PLASMA CONCENTRATIONS OF NELFINAVIR AND VIRAL SUPPRESSION IN HIV-1 INFECTED PREGNANT WOMEN

by

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B.Sc. (H.), McGill University, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(Reproductive and Developmental Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

June 2008

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ABSTRACT

BACKGROUND: Highly active antiretroviral therapy (HAART) is used in pregnancy to suppress viral load (pVL) before delivery, reducing risk of vertical HIV-transmission. Nelfinavir (NFV) containing HAART has been highly used in pregnancy, but dosages may be inadequate due to the physiologic changes that occur. Given concerns regarding optimal viral suppression in pregnancy, drug toxicity and resistance development, NFV levels need to be evaluated in this population to guide dosing recommendations.

METHODS: As part of a prospective cohort study maternal blood was collected at 18-28wks, 32-37wks and at delivery. Times of last medication dose and blood sampling were recorded and drug levels were measured using HPLC MS-MS. NFV concentration-ratios (NFV-CRs) were calculated by dividing individual levels by a time-adjusted population value. Plasma NFV concentrations and NFV-CRs were compared across gestational age and correlated to variables of interest. Rate and maintenance of viral suppression were analyzed in relation to NFV concentrations and CRs. Statistical tests included ANOVA, χ2, linear regression, and Kaplan Meier estimates.

RESULTS: 113 samples were collected from 32 subjects. Samples were eliminated if not in steady state (n=20); 93 samples from 32 subjects were analyzed. Mean NFV-CR at 18-28wks (1.1±0.73) and 32-37wks (0.86±0.73) were not significantly different but were both significantly higher by ANOVA (p=0.049) than the mean NFV-CR at delivery (0.44±0.50). CRs were highly variable. Of 49 antepartum samples, 49% (24) had a CR<0.90 (clinically relevant threshold). Four women reached a pVL <50 copies/mL by 34wks but had a detectable pVL at delivery. One woman never reached an undetectable pVL in pregnancy. Minimum and mean NFV-CRs in these 5 women were
not significantly different than those who achieved and maintained virologic suppression.

Vertical HIV transmission rate was 0%.

CONCLUSIONS: There were no HIV transmissions but 16% (5/32) of women were inadequately suppressed at delivery, which is of concern. Factors associated with inadequate suppression and NFV-CRs need to be explored in conjunction with patient/physician reported adherence and viral resistance profiles. Extreme variability in CRs may limit the potential usefulness of random timed drug levels in all pregnant women.
# TABLE OF CONTENTS

ABSTRACT ................................................................................................................... ii
TABLE OF CONTENTS .................................................................................................... iv
LIST OF TABLES ............................................................................................................. vi
LIST OF FIGURES .......................................................................................................... vii
LIST OF ABBREVIATIONS ............................................................................................. viii
ACKNOWLEDGEMENTS ............................................................................................... ix

INTRODUCTION ............................................................................................................. 1
   BIOLOGY of HIV INFECTION ......................................................................................... 1
   EPIDEMIOLOGY of HIV ................................................................................................. 2
   VERTICAL TRANSMISSION of HIV ............................................................................ 4
   MANAGEMENT of HIV INFECTED PREGNANCIES in HIGH RESOURCE SETTINGS .... 7
   SAFETY of ANTIRETROVIRALS in PREGNANCY .................................................... 10
   PHYSIOLOGICAL CHANGES in PREGNANCY & DRUG DISPOSITION ................. 14
   THERAPEUTIC DRUG MONITORING of ANTIRETROVIRALS ............................... 17
   MEASURES and TARGET THRESHOLDS for ANTIRETROVIRAL TDM ................. 19
   PREGNANCY and ANTIRETROVIRAL PHARMACOKINETICS .................................. 24
   JUSTIFICATION / RATIONALE ..................................................................................... 28
   HYPOTHESIS ................................................................................................................ 29
   OBJECTIVES ................................................................................................................ 29

METHODS ...................................................................................................................... 30
   STUDY DESIGN ........................................................................................................... 30
   STUDY SETTING and POPULATION .......................................................................... 30
   SAMPLE SIZE CALCULATION ..................................................................................... 31
   STUDY VISITS and SAMPLE COLLECTION ................................................................ 32
   DATA COLLECTION ..................................................................................................... 33
   VARIABLE IDENTIFICATION and SELECTION .......................................................... 34
   REPORTING on ADHERENCE ..................................................................................... 34
   DETERMINING DRUG PLASMA CONCENTRATIONS ............................................... 35
   PLASMA CONCENTRATION RATIOS ......................................................................... 36
   DEFINITION of REMAINING VARIABLES ................................................................ 37
   ANALYSIS PLAN ......................................................................................................... 41
   ETHICAL CONSIDERATIONS ....................................................................................... 42

RESULTS ....................................................................................................................... 43
   SUBJECT and SAMPLE INCLUSION .......................................................................... 43
   SAMPLE CHARACTERISTICS ..................................................................................... 44
   BASIC DEMOGRAPHICS ............................................................................................. 45
   HIV RELATED DESCRIPTORS ..................................................................................... 47
   ANTIRETROVIRAL THERAPY in PREGNANCY ........................................................... 49
   NELFINAVIR RAW PLASMA CONCENTRATIONS ...................................................... 50
   NELFINAVIR PLASMA CONCENTRATION RATIOS .................................................... 52
   REPORT on CO-VARIATES ........................................................................................... 56
   CORRELATIONS of COVARIATES and NFV CONCENTRATIONS .............................. 57
   DESCRIPTION of LOPINAVIR/ritonavir CONCENTRATIONS IN PREGNANCY .... 58
LIST OF TABLES

TABLE 1. US FDA PREGNANCY CLASS OF ANTIRETROVIRAL DRUGS (36) .............................................................. 11
TABLE 2. SUBJECT EXCLUSION .......................................................................................................................... 43
TABLE 3. SAMPLE EXCLUSION .......................................................................................................................... 44
TABLE 4. SAMPLE DISTRIBUTION BY TIME POINT .............................................................................................. 44
TABLE 5. MATERNAL GENERAL DEMOGRAPHICS, N=40 ................................................................................. 46
TABLE 6. HIV CHARACTERISTICS, N=40 ........................................................................................................... 48
TABLE 7. NELFINAVIR RAW PLASMA CONCENTRATIONS .................................................................................. 50
TABLE 8. NELFINAVIR PLASMA CONCENTRATION RATIOS ............................................................................. 52
TABLE 9. CO-VARIANTS ACROSS PREGNANCY ............................................................................................... 56
TABLE 10. UNIVARIATE ANALYSIS, CO-VARIATES AND NFV CONCENTRATIONS ........................................ 57
TABLE 11. LOPINAVIR PLASMA CONCENTRATIONS (MG/ML) ......................................................................... 58
TABLE 12. PROPORTIONAL HAZARDS, TIME TO UNDETECTABLE PVL (N=24) ............................................ 64
TABLE 13. CHARACTERISTICS ASSOCIATED WITH LACK OF VIRAL SUPPRESSION AT DELIVERY ......... 65
TABLE 14. VIRAL RESISTANCE AND ADHERENCE FOR PATIENTS WITH DETECTABLE VIRAL LOAD AT DELIVERY .................................................................................................................. 67
LIST OF FIGURES

FIGURE 1. PHARMACOKINETIC MEASURES FOR THERAPEUTIC DRUG MONITORING ........................................... 20
FIGURE 2. ANTIRETROVIRAL THERAPY REGIMENS PRESCRIBED IN PREGNANCY .................................................. 49
FIGURE 3. NELFINAVIR PLASMA CONCENTRATIONS .................................................................................................................. 51
FIGURE 4. NELFINAVIR CONCENTRATIONS RATIOS .................................................................................................................. 53
FIGURE 5. COMPARISON OF TWO ANTEPARTUM CONCENTRATION RATIOS IN RELATED SAMPLES ........... 54
FIGURE 6. CHANGE IN SUBJECTS’ CONCENTRATION RATIO ACROSS GESTATIONAL AGE ........................................... 55
FIGURE 7. MEDIAN LPV & RTV PLASMA CONCENTRATIONS IN PREGNANCY ................................................................. 59
FIGURE 8. RATE OF VIRAL SUPPRESSION BY FIRST ANTEPARTUM NFV CR ................................................................. 61
FIGURE 9. RATE OF VIRAL SUPPRESSION BY MEAN OF ANTEPARTUM NFV CRS ............................................................. 62
FIGURE 10. LACK OF VIRAL SUPPRESSION AT DELIVERY ................................................................................................. 66
FIGURE 11. NELFINAVIR AND M8 METABOLISM BY LIVER ENZYMES ...................................................................................... 70
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACTG</td>
<td>AIDS Clinical Trial Group</td>
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<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
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<tr>
<td>AUC</td>
<td>Area under concentration-time curve</td>
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<tr>
<td>AZT</td>
<td>Zidovudine</td>
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<tr>
<td>BID</td>
<td>Twice daily</td>
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<tr>
<td>CD4</td>
<td>T helper</td>
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<tr>
<td>Cmax</td>
<td>Peak plasma concentration</td>
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<tr>
<td>Cmin</td>
<td>Minimum plasma concentration</td>
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<tr>
<td>CPARG</td>
<td>Canadian Pediatric AIDS Research Group</td>
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<tr>
<td>CR</td>
<td>Concentration Ratio</td>
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<tr>
<td>CWHCBC</td>
<td>Children’s and Women’s Health Centre of British Columbia</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P-450</td>
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<tr>
<td>EI</td>
<td>Entry Inhibitor</td>
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<tr>
<td>GA</td>
<td>Gestational age</td>
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<tr>
<td>H</td>
<td>Hours</td>
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<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
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<td>HIV</td>
<td>Human immunodeficiency virus type 1</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>IC</td>
<td>Inhibitory Concentration</td>
</tr>
<tr>
<td>IDU</td>
<td>Intravenous drug use</td>
</tr>
<tr>
<td>II</td>
<td>Integrase Inhibitor</td>
</tr>
<tr>
<td>IQ</td>
<td>Inhibitory Quotient</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>LPV/r</td>
<td>Lopinavir boosted with ritonavir (Kaletra)</td>
</tr>
<tr>
<td>MEC</td>
<td>Minimum Effective Concentration</td>
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<tr>
<td>MS-MS</td>
<td>Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>NFV</td>
<td>Nelfinavir (Viracept)</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Non-Nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NVP</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>OTC</td>
<td>Oak Tree Clinic</td>
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<tr>
<td>PACTG</td>
<td>Pediatric AIDS Clinical Trial Group</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PLAT</td>
<td>Pregnancy limited antiretroviral therapy</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
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<tr>
<td>PK-PD</td>
<td>pharmacokinetic-pharmacodynamic</td>
</tr>
<tr>
<td>PI</td>
<td>Protease inhibitor</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>The Joint United Nations Programme on HIV/AIDS</td>
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<tr>
<td>US FDA</td>
<td>United States Food and Drug Administration</td>
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<tr>
<td>Wks</td>
<td>Weeks</td>
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<tr>
<td>3TC</td>
<td>Lamuvidine</td>
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<tr>
<td>95%CI</td>
<td>95% confidence interval</td>
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ACKNOWLEDGEMENTS

I would like to give considerable thanks to all of the staff at the Oak Tree Clinic for the support they have given to this project. I am grateful for their patience and enthusiasm. I would particularly like to thank Evelyn Maan for her insight, humor, unflattering encouragement and wisdom. I owe her a debt of gratitude. I would also like to expressly thank my supervisor, Dr. Deborah Money, not only for her time and extensive mentorship, but also for the firm foundation on which to build my future.

My thanks extend to the team studying HAART associated mitochondrial toxicity in pregnancy for the use of the study samples and collected data. This project could also not have been completed without the support of the BC Centre for Excellence in HIV/AIDS for the analyses of antiretroviral drug concentration.

I would like to thank my thesis committee, Drs. Helene Cote, Mary Ensome, Richard Harrigan and Peter Leung for their interest, expertise and contribution to this thesis. Their input has been invaluable in both its preparation and completion.

Finally I would like to thank the Michael Smith Foundation for Health Research for trainee funding support through a Junior Graduate Studentship. Further funding for the study was awarded to Dr. Money from the Canadian Association for AIDS Research (CANFAR) and the Canadian Institutes for Health Research (CIHR).
INTRODUCTION

BIOLOGY of HIV INFECTION

The human immunodeficiency virus (HIV) is an RNA retrovirus that targets the human body's immune system. Infection occurs through the transfer of bodily fluids, with the major routes of transmission being unprotected sexual intercourse, percutaneous exposure to contaminated needles, and transmission from an infected mother to her baby at birth or through breast milk. Initial infection results in high levels of viral replication in the bloodstream (>10^6 virons/mL) and in 80-90% of patients, a seroconversion illness (1).

Acute infection is followed by a period of clinical latency: a strong defence by the body's immune system reduces the amount of circulating virus and infection is established in the cells of the lymphoid system, specifically in T helper (CD4) cells, macrophages and dendritic cells. During cellular infection, viral RNA is converted into DNA using the virus' own reverse transcriptase, which permits the virus' genetic information to be integrated into the cell's DNA using the virally encoded enzyme, integrase. HIV can either then become latent in the cell, with the infected cell continuing to function, or become active, with cell death and the liberation of newly replicated virons into the bloodstream.

