reports which found no association between this polymorphism and LPV concentrations in children\textsuperscript{13} or adults\textsuperscript{14}. We found trends between \textit{SLCO1B1} rs112403792 and rs4149057 with increased LPV C\textsubscript{min} and AUC\textsubscript{0-12}. There are no previous reports regarding these trends and these polymorphisms.

There was no significant association between polymorphisms in \textit{ABCB1} and LPV CL/F, C\textsubscript{min} and AUC\textsubscript{0-12} after adjusting for multiple comparisons, similar to previous reports in children\textsuperscript{13} or adult studies\textsuperscript{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 \textit{CYP3A4} and/or \textit{CYP3A5} genes were associated with LPV CL, C\textsubscript{min} and AUC\textsubscript{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\textsuperscript{13,32}. 

123
Chapter 5

In summary, this is the first study to quantify effects of \textit{ABCB1}, \textit{CYP3A4}, \textit{CYP3A5} and \textit{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 \textbf{Acknowledgements:}

Grant support included AI069439, TR000445, AI077505 (DWH). We thank Paxton Baker and Cara Sutcliffe for genotyping work done at Vanderbilt Technologies for Advanced Genomics (VANTAGE).

5.7 \textbf{References}


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Chapter 5


Chapter 5


30. Hartkoorn RC, Kwan WS, Shallcross V, et al. HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenet Genomics.* 2010;20:112-120.


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

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<th>Characteristic</th>
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### Table 2: Final Model Parameter Estimates

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<th>Parameter</th>
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<tr>
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<td>--------</td>
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<td>26.51(9.68-31.75)</td>
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<tr>
<td>RIF Effect on CL/F [%]</td>
<td>59.5%</td>
<td>59.49%(50.95%-61.77%)</td>
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#### Variability

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<th>IIV CL</th>
<th>44.1%</th>
<th>42.15%(40.22%-46.30%)</th>
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<td>IIV V</td>
<td>28.8%</td>
<td>28.24%(20.08%-31.55%)</td>
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<td>IIV KA</td>
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<tr>
<td>IIV ALAG</td>
<td>26.9%</td>
<td>26.47%(20.06%-26.95%)</td>
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<tr>
<td>IIV F</td>
<td>42.9%</td>
<td>41.15%(41.11%-41.29%)</td>
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<td>IOV KA</td>
<td>32.3%</td>
<td>31.48%(31.39%-31.47%)</td>
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<td>IOV ALAG</td>
<td>46.7%</td>
<td>44.38%(43.68%-44.49%)</td>
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<tr>
<td>IOV F</td>
<td>37.9%</td>
<td>36.89%(36.57%-42.49%)</td>
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#### 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) |
| AUC₀₋₁₂[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
## Chapter 5

### Table 3: Genetic associations with lopinavir CL, C\textsubscript{min} and AUC

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<th>CHR</th>
<th>SNP(Gene)</th>
<th>β(95%CI, P Value)</th>
<th>CHR</th>
<th>SNP(Gene)</th>
<th>β(95%CI, P Value)</th>
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<th>SNP(Gene)</th>
<th>β(95%CI, P Value)</th>
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<td>rs73250843 (SLCO1B1)</td>
<td>0.12(-0.04 to 0.19, P=0.004)</td>
<td>12</td>
<td>rs112403792 (SLCO1B1)</td>
<td>0.73(0.30 to 1.16, P=0.001)</td>
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<td>16.60(7.17 to 26.04, P=0.0007)</td>
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<td>-0.10(-0.17 to -0.02, P=0.02)</td>
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<td>rs11045819 (SLCO1B1)</td>
<td>0.78(0.25 to 1.30, P=0.004)</td>
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<td>rs11045819 (SLCO1B1)</td>
<td>16.86(5.29 to 28.43, P=0.005)</td>
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<td>16.86(4.80 to 26.58, P=0.006)</td>
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<td>rs112108376 (SLCO1B1)</td>
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<td>11.82(-1.17 to 24.81, P=0.08)</td>
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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

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<th>Major Allele</th>
<th>Minor Allele Frequency</th>
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### Supplementary Figure 1: Depicts the goodness of fit plots of the final model

![Graphs showing goodness of fit plots](attachment://image.png)

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CHR, chromosome; SNP, single nucleotide polymorphism
Chapter 5

Supplementary Figure 2: Shows the linkage disequilibrium of ABCB1 gene

Supplementary Figure 3: Presents the linkage disequilibrium of CYP3A4 gene
Chapter 5

Supplementary Figure 3: Demonstrates the linkage disequilibrium of CYP3A5 gene

Supplementary Figure 4: Illustrates the linkage disequilibrium of SLCO1B1 gene
Equation 1

\[
\frac{CL}{Fstd} = CL/Fstd \times \left(\frac{TW\ BT}{12.5}\right)^{0.75}
\]

(1.1)

\[
\frac{V}{Fstd} = V/Fstd \times \left(\frac{TW\ BT}{12.5}\right)^1
\]

(1.2)

Equation 2

\[
PMA = AGE + (9/12)
\]

\[
TVPMA = MEDAGE + (9/12)
\]

\[
F = \frac{1}{\left(1 + \frac{PMA}{TVPMA + PMA50}\right)EXP^{-Hill}}
\]
Author Contributions

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

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

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

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.

Phumlal 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 McIlerson: Oversaw overall analysis strategy planning and mentoring,
guided the writing of the manuscript from beginning to end
Title: Associations between CYP2B6, CY3A4, CYP3A5 and ABCB1 Genotypes and Nevirapine Pharmacokinetics in HIV-Positive South African Children.

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

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

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\textsubscript{min} and AUC\textsubscript{0-12} in a univariate analysis and after adjusting for CYP2B6 516G→T. CYP2B6 15582C→T was associated with CL/F, C\textsubscript{min} and AUC\textsubscript{0-12} after adjusting for CYP2B6 516G→T and 983T→C. Polymorphisms in ABCB1 and CYP3A5 were independently associated with CL/F, C\textsubscript{min} and AUC\textsubscript{0-12}.

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\textsuperscript{1}. The success of NVP in African nations has been partly due its affordability and availability in fixed-dose combinations\textsuperscript{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\textsuperscript{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.

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. Previous studies have shown an association between the CYP2B6 516G→T (rs3745274) polymorphism and NVP concentrations in children and adults. Patients homozygous for CYP2B6 516TT genotype had lower oral clearance and higher trough concentrations. A less frequent CYP2B6 polymorphism, 983T→C (rs28399499), has been associated with increased steady-state exposure of NVP. Both CYP2B6 516T and 983C are more frequent with African ancestry than with European ancestry. 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. 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. CYP2B6 15582C→T has been associated with slower clearance (CL/F) of NVP in Cambodian adults, and longer time for plasma concentrations to fall below the protein-adjusted IC50 in women of African ancestry. 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\textsuperscript{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 \( \leq \) 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\textregistered 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\textsuperscript{+} T lymphocyte percentage (CD4\%). Weight-for-age z-score and height-for-age z-score at randomization were
calculated using WHO software. 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.

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\textsuperscript{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\textsuperscript{24}. A stepwise model building process was guided by changes in the objective function value (OFV; equivalent to \(-2 \log \text{likelihood}\)), 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≤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 $C_{\text{min}}$ and $\text{AUC}_{0-12}$ 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\textsuperscript{11}. One or two compartment disposition models with first-order absorption and elimination were tested, as well as delayed and transit-compartment absorption\textsuperscript{25}. Hepatic elimination of NVP was tested using a previously described semi-mechanistic well-stirred model\textsuperscript{26}. The hepatic model assumed the following parameters: i) fraction unbound (fu) of NVP of 40\%, hepatic plasma flow (QH) of 50 L/h\textsuperscript{27} and a liver volume (VH) of 1L\textsuperscript{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\textsuperscript{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\textsuperscript{29}. Maturation of intrinsic clearance (CL\textsubscript{int}) and pre-hepatic bioavailability (F\textsubscript{PREH}) were tested using power, exponential and sigmoidal functions with or without fixing the Hill coefficient\textsuperscript{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\textsubscript{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 \textit{CYP2B6} 516/983/15582 genotypes were assigned as follows: extensive metaboliser (15582CC-516-GG-983-TT or
Chapter 6

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 r² method in Haploview³⁰ and haplotype phases were inferred using the standard E-M algorithm in PLINK³¹. 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 in 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, 1 in 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. 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 $P \leq 0.001$. Nonetheless, we found nominal associations with $ABCB1$ rs1002204 ($\beta$:$0.32[0.05-0.59, P=2.29 \times 10^{-2}$]), $CYP2B6$ 983T$\rightarrow$C ($\beta$:$-0.26([-0.50 to -0.02, P=3.91 \times 10^{-2}$]), 516G$\rightarrow$T ($-0.16[-0.31 to -0.01, P=4.15 \times 10^{-2}$]), and rs7250597 ($\beta$:$0.17[0.01-0.33, P=4.20 \times 10^{-2}$]), $CYP3A5$ rs185690 ($\beta$:$-0.18[-0.34 to -0.01, P=4.35 \times 10^{-2}$]) and rs15524 ($\beta$:$-0.18[-0.34 to -0.01, P=4.35 \times 10^{-2}$]), respectively. After adjusting for $CYP2B6$ 516G$\rightarrow$T, we found significant nominal associations between NVP CL/F and $ABCB1$ rs1002204 ($\beta$:$-0.14[-0.28 to 0.01, P=4.93 \times 10^{-2}$]), $CYP2B6$ 983T$\rightarrow$C ($\beta$:$-0.19[-0.33 to -0.04, P=1.38 \times 10^{-2}$]) and $CYP2B6$ 15582C$\rightarrow$T ($\beta$:$-0.16[-0.31 to 0.01, P=4.84 \times 10^{-2}$]). 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$\rightarrow$T ($\beta$:$-0.21[-0.37 to -0.05, P=1.33 \times 10^{-2}$]). Similarly, after adjusting for composite
Chapter 6

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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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[95%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, \( P = 2.97 \times 10^{-2} \). After adjusting for \( \text{CYP2B6} \, 516\text{G}\rightarrow\text{T} \), there was a significant association with \( \text{CYP2B6} \, 983\text{T}\rightarrow\text{C} \) (\( \beta: 21.9 [95\% \text{CI}: 9.6-34.2, \, P = 9.96 \times 10^{-4}] \)), and nominal associations with \( \text{CYP2B6} \, \text{rs8100458} \) \( \beta: 15.7 [95\% \text{CI}: 1.7-29.6, \, P = 3.23 \times 10^{-2}] \) and \( \text{CYP2B6} \, 15582\text{C}\rightarrow\text{T} \) (\( \beta: 15.1 [95\% \text{CI}: 1.1-29.2, \, P = 4.02 \times 10^{-2}] \)), \( \text{CYP2A6} \, -48\text{T}\rightarrow\text{G} \) (\( \beta: 15.5 [95\% \text{CI}: 1.9-29, \, P = 2.99 \times 10^{-2}] \)), and \( \text{CYP2B7} \, \text{rs4124633} \) (\( \beta: 13.9 [95\% \text{CI}: 0.1-27.8, \, P = 5.48 \times 10^{-2}] \)). After adjusting for composite \( \text{CYP2B6} \, 516/983 \) genotype, there were significant associations with all polymorphisms in the initial model including \( \text{CYP2B6} \, 15582\text{C}\rightarrow\text{T} \) (\( \beta: 32.2 [95\% \text{CI}: 11.9-52.3, \, P = 2.93 \times 10^{-3}] \)). Similarly, after adjusting for composite \( \text{CYP2B6} \, 15582/516/983 \) 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\textsuperscript{11} and lower than other studies in children and adults. V/F of 19.7L was comparable to previously published data\textsuperscript{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\textsuperscript{11}.

We evaluated associations with genetic polymorphisms in \( \text{ABCB1} \), \( \text{CYP2A6} \), \( \text{CYP2B7} \), \( \text{CYP2B6} \), \( \text{CYP3A4} \) and \( \text{CYP3A5} \) using a candidate gene approach. Previous studies have shown associations between NVP pharmacokinetics and \( \text{CYP2B6} \, 516\text{G}\rightarrow\text{T} \textsuperscript{10,12} \) and \( 983\text{T}\rightarrow\text{C} \textsuperscript{30-33}. \)
Chapter 6

CYP2B6 15582C→T has also been associated with NVP PK in Cambodian and African adults\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsubscript{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\textsubscript{min}. These findings are consistent with previous reports from our group with regards the relationship between CYP2B6 983T→C and NVP C\textsubscript{min}\textsuperscript{11}. Similarly, in a univariate analysis, we found significant association with NVP AUC\textsubscript{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 \textit{CYP2B6} 516/983 or \textit{CYP2B6} 15582/516/983 genotype, all the aforementioned polymorphisms were significantly associated with NVP AUC\textsubscript{0-12}.

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


Chapter 6


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Chapter 6

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plasma nevirapine exposure following an intrapartum dose to prevent mother-to-

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### Table 1: Summary of the data

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<tr>
<th>Characteristic</th>
<th>Median(IQR)</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age(Months)</td>
<td>30(24-42)</td>
<td>10-77</td>
</tr>
<tr>
<td>BW(Kg)</td>
<td>13(11-15)</td>
<td>6.6-20</td>
</tr>
<tr>
<td>Dose(mg/m²)</td>
<td>105(90-120)</td>
<td>55-165</td>
</tr>
<tr>
<td>CD4%</td>
<td>32(24-39)</td>
<td>7.86-64</td>
</tr>
<tr>
<td>VL</td>
<td>50(25-73)</td>
<td>25-5.6 x 10⁶</td>
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### Table 2: Final Model Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical Values (For 13KG Child)</th>
<th>Bootstrap Values [Median (95%CI)]</th>
</tr>
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<tbody>
<tr>
<td><strong>CLini [L/hr]</strong></td>
<td>2.07</td>
<td>2.08 (1.38-4.74)</td>
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<tr>
<td><strong>V/F[L]</strong></td>
<td>19.7</td>
<td>19.70 (13.73-26.86)</td>
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<tr>
<td><strong>Ka [hr⁻¹]</strong></td>
<td>0.84 FIX</td>
<td></td>
</tr>
<tr>
<td><strong>MTT[hr]</strong></td>
<td>0.56 FIX</td>
<td></td>
</tr>
<tr>
<td><strong>NN</strong></td>
<td>3 FIX</td>
<td></td>
</tr>
<tr>
<td><strong>F at Birth [%]</strong></td>
<td>50.4</td>
<td>50.35 (44.81-55.08)</td>
</tr>
<tr>
<td><strong>KBIO[Years]</strong></td>
<td>0.84</td>
<td>0.84 (0.50-0.95)</td>
</tr>
</tbody>
</table>

#### 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; PMAS0, post menstrual age; Prop, proportional error; RUV, residual unexplained variability; V/F, volume
Chapter 6

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

<table>
<thead>
<tr>
<th>CHR</th>
<th>SNP (Gene)</th>
<th>Unadjusted β(95%C.I,P)</th>
<th>516G→T Adjusted β(95%C.I,P)</th>
<th>516G→T, 983T→C Adjusted β(95%C.I,P)</th>
<th>516G→T, 983T→C and 15582C→T Adjusted β(95%C.I,P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>rs1002204 (ABCB1)</td>
<td>0.32(0.05-0.59,P=0.02)</td>
<td>-0.14(-0.28 to 0.01,P=0.05)</td>
<td>-0.21(-0.36 to -0.07,P=0.007)</td>
<td>-0.09(-0.13 to -0.05,P=9.3E-06)</td>
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<tr>
<td>19</td>
<td>983T→C (CYP2B6)</td>
<td>-0.26(-0.50 to -0.02,P=0.04)</td>
<td>-0.19(-0.33 to -0.04,P=0.01)</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>19</td>
<td>516G→T (CYP2B6)</td>
<td>-0.16(-0.31 to -0.01,P=0.04)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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<td>19</td>
<td>rs7250597 (CYP2B6)</td>
<td>0.17(0.01-0.33,P=0.04)</td>
<td>-0.11(-0.25 to 0.04,P=0.16)</td>
<td>-0.19(-0.34 to -0.03,P=0.02)</td>
<td>-0.08(-0.12 to -0.04,P=0.0001)</td>
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<td>7</td>
<td>rs1859690 (CYP3A5)</td>
<td>-0.18(-0.34 to -0.01,P=0.05)</td>
<td>-0.13(-0.28 to 0.02,P=0.10)</td>
<td>-0.17(-0.34 to 0.01,P=0.04)</td>
<td>-0.09(-0.13 to -0.05,P=0.0002)</td>
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<tr>
<td>7</td>
<td>rs15524 (CYP3A5)</td>
<td>-0.18(-0.34 to -0.01,P=0.05)</td>
<td>-0.13(-0.28 to 0.02,P=0.10)</td>
<td>0.17(-0.34 to 0.01,P=0.04)</td>
<td>-0.09(-0.13 to -0.05,P=0.0002)</td>
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<tr>
<td>7</td>
<td>rs17064 (ABCB1)</td>
<td>0.18(0.01-0.37,P=0.06)</td>
<td>-0.13(-0.28 to 0.01,P=0.07)</td>
<td>-0.21(-0.36 to -0.06,P=0.009)</td>
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</tr>
<tr>
<td>7</td>
<td>rs2687134 (CYP3A5)</td>
<td>-0.15(-0.31 to 0.01,P=0.06)</td>
<td>-0.13(-0.28 to 0.02,P=0.09)</td>
<td>-0.18(-0.34 to 0.01,P=0.04)</td>
<td>-0.09(-0.13 to -0.05,P=0.0001)</td>
</tr>
<tr>
<td>7</td>
<td>rs1922240 (ABCB1)</td>
<td>-0.19(-0.40 to 0.01,P=0.06)</td>
<td>-0.09(-0.24 to 0.06,P=0.23)</td>
<td>-0.18(-0.34 to -0.02,P=0.03)</td>
<td>-0.09(-0.13 to -0.05,P=0.0002)</td>
</tr>
<tr>
<td>7</td>
<td>rs10211 (CYP3A5)</td>
<td>-0.18(-0.33 to 0.01,P=0.07)</td>
<td>-0.13(-0.28 to 0.02,P=0.09)</td>
<td>-0.17(-0.34 to -0.03,P=0.03)</td>
<td>-0.09(-0.13 to -0.05,P=0.0002)</td>
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<tr>
<td>19</td>
<td>15582C→T (CYP2B6)</td>
<td>0.13(-0.05 to 0.31,P=0.16)</td>
<td>-0.16(-0.31 to 0.01,P=0.05)</td>
<td>-0.21(-0.37 to -0.05,P=0.01)</td>
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CHR, chromosome; SNP, single nucleotide polymorphism.
## Chapter 6