Over the course of the clinical latent period, the body's immune system deteriorates. The CD4 count depletes through three main mechanisms: CD4 cell death by apoptosis (programmed cell death), by direct viral killing, and by killing by CD8 cytotoxic lymphocytes. Patients transition to symptomatic HIV infection and finally to Acquired Immunodeficiency Syndrome (AIDS). The body is then prone to a wide range of opportunistic infections such as tuberculosis, Pneumocystis pneumonia, toxoplasmosis, Mycobacterium avium complex, Cryptococcal meningitis, etc. and malignant cancers including Kaposi’s sarcoma, cervical cancer and high grade B-cell lymphomas.
EPIDEMIOLOGY of HIV

At the end of 2007, the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported that an estimated 33 million people worldwide were living with HIV (2). More than two million AIDS related deaths and 2.5 million new infections occur annually, the majority in Sub-Saharan and North Africa, the Middle East, and South and South East Asia (2). Those newly HIV infected represent diverse ethnicities and nationalities, and include over 50% women and just under half a million children under the age of 15 (2). Equal gender distribution of disease has meant an increase in AIDS-related illness and death in women, while mother to child transmission (vertical transmission) causes the vast majority (>90%) of childhood HIV infections (2).

Initial HIV seropositivity rates among women in the developed world were low; however, over the past decade there has been a consistent rise in incidence and prevalence among women of reproductive age. In 2006, 9,500 of the 58,000 Canadians diagnosed with HIV were women (3). This reflected a 15% increase in new diagnoses among women from the previous year. Heterosexual contact (61.1%) and injection drug use (30.7%) were named as the primary acquisition risk factors in this population (3). Women also accounted for 45% of positive test results in people between the ages of 15-29 (3).

In British Columbia specifically, the total number of women diagnosed with HIV in the province reached 1,758 in 2006, as 72 women were newly diagnosed (4). Of the 72 new infections, 28% were Caucasian, 36% First Nations/Metis, 9.7% Black, and 13% Asian/South Asian (4). Heterosexual contact (39%), intravenous drug use (IDU) (25%), and sex trade work in combination with IDU (26%) were noted as the major risk categories (4).
As women represent an increasing proportion of the HIV infected population, both provincially and nationally, there is significant concern about perinatal HIV infection. A study to examine the fertility intentions of HIV infected women living in British Columbia between November 2003 and December 2004 found that of the 230 women who completed the survey, 79% were of reproductive age, and 26% indicated an intention to have children (5). Additionally, a significant number of women are diagnosed with HIV in the antenatal period (5).

All of the Canadian provinces have now incorporated HIV testing as part of recommended prenatal blood work, and from October 2003 to October 2005, 83% of pregnant women in British Columbia were tested. HIV seroprevalence among this population was found to be 9.0 cases per 10,000 pregnant women (6) compared to 3.0 cases per 10,000 in Alberta (2000), 2.3 in Ontario (2003) and 5.5 in Quebec (1990) (3). By the end of 2005, 2,206 Canadian children had been exposed to HIV in utero since the start of the epidemic in the early 1980s, and 496 were perinatally infected (3), the majority prior to routine treatment with ART in pregnancy.

Fortunately, these recent elevated rates of adult infection and HIV infected pregnancies have not translated into increased neonatal infections in high-resource settings. ART and comprehensive antenatal care has dramatically reduced vertical transmission rates in Canada, from 30% (in 1995) to less than one percent in 2005 (3). The Canadian Pediatric AIDS Research Group (CPARG) reported that of the 195 known infants born to HIV infected mothers in 2007, one was infected (7).
VERITCAL TRANSMISSION of HIV

Three different routes or different timings of vertical transmission have been identified: antepartum/ pregnancy, intrapartum/delivery and postpartum/breastfeeding. Kourtis et al. developed a hypothetical model to describe the temporal distribution of transmission, and suggested that the majority of infections occur during late pregnancy at the onset of placental separation, between 36wks gestational age (GA) and start of labour (8). The delay of viral detection in infants’ bloodstream by PCR (polymerase chain reaction) until after 7 days of age alternatively suggests that the vast majority (50-80%) of infections occur in the intrapartum period (9;10).

Several studies of women in both developed and developing nations have showed inconsistencies as to whether untreated HIV affects pregnancy outcomes. A study of HIV infected and uninfected intravenous drug users in Italy found no statistical difference in obstetrical outcomes, although the control group demonstrated unusually high rates of adverse events including pre term birth, low birth weight, and poor infant health in the 5 minutes after birth (APGAR score) (11). Increased rates of preterm birth, intrauterine growth restriction, and low birth weight were seen among untreated HIV infected women in Rwanda when compared to a control population (12). In both studies, advanced HIV disease was associated with increased adverse outcomes.

The effect of pregnancy on maternal HIV progression has also been explored. In a large cohort study, the relative risk of progression from HIV infection to an AIDS diagnosis associated with pregnancy was 0.7 (95% confidence interval (95%CI) 0.4-1.2) (13). Two other studies in North America and Europe found no alternation in the course of disease (14;15). A more recent study of pregnant and non-pregnant women receiving ART, found that pregnancy was associated with a
lower risk of disease progression when conducting both a large cohort analyses (Cox proportional hazard ratio (HR) 0.4 – 95%CI 0.20-0.79; p=0.009) and matched-pair analyses (Cox HR 0.44 – 95%CI 0.19-1.00; p=0.05) (16).

Despite exposure to maternal HIV, most infants are not infected. Numerous studies have investigated factors associated with differing transmission rates. Advanced HIV disease, namely low CD4 counts or an AIDS diagnosis, was initially associated with increased perinatal infection (17). Five years later, two landmark studies in the New England Journal of Medicine identified high plasma HIV viral load to be an independent predictive factor for transmission. Garcia et al. reported that vertical transmission rates in 64 women with viral loads >100,000 copies/mL was 63.3%, compared with 0% in 57 women with viral loads of <1,000 copies/mL (18). Similarly, Mofenson et al. found that for each log increment of HIV viral load at delivery, the adjusted odds ratio for transmission increased by 3.4 (95% CI 1.7-6.8, p 0.001) (19).

Levels of plasma viral load have shown to be a predictor for the presence of HIV-RNA in cervical and vaginal secretions (20), also identified as a risk factor for transmission. Transmission was 26.3% among Thai women with detectable virus by cervicovaginal lavage, compared to 7.9% in women with no detectable virus (21). Similarly, the presence of cervical and vaginal HIV-RNA in Kenyan patients was shown to be a risk factor for transmission, statistically independent of plasma viral load (22).

Beyond maternal HIV parameters, different modes of delivery have been investigated in relation to perinatal infection rates. A meta-analysis of 8,533 women from 15 study groups found a 50% reduction in transmission when infants were delivered by caesarean section prior to the onset of labour (23): 8.2% of infants delivered by caesarean section were infected, as compared to 16.7% of those delivered vaginally. This protective effect, however, was shown to lessen when the
patient was treated with zidovudine (AZT), and even more so in women treated effectively with combination ART (23). Increased transmission rates have also been noted with increased length of time between rupture of membranes and delivery, instrumental or operative vaginal deliveries, and the use of obstetrical devices such as scalp electrodes (24).

Breastfeeding is also a known risk factor for pediatric infection. In a study of 4,085 breastfed children, 42% of infections were determined as being late-postnatal, and attributed to HIV acquisition through breast milk (25). This risk factor is largely minimized in high resource settings with ready access to infant formula, but remains of significant issue in developing countries where formula is prohibitively expensive and is associated with widespread stigma. This problem is further compounded by irregular access to clean drinking water and mixed infant feeding practices (giving other foods or liquids as well as breast milk) which has been associated with increased rates of infant HIV infection and death (26).

Finally, the role of ART in reducing vertical transmission is paramount. In 1994 the New England Journal of Medicine published the landmark results of the Pediatric AIDS Clinical Trial Group (PACTG) 076 Study, which introduced the use of AZT to prevent perinatal HIV infection (27). The PACTG 076 regimen included a three part monotherapy series: pregnant women were initiated on AZT from 14-34 wks GA, received intravenous AZT during labour, and infants received up to six wks of AZT prophylaxis after birth. The trial was halted after an interim analysis; AZT was shown to reduce transmission by 66%, from 25.5% in the placebo arm, to 8.3% in the AZT arm. Since this seminal paper was published, monotherapy has been replaced by combinations of three or more unique drugs, known collectively as highly active antiretroviral therapy (HAART). When antenatal HAART was combined with IV AZT in the intrapartum period, as well as neonatal prophylaxis and infant formula, published vertical transmission rates ranged from 1.2-1.7% (28;29).
MANAGEMENT OF HIV INFECTED PREGNANCIES IN HIGH RESOURCE SETTINGS

Between 1996-2000 HAART became the standard of care for adult therapy. By minimizing viral replication and permitting immune reconstitution, treatment dramatically decreases time to symptomatic HIV, AIDS and death (30;31). In non-pregnant adults, HAART is initiated for two main reasons: symptomatic HIV infection (including a clinical diagnosis of AIDS), or asymptomatic HIV disease with a low CD4 cell count. The CD4 cell count cut off at which to initiate treatment remains controversial; current Therapeutic Guidelines published by the BC Centre for Excellence in HIV/AIDS details that patients with a CD4 <200 x10^6 cells/L should commence treatment, while patients with a CD4 >200 x10^6 cells/L but <350 x10^6 cells/L should be closely evaluated, taking into consideration clinical and laboratory parameters (including CD4 cell fraction and pVL) as well as the patient’s preference (32). First line therapies in this population take advantage of newer fixed dose combinations of drugs because of the low pill burden and the associated facilitation of adherence. Common combinations include two nucleoside reverse transcriptase inhibitors (NRTIs) partnered with either a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) (32).

Consistent with the standard of care for adults, HAART is used in HIV infected pregnant women to treat both underlying maternal disease (33) and to prevent perinatal infection (34). In 2003, the Canadian HIV Trials Network Working Group on Vertical HIV Transmission published the Canadian consensus guidelines for the management of pregnancy, labour and delivery and for postpartum care of HIV infected pregnant women and their offspring (35). The guidelines recommend ART in pregnancy to ensure maximal viral suppression while maintaining a woman’s long-term treatment options. Efforts are made to avoid potentially toxic medications, especially since it is suspected that pregnancy increases the risk of toxic effects due to nucleoside analogues, such as lactic acidosis.
Similar recommendations made by the United States Public Health Service Task Force are revised bi-yearly to accommodate new therapies or investigational results (36). Based largely on the three part (antepartum, intrapartum and neonatal) PACTG 076 regimen (27), the recommendations suggest that AZT should be included as a component of antenatal HAART whenever possible. AZT is known to readily cross the placenta, and the fetus may have inhibitory levels of therapy present during the birthing process (37).

If not required for maternal health, HAART used in pregnancy primarily to prevent vertical transmission has recently been termed Pregnancy Limited Antiretroviral Therapy (PLAT) and is distinct from HAART used for adult therapy for two main reasons. To avoid exposing the fetus to ART during major organogenesis and also to minimize toxicities, PLAT is often initiated following a detailed ultrasound at 18-19 wks of GA. Treatment is discontinued immediately following delivery (at approximately 40 wks GA), and so mother and fetus experience a relatively brief period of drug exposure, as compared to patients who may be on HAART for many years.

The combinations of unique drugs used in pregnancy are also generally different from the first line of drugs recommended for non-pregnant adults. Much uncertainty remains about the safety of newer HAART regimens in pregnancy, and obstetricians often choose to prescribe older regimens, with which they have had more clinically experience (38), with the longest experience in pregnancy. The most common combination of therapy prescribed for pregnant women in British Columbia during the study period was a PI such as nelfinavir (NFV) or lopinavir boosted with ritonavir (LPV/r), with a backbone of two NRTIs, most often AZT and lamuvidine (3TC). Recently, availability of NFV was affected by evidence of a contaminant in the production of the drug that temporarily ceased usage in all populations. Prescribed dosages in pregnancy are the
same as for all non-pregnant adults of both sexes, which were previously determined by pharmaceutical licensure trials in largely male populations.

Of note, the HAART regimen of those women who are already on antiretroviral medications at conception, for their own health, is reviewed to make sure it is as “pregnancy-friendly” as possible.

Standard clinical care of an HIV infected pregnant woman also includes monitoring of CD4 and HIV viral load, and other HIV and pregnancy associated lab evaluations every 4-6 wks throughout the pregnancy. Prophylaxis against opportunistic infections, such as *Pneumocystis carinii* (PCP) and *Mycobacterium avium complex* (MAC) is offered to women who are significantly immunocompromised with CD4 counts of <200x10^6 cells/L. Screening for other sexually transmitted infections and for cervical cytologic abnormalities (Papanikolaou test) is also recommended.

If there are no other obstetrical indications, a vaginal delivery is offered to women whose viral load is <50 copies/mL at the onset of labour. Alternatively, if either obstetrical indication exists or the viral load is not fully suppressed, a caesarean section is offered, and then completed at approximately 38-39 wks GA. Oral HAART medications are continued throughout labour, and IV AZT is initiated either at the onset of regular contractions, at the time of ruptured membranes, or 2 hours (h) prior to a planned caesarean section. Additionally, a single oral dose of nevirapine (NVP) is administered intrapartum to women without adequate antenatal therapy.

Neonates are started on oral AZT prophylaxis within 6-12 hour of birth and continue for up to 6 wks if the drug is well tolerated. In addition, a single dose of NVP is given to an infant of a mother who did not receive adequate antenatal prophylaxis. Breastfeeding is contraindicated. Infants
are tested regularly for the presence of HIV DNA by PCR; three consecutive negative PCRs before six months of age is generally considered confirmation of the absence of HIV infection. Maternal antibodies, however, can be detected in the newborn up to 2 years of age.