### Table 4: Summary of Selected Genotypes Associated with Nevirapine C\textsubscript{min}

<table>
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<tr>
<th>CHR (Gene)</th>
<th>Unadjusted β(95%CI,P)</th>
<th>516G→T Adjusted β(95%CI,P)</th>
<th>516G→T, 983T→C Adjusted β(95%CI,P)</th>
<th>516G→T, 983T→C and 15582C→T Adjusted β(95%CI,P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 983T→C (CYP2B6)</td>
<td>6.07(3.14-9.01,P=0.0002)</td>
<td>3.24(1.53-4.94,P=0.005)</td>
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<tr>
<td>19 -48T→G (CYP2A6)</td>
<td>7.14(1.92-12.37,P=0.009)</td>
<td>2.16(0.27-4.05,P=0.03)</td>
<td>3.29(1.41-5.18,P=0.001)</td>
<td>1.30(0.79-1.82,P=7.0E-06)</td>
</tr>
<tr>
<td>7 rs10211 (CYP2A6)</td>
<td>2.74(0.54-4.94,P=0.02)</td>
<td>1.16(-0.90 to 3.22,P=0.27)</td>
<td>2.87(0.82-4.93,P=0.008)</td>
<td>131(0.77-1.85,P=1.4E-0.5)</td>
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<tr>
<td>7 rs1859690 (CYP3A5)</td>
<td>2.70(0.49-4.91,P=0.02)</td>
<td>1.24(-0.80 to 3.28,P=0.24)</td>
<td>2.93(0.91-4.93,P=0.006)</td>
<td>1.31(0.79-1.84,P=9.4E-0.6)</td>
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<td>7 rs15524 (CYP3A5)</td>
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<td>1.24(-0.80 to 3.28,P=0.24)</td>
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<td>7 rs113539362 (CYP3A5)</td>
<td>4.44(0.51-8.36,P=0.03)</td>
<td>1.71(-0.24 to 3.66,P=0.09)</td>
<td>3.13(1.16-5.10,P=0.003)</td>
<td>1.33(0.81-1.85,P=6.9E-0.6)</td>
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<td>19 rs4124633 (CYP2B7)</td>
<td>2.98(0.30-5.65,P=0.03)</td>
<td>1.51(-0.48 to 3.49,P=0.14)</td>
<td>3.12(1.15-5.10,P=0.003)</td>
<td>1.34(0.82-1.86,P=4.5E-0.6)</td>
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<tr>
<td>19 rs8100458 (CYP2B6)</td>
<td>3.36(-0.14 to 6.86, P=0.07)</td>
<td>1.56(-0.44 to 3.57,P=0.13)</td>
<td>3.05(0.98-5.12,P=0.006)</td>
<td>1.36(0.81-1.90,P=1.0E-0.5)</td>
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<tr>
<td>19 516G→T (CYP2B6)</td>
<td>1.81(-0.21 to 3.82, P=0.08)</td>
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<td>N/A</td>
<td>N/A</td>
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<td>19 15582C→T (CYP2B6)</td>
<td>-1.97(-3.46 to 1.41, P=0.41)</td>
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CHR, chromosome; SNP, single nucleotide polymorphism.
### Table 5: Summary of Genetic associations with Nevirapine AUC

<table>
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<tr>
<th>CHR</th>
<th>SNP (Gene)</th>
<th>Unadjusted β(95%CI,P)</th>
<th>516G→T Adjusted β(95%CI,P)</th>
<th>516G→T, 983T→C Adjusted β(95%CI,P)</th>
<th>516G→T, 983T→C and 15582C→T Adjusted β(95%CI,P)</th>
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<tbody>
<tr>
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<td>983T→C (CYP2B6)</td>
<td>59.3(30.6-88.2, P=0.0002)</td>
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<td>N/A</td>
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<td>15.5(1.9-29, P=0.03)</td>
<td>32.3(13.6-50.6, P=0.001)</td>
<td>12.7(7.7-17.7, P=8.4E-06)</td>
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<tr>
<td>7</td>
<td>rs10211 (CYP3A5)</td>
<td>26.9(5.4-48.4, P=0.02)</td>
<td>13.7(-0.70 to 28.10, P=0.07)</td>
<td>27.9(7.8-48.1, P=0.009)</td>
<td>12.7(7.5-17.9, P=1.6E-05)</td>
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<tr>
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<td>rs1859690 (CYP3A5)</td>
<td>26.5(4.8-48.2, P=0.02)</td>
<td>13.8(-0.50 to 28, P=0.06)</td>
<td>28.6(8.7-48.4, P=0.007)</td>
<td>12.8(7.6-17.9, P=1.1E-05)</td>
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<td>13.8(-0.50 to 28, P=0.06)</td>
<td>28.6(8.7-48.4, P=0.007)</td>
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<td>12.9(7.8-18.1, P=8.3E-06)</td>
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<tr>
<td>19</td>
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<td>15.7(1.7-29.6, P=0.03)</td>
<td>29.6(9.3-49.9, P=0.006)</td>
<td>13.5(7.8-18.5, P=1.3E-05)</td>
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**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

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<th>Gene</th>
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<th>Minor Allele</th>
<th>Major Allele</th>
<th>Minor Allele Frequencies</th>
<th>Genotype Frequencies</th>
<th>P-value</th>
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Chapter 6

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

Supplementary Figure 2: Illustrates the linkage disequilibrium plot for CYP2B6
Chapter 6

**Supplementary Figure 3:** Presents linkage disequilibrium plot for ABCB1

**Supplementary Figure 4:** Demonstrates the linkage disequilibrium plot for CYP3A4
Chapter 6

Supplementary Figure 5: Shows the linkage disequilibrium plot for CYP3A5

Equation 1

\[
\frac{CL}{Fstd} = \frac{CL}{Fstd} \times \left(\frac{TWBT}{12.5}\right)^{0.75}
\]  

(1.1)

\[
\frac{V}{Fstd} = \frac{V}{Fstd} \times \left(\frac{TWBT}{12.5}\right)^{1}
\]  

(1.2)

Equation 2

\[
F_{preH} = 1 - (1 - F_{preH\,Birth}) \times \exp^{-K_{preH\,AGE}}
\]
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
Chapter 7
Overall Discussions and Conclusions

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 (Cmin) and area under the concentration time curve (AUC0-12), 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 adults5 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.
Chapter 7

Overall Discussions and Conclusions

reported elsewhere\textsuperscript{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\textsuperscript{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
Overall Discussions and Conclusions

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.

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. 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.

From the final model, individual CL/F, Cmin, and AUC0-12 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. When adjusting for multiple comparisons, there were no significant associations between 56 SLCO1B1 polymorphisms and lopinavir CL/F, Cmin or AUC0-12, 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. There were significant nominal associations between SLCO1B1 463C→A (rs11045819) and decreased lopinavir CL/F or increased Cmin and AUC0-12, however the results are in contrast with previous reports. Furthermore, there were nominally significant associations with SLCO1B1 388A→G (rs2306283) and increased lopinavir Cmin or AUC0-12, contrasting previous reports. Additionally, there were trends of increased Cmin and AUC0-12 with SLCO1B1 rs112403792 and rs4149057, however this have not been reported previously.
Chapter 7  
Overall Discussions and Conclusions

Similarly, when adjusting for multiple comparisons, there were no significant associations between \textit{ABCB1} polymorphisms and lopinavir CL/F, \textit{C}_\text{min} or \textit{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 \textit{CYP3A4} and \textit{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\textsuperscript{18}. There was no maturation effect on CL/F, however there was an age-driven effect on relative bioavailability, similar to previous reports\textsuperscript{18}. From the final model, individual CL/F, \textit{C}_\text{min} and \textit{AUC}_{0-12} were obtained and were used to evaluate for associations with genetic polymorphisms in \textit{ABCB1}, \textit{CYP2A6}, \textit{CYP2B7}, \textit{CYP2B6}, \textit{CYP3A4} and \textit{CYP3A5}.