SAFETY OF ANTIRETROVIRALS IN PREGNANCY

The decreased rate of vertical HIV transmission to less than one percent (3) through the use of HAART, has shifted the emphasis of concern to the possible toxicity, teratogenicity and general safety of these agents in pregnancy. Data originating from a few publications describing prospective and retrospective HIV infected ART exposed pregnancy cohorts have identified some associated outcomes; however, the macro-level initiative of the Antiretroviral Pregnancy Registry has also been valuable in providing “an early signal of any major teratogenic effect associated with a prenatal exposure,” that might have been missed in individual cohort studies due to low levels of incidence. The Registry depends on the voluntary efforts of physicians to collect and evaluate outcomes associated with non-experimental pregnancy exposures to antiretroviral products and collects information from all over North America and Europe (39).

Another description of a drug’s safety profile in pregnancy comes from the United States Food and Drug Administration (US FDA). Antiretrovirals available for use in the United States are designated a specific class, depending on what is known from animal toxicity data, case reports, or registry data (Table 1); this classification may guide recommendations for administration to pregnant women.
Table 1. US FDA Pregnancy Class of Antiretroviral Drugs (36)

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<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>ART</th>
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<td>A</td>
<td>Adequate and well-controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters).</td>
<td>none</td>
</tr>
<tr>
<td>B</td>
<td>Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women OR Animal studies which have shown an adverse effect, but adequate and well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester.</td>
<td>NRTI Didanosine (ddI) Emtricitabine (FTC) Tenofovir (TDF) NNRTI Nevirapine (NVP) PI Atazanavir (ATZ) EI* Darunavir Nelfinavir (NFV) Ritonavir (RTV) Saquinavir (SQV) Enfuvirtide Maravaron</td>
</tr>
<tr>
<td>C</td>
<td>Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.</td>
<td>NRTI Abacavir (ABC) Lamuvidine (3TC) NNRTI Staduvine (d4T) Zalcitabine (ddC) PI Zidovudine (AZT) Delavirdine Amprenavir Fosamprenavir Indinavir (IDV) II# Lopinavir (LPV) Raltegravir</td>
</tr>
<tr>
<td>D</td>
<td>There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.</td>
<td>NNRTI Efavirenz (EFV)</td>
</tr>
<tr>
<td>X</td>
<td>Studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits.</td>
<td>none</td>
</tr>
</tbody>
</table>

*EI: Entry Inhibitors including Fusion Inhibitors and CCR5 inhibitors  #II: Integrase Inhibitors

One drug, efavirenz (EFV), has been strongly associated with central nervous system malformations in newborns when administered to pregnant cynomolgus monkeys (40) and case reports have identified human malformations (including neural tube defects) as a result of 1st trimester in utero exposure (41;42). The drug thus has an US FDA classification of D and is contraindicated for use in the first trimester of pregnancy (36).
All other ART medications fall into either US FDA classification B or C (36), indicating that possibility of risk associated with use during the first (and second or third) trimester of pregnancy has not been eliminated. Of note, NVP is now indicated for use in pregnant women with CD4 counts below 250x10⁶ cells/L, as it was shown to have associations with high incidences of hepatotoxicity and Stevens Johnson syndrome in women whose CD4 was greater than 250x10⁶ cells/L (43). Maternal mortality due to these toxicities resulted in an FDA warning, but NVP remains labelled as a US FDA class B drug (36).

Beyond the US FDA classifications, data about various cellular toxicities related to antiretroviral exposure originates almost singularly from non-pregnant adults; this depth of research is generally not available for pregnant women or for exposed infants. Of note, in the effort to prevent HIV transmission, fetuses are being exposed in utero for up to 40 wks to HIV therapies that generally are known to readily cross the placenta (44). Varying HAART-associated toxicities during pregnancy have been described:

i. **HYPERTENSION** – several studies, including Bucceri et al. 2002, have shown an elevated rate of pregnancy induced hypertension (PIH) in HAART treated HIV infected pregnancies (45-47). Previously, data demonstrated a higher rate of PIH only in HAART treated women vs. HIV infected untreated women, but rates were similar to HIV negative controls (48). Most recently, a study from Barcelona noted increased rates of HAART related pre-eclampsia when compared to the general population of pregnant women (49).

ii. **DIABETES** – the stated risk of gestational diabetes in HIV infected HAART treated pregnancies is 20-25% (50;51). By comparison, the rate of gestational diabetes for the general pregnant population in British Columbia is 6.7% (52).
iii. PRE TERM DELIVERY – Given that pre-term infants have higher rates of complications and poorer neonatal outcomes the lowest proportion of pre term deliveries is desirable. Published data, although varied, has associated HAART with inappropriate rates of pre term delivery. A small Swiss cohort study originally showed that therapy was associated with a 33% risk of pre term delivery (53), while the Pediatric AIDS Clinical Trials Group found a rate of 66% (54). A more recent publication linked only PI containing regimens with pre term delivery (55), supported by another cohort that associated NFV only to pre term delivery (56). Alternatively, an American meta-analysis reported that no association was found between HAART and pre term delivery (57).

iv. LOW BIRTH WEIGHT – low birth weight in ART exposed infants has been seen throughout the literature (53-55). While several studies have associated PIs with very low birth weight, authors have also consistently stated their belief that it is the severity of HIV infection in the PI treated women that is responsible for this outcome. A large (645 women) multicentre study recently reported that HAART is associated with low birth weight when compared to mono or dual therapy (56).

v. HEPATOTOXICITY – recent studies have reported a significant rate of abnormal transaminases while using saquinavir/ritonavir during pregnancy. A total of 31% of subjects in an Irish cohort had abnormal lab results, ranging from grade one to three, within two to four wks of initiating treatment, and necessitating regimen changes in a fraction of the group (58).

vi. MITOCHONDRIAL TOXICITY – NRTIs are known to inhibit mitochondrial DNA polymerase gamma, which may result in mitochondrial DNA/RNA depletion and possible mitochondrial dysfunction (59;60) and clinical conditions such as myopathy, neuropathy,
hyperlatatemia or fatty liver (37). Case reports suggest that these toxic effects are more pronounced in pregnant women (61) and may also affect exposed infants.

These outcomes are of significant concern in HAART treated pregnancies, but the role of other risk factors should also be considered. Smoking and injection drug use are known to be confounders of pregnancy outcomes including low birth weight and HIV clinical status might also impact rates of complications or differing responses with HAART during pregnancy.

**PHYSIOLOGICAL CHANGES IN PREGNANCY & DRUG DISPOSITION**

Outside the context of HIV, the use of many medications during pregnancy has long necessitated consideration of the general safety and toxicity of drugs for both mother and fetus. These potential toxicities are directly related to drug exposure, causing maternal drug levels as well as transplacental transfer to become relevant. In general, drug concentrations in pregnant women are not known; clinical thresholds have traditionally been determined in non-pregnant adult populations, but it is evident that the physiological changes associated with pregnancy may alter the pharmacokinetics (PKs) of drugs. These changes can result in both lower levels (necessitating higher, or more frequent dosing) and higher levels (requiring decreased dosing), and become especially pronounced from the end of the second trimester onwards. The alterations in the body’s physiology that can result in changes in drug concentration can be categorized in four areas: absorption, distribution, metabolism and excretion.

**ABSORPTION:** A wide range of changes can alter the body’s uptake of orally dosed drugs. Vomiting and nausea that accompany the first trimester may simply lower gastrointestinal absorption, while heightened cardiac output may increase absorption from the stomach and small intestine...
due to increased blood flow (62). Progesterone is known to relax smooth muscles, resulting in reduced gastrointestinal motility and increased emptying time. Pregnant women also have a 40 percent decrease in gastric acid secretion (when compared to non-pregnant women), and increased mucus secretion, causing the ionization, and absorption of weak acids and bases to be altered through changes in pH and buffering capacity (63), also decreasing absorption. Although no antiretroviral drugs are inhaled, the increased pulmonary blood flow associated with pregnancy favours the absorption of drugs taken by inhalation.

**DISTRIBUTION:** The increase in plasma volume by 50 percent, and total water content of the body by 8 litres, causes the peak serum concentration (Cmax) of many drugs to decrease in pregnancy (63). The rate of distribution, however, is generally increased (62) due to higher cardiac output and increased blood flow. There are also increased stores of body fat (64). Protein binding during pregnancy is also affected by the large change in plasma volume. As plasma volumes rapidly increase, the production of maternal serum albumin and other plasma proteins cannot keep pace, and the proportion of circulating proteins decreases. This often reduces a drug’s binding affinity, and results in higher serum concentrations of the drug’s unbound or free fraction. The free concentration is the pharmacologically active form and is also available for biotransformation and elimination (64).

**METABOLISM:** Hepatic metabolism is variably affected during pregnancy. Progesterone is thought to induce a higher rate of metabolism in the liver by stimulating hepatic microsomal enzyme activity such as cytochrome P-450 (CYP) 3A4 and CYP2D6. Alternatively, it acts through competitive inhibition of microsomal oxidases to hinder the elimination of other drugs through the down-regulation of CYP1A2. Estrogen may also affect hepatic drug metabolism through its cholestatic properties (63).
EXCRETION: Renal excretion of certain drugs is augmented due to higher renal plasma (increased by 25-50% (64)) and glomerular filtration rate (increases progressively by up to 50% through pregnancy) in the kidneys (62). Drug elimination also occurs through respiration and the increased pulmonary function during gestation makes this route more important (62).

PK data on pregnant women is necessary to understand altered drug disposition, and to determine if a drug's potential toxicities increase due to these changes. It is unknown whether the magnitude of these changes warrants a change in dosing for many commonly used drugs, as they may have a negating effect on each other. Previous studies have shown that pregnant women with epilepsy experience an increased frequency of seizures thought to be associated with subtherapeutic drug levels (64); similarly, pregnant women on an antidepressants require increased doses to maintain adequate serum drug concentrations (64).

A review by Little (62) looked at a number of studies and found that information on PK in pregnancy is challenging to interpret because explicit quantitative dosing or scheduling recommendations are not given and there is often conflicting data on the same therapies. Little also highlights the implications of these studies by showing that the reported peak plasma concentration, steady state, and drug half life all decreased in pregnancy, while both the reported volume of distribution and drug clearance increased, in more than 30 percent of the investigations reviewed.

The physiologic changes of pregnancy and their impact on drug disposition are of great concern in the context of HIV as the negative implications of varied antiretroviral PKs in non-pregnant adult populations has been well described in the literature.
THERAPEUTIC DRUG MONITORING of ANTIRETROVIRALS

Adequate plasma levels of ART are required to effectively suppress the virus and permit immune reconstitution (65). Those individuals with drug levels that fall below certain parameters when measuring peak plasma concentration (Cmax), minimum plasma concentration (Cmin), area under concentration-time curve (AUC) etc., have increased risk for treatment failure (incomplete viral suppression) and for developing drug resistance (66;67). Previous studies, using early antiretroviral drugs, showed that up to 50% of patients failed to reach viral suppression on various therapies (68), and of those that did suppress, between 10-50% rebounded within one year of follow-up (69;70). Increased knowledge of drug interactions and resistance, as well as newer, highly potent drugs have improved those outcomes. Drug concentrations that exceed recommended levels may also be associated with increased toxicity and incidence of adverse events (71;72). The role of therapeutic drug monitoring (TDM) of ART is therefore to assess whether measured plasma concentrations fall within an optimum therapeutic window (73).

While the cause of treatment failure is generally multifactorial and may include viral resistance to therapy, adherence, food (absorption), liver function, GI abnormalities, toxicities, and concomitant medications, the information garnered through TDM ultimately allows an initial insight into a patient’s interaction with the drugs and how individualization of treatment through dose adjustments or change of therapy could be approached (74).

Given the increasing complexity of HAART regimens (there are currently 25 US FDA approved drugs in 5 classes and many new drugs in development), it is important to consider the utility of TDM for different drugs. NRTIs are often used in combination to act as a therapy’s backbone. The active moiety of this class is a triphosphate anabolite that causes the termination of DNA elongation through the absence of the 3’-hydroxyl group. These drugs require intracellular
phosphorylation to become substrates for the reverse transcriptase, and the intracellular levels of two NRTIs, AZT and 3TC, have shown only a weak correlation to nucleoside plasma concentrations (75;76). The extensive procedure required to measure intracellular triphosphate levels makes its use, and TDM of NRTIs in general, impractical in the clinical setting (77).

The NNRTIs also target the HIV-1 reverse transcriptase by binding close to the enzyme’s catalytic active site and inhibiting reverse transcription. NNRTIs generally have prolonged half-lives, sufficient maintenance of steady state concentrations, and very little PK variability. The consistency and PK robustness of these drugs have previously lowered the need for TDM.

The third, widely used, class of ART is the PIs. PIs prevent the cleavage of nascent viral proteins for viron assembly, and lead to the production of non-infectious viral particles. Generally, better and more durable clinical outcomes are seen when most PIs are boosted with a low dose of a second PI, ritonavir, and one pharmaceutical formulation combines both drugs in a single pill. As Van Heeswijk describes, PIs satisfy the four requirements for TDM (78):

1. There is a relatively simple method to measure PI plasma levels.
2. A strong correlation between PI plasma concentrations and virologic response has been described.
3. There is large intra and inter patient variability of PI plasma concentrations, despite standard dosing and regimens.
4. The short-term direct clinical effect of PIs is difficult to assess given the slow progression of HIV.
The clinical utility of TDM for PIs, however, is highly dependant on choosing an appropriate PK parameter and establishing a target drug level for patient populations. Altering drug levels predictably with changes in dosing, and achieving an appropriate virologic response is also necessary.