Nevirapine is substrate of \textit{CYP2B6}, previous studies have shown associations between \textit{516G}→\textit{T}\textsuperscript{18–20} and \textit{983T}→\textit{C}\textsuperscript{21–23} variants and nevirapine pharmacokinetics. When adjusting for multiple comparisons in a univariate analysis, there were nominal associations between nevirapine CL/F and \textit{ABCB1} rs1002204 and \textit{CYP2B6} rs7250597, \textit{CYP3A4} rs1859690, \textit{CYP3A5} rs15524, \textit{CYP2B6} 516G→T and 983T→C. Adjusting for \textit{CYP2B6} 516G→T genotype resulted in nominal associations with \textit{ABCB1} rs1002204, \textit{CYP2B6} 983T→C and \textit{CYP2B6} 15582 C→T. When adjusting for \textit{CYP2B6} 516/983 haplotype and the composite \textit{CYP2B6} 516/983/15582 haplotype, there were significant associations with the above-mentioned polymorphisms.
Chapter 7
Overall Discussions and Conclusions

Regarding nevirapine $C_{\text{min}}$, when adjusting for multiple comparison by univariate analysis, there was a significant association with $\text{CYP}2\text{B}6$ $983\text{T} \rightarrow \text{C}$ and nominal associations with $\text{CYP}3\text{A}5$ rs10211, $\text{CYP}3\text{A}5$ rs1859690, $\text{CYP}3\text{A}5$ rs15524, $\text{CYP}3\text{A}5$ rs113539362, $\text{CYP}2\text{B}6$ rs8100458, $\text{CYP}2\text{A}6$ -48T$ \rightarrow $G, and $\text{CYP}2\text{B}7$ rs4124633. When adjusting $\text{CYP}2\text{B}6$ 516G$ \rightarrow $T, there were nominal associations with only $\text{CYP}2\text{B}6$ 983T$ \rightarrow $C and $\text{CYP}2\text{A}6$ -48T$ \rightarrow $G. After adjusting for $\text{CYP}2\text{B}6$ 516/983 haplotype there were nominal association between the aforementioned genotypes in the first model and nevirapine $C_{\text{min}}$, whereas adjusting composite $\text{CYP}2\text{B}6$ 516/983/15582 haplotype, they were significantly associated with nevirapine $C_{\text{min}}$. 

For nevirapine $\text{AUC}_{0-12}$, in a univariate analysis, when adjusting for multiple comparisons, there was a significant association between nevirapine $\text{AUC}_{0-12}$ and $\text{CYP}2\text{B}6$ 983T$ \rightarrow $C and associations nominally with $\text{CYP}3\text{A}5$ rs10211, $\text{CYP}3\text{A}5$ rs1859690, $\text{CYP}3\text{A}5$ rs15524, $\text{CYP}3\text{A}5$ rs113539362, $\text{CYP}2\text{A}6$ -48T$ \rightarrow $G, $\text{CYP}2\text{B}7$ rs4124633 and $\text{CYP}2\text{B}6$ rs8100458. After adjusting for $\text{CYP}2\text{B}6$ 516G$ \rightarrow $T, there were nominal associations between nevirapine $\text{AUC}$ and $\text{CYP}2\text{B}6$ 983T$ \rightarrow $C, rs8100458 and 15582C$ \rightarrow $T and/or $\text{CYP}2\text{A}6$ -48T$ \rightarrow $G. After adjusting for $\text{CYP}2\text{B}6$ 516/983 haplotype, there nominal associations with polymorphisms mentioned in the first model including 15582C$ \rightarrow $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
Chapter 7  
Overall Discussions and Conclusions

was accounted by multiple imputation, as this approach has been shown to be superior to complete case analysis in which subjects who do not have missing values are analysed\textsuperscript{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\textsuperscript{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\textsuperscript{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.
Chapter 7

Overall Discussions and Conclusions

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 \textit{SLCO1B1} and \textit{ABCB1}. This thesis confirmed significant associations between nevirapine pharmacokinetics and \textit{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.
Chapter 7
Overall Discussions and Conclusions

Moreover, more pharmacogenetic studies are needed to confirm the influence of genetic polymorphisms in \textit{SLCO1B1} and \textit{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.
Chapter 7
Overall Discussions and Conclusions

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


Chapter 7

Overall Discussions and Conclusions


178
Chapter 7

Overall Discussions and Conclusions


16. Hartkoorn RC, Kwan WS, Shallcross V, et al. HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenet Genomics.* 2010;**20**:112-120.

Chapter 7

Overall Discussions and Conclusions


Chapter 7

Overall Discussions and Conclusions


APPENDIX I

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

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,baselinevfa,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","whostage2","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,splintime=3,empr=1000)

ameliabind(myimp)
plot(myimp)
or
par(mfrow=c(2,2))

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

compare.density(myimp,var="logbaselinevl")
APPENDIX I

```r
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$imputations[[i]])[17]<-c("hfacat")
}
```
myimp$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")
APPENDIX I

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],summary(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")
APPENDIX I

m3_5<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[5]],method="breslow")
m3_6<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[6]],method="breslow")
m3_7<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[7]],method="breslow")
m3_8<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[8]],method="breslow")
m3_9<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[9]],method="breslow")
m3_10<-coxph(Surv(X_t0,X_t,vlssupp) ~ lpvcat1 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$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],summary(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=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, vlssupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[1]],method="breslow")
m4_2<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[2]],method="breslow")
m4_3<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[3]],method="breslow")
m4_4<-coxph(Surv(X_t0,X_t, vlssupp) ~ lpvcat4 + as.factor(cd4pccat) + as.factor(logvlcat) + as.factor(agecat) + as.factor(whostage2) + as.factor(wfacat) + as.factor(hfacat)+ cluster(id),data=myimp$imputations[[4]],method="breslow")
APPENDIX I

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]",summary(m2_2)"[7]",summary(m2_3)"[7]",summary(m2_4)"[7]",summary(m2_5)"[7]",summary(m2_6)"[7]",summary(m2_7)"[7]",summary(m2_8)"[7]",summary(m2_9)"[7]",summary(m2_10)"[7]")

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")
APPENDIX I

m2_101<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpv + cluster(id),data=myimp$imputations[[10]],method="breslow")
myest1 <- list(coef(m2_11),coef(m2_21),coef(m2_31),coef(m2_41),coef(m2_51),coef(m2_61),coef(m2_71),coef(m2_81),
coef(m2_91),coef(m2_101))
mystd1 <- list(summary(m2_11)[[7]][,4],summary(m2_21)[[7]][,4],summary(m2_31)[[7]][,4],summary(m2_41)[[7]][,4],summary(m2_51)[[7]][,4],summary(m2_61)[[7]][,4],summary(m2_71)[[7]][,4],summary(m2_81)[[7]][,4],summary(m2_91)[[7]][,4],summary(m2_101)[[7]][,4])
my2a1 <- mi.inference(myest1, mystd1, confidence=0.95)
my_21 <- round(cbind(exp(my2a1$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],summary(m2_53)[[7]][,4],summary(m2_63)[[7]][,4],summary(m2_73)[[7]][,4],summary(m2_83)[[7]][,4],summary(m2_93)[[7]][,4],summary(m2_103)[[7]][,4])
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)[[1]][4],summary(m2_24)[[1]][4],summary(m2_34)[[1]][4],summary(m2_44)[[1]][4],summary(m2_54)[[1]][4],summary(m2_64)[[1]][4],summary(m2_74)[[1]][4],summary(m2_84)[[1]][4],summary(m2_94)[[1]][4],summary(m2_104)[[1]][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")
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")
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")
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")

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],sum
mary(m2_56)[[7]][,4],summary(m2_66)[[7]][,4],summary(m2_76)[[7]][,4],summary(m2_86)[[7]][,4],summary(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")
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]],summary(m2_57)[[7]][[4]],summary(m2_67)[[7]][[4]],summary(m2_77)[[7]][[4]],summary(m2_87)[[7]][[4]],summary(m2_97)[[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)

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]],summary(m2_58)[[7]][[4]],summary(m2_68)[[7]][[4]],summary(m2_78)[[7]][[4]],summary(m2_88)[[7]][[4]],summary(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)
APPENDIX I

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],summary(m2_59)[[7]][,4],summary(m2_69)[[7]][,4],summary(m2_79)[[7]][,4],summary(m2_89)[[7]][,4],summary(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$imputations[[3]],method="breslow")
m2_51<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(lpvcat4) +
cluster(id),data=myimp$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],summary(m2_59)[[7]][,4],summary(m2_69)[[7]][,4],summary(m2_79)[[7]][,4],summary(m2_89)[[7]][,4],summary(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)))
APPENDIX I