**MEASURES AND TARGET THRESHOLDS FOR ANTIRETROVIRAL TDM**

Published studies of antiretroviral PKs in the clinical setting have generally utilized four measures: AUC, Cmax, Cmin, and concentration ratio (CR) (Figure 1). Each measure and its associated target threshold has been correlated to clinical outcomes in specific patient populations (virologic suppression, development of resistance, etc.) with varying results, but it remains unclear how drug concentrations should be interpreted and at what time they should be measured (78). The four options and supporting body of literature are explored below.
Figure 1. Pharmacokinetic Measures for Therapeutic Drug Monitoring

Area under the time-concentration curve is found by plotting the concentrations of serial blood samples versus the time in minutes after dosing and then calculating the area that falls below the trend line. Cmax represents the concentration drawn at the time of the dosing interval where the most amount of drug is thought to be present in the plasma. Similarly, Cmin is drawn when the least amount of drug is present. A concentration ratio is found by dividing the patient’s concentration by the concentration found at the exact same time on a constructed population curve.
Area Under the Curve (AUC) is determined from a full PK profile, with serial blood sampling over a dosing interval (approximately a 12h period for twice daily (BID) regimens). The plasma concentration at each time point is then plotted, connected via a curve, and the area under the curve calculated. An AUC value represents the total amount of drug in the bloodstream after a specific dose, and is a useful measure to look at drug-drug and drug-food interactions and how a patient generally handles a medication. From the AUC, other parameters can also be calculated including clearance, half-life and volume of distribution. The literature relating AUC values to virologic response to therapy is sparse; an investigation of monotherapy saquinavir established which AUC gave maximal viral suppression, (79) and one further study did show a statistically significant correlation between AUCs of NVP and IDV and rate of HIV RNA decline (80).

AUC, Cmax and Cmin are often closely correlated, but evidence from the study of other antimicrobial agents show a distinction between these parameters in terms of pharmacokinetic-pharmacodynamic relationships (PK-PD) (78). Cmax is the highest concentration of drug in the plasma after a dose, and is thought to occur at a specific time after dosing for each drug. It is most often correlated to rates of adverse events: the Cmax from patients experiencing side effects on ritonavir were found to be significantly higher than those of control patients not experiencing side effects (81). Similarly, high levels of EFV have been linked to development of insomnia (82), and increased levels of IDV associated with increased urological complaints (71). As for AUC, published literature has failed to widely demonstrate a relationship between Cmax and virologic response.

Alternatively, the Cmin, or trough concentration, represents the point over a dosing interval with the lowest plasma concentration of a drug, and therefore the point at which the plasma concentration may be insufficient to prevent viral replication. It is widely accepted in the
literature that in order to prevent virologic failure, a patient’s Cmin should always be greater than a clinically relevant threshold.

Defining the necessary clinically relevant threshold, however, is difficult, as the potency of each antiretroviral drug has traditionally been established in vitro. Inhibitory Concentrations (IC), or IC$_{95}$ and IC$_{50}$ values, are the in vitro concentrations needed to inhibit 95% and 50% of wild-type viral replication respectively. Many antiretrovirals, however, bind to plasma proteins leaving only the free concentration to inhibit viral replication. To adjust for protein binding, novel IC$_{95}$ and IC$_{50}$ values have been measured in the presence of 50% human sera. These values, named minimum effective concentration (MEC) remain only approximations of in vivo ICs and there are irregularities among reported values. The simplest in vivo translation (and threshold for TDM) is that the Cmin should be higher than the designated IC (83) or MEC (84) values.

Cmins have also been directly correlated to virologic response, and were found to be more predictive than either AUC or Cmax. In a study of 156 HIV-1 infected NFV treated patients, Pellegrin et al. found that Cmin was associated with virologic success, but AUC was similar between success or failure (85). Similar results were found with both indinavir (86) and atazanavir (87-89).

The Inhibitory Quotient (IQ) is an alternate adaptation of Cmin for TDM that corrects for viral resistance (90). The IQ can be calculated several different ways using the patient’s Cmin value over a resistance measure related to specific viral isolates (IQ=Cmin/Resistance Measure). Resistance measures originate from the individual’s genotype or phenotype resistance report or from a calculated normalized population value and might include the total number of minor and major PI mutations or the fold change (degree of difference in sensitivity between wild-type virus and the patient’s virus) (90). However, there has been a lack of standardization in the
calculation of IQ (90), and the potential variability of IQ between patients could be as large as 100% (84). The majority of publications demonstrate a statistical correlation between IQ and virologic response, especially in treatment experienced patients (88;91;92).

The final measure, the CR, uses a “time matched population value” to determine if drug plasma concentrations are adequate. HIV-1 infected, control patients, completed full twelve hour PK profiles the medians were used to construct a standardized population PK curve. Random samples from a larger population were collected, and the time from ingestion of dose to blood draw recorded. The concentrations of these samples (patient values) were then divided by the value taken from the point on the population curve that matched the time after drug administration (time adjusted population value) (see Figure 1). This ratio was named the CR and an original publication documented that NFV and SQV CRs were statistically correlated with more rapid viral decay, and were independent of baseline CD4 values (93).

The considerable discrepancies among assays used for TDM, as well as the marked inter- and intra-patient variability (upwards of 65%) of selected parameters, make it difficult to determine which parameters (AUC, Cmax, Cmin, or CR) are best correlated to clinical outcomes (78). A single target threshold value also assumes that the susceptibility of all viral isolates is similar, and does not adjust for drug resistance (with the exception of the IQ). Moreover, the logistical difficulties associated with AUC, Cmin (especially with NFV, where trough values often do not accurately reflect the dosing Cmin (94) or Cmax (difficult to measure in reality because it is impossible to predict when it is reached in a particular patient on any given day (95)) make their widespread use difficult. These issues are not unique to ART, and persist in sectors where TDM has been employed for many years (78).
PREGNANCY AND ANTIRETROVIRAL PHARMACOKINETICS

The possibility of altered ART PKs becomes particularly relevant in the context of pregnancy, especially given the accompanying physiologic changes. Inadequate levels of ART may result in slow and incomplete viral suppression, increasing the risk of vertical transmission and development of viral resistance. Alternatively, excess drug concentrations may result in increased toxicities to both mother and infant.

Exploring ART PKs in this population is a relatively new field; there are only a few published studies and abstracts, with only one considering the PK-PD effects. Emphasis in the literature has been placed on investigating the drugs that are most widely used in pregnancy, such as AZT, NVP, NFV and LPV/r. Current knowledge about each of these drugs’ disposition in pregnancy is explored below.

AZT
AZT is perhaps the antiretroviral drug used most in pregnancy and best described in the literature, as it has been extensively used in clinical settings. Two different groups specifically studied AZT PK in pregnancy with conflicting results, as one study reported that AZT PK in pregnancy was no different from non pregnant adults (96), while the other found a significant decrease of 33 percent for the AUC, when samples taken during pregnancy were compared against those from post partum (97). Despite unresolved discrepancies, the recommended dosing of AZT for pregnant women remains the same as for non-pregnant adults.

3TC
The pharmacokinetics of 3TC were studied in the context of a short course of monotherapy, or in combination with AZT. Ten women were enrolled in each arm. Plasma concentrations of 3TC
drawn at 38 wks were statistically similar in study both arms (mono versus dual therapy), and were also statistically similar to concentrations drawn at one week post partum (98). Prescribed dosing of 3TC for pregnant women is standard adult dosing.

NEVIRAPINE

NVP was used widely in pregnancy because of its fast absorption and prolonged elimination; however, it was associated with numerous cases of life threatening toxicities Stevens-Johnson Disease (rash and liver toxicity) (43), and is now indicated only for individuals with CD4 counts of less than 250x10^6 cells/L that require potent NNRTIs.

A study of 18 pregnant women showed steady state NVP concentrations similar to the general adult population (99) while recently published investigations in 26 women showed that antepartum levels were similar to those postpartum (100). Both these studies suggest that NVP PKs are not significantly altered by pregnancy. Alternatively, a study of 45 pregnant women and 152 non-pregnant women receiving NVP found that pregnancy has a moderate but significant lowering effect on plasma concentrations (101).

NELFINAVIR

NFV has been widely used in pregnancy as it is well tolerated with little side effects, and has a favorable safety and efficacy profile. Standard dosing is 1250 mg NFV BID. Of note, 50% of circulating NFV is metabolized into the active metabolite hydroxy-tert-butylamide (M8).

Two case studies documenting TDM of NFV in pregnancy presented women with consistently sub-optimal plasma concentrations, who initially had good viral suppression, but ultimately developed virologic breakthrough. In one case, the woman's NFV dose was incrementally increased, and the virus became undetectable for the remainder of the pregnancy, through to
delivery (102). Despite dosing interventions, the other case maintained detectable viremia until delivery; mutations associated with all three drugs used for therapy were noted shortly after delivery (103).

There have been several studies documenting 12h PK profiles of NFV in pregnant women. An early study found that 14/17 women met an AUC target of >15mg/h/mL antepartum, while 10/11 women met the target 6 wks post partum. Post partum AUCs were higher than antepartum AUCs (104). Conversely, another study with 9 women (14 visits) during the third trimester found that diminished NFV AUC was not evident, and that plasma values were widely variable (105). When samples drawn from the same woman ante- and postpartum were compared, there was no consistency in the magnitude or sign of the measured differences (106).

Nellen et al. (107) showed that pregnant women generally have NFV CRs that are lower than non-pregnant women, and often also fall below the established clinically relevant threshold of 0.9 (see methods section). As NFV plasma concentrations have previously been shown to be independent of sex, age and body weight (94) the authors suggest that this threshold is also relevant for pregnancy. In samples taken from 21 patients during the third trimester, the mean CR was found to be 0.84 ± 0.51, the median 0.88 (0.38-1.13), and the percent of the population with CR <0.9, 52.4% (107). CR's from pregnant patients were on average 34% lower than those of non-pregnant women after adjusting for HIV-RNA load, CD4 count and HCV infection (107). Of note, all but one of the women with a CR <0.9 had an undetectable viral load at delivery, and none of the infants were HIV infected.

A more recent study also documented reduced NFV exposure in the third trimester. Eight of 11 women who completed a 12h PK profile had subtherapeutic trough concentrations, and the Cmin value was statistically lower than in non-pregnant adults (108). Abstracts presented at the 14th
Conference on Retroviruses and Opportunistic Infections in 2007, however, presented conflicting results: Read et al. (109) showed decreased exposure to NFV in the third trimester when compared to 6-12 wks post partum, while Aweeka et al.’s (110) findings did not support this trend.

To further investigate NFV PKs in pregnancy and identify patient characteristics that influence NFV (and M8) concentrations Hirt et al. developed an integrated PK model (111). Sixty nine non pregnant women (129 samples), 60 pregnant women (87 samples), and 42 women (43 samples) at the time of delivery were included in the population study. Data was not included from patients when repeated low compliance was suspected or if time since dosing exceeded 15h for the BID regimen (or 11h for TID). The model showed that mean plasma clearance, apparent plasma clearance and NFV/M8 ratios were consistent with previously published data from studies in pregnant women, but that the percentage of women with NFV plasma concentrations above 1mg/mL was not significantly different between pregnant and non-pregnant women (111). The exception was day of delivery. The authors concluded that NFV dosage should not be changed in pregnancy, but might be doubled on the day of delivery (111).

**LOPINAVIR**

The boosted lopinavir concentrations of a cohort of 101 pregnant women were compared against those of matched non-pregnant controls, and found to be statistically lower. The data from this study also suggested that inadequate viral suppression by delivery was directly related to a low lopinavir Cmin (112). A second study compared samples taken at thirty-six wks GA and those taken at 6 wks post partum. The trend showed that mean antepartum drug levels were lower than those from post partum, although this difference did not reach significance (110).
JUSTIFICATION / RATIONALE

The physiological changes in pregnancy may result in considerable alterations in ART disposition, and published data on PK of ART in pregnancy remains inconsistent. Despite this, clinicians are already prescribing increased doses to pregnant women, which may pose unnecessary risk to the fetus. Before considering a clinical trial of dose adjustments in pregnancy, the significant limitations of the literature must be addressed. Variation of plasma concentrations across GA has not been explored, a concentration–response (rate and maintenance of viral suppression) relationship has not been developed, and there has been no formal consideration of adherence.

It is also important to note that while early clinical trials showed a benefit of TDM (113;114), two recent randomized studies found no overall difference in the clinical outcomes of the TDM vs standard clinical care groups in non pregnant adults (115;116). Median trough concentrations increased significantly more in the TDM arms, but there was no difference in the time to virologic failure or in the proportions of patients achieving an undetectable viral load (115;116).

The complexities of using ART in pregnancy will only increase in years to come. HIV infected women are living longer, healthier lives, and in the process are being exposed to long periods of therapy as well as potential multiple, short-courses during pregnancies. These exposures increase the risk of resistance development, and may necessitate novel combinations of drugs in future pregnancies to fully suppress the virus. A greater understanding of the disposition of these agents in pregnancy will ultimately permit a balance between effective therapy to protect long term maternal treatment options, while minimize the risk of perinatal infection and potential toxicities to mother and fetus.
The impact of this greater understanding will extend beyond Canada to the developing world, as every year 2.5 million HIV infected women deliver infants worldwide. Currently less than five percent of these women receive some form of antiretroviral treatment (2); however, with increased international initiatives and commitment from the United Nations General Assembly, ART rollouts continue to escalate towards universal use.

HYPOTHESIS

The plasma concentrations of NFV vary across GA and show correlation to optimal viral suppression in HIV-1 infected pregnant women.

OBJECTIVES

To investigate the utility of random timed monitoring of PIs in pregnancy by:

1. evaluating plasma concentrations of NFV and LPV/r in HIV-1 infected pregnant women
2. exploring correlations between plasma concentrations and maternal characteristics
3. determining if plasma NFV concentrations in pregnancy affect the rate and maintenance of viral suppression in pregnancy
METHODS

STUDY DESIGN

This research paper examines the plasma concentrations of PIs in HIV-1 infected pregnant women accessing care from the Oak Tree Clinic (OTC) between December 2004 and September 2006. Samples were collected as part of a prospective cohort study evaluating the mitochondrial toxicity of HAART in pregnancy.

Random timed blood samples were chosen for collection because they best reflect the realistic sampling in a population of diverse pregnant women with multiple social issues, including demanding family responsibilities to partners and children. Given these considerations, and the already considerably frequent schedule of visits for care of HIV in pregnancy, it would be impracticable to add separate or very specifically timed visits for PK studies in the standard clinical care.

STUDY SETTING AND POPULATION

Patients were recruited for this study at OTC the Women and Family HIV Centre at the Children’s and Women’s Health Centre of British Columbia (CWHCBC). OTC is the tertiary referral outpatient centre for all HIV infected children and pregnant women in the province, and also provides care to HIV infected women, as well as their children and partners (117).

Established in 1994, the Oak Tree program utilizes a multidisciplinary team approach and includes clinical team members in varying disciplines: adult and paediatric infectious disease
specialists, obstetrical and gynaecological infectious disease specialists, a nurse practitioner and clinical nurse specialist, pharmacists, dieticians, social workers and outreach staff.

Currently, more than 500 adults (over 80% women) and 150 exposed and infected children access care through OTC. The clinic also manages approximately 30 HIV infected pregnancies each year; either directly through clinic visits in Vancouver or in partnership with care providers in more remote regions of the province.

The patients at OTC are diverse. Patients include new Canadians from endemic regions such as refugees from Africa and immigrants from South Asia, as well individuals actively using substances of addiction, and those with no discernable HIV acquisition risk factors apart from being sexually active. Local women in correctional institutions who are infected also receive care at the clinic. Persons of aboriginal descent are over represented in the patient population, when compared to provincial statistics.

**SAMPLE SIZE CALCULATION**

The major evaluation for this thesis is the comparison of plasma concentrations at different GAs in pregnancy and their affect on rate and maintenance of viral suppression. To establish a medium effect size (0.25) difference in two groups, using an $\alpha$ error of 0.05 and a power of 0.80, the total sample size needed is 128, with 64 subjects/samples per arm. G*Power 3 was used for the calculation, set to F tests: Fixed effects, omnibus, one-way.
STUDY VISITS AND SAMPLE COLLECTION

Patients were introduced to the study during a visit to OTC for obstetrical care, and were then enrolled and consented by research staff. In the months leading up to delivery, several study visits were conducted for each subject, the vast majority concurrent to a regular clinic visit.

The first study visit for women not on therapy at conception aimed to capture baseline data for the mitochondrial toxicity portion of the study, and when possible occurred prior to HAART initiation. The blood test results and data collected at this visit are not pertinent to this thesis, and were not included. Antenatal maternal blood samples for analysis of plasma drug concentration were therefore collected twice with routine clinical blood work: once in late second trimester / very early third trimester (between 18-28 wks GA) and then again during mid third trimester (between 32-37 wks GA).

Maternal venous delivery samples were collected peripartum, and cord blood samples were collected immediately following delivery. Postpartum samples were collected with regular clinical blood work. All venous samples were drawn by phlebotomists at the accessioning lab at Children’s & Women’s Health Centre of British Columbia using a butterfly catheter.

During study visits, each subject had an extensive conversation with OTC pharmacists to discuss side effects associated with their HAART regimen, as well as any challenges they had encountered in taking their medications. As for all patients at the clinic, pillboxes and medication timers were offered to study subjects to aid with their adherence.

An extended amount of time was also spent with the research staff, who established a positive and trustful rapport with all the women. During this time, subjects were asked to self describe
some demographic data, detail any missed antiretroviral doses or side effects they experienced, as well as report the time of their last medication dose. This last data variable was of particular value to the study, and subjects were closely questioned as to whether the time of last dose had actually been 10-15 minutes earlier or later than reported. All specimens included in the analysis were associated with dosing times for which the subject was certain.

DATA COLLECTION

Data for this study was collected by patient self-report during study visits and then verified and supplemented using prospective chart review by research staff. Two sets of charts were used to complete the data set: standard maternal and infant hospital charts from CWHCBC and the maternal and infant charts maintained independently at OTC.

OTC charts provide comprehensive information on a patient's medical history and HIV status, as updates by clinic physicians are documented after each medical assessment. These updates capture patient demographics, current and past clinical interventions, alcohol and drug use, physical examination results, social circumstances etc. During pregnancy this information is recaptured and updated in great detail on the British Columbia Reproductive Care Program standard antenatal records, Part 1 & 2. Further insight on patients' well being is found in the reports from the other OTC clinical services, reflecting the multidisciplinary team approach of the clinic. Copies of lab reports, specialized consults and letters from community service organisations are also included. OTC charts were thus the source for the majority of study data. Delivery and in-patient data (time of medication dosing, concomitant medications or medical conditions etc), however, were collected for all patients from main hospital charts.
Demographic and clinical data was recorded on study-specific data collection forms. The data was then inputted into the Smartlist To Go version 2.002, a data management program, on a Palm® handheld device, and then synched into a Microsoft Access™ database.

**VARIABLE IDENTIFICATION AND SELECTION**

Variables included in the overall study database were established through a comprehensive review of the relevant medical literature and discussion between the team of experienced clinicians and scientists involved in the study. Since the variables selected were relevant to the clinical and molecular outcomes of the HAART toxicity component of the study, they were further refined for the purpose of this thesis. Factors that have previously been demonstrated to impact upon the plasma concentrations of adults and rate and maintenance of viral suppression were identified.

Considering the small sample size, the relatively low event rate, and the large number of selected variables, the possibility of missing relationships between variables (Type II errors / false negatives) exists. The data analysis therefore attempted to consider only those questions or trends that were theory based.

**REPORTING ON ADHERENCE**

Patient self-reporting of adherence at each visit was completed using an AIDS Clinical Trial Group (ACTG) Adherence questionnaire. This questionnaire gathers information on multiple aspects of adherence including doses missed in the last few days, doses missed in the last four months, how
regularly doses were taken on schedule during the day and how often doses were taken following particular instructions, such as with or without food (Appendix 1).

An adherence index was calculated using the formula from a cross-protocol analysis of the questionnaire by Reynolds et al. (118). This index optimizes the variation between subject reports, and is normed to give a range of adherence between 0 and 100. This tool was found to be strongly associated with pVL outcomes, and compare well with estimates based on medication event monitoring system data (118).

**DETERMINING DRUG PLASMA CONCENTRATIONS**

The plasma concentrations of PIs and NNRTIs were simultaneously assayed by a validated and sensitive method using high-pressure liquid chromatography coupled with tandem mass spectrometry (HPLC MS-MS) (119;120) by technicians at the BC Centre for Excellence in HIV/AIDS. Reverse-phase HPLC was completed using the Zorbax XDB-C18 column from Agilent Technologies and MS-MS by the API-2000 system from Applied Biosystems.

Acetonitrile was used to precipitate out proteins from the plasma, and the sample was centrifuged. An aliquot was then injected into the HPLC column with ammonium acetate for the online extraction. The second mobile phase used was methanol, which eluted the desired drugs. The analytes were then analyzed by MS-MS.

Reserpine (Sigma-Aldrich) was used as the internal standard. The quality control standard curve accuracy for NFV ranged from 86.0-111%, for LPV it was 90.8-102%, and for RTV it was 95.2-
113%. The lower limit of quantification for NFV was 56ng/mL, for LPV was 98 ng/mL, and RTV 102 ng/mL.

The clinically relevant threshold for the random timed NFV plasma concentrations was set at 0.8 ug/L. This is lower than the NFV Cmin efficacy threshold of >1.0 ug/L found by Pellegrin et al. (85) for non-pregnant adults in the VIRAPHAR study. It is consistent with established guidelines and published research on improved virologic response in paediatric patients (121), and is more discriminating than the value used for associating un-timed random samples to virologic failure (120) or that found by Duval et al. in a study of 68 NFV treated patients (122).

**PLASMA CONCENTRATION RATIOS**

NFV CRs were found using a protocol first published by Burger et al. (66;114) and then further developed by Baede-van Dijk et al. (94).

The published PK population curve was constructed using blood samples taken between 0-12h following morning dosing. The median plasma concentrations from 30 min time groups were used for the fitting procedure, as well as additional knowledge about previously constructed plasma concentration-time curves (94). The curve was derived from 618 samples obtained from 355 patients taking NFV at 1250 mg BID. The patients were 80% male, an average age of 38 (range 18-74) years old, and had an average weight of 74 (range 37-119) kg.

For each sample collected in this study, the ratio of the plasma NFV concentration determined by HPLC MS-MS, to the point on population curve matching the time since last dose, was calculated. This represented the NFV CR.
The clinically relevant threshold was set at a CR of 0.9, as initially established through a receiver operating characteristic (ROC) curve analysis using samples from 48 patients (66). It was further verified in more extensive studies, and predicts virologic failure with a sensitivity of 64% and a specificity of 74% (p=0.014) (66;94;114). This threshold has been previously used for NFV CRs in pregnancy, allowing comparisons to published data (107).

**DEFINITION OF REMAINING VARIABLES**

Maternal demographic and clinical data was abstracted from a combination of OTC and BC Women's Hospital Charts or self-described by the patient.

**Maternal HIV Serostatus.** All HIV blood testing in the province is completed by the BC Centre for Disease Control (BCCDC) and includes an enzyme linked immunosorbant assay (ELISA) and a Western Blot test. Confirmation of maternal HIV serostatus was obtained, and copy of positive tests results were placed in the patient’s clinic chart.

**Ethnicity** was self-described by each woman. Categories were those used by Health Canada for positive HIV test results and AIDS reporting. “ Aboriginal” includes all women of First Nation, Metis and Inuit descent. “ Black” describes all African, Caribbean, and African-Canadian women in the study. “ Asian” women include those from Asia (Japan, Korea, Thailand, Philippines etc.), as well as from South and West Asia (India, Pakistan etc.) (123).

**Maternal Age (years)** at the time of delivery was calculated from the recorded maternal date of birth.
**HIV Exposure Category.** Probable mode of HIV acquisition was self-reported by each woman. Exposure categories include IDU, unprotected heterosexual intercourse, iatrogenically by infected blood products or needles, perinatal transmission and unknown. If more than one exposure category was reported, a woman was assigned to a single exposure category according to a slightly modified version of Health Canada’s established hierarchy of HIV-related risk factors. This approach is used for the national reporting of each positive HIV test or AIDS case report, where a case is classified by the highest category of the hierarchy (Appendix 2) (123).

**Pre-Pregnancy Weight.** A patient’s most recent weight (kg) prior to conception was abstracted from clinic charts. If data was unavailable from the chart, the patient self-reported this value.

**History of Exposure to ART.** Detailed information on previous exposure to ART was abstracted from physician reported histories and pharmacy order sheets from the BC Centre for Excellence in HIV/AIDS Drug Treatment Program (provides all ART to patients in BC). Names of all specific medications, regimens/combinations and length of exposure were recorded.

**Gestational Age of events (GA).** The estimated date of delivery (EDD) for each patient was abstracted from the BC Women’s Hospital Ultrasound report closest to delivery. GA (wks) for each event was then calculated with the formula: GA=40-(EDD-date of event)/7.

**Current Weight in Pregnancy.** Patients’ weight (kg) was measured at each study visit and closest to delivery, and recorded in the clinic chart and on study data collection forms.

**Current Use of Substances of Addiction.** Use of substances of addiction (y/n) was self-described by each woman. Time periods of use were categorized as i) use ever (prior to
pregnancy), ii) use in pregnancy prior to 1st study visit, iii) use between study visits. Substances of addiction included tobacco, alcohol, marijuana, cocaine, heroin, and methamphetamines.

**Concomitant Medications of Interest.** All concomitant medications, including vitamins, taken by a patient during pregnancy were recorded on study data collection forms. Medications of interest, known to have drug-drug interactions with NFV, were then identified through the Thompson MICROMEDEX database (Appendix 3). Patients were categorized as having taken any of these medications or not.

**Plasma HIV-1 RNA levels.** pVL (copies/mL) were determined using the Roche Amplicor Monitor assay (versions 1.5; Roche Diagnostics) via the UltraSensitive preparation, at the UBC Virology Lab. The assay’s quantification limit was ≤50 copies/mL.

**Steady state.** Samples were determined to be in steady state from the patient-reported questionnaire on adherence, representing the point at which drug absorption is approximately the same as drug elimination, which is theoretically established after 5-6 half-lives of most medications. The drug terminal half-life of NFV is 2.5-5h, and is 5-6h for LPV/r. In practical terms, a sample was considered to be in steady state if, at the time of blood draw, there had been no missed doses in the previous three days and if the regimen had been initiated more than two wks prior.

**Viral Resistance Profile.** Viral resistance phenotypes for some patients were determined through the identification of mutations (base variation in target amino acid sequences) encoded in the viral genetic information. The mutations were correlated to a change in effectiveness of drug potency, permitting the development of a phenotypic profile for each antiretroviral agent. Profiles were completed at the BC Centre for Excellence in HIV/AIDS.
**Alanine Aminotransferase**, ALT (Normal Reference Range 10-55 U/L). No patients had clinical manifestation due advanced Hepatitis B or C disease, and, as such ALT was used as a marker of active liver inflammation in the context of chronic Hepatitis B or C infection. Results were available from the hospital lab departments at CWHCBC.