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, , stage, HFA
stepAIC(m2_8, direction = c("both"))  # LPV, stage, HFA
stepAIC(m2_9, direction = c("both"))  # LPV, stage, HFA
stepAIC(m2_10, direction = c("both"))  # LPV, VL,
APPENDIX I

# 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) + as.factor(hfacat) + cluster(id), data=seldat, weights=myweights+1e-08, method="breslow")
m5_4 <- 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_5 <- 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_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
APPENDIX I

# 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], summary(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 #

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

Mlsummary[,1:4] <- my_2

Mlsummary[1:5,8] <- my_21
APPENDIX I

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")
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],summary(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)

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

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

Msummary[,1:4] <- my_2
Msummary[1,5:8] <- my_21
Msummary[2,5:8] <- my_23
Msummary[3,5:8] <- my_24
Msummary[4,5:8] <- my_25
Msummary[5,5:8] <- my_26
Msummary[8:9,5:8] <- my_28
Msummary[c(1,3,8:9),9:12] <- my_4

rownames(Msummary) <- 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(Msummary) <- c("Adj.",",",",",","Crude","",",","AIC sel.",",",")

Msummary

write.csv(round(Msummary, 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")
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],summary(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

Mlsummary <- matrix(rep(NA,12*9),nrow=9,ncol=12)
Mlsummary[,1:4] <- my_2
Mlsummary[1:5,8] <- my_21
Mlsummary[2:5:8] <- my_23
Mlsummary[3:5:8] <- my_24
Mlsummary[4:5:8] <- my_25
Mlsummary[5:5:8] <- my_26
Mlsummary[6:7:5:8] <- my_27
Mlsummary[8:9,5:8] <- my_28
Mlsummary[c(1,3,8:9),9:12] <- my_5
APPENDIX I

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")
APPENDIX I

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$imputations[[23]], robust=TRUE, method="breslow")
Lpvm324 <- coxph(Surv(X_t0,X_t,vlsupp) ~ pspline(lpv, df=4) + as.factor(logvlcat) + as.factor(whostage2) + as.factor(cd4pccat) + as.factor(hfacat) + as.factor(agecat) + as.factor(wfacat)+ cluster(id),
data=myimp$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)
APPENDIX I

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]
ejectAIC(m4_0b)[2]
ejectAIC(m4_0c)[2]
ejectAIC(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")

205
APPENDIX I

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")
APPENDIX I

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]
APPENDIX I

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")
APPENDIX I

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")
APPENDIX I

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")
APPENDIX I

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_40<-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-randoanimal 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)
APPENDIX II

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){
    myimpSimputations[[m]]<-transform(myimpSimputations[[m]], vlcat=cut(myimpSimputations[[m]]$t0rvl, breaks=c(-1,51,1000000000)))
    myimpSimputations[[m]]<-transform(myimpSimputations[[m]], cd4pccat=cut(myimpSimputations[[m]]$t0rcd4pc, breaks=c(-1,25,1000000000)))
    myimpSimputations[[m]]<-transform(myimpSimputations[[m]], agecat=cut(myimpSimputations[[m]]$ageatran, breaks=c(-1,20,1000000000)))}
myimp$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$imputations[[m]], lpvcat1=cut(myimp$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)
APPENDIX II

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){
  myimpSimputations[[m]]<-transform(myimpSimputations[[m]],lpvre=shift.l(myimpSimputations[[m]]$lpvcorr,myimpSimputations[[m]]$id))
  myimpSimputations[[m]]$lpvre <- replace(is.na(myimpSimputations[[m]]$lpvre),0)
  myimpSimputations[[m]] <- transform(myimpSimputations[[m]],lpave=((lpvcorr+lpvre)/2))
}

head(myimpSimputations[[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=myimpSimputations[[1]],robust=TRUE,method="breslow")

m1_2<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus + cluster(id),data=myimpSimputations[[2]],robust=TRUE,method="breslow")

m1_3<-coxph(Surv(X_t0,X_t,vlsupp) ~ adstatus + cluster(id),data=myimpSimputations[[3]],robust=TRUE,method="breslow")
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],summary(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$imputations[[7]],robust=TRUE,method="breslow")
m1b_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr +
cluster(id),data=myimp$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
coef(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], summary(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(myst1c, 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], summary(m1d_5)[7][4], summary(m1d_6)[7][4], summary(m1d_7)[7][4], summary(m1d_8)[7][4], summary(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
APPENDIX II

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],summary( 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")
APPENDIX II

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(m1df_1)[7][,4],summary(m1f_2)[7][,4],summary(m1f_3)[7][,4],summary(m1f_4)[7][,4],summary(m1f_5)[7][,4],summary(m1f_6)[7][,4],summary(m1f_7)[7][,4],summary(m1f_8)[7][,4],summary(m1f_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")
\[
m_2_9<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(vlcat)} + \text{cluster(id)}, \text{data=myimp$imputations[[9]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_2_10<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(vlcat)} + \text{cluster(id)}, \text{data=myimp$imputations[[10]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
\text{myest2 <- list(coef(m2_1), coef(m2_2), coef(m2_3), coef(m2_4), coef(m2_5), coef(m2_6), coef(m2_7), coef(m2_8), coef(m2_9), coef(m2_10))}
\]
\[
\text{mystd2 <- list(summary(m2_1)[[7]][,4], summary(m2_2)[[7]][,4], summary(m2_3)[[7]][,4], summary(m2_4)[[7]][,4], summary(m2_5)[[7]][,4], summary(m2_6)[[7]][,4], summary(m2_7)[[7]][,4], summary(m2_8)[[7]][,4], summary(m2_9)[[7]][,4], summary(m2_10)[[7]][,4])}
\]
\[
\text{my2a <- mi.inference(myest2, mystd2, confidence=0.95)}
\]
\[
\text{my_2 <- round(cbind(exp(my2a$est), exp(my2a$lower), exp(my2a$upper), my2a$signif), digits=3)}
\]
\[
m_3_1<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[1]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_2<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[2]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_3<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[3]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_4<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[4]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_5<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[5]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_6<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[6]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_7<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[7]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_8<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[8]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_9<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[9]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
m_3_10<-\text{coxph}(\text{Surv}(X_{t0},X_{t}, \text{vlsupp}) ~ \text{as.factor(cd4pccat)} + \text{cluster(id)}, \text{data=myimp$imputations[[10]]}, \text{robust=TRUE}, \text{method="breslow"})
\]
\[
\text{myest3 <- list(coef(m3_1), coef(m3_2), coef(m3_3), coef(m3_4), coef(m3_5), coef(m3_6), coef(m3_7), coef(m3_8), coef(m3_9), coef(m3_10))}
\]
\[
\text{mystd3 <- list(summary(m3_1)[[7]][,4], summary(m3_2)[[7]][,4], summary(m3_3)[[7]][,4], summary(m3_4)[[7]][,4], summary(m3_5)[[7]][,4], summary(m3_6)[[7]][,4], summary(m3_7)[[7]][,4], summary(m3_8)[[7]][,4], summary(m3_9)[[7]][,4], summary(m3_10)[[7]][,4])}
\]
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)

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], summary(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_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],summary(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_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],summary(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$imputations[[7]],robust=TRUE,method="breslow")
m9_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ as.factor(postrantb) +
cluster(id),data=myimp$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],summary(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 continuous 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$imputations[[7]],robust=TRUE,method="breslow")
m11_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcorr + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor(wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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],summary(m11_5)[[7]][4],summary(m11_6)[[7]][4],summary(m11_7)[[7]][4],summary(m11_8)[[7]][4],summary(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$imputations[[7]],robust=TRUE,method="breslow")
m14_8<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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_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],summary(m14_5)[7][,4],summary(m14_6)[7][,4],summary(m14_7)[7][,4],summary(m14_8)[7][,4],summary(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")
APPENDIX II

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],summary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summary(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 Additive 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")
APPENDIX II

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)
APPENDIX II

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
# 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?

```r
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

```r
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")
```

```r
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")
```
APPENDIX II

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)
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(co effdiff,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)
APPENDIX II

```r
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.tif2", res=600, height=4, width=4, units="p")

tiff(file = "temp.tif", width =672, height = 672, units = "px", res = 800,type = c("windows", "cairo"),family = "", restoreConsole = TRUE,antialias="cleartype")

tiff(file = "temp.tif", 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
```
APPENDIX II

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

# 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],summary(m12_5)[[7]][,4],summary(m12_6)[[7]][,4],summary(m12_7)[[7]][,4],summary(m12_8)[[7]][,4],summary(m12_9)[[7]][,4],summary(m12_10)[[7]][,4],su
APPENDIX II

mmary(m12_5)[[7]][4], summary(m12_6)[[7]][4], summary(m12_7)[[7]][4], summary(m12_8)[[7]][4], summary(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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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) ~ lqvcat4 + 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))
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-values

# 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_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], summary(m14_5)[[7]][,4], summary(m14_6)[[7]][,4], summary(m14_7)[[7]][,4], summary(m14_8)[[7]][,4], summary(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-

#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")
APPENDIX II

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],summary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summary(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)

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

# 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")
APPENDIX II

m16_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")
ymyst <- 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))

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)

#Using Lopinavir 5 mg/L Cut off

m17_1c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")
m17_2c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")
m17_3c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")
m17_4c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")
m17_5c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")
m17_6c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")
m17_7c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")
m17_8c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")
m17_9c-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcatto) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor (postrantb) + cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")
m17_10<-coxph(Surv(X_t0,X_t,vlsupp) ~ lpvcat3.5 + as.factor(vlcat)+ as.factor(cd4pcat) + as.factor(agecat) +
 as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) +
 cluster(id),data=myimp$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],sum
mary(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

###################################
#
#
#
#
#
#
# Michael 17: I chose LPV cat1

MMsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)
APPENDIX II

Mls summary[1,1:4] <- my_1
Mls summary[2,1:4] <- my_2
Mls summary[4,1:4] <- my_4
Mls summary[6,1:4] <- my_6
Mls summary[7,1:4] <- my_7
Mls summary[8,1:4] <- my_9

rownames(Mls summary) <- c("LPV conc.", "VL (>50)", "CD4% (Low)", "Age (>1/2 yr.)", "WFA (Advanced)", "HFA (Advanced)", "Postrantab(Yes)")

colnames(Mls summary) <- c("Crude", "", "", "Adj.", "", "")

Mls summary
write.csv(round(Mls summary, digits=3),file="Cox_LPVCONT.csv")

Mls summary <- matrix(rep(NA,8*10),nrow=10,ncol=8)
Mls summary[1,1:4] <- my_1b
Mls summary[2,1:4] <- my_2
Mls summary[4,1:4] <- my_4
Mls summary[6,1:4] <- my_6
Mls summary[7,1:4] <- my_7
Mls summary[8,1:4] <- my_9

rownames(Mls summary) <- c("LPVCAT1","VL (>50)", "CD4% (Low)", "Age (>1/2 yr.)", "WFA (Advanced)", "HFA (Advanced)", "Postrantab(Yes)")

colnames(Mls summary) <- c("Crude", "", "", "Adj.", "", "")

Mls summary
write.csv(round(Mls summary, digits=3),file="Cox_LPVCAT1.csv")

Mls summary <- matrix(rep(NA,8*10),nrow=10,ncol=8)
Mls summary[1,1:4] <- my_1c
APPENDIX II

MIsummary[2,1:4] <- my_2
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_8
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[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

```r
MIsummary <- matrix(rep(NA, 8*10), nrow=10, ncol=8)
MIsummary[1,1:4] <- my_1e
MIsummary[2,1:4] <- my_2
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.",&quot;&quot;)
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 =24 weeks
# 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)+
```
APPENDIX II

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]]$X_t < 53,], robust=TRUE, method="breslow")
m4_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr) + as.factor(cd4pccat) + as.factor(hfacat) + as.factor(postrantb) + cluster(id),data=(myimp$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(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(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(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(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(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(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(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(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>30]) # not much data above 30

m4_1c<-coxph(Surv(X_t0,X_t, vlsupp) ~ pspline(lpvcorr,df=2) + as.factor(cd4pccat) + 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(hfacat) + as.factor(postrantb)+

APPENDIX II
APPENDIX II

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(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 #
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$imputations[[3]],method="breslow")
m5_2b<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$imputations[[3]],method="breslow")

m5_2c<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpace + 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) ~ lpcorr + 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) ~ lpace + 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) ~ lpcorr + 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) ~ lpace + 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")
APPENDIX II

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")
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")
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) ~ 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")
APPENDIX II

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(m5_9a)[2]

0.1*extractAIC(m5_0b)[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_0c)[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")
APPENDIX II

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]
m4_6a<-coxph(Surv(X_t0,X_t, vlsupp) ~ lpvcat1 + as.factor(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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(ctlcat)+ as.factor(cd4pcat) + 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 #
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$imputations[[2]], method="breslow")
m5_43 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[3]], method = "breslow")

m5_44 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[4]], method = "breslow")

m5_45 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[5]], method = "breslow")

m5_46 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[6]], method = "breslow")

m5_47 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[7]], method = "breslow")

m5_48 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[8]], method = "breslow")

m5_49 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + as.factor(postrantb) +
+ cluster(id), data = myimp$imputations[[9]], method = "breslow")

m5_410 <- coxph(Surv(X_t0, X_t, vl supp) ~ adstatus + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(gecat) +
+ as.factor(wfcat) + as.factor(hfcat) + 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], summary(m5_45)[[7]][4], summary(m5_46)[[7]][4], summary(m5_47)[[7]][4], summary(m5_48)[[7]][4], summary(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
Cox Proportional Hazards Multiple failure Event Model of Nevirapine During The Post-randomization Phase

```r
library(splines)
library(survival)
library(pspline)
NVP<-read.csv("NVP_02_09_2014.csv",header=T,sep=";",stringsAsFactors=FALSE)
attach(NVP)

 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=ct("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=ct("nvp","adstatus","X_t"),polytime=3,splinetime=3,empri=5,incheck=TRUE,tolerance=0.001)
plot(myimp)
```
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){
APPENDIX III

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]],cd4pcat=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)
}

260
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],summary(m1_5)[[7]]
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], summary(m1b_5)[[7]][,4], summary(m1b_6)[[7]][,4], summary(m1b_7)[[7]][,4], summary(m1b_8)[[7]][,4], summary(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$imputations[[1]], robust=TRUE, method="breslow")
m1c_2<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre +
cluster(id),data=myimp$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),
     coef(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],summary(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$imputations[[5]],robust=TRUE,method="breslow")
m1d_6<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpave +
  cluster(id),data=myimp$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")
APPENDIX III

```r
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],summary(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
```

```r
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],summary(m1f_5)[[7]][,4],summary(m1f_6)[[7]][,4],summary(m1f_7)[[7]][,4],summary(m1f_8)[[7]][,4],summary(m1f_9)[[7]][,4],summary(m1f_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

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),coef(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],summary(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")
APPENDIX III

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],summary(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")
APPENDIX III

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], summary(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")
APPENDIX III

\[ m_4_{-10}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(agecat) + cluster(id),data=myimp$imputations[[10]],method="breslow")} \]

\[ \text{myest4 <- list(coef(m4_1),coef(m4_2),coef(m4_3),coef(m4_4),coef(m4_5),coef(m4_6),coef(m4_7),coef(m4_8),coef(m4_9),coef(m4_{10}}) \]

\[ \text{mystd4 <- list(summary(m4_1)[[7]][,4],summary(m4_2)[[7]][,4],summary(m4_3)[[7]][,4],summary(m4_4)[[7]][,4],summary(m4_5)[[7]][,4],summary(m4_6)[[7]][,4],summary(m4_7)[[7]][,4],summary(m4_8)[[7]][,4],summary(m4_9)[[7]][,4],summary(m4_{10})[[]][,4])} \]

\[ \text{my4a <- mi.inference(myest4, mystd4, confidence=0.95)} \]

\[ \text{my_4 <- round(cbind(exp(my4a$est),exp(my4a$lower),exp(my4a$upper),my4a$signif),digits=3)} \]

\[ \text{my_4} \]

\[ m_{6\_1}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[1]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_2}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[2]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_3}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[3]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_4}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[4]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_5}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[5]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_6}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[6]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_7}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[7]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_8}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[8]],robust=TRUE,method="breslow")} \]

\[ \text{m}_{6\_9}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[9]],robust=TRUE,method="breslow")} \]

\[ m_{6\_10}<-\text{coxph(Surv(X}_{t0}\text{,X}_{t},vlsupp) \sim as.factor(wfacat) + cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")} \]

\[ \text{myest6 <- list(coef(m6_{-1}),coef(m6_{-7}),coef(m6_{-3}),coef(m6_{-4}),coef(m6_{-5}),coef(m6_{-6}),coef(m6_{-7}),coef(m6_{-8}),coef(m6_{-9}),coef(m6_{-10}}) \]

\[ \text{mystd6 <- list(summary(m6_{-1})[[7]][,4],summary(m6_{-7})[[7]][,4],summary(m6_{-3})[[7]][,4],summary(m6_{-4})[[7]][,4],summary(m6_{-5})[[7]][,4],summary(m6_{-6})[[7]][,4],summary(m6_{-7})[[7]][,4],summary(m6_{-8})[[7]][,4],summary(m6_{-9})[[7]][,4],summary(m6_{-10})[[]][,4])} \]
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(hcat) + 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_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], summary(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
APPENDIX III