**Absolute CD4 Count**, (Normal Reference Range 300-1400 x10^6 cells/L) was used as a measure of immune status. Results were available from the hospital lab departments at CWHCBC and were determined by flow cytometry.

**Hepatitis Serology**. All testing for Hepatitis is conducted at the BCCDC and includes testing for active Hepatitis C virus (by PCR) or Hepatitis B virus (by detection of surface antigen) infections, as well as previous natural exposure (HCV antibody, HBV core antibody). Confirmation of maternal serostatus was obtained, and copy of positive tests results were placed in the patient’s clinic chart.

**Infant Birth Weight** was available from the Provincial Labour and Delivery Summary or the Provincial Neonatal Record Part 1.

**Infant HIV Serostatus**. Infant infection was determined by PCR detection of HIV-RNA. Two positive results on different occasions indicated a HIV infection in the infant. Alternatively, two negative PCRs taken on different occasions at more than 1 month of age is indicative that the infant is not HIV infected. A final negative PCR and a negative ELISA/Western Blot at 18 months of age, confirms an absence of infection and demonstrates the loss of maternal antibodies.
ANALYSIS PLAN

Data analysis was conducted using Microsoft Excel™ and SPSS® statistical software.

| Objective 1: | • compare plasma concentrations and CRs from 18-28 wks to those from 32-37 wks and time of delivery |

A one-way analysis of variance (ANOVA) with repeated measures was used to test for a difference in the means of the three groups. Differences in the medians were tested using the Mann Whitney U Test, and comparison of related samples was done using the Wilcoxon signed-rank test. Pearson Chi square tests were used to compare the number of subjects at each time point that had a plasma concentration or CR above the clinically relevant threshold.

| Objective 2: | • identify associations between variables of interest and plasma concentrations at each time point |

Univariate and multivariate linear regression models were used to investigate if variables of interest were associated with differing plasma levels. Both raw plasma concentrations and CRs were used as the dependent variable.

| Objective 3: | • evaluate association of plasma NFV concentrations and CRs to rate of viral suppression |
|            | • investigate the relationship between time to undetectable plasma viral load and possible co-variants |
|            | • compare variables of patients who did not achieve or maintain suppression to those who were fully suppressed through to delivery |

To evaluate the association of plasma NFV concentrations and CRs to rate of viral suppression, stratified survival curves with Kaplan Meier estimate, were used. The number of days to pVL <50
copies/mL was the dependent variable, and the subjects were stratified by whether their mean antepartum concentrations and first antepartum concentration after HAART initiation were above or below the clinically relevant threshold.

To investigate the relationship between time to undetectable plasma viral load and possible co-variants, Cox proportional hazard models were used. Again, using days to pVL <50 copies/mL as the dependent variable.

Finally, the analysis to compare patients who did not achieve or maintain suppression to those whose virus was fully suppressed through to delivery included the Student’s t-test and Mann-Whitney U test to compare respectively, the means and medians of continuous data, and the Pearson Chi square test was used to compare the number of subjects that had a plasma concentration or CR above the established clinically relevant threshold.

**ETHICAL CONSIDERATIONS**

This study was approved by the Clinical Research Ethics Board (#C04-0540), Faculty of Medicine, University of British Columbia (Appendix 4), and the Clinical Research Review Committee (#CW04-0212), CWHCBC, in Vancouver, Canada.
RESULTS

SUBJECT AND SAMPLE INCLUSION

Of the 52 women who enrolled in the study from Dec 2004 to September 2007, 40 subjects were included in the final analysis and 12 subjects did not meet the inclusion criteria (Table 2). We restricted our analysis to HIV-infected women who were treated with a NFV or LPV based regimen and from whom at least one sample was collected in steady state, prior to delivery.

Table 2. Subject Exclusion

<table>
<thead>
<tr>
<th># of Subjects</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Medication or PI other than NFV or LPV/r</td>
</tr>
<tr>
<td>1</td>
<td>Spontaneous abortion at 20 wks</td>
</tr>
<tr>
<td>4</td>
<td>No samples obtained in steady state</td>
</tr>
<tr>
<td>1</td>
<td>Patient not initiated on ART prior to delivery</td>
</tr>
<tr>
<td>2</td>
<td>Patients received majority of care outside OTC, insufficient samples</td>
</tr>
<tr>
<td><strong>12</strong></td>
<td><strong>TOTAL</strong></td>
</tr>
</tbody>
</table>

Samples were excluded from analysis if they were considered not to be in steady state or if the blood collection occurred >14h after the last medication dose. One hundred and forty six samples were collected and 126 (1-6 samples per subject) samples were included in the final analysis (Table 3).
Table 3. Sample Exclusion

<table>
<thead>
<tr>
<th># of Samples</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Not in steady state from patient reported questionnaire</td>
</tr>
<tr>
<td>11</td>
<td>Blood draw &gt;14h after last medication dose</td>
</tr>
<tr>
<td><strong>20</strong></td>
<td><strong>TOTAL</strong></td>
</tr>
</tbody>
</table>

SAMPLE CHARACTERISTICS

Eighty-nine of the 126 (70.6%) total samples collected were of NFV. Forty nine antepartum NFV samples were collected; 23 were drawn between 18-28 wks and 26 were drawn between 32-37 wks GA (Table 4).

The 27 delivery samples were drawn peripartum; 48.1% (13) were drawn prior to delivery, 9/13 within 24h. Of the 14 samples drawn after delivery, 13 were drawn within 24h.

Only 15 antepartum LPV/r samples were drawn; the sample size was too small for a complete analysis, but allowed for some description. Similarly, since no venous blood was drawn at the exact same time that the cord samples were taken, no ratio of cord level : plasma level can be calculated. No analysis was completed using the cord samples.

Table 4. Sample Distribution by Time Point

<table>
<thead>
<tr>
<th></th>
<th>18-28 wks</th>
<th>32-37 wks</th>
<th>Delivery</th>
<th>Cord</th>
<th>Post Partum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFV</td>
<td>23</td>
<td>26</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>LPV/r</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>37</td>
</tr>
<tr>
<td>ALL</td>
<td>30</td>
<td>34</td>
<td>27</td>
<td>23</td>
<td>11</td>
<td>126</td>
</tr>
</tbody>
</table>
**BASIC DEMOGRAPHICS**

The subjects’ demographics show a diverse study population (Table 5). Women self-described their ethnicity as Aboriginal (30.0%), Caucasian (30.0%), Black (25.0%) or Asian (15.0%) and were born in Canada (62.5%), Africa (25.0%) or Asia (12.5%). Women of visible minority or aboriginal identity were over-represented in the study population, compared to the provincial population.

Twelve (30.0%) subjects had completed some college or university education and an additional six (15.0%) had graduated from high school or received their General Educational Diploma. Of those remaining, 4 (10.0%) completed some grade school only, and 16 (40.0%) completed some high school.

Half of the subjects were receiving social assistance at the time of the study; 7 (17.5%) were employed in any way, and 13 (32.5) were unemployed and not receiving assistance. Just over half (52.5%) had an annual household income of <$15000.

The majority of study subjects reported a history of use of drugs of dependency at one point in their lives. When questioned about use of drugs of dependency in the current pregnancy, 16 (40%) admitted to using alcohol, and 16 (40%) to using illicit drugs. Half of the patients (50%) admitted to smoking in pregnancy, compared to 10.0% of pregnant women in BC (52).

Subjects were a mean age of 29.4 years of age at the time of delivery, and ranged from 16.7-40.4 years, which was slightly lower than the provincial average for age at delivery of 30.4 years. The mean maternal weight pre-pregnancy was 67.4 kg, and ranged from 45-101 kg.
One quarter of the study subjects had ever been infected with the Hepatitis C Virus (HCV antibody positive), and ten percent had an active infection in pregnancy (HCV PCR positive). Two subjects (5.0%) were actively infected with the Hepatitis B Virus (HepB surface antigen positive).

Table 5. Maternal General Demographics, n=40

<table>
<thead>
<tr>
<th></th>
<th># (%)</th>
<th>Population Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MATERNAL RACE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aboriginal</td>
<td>12 (30.0)</td>
<td>4.8 % ¥</td>
</tr>
<tr>
<td>Caucasian</td>
<td>12 (30.0)</td>
<td>70 % ¥</td>
</tr>
<tr>
<td>Black</td>
<td>10 (25.0)</td>
<td>0.7 % ¥</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (15.0)</td>
<td>20 % ¥</td>
</tr>
<tr>
<td><strong>COUNTRY OF ORIGIN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>25 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>10 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>5 (12.5)</td>
<td></td>
</tr>
<tr>
<td><strong>MATERNAL EDUCATION</strong> (highest level completed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade school</td>
<td>4 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Some high school</td>
<td>16 (40.0)</td>
<td></td>
</tr>
<tr>
<td>High school grad/GED</td>
<td>6 (15.0)</td>
<td></td>
</tr>
<tr>
<td>Any college/university</td>
<td>12 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (5.0)</td>
<td></td>
</tr>
<tr>
<td><strong>MATERNAL EMPLOYMENT STATUS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed in any way</td>
<td>7 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Not employed</td>
<td>13 (32.5)</td>
<td></td>
</tr>
<tr>
<td>Social Assistance</td>
<td>20 (50.0)</td>
<td></td>
</tr>
<tr>
<td><strong>ANNUAL HOUSEHOLD INCOME</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; $15000</td>
<td>19 (47.5)</td>
<td></td>
</tr>
<tr>
<td><strong>USE OF SUBSTANCES OF ADDICTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illicit Drugs (EVER)</td>
<td>23 (57.5)</td>
<td></td>
</tr>
<tr>
<td>Alcohol in Pregnancy</td>
<td>16 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Smoking in Pregnancy</td>
<td>20 (50.0)</td>
<td>10.7% ‡</td>
</tr>
<tr>
<td>Illicit Drugs in Pregnancy</td>
<td>16 (40.0)</td>
<td></td>
</tr>
<tr>
<td><strong>AGE</strong></td>
<td>29.4 (16.7-40.4)</td>
<td>30.4 yrs ‡</td>
</tr>
<tr>
<td><strong>PRE-PREGNANCY WEIGHT</strong></td>
<td>67.4 (45.0-101.0)</td>
<td></td>
</tr>
<tr>
<td><strong>CO-INFECTIONS (CURRENT)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV antibody +ve</td>
<td>10 (25.0)</td>
<td></td>
</tr>
<tr>
<td>HCV RNA PCR +</td>
<td>4 (10.0)</td>
<td>66.8 per 100,000 population †</td>
</tr>
<tr>
<td>HBV surface antigen +ve</td>
<td>2 (5.0)</td>
<td>0.9 per 100,000 population †</td>
</tr>
</tbody>
</table>

Reference Population Definition:
‡ Pregnant Women in British Columbia, 2006/2007 (52)
† Reported cases in British Columbia, 2006 (124)
¥ British Columbia Population, 2006 (125)
**HIV RELATED DESCRIPTORS**

Twenty-five (62.5%) woman described their HIV exposure category as heterosexual contact, nine (22.5%) as having most likely contracted HIV through IDU, and 6 (15.0%) iatrogenically through infected needles or blood products (Table 6).

Twenty-one (52.5%) subjects had exposure to ART prior to the current pregnancy, and 19 (47.5%) were ART naïve. Seven women had previously been exposed to NFV, similarly, 7 women had previously been exposed to LPV/r. Seven women were on therapy at the time of conception; for the remaining 33, the median GA at HAART initiation in pregnancy was 22.4 wks (IQR 19.6-24.9 wks).

Baseline labs describe the status of the subject’s HIV infection. The median CD4 nadir was 300 x 10^6 cells/L (IQR 170-445 x 10^6 cells/L). The first CD4 count drawn in pregnancy for each subject was determined and the median count was found: 365 x10^6 cells/L (IQR 278-560 x10^6 cells/L). The baseline median viral load was similarly established, and found to 3.58 log_{10} copies/mL (IQR of 2.62-4.22 log_{10} copies/mL).

Adherence was self-described by each subject at each study visit. The mean adherence was 94.8% with the highest adherence reported as 100%, and the lowest as 72.9%.

There were no cases of HIV vertical transmission.
Table 6. HIV Characteristics, n=40

<table>
<thead>
<tr>
<th>MODE OF HIV ACQUISITION</th>
<th>#</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterosexual contact</td>
<td>25</td>
<td>(62.5)</td>
</tr>
<tr>
<td>IDU</td>
<td>9</td>
<td>(22.5)</td>
</tr>
<tr>
<td>Blood products/Percutaneous</td>
<td>6</td>
<td>(15.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ART HISTORY</th>
<th>#</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experienced</td>
<td>21</td>
<td>(52.5)</td>
</tr>
<tr>
<td>Naïve</td>
<td>19</td>
<td>(47.5)</td>
</tr>
<tr>
<td>Previous exposure to NFV</td>
<td>7</td>
<td>(17.5)</td>
</tr>
<tr>
<td>Previous exposure to LPV/r</td>
<td>7</td>
<td>(17.5)</td>
</tr>
<tr>
<td>On therapy at conception or GA at HAART initiation</td>
<td>7</td>
<td>(17.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LABORATORY</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 Nadir (x10^6 cells/L)</td>
<td>300 (170-445)</td>
</tr>
<tr>
<td>Baseline CD4</td>
<td>365 (278-560)</td>
</tr>
<tr>
<td>Baseline VL (log10 copies/mL)</td>
<td>3.58 (2.62-4.22)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADHERENCE</th>
<th>Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>94.8% (72.9-100%)</td>
</tr>
</tbody>
</table>

| VERTICAL TRANSMISSION, no. (%)          | 0 (0)         |
ANTIRETROVIRAL THERAPY IN PREGNANCY

All 40 women included in this analysis received ART during pregnancy; 29 (72.5%) received a NFV based regimen, 9 (22.5%) received a LPV/r based regimen, and 2 (5.0%) were initiated on NFV and then switched to LPV/r over the course of pregnancy (Figure 2).