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],summary(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$imputations[[2]],robust=TRUE,method="breslow")
m11_3<coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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],summary(m11_5)[[7]][4],summary(m11_6)[[7]][4],summary(m11_7)[[7]][4],summary(m11_8)[[7]][4],summary(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$imputations[[2]],robust=TRUE,method="breslow")
m14_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpre + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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],summary(m14_5)[[7]][,4],summary(m14_6)[[7]][,4],summary(m14_7)[[7]][,4],summary(m14_8)[[7]][,4],summary(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")
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],summary(m15_5)[[7]][,4],summary(m15_6)[[7]][,4],summary(m15_7)[[7]][,4],summary(m15_8)[[7]][,4],summary(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")
APPENDIX III

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$imputations[[2]],robust=TRUE,method="breslow")
m17_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat10 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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,valsup) ~ nvpcat10 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$imputations[[10]],robust=TRUE,method="breslow")
mystd <- list(summary(m17_1)[[7]][,4],summary(m17_2)[[7]][,4],summary(m17_3)[[7]][,4],summary(m17_4)[[7]][,4],summary(m17_5)[[7]][,4],summary(m17_6)[[7]][,4],summary(m17_7)[[7]][,4],summary(m17_8)[[7]][,4],summary(m17_9)[[7]][,4],summary(m17_10)[[7]][,4])
my17a <- mi.inference(mystd, confidence=0.95)
m18_3<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat15 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb)+ cluster(id),data=myimp$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],summary(m18_5)[[7]][4],summary(m18_6)[[7]][4],summary(m18_7)[[7]][4],summary(m18_8)[[7]][4],summary(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")
APPENDIX III

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],summary(m19_5)[[7]][4],summary(m19_6)[[7]][4],summary(m19_7)[[7]][4],summary(m19_8)[[7]][4],summary(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")
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],summary(m20_5)[[7]][,4],summary(m20_6)[[7]][,4],summary(m20_7)[[7]][,4],summary(m20_8)[[7]][,4],summary(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")
APPENDIX III

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],summary(m21_5)[[7]][4],summary(m21_6)[[7]][4],summary(m21_7)[[7]][4],summary(m21_8)[[7]][4],summary(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")]

280
# 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)
# 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)
APPENDIX III

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(agenet)+as.factor(wfacat)+as.factor(hfacat)+as.factor(postrantb),data=myimp$imputations[[1]],random=~(1|id),family=binomial)
mi1_2 <- gam(vlsupp ~ cut(nvp, breaks=c(0,7.5,100))+s(X_t)+as.factor(vlcat)+as.factor(cd4pccat)+as.factor(agenet)+as.factor(wfacat)+as.factor(hfacat)+as.factor(postrantb),data=myimp$imputations[[2]],random=~(1|id),family=binomial)
APPENDIX III

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$imputations[[5]],random=~(1|id),family=binomial)
mi3_1$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)

mi6_1$gcv+mi6_2$gcv
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

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)

mi8.1$gcv+mi8.2$gcv+mi8.3$gcv+mi8.4$gcv+mi8.5$gcv
APPENDIX III

\[\text{mi8}_1\text{gcv}+\text{mi8}_2\text{gcv}+\text{mi8}_3\text{gcv}+\text{mi8}_4\text{gcv}+\text{mi8}_5\text{gcv}\]

\[\text{mi9}_1 \leftarrow \text{gam(vlsupp} \sim \text{cut(nvp, breaks} = (0,25,100)) + \text{s(X}_t) + \text{as.factor(vlcat)} + \text{as.factor(cd4pccat)} + \text{as.factor(agecat)} + \text{as.factor(wfacat)} + \text{as.factor(hfacat)} + \text{as.factor(postrantb)}, \text{data=myimp}\text{imputations[1]}, \text{random} = \sim(1|id), \text{family=binomial})\]

\[\text{mi9}_2 \leftarrow \text{gam(vlsupp} \sim \text{cut(nvp, breaks} = (0,25,100)) + \text{s(X}_t) + \text{as.factor(vlcat)} + \text{as.factor(cd4pccat)} + \text{as.factor(agecat)} + \text{as.factor(wfacat)} + \text{as.factor(hfacat)} + \text{as.factor(postrantb)}, \text{data=myimp}\text{imputations[2]}, \text{random} = \sim(1|id), \text{family=binomial})\]

\[\text{mi9}_3 \leftarrow \text{gam(vlsupp} \sim \text{cut(nvp, breaks} = (0,25,100)) + \text{s(X}_t) + \text{as.factor(vlcat)} + \text{as.factor(cd4pccat)} + \text{as.factor(agecat)} + \text{as.factor(wfacat)} + \text{as.factor(hfacat)} + \text{as.factor(postrantb)}, \text{data=myimp}\text{imputations[3]}, \text{random} = \sim(1|id), \text{family=binomial})\]

\[\text{mi9}_4 \leftarrow \text{gam(vlsupp} \sim \text{cut(nvp, breaks} = (0,25,100)) + \text{s(X}_t) + \text{as.factor(vlcat)} + \text{as.factor(cd4pccat)} + \text{as.factor(agecat)} + \text{as.factor(wfacat)} + \text{as.factor(hfacat)} + \text{as.factor(postrantb)}, \text{data=myimp}\text{imputations[4]}, \text{random} = \sim(1|id), \text{family=binomial})\]

\[\text{mi9}_5 \leftarrow \text{gam(vlsupp} \sim \text{cut(nvp, breaks} = (0,25,100)) + \text{s(X}_t) + \text{as.factor(vlcat)} + \text{as.factor(cd4pccat)} + \text{as.factor(agecat)} + \text{as.factor(wfacat)} + \text{as.factor(hfacat)} + \text{as.factor(postrantb)}, \text{data=myimp}\text{imputations[5]}, \text{random} = \sim(1|id), \text{family=binomial})\]

\[\text{mi9}_1\text{gcv}+\text{mi9}_2\text{gcv}+\text{mi9}_3\text{gcv}+\text{mi9}_4\text{gcv}+\text{mi9}_5\text{gcv}\]

# For figure: What is better: average or current?

\[\text{m\_current} \leftarrow \text{gam(vlsupp} \sim \text{s(nvp)} + \text{s(X}_t), \text{data=myimp}\text{imputations[1]}, \text{family=binomial})\]

\[\text{m\_average} \leftarrow \text{gam(vlsupp} \sim \text{s(nvpave)} + \text{s(X}_t), \text{data=myimp}\text{imputations[1]}, \text{family=binomial})\]

\[\text{m\_current}\text{gcv} \quad \text{# Michael 5: current NVP better, but to discuss with Ray, because method limited}\]

\[\text{m\_average}\text{gcv}\]

# Figure ####

# Spline representation

# Imputation based approach

\[\text{Lpvm31} \leftarrow \text{coxph(Surv(X}_0, X\_t, \text{vlsupp})} \sim \text{pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(hfacat) + as.factor(agecat) + as.factor(wfacat) + as.factor(postrantb) + cluster(id)}, \text{data=myimp}\text{imputations[1]}, \text{robust=TRUE, method="breslow")}\]

\[\text{Lpvm32} \leftarrow \text{coxph(Surv(X}_0, X\_t, \text{vlsupp})} \sim \text{pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(hfacat) + as.factor(agecat) + as.factor(wfacat) + as.factor(postrantb) + cluster(id)}, \text{data=myimp}\text{imputations[2]}, \text{robust=TRUE, method="breslow")}\]

\[\text{Lpvm33} \leftarrow \text{coxph(Surv(X}_0, X\_t, \text{vlsupp})} \sim \text{pspline(nvp, df=4) + as.factor(vlcat) + as.factor(cd4pccat) + as.factor(hfacat) + as.factor(agecat) + as.factor(wfacat) + as.factor(postrantb) + cluster(id)}, \text{data=myimp}\text{imputations[3]}, \text{robust=TRUE, method="breslow")}\]
APPENDIX III

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)

# Main Figure:

tiff("NVP3.tif", res=600, compression = "lzw", height=5, width=5, units="in")

dev.off()}
APPENDIX III

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

MIsummary <- matrix(rep(NA,8*10),nrow=10,ncol=8)
MIsummary[1,1:4] <- my_1b
MIsummary[2,1:4] <- my_2
MIsummary[4,1:4] <- my_4
MIsummary[6,1:4] <- my_6
MIsummary[7,1:4] <- my_7
APPENDIX III

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[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[,] <- 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[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[,] <- my_12
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[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“)
APPENDIX III

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")
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")
extractAIC(m5_2a)[2]
extractAIC(m5_2b)[2]
extractAIC(m5_2c)[2]

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]
APPENDIX III

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) ~ 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_3c<-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_3d<-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")

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) ~ 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_4c<-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_4d<-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")

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")
APPENDIX III

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")
extraitAIC(m5_5a)[2]
extraitAIC(m5_5b)[2]
extraitAIC(m5_5c)[2]
extraitAIC(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")
extraitAIC(m5_6a)[2]
extraitAIC(m5_6b)[2]
extraitAIC(m5_6c)[2]
extraitAIC(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")

295
APPENDIX III

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]
APPENDIX III

extractAIC(m5_9d)[2]

m4_0a<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvpcat3 + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(gecat) +
               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(gecat) +
               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(gecat) + 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(gecat) + 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(gecat) + 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(gecat) + 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(gecat) +
               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(gecat) + 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")

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")
APPENDIX III

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$imputations[[8]],method="breslow")
m5_39<-coxph(Surv(X_t0,X_t, vlsupp) ~ nvp + as.factor(vlcat)+ as.factor(cd4pccat) + as.factor(agecat) + as.factor (wfacat) + as.factor (hfacat) + as.factor(postrantb) + cluster(id),data=myimp$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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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, vlcat) ~ 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])
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=10000000

$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 WFMF 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 (0.1,0.04989) ; 1 CL [L/h]
$THETA (0.8,0.03299) ; 2 V [L]
$THETA (0.74) FIX ; 3 KA [1/h]
$THETA (0.004,0.103454,5) ; 4 ADD [mg/L]
$THETA (0.11682) ; 5 PROP[%]
$THETA (0.26,6.646) ; 6 PMA50 [Months]
$THETA (0.594982,1) ; 7 RIFCL
$THETA (0.37) FIX ; 9 TVLAG

$OMEGA BLOCK(1)
0.177698 ; 1 IIV_CL

$OMEGA BLOCK(1)
0.07973 ; 2 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
0.169358 ; 22 IIV BIO

0.133771 ; 23 IOV BIO
APPENDIX IV

$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) FIX
0 ; 42 IOV CL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
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
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APPENDIX IV

$\text{OMEGA } \text{BLOCK(1) SAME}$
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$\text{PK}$

;--------------------------------------------------------------------------------------------------------------------------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

ENDIF
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
;SOCOVARIANCE 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 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 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)
APPENDIX V

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)
APPENDIX V

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 ; bioavailability 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 -

;----------------------------------------------------------------------------------------------- TRANSFER 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 ; bioavailability after first pass

BIO_BIRTH = THETA(8) ; BIO AT BIRTH
APPENDIX V

\[
\text{KBIO} = \text{THETA}(9) \ ; \ \text{AGE EFFECT CONSTANT}
\]

\[
\text{AGEBIO} = 1 - ((1 - \text{BIO}_\text{BIRTH})^\text{VISAGE} \times \text{KBIO}) ; \ \text{AGE EFF ON F1 AS INVERSE EXP WITH INTERCEPT; AGE IN YEARS FROM BIRTH}
\]

\[
\text{BIO} = \text{TVBIO} \times \text{EXP}(\text{BSV}_\text{BIO} + \text{BOV}_\text{BIO}) \times \text{AGEBIO} \ ; \ \text{PRE-HEPATIC BIO}
\]

\[
S3 = V3
\]

; Transit Compartment

\[
F1 = 0
\]

\[
\text{KTR} = (\text{NN} + 1) / \text{MTT}
\]

\[
\text{IF (NEWIND} = 2 \text{OR.EVID} = 3) \text{ THEN ; new individual, or reset event}
\]

; The values read here will be stored in TDOS and PD in this very PK call.

\[
\text{TNXD} = \text{TIME} \ ; \ \text{Time of the dose}
\]

\[
\text{PNXD} = \text{AMT} \ ; \ \text{Amount. If it's zero, the DE is deactivated.}
\]

ENDIF

\[
\text{TDOS} = \text{TNXD} \ ; \ \text{This will either save here the temporary values if it's a new individual...}
\]

\[
\text{PD} = \text{PNXD} \ ; \ ... \text{or the values which were read one record ahead during the execution of the previous record.}
\]

IF(\text{AMT.GT.0}) \text{ THEN ; This reads one record ahead and stores the data to be used when running the following record}

; \text{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}

\[
\text{TNXD} = \text{TIME}
\]

\[
\text{PNXD} = \text{AMT}
\]

ENDIF

\[
\text{LNGAM} = \text{NN} \times \text{LOG}(\text{NN}) - \text{NN} + \text{LOG}((\text{NN} + 1) \times (1 + 4 \times \text{NN} \times (1 + 2 \times \text{NN}))) / 6 + 0.572364942 \ ; \ \text{approximation of log of gamma(n), 0.572364942 is LOG(Pi)/2}
\]

; To speed up the computation, I calculate here all the non-time-varying quantities used in $DES

\[
\text{PIZZA} = \text{LOG}((\text{BIO} \times \text{PD} \times \text{KTR} + 0.00001) - \text{LNGAM}) \ ; \ \text{without +0.00001, it won't work with ETAs in bioavailability}
\]

; Dosing Compartment Initialization

\[
\text{TAU_EQ} = \text{MTT} + 1 / \text{KA}
\]
APPENDIX V

$\text{KA}_\text{EQ} = 1/\text{TAU}_\text{EQ}$

; DOSING IS EVERY 12H, HERE USE HF THE TOTAL DOSE

$\text{BASELINE} = \left(\frac{((\text{BIO} \times \text{AMT}) \times \text{KA}_\text{EQ})}{(\text{KA}_\text{EQ} - K)}\right) \times \left(\frac{1}{1 - \exp(-K \times 12)}\right) - \left(\frac{1}{1 - \exp(-\text{KA}_\text{EQ} \times 12)}\right)$

$A_0(1) = 0.0001$

$A_0(2) = \text{BASELINE} \times VH$

$A_0(3) = \text{BASELINE} \times V3$

; Calculating $C_{\text{min}}$

$\text{C}_{\text{MIN}} = \left(\frac{((\text{BIO} \times \text{PNXD}/2) \times \text{KA}_\text{EQ})}{(V3 \times (\text{KA}_\text{EQ} - K))}\right) \times \left(\frac{1}{1 - \exp(-K \times 12)}\right) - \left(\frac{1}{1 - \exp(-\text{KA}_\text{EQ} \times 12)}\right)$

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

$\text{TDOS} = T - \text{TDOS}$ ; this is time after dose, it should always be $\geq 0$

$\text{KTT} = 0$

$\text{CP} = A(3)/V3$

IF (CP.GE.COM(1)) THEN

COM(1) = CP ; $C_{\text{MAX}}$

COM(2) = T - TDOS ; TIME OF $C_{\text{MAX}}$

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

$\text{KTT} = \text{KTR} \times (\text{TEMPO})$

$\text{DADT}(1) = \exp(\text{PIZZA} + \text{NN} \times \log(\text{KTT}) - \text{KTT}) - \text{KA} \times A(1)$

ENDIF

DADT(2) = $\text{KA} \times A(1) - \text{K} \times A(2) - \text{K23} \times A(2) + \text{K32} \times A(3)$
APPENDIX V

\[
DADT(3) = K23*A(2) - K32*A(3)
\]

;-----------------------------------------------------------------------------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
$OMEGA BLOCK(1) SAME
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$OMEGA BLOCK(1) SAME
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\$\text{OMEGA BLOCK(1)}
0.23842 ; 21 BSV\_KA
\$\text{OMEGA BLOCK(1)}
0.173174 ; 22 BOV\_KA

\$\text{OMEGA BLOCK(1) SAME}
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$\text{BLOCK(1)} \quad \text{SAME}

$\text{BLOCK(1)} \quad \text{SAME}

$\text{BLOCK(1)} \quad \text{0.0285655} ; \text{40 BSV\_BIO}$

$\text{BLOCK(1)} \quad \text{0.1995301} ; \text{41 BOV\_BIO}$

$\text{BLOCK(1)} \quad \text{SAME}

$\text{BLOCK(1)} \quad \text{SAME}

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$\text{BLOCK(1)} \quad \text{SAME}

$\text{BLOCK(1)} \quad \text{FIX}

\text{0} ; \text{59 BSV\_MTT}

$\text{BLOCK(1)} \quad \text{1.16439} ; \text{60 BOV\_MTT}$

$\text{BLOCK(1)} \quad \text{SAME}

$\text{BLOCK(1)} \quad \text{SAME}

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$\text{BLOCK(1)} \quad \text{SAME}$
APPENDIX V

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$SIGMA 1 fix

$ESTIMATION maxeval=0 sigdig=3 print=1 noabort method=cond interp msfo=msfo190 mceta=100 ranmethod=4P atol=9

$ESTIMATION maxeval=9999 sigdig=3 print=1 noabort method=cond interp 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 ipredires 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 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 wfh bmifa cd4cnt cd4pc viraload noprint noappend oneheader format=,

$TABLE file=catab190.csv id tb resistance noprint noappend oneheader format=,