The most common pair of NRTIs used for the regimen backbone was AZT and 3TC, known as Combivir® when formulated together. Three other NRTIs were prescribed in pregnancy: DDI, D4T, and ABC, each used in combination with AZT and/or 3TC (Figure 2).

FIGURE 2. Antiretroviral Therapy Regimens Prescribed in Pregnancy

The HAART combination of three to five unique drugs received by each subject in the study is described. The PI is captured first, followed by the NRTI backbone, and finally by the number of subjects on that combination.
NELFINAVIR RAW PLASMA CONCENTRATIONS

The mean NFV concentrations at 18-28 wks and 32-37 wks were 2.64 and 2.05 μg/mL (p=0.138), respectively; the median concentrations were 2.88 and 1.18 μg/mL (p=0.113), respectively. Six (26.1%) of 23 pregnant women had a concentration of <0.8μg/mL at 18-28 wks, compared with 11 (42.3%) of 26 at 32-37 wks. Delivery samples had a mean of 1.42 μg/mL, a median of 1.21μg/mL, and 40.0% of samples were below the threshold (Table 7, Figure 3).

Table 7. Nelfinavir Raw Plasma Concentrations

<table>
<thead>
<tr>
<th></th>
<th>18-28wks (n=23)</th>
<th>32-37wks (n=26)</th>
<th>p*</th>
<th>Delivery (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFV mean, μg/mL (±SD)</td>
<td>2.64 (±1.93)</td>
<td>2.05 (±1.87)</td>
<td>.138</td>
<td>1.42 (±1.13)</td>
</tr>
<tr>
<td>NFV median, μg/mL (IQR)</td>
<td>2.88 (0.79-4.25)</td>
<td>1.18 (0.62-3.56)</td>
<td>.113</td>
<td>1.21 (0.58-1.93)</td>
</tr>
<tr>
<td>&lt;0.8μg/mL, no. (%) of samples</td>
<td>6 (26.1%)</td>
<td>11 (42.3%)</td>
<td>.235</td>
<td>8 (40.0%)</td>
</tr>
</tbody>
</table>

NOTE. IQR, interquartile range. SD, standard deviation.
* Student’s t test (mean), Mann-Whitney U test (median) for continuous data, and χ² test for categorical data.

A one-way repeated measures ANOVA found that samples drawn between 18-28 wks and 32-37 to be significantly higher than at delivery (p=.048).
FIGURE 3. Nelfinavir Plasma Concentrations

The concentration from each sample is plotted by time since last medication dose. Clinically relevant thresholds for raw concentrations and concentration ratios are indicated by dotted lines.
NELFINAVIR PLASMA CONCENTRATION RATIOS

The mean NFV CRs at 18-28 wks and 32-37 wks were 1.09 and 0.86, (p=0.133), respectively; the median CRs were 1.05 and 0.70 (p=0.102), respectively. Ten (43.1%) of 23 pregnant women had a CR of <0.9 at 18-28 wks, compared with 14 (53.8%) of 26 at 32-37 wks. Delivery samples had a mean of 0.44, a median of 0.27, and 85.7% of samples were less than the clinically relevant threshold (0.9) (Table 8, Figures 3,4).

Table 8. Nelfinavir Plasma Concentration Ratios

<table>
<thead>
<tr>
<th></th>
<th>18-28wks (n=23)</th>
<th>32-37wks (n=26)</th>
<th>p*</th>
<th>Delivery (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFV mean CR, (±SD)</td>
<td>1.09 (± 0.73)</td>
<td>0.86 (±0.73)</td>
<td>.133</td>
<td>0.44 (±0.50)</td>
</tr>
<tr>
<td>NFV median CR, (IQR)</td>
<td>1.05 (0.39-1.64)</td>
<td>0.70 (0.21-1.37)</td>
<td>.102</td>
<td>0.27 (0.09-0.62)</td>
</tr>
<tr>
<td>&lt;0.9, no. (%) of samples</td>
<td>10 (43.5%)</td>
<td>14 (53.8%)</td>
<td>.471</td>
<td>18 (85.7%)</td>
</tr>
</tbody>
</table>

NOTE. IQR, interquartile range. SD, standard deviation.
* Student’s t test (mean), Mann-Whitney U test (median) for continuous data, and χ² test for categorical data.

A one-way repeated measures ANOVA found that samples drawn between 18-28 wks and 32-37 wks had significantly higher CRs than at delivery (p=.049). A Wilcoxon signed-rank test was also completed to compare the CRs of related samples. Nineteen subjects had samples from both antepartum time points and were included in the test (Figure 5). The Wilcoxon signed ranked statistic W was found to be -32, indicating that 32-37 wk CRs were smaller than at 18-28 wks, but was not found to be statistically significant (p=0.26). Fourteen subjects had two antepartum samples and a delivery sample (n=14); Figure 6 shows the related samples.
Figure 4. Nelfinavir Concentrations Ratios
Concentration Ratios were plotted across GA. Dotted lines show mean value for each group.
Figure 5. Comparison of Two Antepartum Concentration Ratios in Related Samples
Coloured lines represent the change in antepartum CRs for each patient. The subjects were split into two groups based on whether CRs increased (upper graph) or decreased (lower graph) across pregnancy.
Figure 6. Change in Subjects’ Concentration Ratio Across Gestational Age
REPORT ON CO-VARIATES

Variables that could affect NFV plasma concentrations were investigated to determine if they changed over the course of pregnancy. Time from last medication dose to blood draw was a mean of 305, 272 and 341 minutes at 18-28 wks, 32-37 wks and at the time of delivery, respectively.

Subjects’ weight, as expected, showed an increasing trend over the course of pregnancy. Between 18-28 wks, subjects had a mean weight of 73.0 kg, which reached 81.7 kg by the time of delivery.

The lab test used as a marker for liver inflammation, alanine aminotransferase, showed little to no change in mean over the second and third trimesters.

Table 9. Co-Variants Across Pregnancy

<table>
<thead>
<tr>
<th></th>
<th>18-28wks (n=23)</th>
<th>32-37wks (n=26)</th>
<th>Delivery (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from last medication dose to blood draw (mins) Mean (95% CI)</td>
<td>305 (224-386)</td>
<td>272 (208-336)</td>
<td>341 (254-428)</td>
</tr>
<tr>
<td>Weight (kg) Mean (95% CI)</td>
<td>73.0 (63.8-82.2)</td>
<td>79.9 (74.0-85.8)</td>
<td>81.7 (74.1-89.3)</td>
</tr>
<tr>
<td>Alanine Aminotransferase (U/L) Median (range)</td>
<td>13 (&lt;3-214)</td>
<td>16 (&lt;3-101)</td>
<td>13 (&lt;3-58)</td>
</tr>
</tbody>
</table>

**NOTE.** 95% CI, 95% Confidence Interval.
CORRELATIONS OF COVARIATES AND NFV CONCENTRATIONS

Univariate linear regression models were completed to determine if any demographic or pregnancy related factors might predict or impact on raw NFV plasma concentrations (Table 10). The statistical correlations found, however, were inconsistent across GA, and Multivariate linear regression models found no significant predictors of raw NFV concentrations or NFV-CRs.

Table 10. Univariate Analysis, Co-Variates and NFV Concentrations

<table>
<thead>
<tr>
<th>Factor</th>
<th>18-28 wks</th>
<th></th>
<th>32-37 wks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression co-efficient</td>
<td>P</td>
<td>Regression co-efficient</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.120</td>
<td>.290</td>
<td>0.477</td>
<td>.007</td>
</tr>
<tr>
<td>Pre-pregnancy weight (kg)</td>
<td>-0.231</td>
<td>.144</td>
<td>-0.145</td>
<td>.245</td>
</tr>
<tr>
<td>Previous exposure to NFV</td>
<td>-0.162</td>
<td>.230</td>
<td>0.323</td>
<td>.006</td>
</tr>
<tr>
<td>Baseline CD4 (x10^6 cells/L)</td>
<td>0.207</td>
<td>.172</td>
<td>-0.090</td>
<td>.331</td>
</tr>
<tr>
<td>Drugs of addiction in pregnancy</td>
<td>-0.147</td>
<td>.252</td>
<td>-0.331</td>
<td>.049</td>
</tr>
<tr>
<td>Concomitant medications (CYP)</td>
<td>-0.181</td>
<td>.205</td>
<td>-0.400</td>
<td>.021</td>
</tr>
<tr>
<td>Adherence (%)</td>
<td>0.262</td>
<td>.113</td>
<td>0.359</td>
<td>.035</td>
</tr>
<tr>
<td>Current weight (kg)</td>
<td>-0.136</td>
<td>.274</td>
<td>-0.360</td>
<td>.035</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>-0.332</td>
<td>.060</td>
<td>0.087</td>
<td>.342</td>
</tr>
<tr>
<td>Time since dose (min)</td>
<td>-0.094</td>
<td>.336</td>
<td>-0.255</td>
<td>.105</td>
</tr>
</tbody>
</table>
DESCRIPTION of LOPINAVIR/ RITONAVIR CONCENTRATIONS IN PREGNANCY

Plasma concentrations of LPV/r were highly variable in pregnancy, and both mean and median lopinavir and ritonavir levels appeared to be higher post partum (Table 8, Figure 7).

Mean lopinavir levels were above the clinical threshold for Cmin (3.00 μg/mL) at all three antepartum time points (18-21, 23-27 and 31-36 wks) and at delivery. The IQR at each time point also show that at least 75% of all samples were above this threshold.

Table 11. Lopinavir Plasma Concentrations (μg/mL)

<table>
<thead>
<tr>
<th></th>
<th>18-21wks n=5</th>
<th>23-27wks n=7</th>
<th>31-36wks n=8</th>
<th>delivery n=7</th>
<th>post partum n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean LPV (95% CI)</td>
<td>5.90 (4.48-7.32)</td>
<td>5.42 (4.43-6.41)</td>
<td>6.56 (4.89-8.22)</td>
<td>6.83 (2.91-10.7)</td>
<td>9.41 (5.42-13.4)</td>
</tr>
<tr>
<td>median LPV (IQR)</td>
<td>5.84 (5.05-7.26)</td>
<td>5.74 (4.95-6.04)</td>
<td>6.40 (4.88-7.65)</td>
<td>5.13 (3.76-7.10)</td>
<td>11.00 (7.65-13.0)</td>
</tr>
<tr>
<td>mean RTV (95% CI)</td>
<td>0.28 (0.19-0.37)</td>
<td>0.27 (0.20-0.32)</td>
<td>0.46 (0.12-0.79)</td>
<td>0.43 (0.18-0.67)</td>
<td>1.08 (0.12-2.03)</td>
</tr>
<tr>
<td>median RTV (IQR)</td>
<td>0.26 (0.24-0.32)</td>
<td>0.29 (0.25-0.31)</td>
<td>0.28 (0.22-0.47)</td>
<td>0.24 (0.21-0.58)</td>
<td>0.70 (0.26-1.41)</td>
</tr>
</tbody>
</table>

NOTE. 95% CI, 95% Confidence Interval, IQR, interquartile range.
Figure 7. Median LPV & RTV Plasma Concentrations in Pregnancy

Plasma concentrations were plotted at five different time points, including post partum. Medians are plotted with 25th and 75th percentile markers (IQR).


**TIME TO UNDETECTABLE VIRAL LOAD AND NFV CRs**

Twenty-eight (96.5%) of the 29 patients treated with only NFV had an undetectable viral load at one point during pregnancy. Twenty-four of these subjects were not on therapy at conception and had detectable viral loads prior to treatment initiation. One patient was considered an “elite suppressor” as no virus was detected using standard assays while the patient was not receiving any ART, and therefore, was not included in this analysis. This woman was still treated with HAART in pregnancy to prevent vertical transmission as a precaution.

The mean number of days to undetectable viral load in the twenty-four patients who suppressed in pregnancy was 61 (range 16-126), and was reached in all subjects prior to 34 wks GA. The median number of virological measurements per woman was 5 (range 3-8) with a similar interval between successive tests (mean of 26 days).

Figure 8 displays estimated proportions of women achieving undetectable viral loads, beginning at the time of initiation of therapy, stratified by whether the first antepartum NFV CR drawn was above or below the clinically relevant threshold of 0.9. Twelve subjects were classified into the sub-therapeutic group (NFV-CR <0.9) and twelve were considered therapeutic (NFV-CR >0.9). Twenty five percent of the subtherapeutic group and 30.8% of the therapeutic group were undetectable 30 days after HAART initiation, 50.0% of the sub-therapeutic group and 53.9% of the therapeutic group by 60 days, and 75.0% of the sub-therapeutic group and 77.0% of the therapeutic achieved an undetectable viral load by 80 days, indicating a similar response by initial NFV exposure.

Figure 9 displays an additional survival curve; the mean of each subject’s antepartum NFV CRs was found and used to stratify the group based again on the clinically relevant threshold of 0.9.
Figure 8. Rate of Viral Suppression by First Antepartum NFV CR

Survival curves for the time from initiation of HAART to achievement of an undetectable viral load, by first antepartum NFV CR.
Figure 9. Rate of Viral Suppression by Mean of Antepartum NFV CRs

Survival curves for the time from initiation of HAART to achievement of an undetectable viral load, by mean of antepartum NFV CRs.
Nine subjects (36.5%) had an antepartum NFV-CR mean below 0.9, and the remaining 15 had a NFV-CR mean above 0.9. This stratification showed a differing response between subject groups. Three subjects (33.3%) of the subtherapeutic group and 20.0% of the therapeutic group were undetectable 30 days after HAART initiation, 66.7% of the sub-therapeutic group and 33.3% of the therapeutic group by 60 days, and 88.9% of the sub-therapeutic group and 46.7% of the therapeutic achieved an undetectable viral load by 80 days.

The relationship between several co-variants and days to undetectable viral load following HAART initiation was considered (Table 11). Baseline CD4 (HR: 1.001, p=0.416) and patient-reported adherence (HR: 0.977, p=0.465) were found not to have a significant effect, while low baseline pVL was associated with a significant decrease in the number of days to reach an undetectable pVL (HR: 0.524, p=0.026).

The effects of mean antepartum raw NFV concentration (RH: 0.810, p=0.229) and NFV CRs (RH: 0.497, p=0.137) were not associated with a change in time to viral suppression.
Table 12. Proportional Hazards, Time to Undetectable pVL (n=24)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Univariate analysis hazard ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence (%)</td>
<td>0.977 (0.917-1.041)</td>
<td>.465</td>
</tr>
<tr>
<td>Baseline CD4 (x10^6 cells/L)</td>
<td>1.001 (0.999-1.003)</td>
<td>.416</td>
</tr>
<tr>
<td>Baseline logged pVL</td>
<td>0.524 (0.296-0.925)</td>
<td>.026</td>
</tr>
<tr>
<td>Mean antepartum raw NFV concentration (μg/mL)</td>
<td>0.810 (0.575-1.142)</td>
<td>.229</td>
</tr>
<tr>
<td>Mean antepartum NFV CR</td>
<td>0.497 (0.197-1.250)</td>
<td>.137</td>
</tr>
</tbody>
</table>

**LACK of VIRAL SUPPRESSION AT DELIVERY**

The median GA at delivery was 38.6 wks (range 31-41), and 24 (82.8%) of 29 NFV only treated women had undetectable viral loads. Five subjects had HIV viral loads which were not completely suppressed. Four women reached a pVL <50 copies/mL (log10pVL <1.70) by 34 wks, but broke through and had a detectable pVL at delivery. One woman never suppressed completely (Figure 10).

The characteristics of both groups (suppressed vs. unsuppressed at delivery) were compared (Table 12). The median baseline CD4 was significantly higher in the suppressed group 490 x 10^6 cells/L (IQR 310-740) compared to the unsuppressed group 340 x 10^6 cells/L (IQR 250-360), p=0.026. Patient reported adherence (p=0.325) and illicit drug use (p=0.498) were statistically similar. When considering raw plasma concentrations of NFV drawn at 32-37 wks GA, there was
no statistical significant difference between the means, medians and percent below the clinically relevant threshold of the two groups, and the results were highly variable. The means of the NFV levels of the five subjects who were unsuppressed and the 24 who remained suppressed, were, 2.06 μg /mL (SD: 0.08-4.06) and 1.95 μg /mL (SD: 0.54-3.36), respectively, p=0.448; the medians were 2.21 ug/mL (1.14-2.83) and 0.81 μg /mL (0.62-3.57), respectively, p=0.488; the percent of samples below 0.8 μg/mL were, 20.0% and 47.6%, respectively, p=0.279.

Table 13. Characteristics Associated with Lack of Viral Suppression at Delivery

<table>
<thead>
<tr>
<th></th>
<th>breakthrough/lack of suppression n=5</th>
<th>suppressed viral load through to delivery n=24</th>
<th>P#</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline CD4 (x10^6 cells/L) median (IQR)</td>
<td>340 (250-360)</td>
<td>490 (310-740)</td>
<td>.026</td>
</tr>
<tr>
<td>adherence % mean (±SD)</td>
<td>93.02% (±5.38)</td>
<td>89.27% (±7.9%)</td>
<td>.325</td>
</tr>
<tr>
<td>Illicit drug use, no. (%)</td>
<td>2 (40%)</td>
<td>6 (28.6%)</td>
<td>.498</td>
</tr>
<tr>
<td>mean NFV at 32-37wks (±SD)</td>
<td>2.07 (±1.99)</td>
<td>1.95 (±1.41)</td>
<td>.448</td>
</tr>
<tr>
<td>median NFV at 32-37wks (IQR)</td>
<td>2.21 (1.14-2.83)</td>
<td>0.81 (0.62-3.57)</td>
<td>.488</td>
</tr>
<tr>
<td>&lt;0.8μg/mL, no. (%) of samples</td>
<td>1 (20%)</td>
<td>10 (47.6%)</td>
<td>.279</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range. SD, standard deviation.
# Student’s t test (mean), Mann-Whitney U test (median) for continuous data, and χ² test for categorical data.
Figure 10. Lack of Viral Suppression at Delivery

The log of plasma viral loads (pVL) were plotted across gestational age for the five subjects who did not achieve or maintain viral suppression (pVL<50 copies/ml, log10 pVL<1.70) during pregnancy, prior to delivery.
Further insight on the five patients who had a detectable viral load at delivery was gained by considering the viral resistance profiles close to the time of delivery (Table 14). Three of the five patients showed considerable resistance to 3TC, and two showed some resistance to NFV. The profiles of two subjects showed pan-susceptible viruses.

Table 14. Viral Resistance and Adherence for Patients with Detectable Viral Load at Delivery

<table>
<thead>
<tr>
<th>case</th>
<th>Regimen</th>
<th>Relevant Resistance Analysis</th>
<th>Adherence in Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>105</td>
<td>COM/NFV</td>
<td><strong>35 wks GA</strong>&lt;br&gt;AZT: maximal response&lt;br&gt;3TC: minimal response&lt;br&gt;NFV: reduced response</td>
<td>patient report: 94.7%</td>
</tr>
<tr>
<td>108</td>
<td>COM/NFV</td>
<td><strong>34 wks GA</strong>&lt;br&gt;AZT: maximal response&lt;br&gt;3TC: minimal response&lt;br&gt;NFV: reduced response</td>
<td>patient report: 96.9%</td>
</tr>
<tr>
<td>122</td>
<td>COM/NFV</td>
<td><strong>37 wks GA</strong>&lt;br&gt;AZT: maximal response&lt;br&gt;3TC: minimal response&lt;br&gt;NFV: maximal response</td>
<td>patient report: 83.9%</td>
</tr>
<tr>
<td>123</td>
<td>COM/NFV</td>
<td><strong>36 wks GA</strong>&lt;br&gt;AZT: maximal response&lt;br&gt;3TC: maximal response&lt;br&gt;NFV: maximal response</td>
<td>patient report: 92.6%</td>
</tr>
<tr>
<td>125</td>
<td>COM/NFV</td>
<td>day of delivery&lt;br&gt;AZT: maximal response&lt;br&gt;3TC: maximal response&lt;br&gt;NFV: maximal response</td>
<td>patient report: 96.9%</td>
</tr>
</tbody>
</table>
DISCUSSION

NELFINAVIR CONCENTRATIONS in PREGNANCY

NFV plasma concentrations and NFV-CRs were found to be statistically higher antepartum compared to day of delivery. Although concentrations were statistically similar at 18-28 wks and 32-37 wks, mean and medians demonstrated a trend towards decreased drug exposure as gestation increased. An increase in sample size may be able to characterize differences more definitively. A large portion of subjects also had concentrations that fell below the therapeutic threshold. The percentage of samples considered sub-therapeutic showed trends of increasing across pregnancy. Pregnant women between 18-28 wks had similar NFV-CRs (1.09 ± 0.73) to those published from forty-eight non-pregnant adult women (1.19 ± 0.67) and the reference population (107). The NFV-CRs at 32-37 wks (0.86 ± 0.73) were also consistent with previously reported values of twenty-one women (0.84 ± 0.51) tested during the third trimester (107). The percentage of patients with samples below the clinically relevant threshold at the same time point (53.8%) was also similar to that of the published pregnant population (52.4%).

The majority of women in the study had no detectable virus at the time of delivery and there were no cases of vertical transmission, suggesting that drug levels of NFV from current dosing may be sufficient for viral suppression for the purposes of prevention of vertical transmission. The development of viral resistance due to low drug exposure should be further explored.
VARIABILITY OF NELFVINA VIR CONCENTRATIONS

Concentrations of NFV were also found to be highly variable antepartum and at time of delivery. Inconsistent adherence, decreased bioavailability due to not taking the drug with food, and inaccurate recall of time of last dose are potential factors explaining the variability. Vrijens et al. suggest “patients’ variable exposure to drugs, created by their diversely erratic execution of protocol-specified dosing regimens, is generally the single largest source of variance in drug responses,”(126). While a dose may be kept constant, the wide range of concentrations is achieved by varying the dosing interval. Indeed, by monitoring electronically the times of dosing, forty percent of the apparent inter-patient variability seen when assuming the samples were drawn at trough, was explained (126).

Using univariate and multivariate linear regressions co-variants of NFV concentrations and NFV CRs were explored. Chronic liver disease with Hepatitis B or C Viruses is known to cause a decrease in hepatic clearance increasing plasma levels; however, univariate linear analysis using the liver enzyme ALT as a marker for liver inflammation found no statistical correlation. Body weight, both prior to conception and during pregnancy was also not predictive of plasma concentrations or CRs, consistent with published literature suggesting CRs are independent of sex, age, body weight, (93). Use of concomitant medications that have drug-drug interactions with NFV did not show any significant effect.

Variation in plasma concentrations might also arise from differing placental response between individuals to the drug. Placental transfer of PIs is known to be quite low, and there is little accumulation in the amniotic fluid (127). The variation in size and weight of placentas suggest that drug may accumulate in the organ and contribute to variability in plasma levels.
NFV is metabolized into M8 by the liver enzyme CYP2C19, and both drugs are metabolized by liver enzyme CYP3A4 (Figure 11). The drugs behave similarly in vitro, however there is little data about the contribution of M8 to virologic response.

Figure 11. Nelfinavir and M8 Metabolism by Liver Enzymes

Fifty percent of circulating NFV is metabolized by liver enzyme CYP 2C19 into the active metabolite hydroxy-tert-butylamide (M8). Both drugs are metabolized by liver enzyme CYP 3A4.

Previously published papers have suggested that an alteration of the regulation of these two enzymes in pregnancy may account for the decreased plasma levels of NFV compared to non-pregnant adult populations. CYP 3A4 is thought to be upregulated in pregnancy (107) and data has shown that decreased IDV AUCs were correlated to increases in secretion of 6β-OHF, an in vivo marker for CYP 3A4 (128). The trend towards lower drug concentrations late in pregnancy compared to the second trimester might be explained by the increase CYP 3A4 activity as gestation increases.
The regulation of CYP 219 in pregnancy is less clear. It is well established that the M8:NFV ratio is constant at 0.29 in non-pregnant adults (94), but M8 : NFV ratios were found to be lower in pregnant women (111). M8 concentrations were also found to be 70% lower during pregnancy compared to post partum (129), suggesting either an induction of CYP 3A4 or an inhibition of CYP 2C19, or both. While it is possible that the decreased M8:NFV ratio is consistent with reduction of CYP2C19 activity during pregnancy(109), population PK from Hirt et al. did not support change in CYP 2C19 metabolism (111).

Plasma protein concentrations are reduced during the third trimester. NFV is highly (99%) protein bound, and this absence of available serum proteins may mean that the drug’s free fraction (ratio of unbound to bound drug) will increase. As the free fraction increases, more unbound drug (pharmacologically active form) is available for hepatic metabolism. While the actual free concentration of drug is not altered do to this increased clearance, changes in protein binding could affect the half-life of NFV, as bound drug is constantly released to maintain the new equilibrium of the increased free fraction.

Protein binding of lopinavir has been investigated in pregnancy (110). Alpha-1-acid glycoprotein (AAG) was found to strongly correlate to the extent of lopinavir binding (when albumin was not) and an increase in the free fraction of the drug was found (110). An animal model of PI metabolism showed similar results (130). Pregnant and non-pregnant mice were treated either orally or intravenously with NFV, and the mean NFV unbound oral plasma clearance of pregnant mice was found to be approximately five-fold that of non-pregnant mice. The terminal half-life of NFV was not significantly different (p>0.05) however, and changes in drug clearance and AUC were strongly correlated to changes in CYP 3A metabolism and not to the increase in NFV free fraction (130). The decrease in plasma protein concentrations over pregnancy may still
contribute to the low NFV levels seen between 32-37 wks GA and should be further explored in humans.

Finally, changes in drug protein binding may also account for some of the inter-patient variability that was seen in this study. A previous investigation found that plasma protein concentration differs between healthy volunteers and HIV infected patients, and this change (up to four time the AAG’s) is related to the stage of HIV disease (131).

**VIRAL SUPPRESSION**

Effective use of HAART in pregnancy is used to suppress HIV RNA load below detectable limits for both maternal health and the prevention of vertical transmission. Twenty-nine women received NFV based HAART in pregnancy, 24 women of which initiated therapy antenatally and reached an undetectable pVL in a mean of 61 days. Four of these women, however, broke through later in pregnancy and were detectable at delivery; an additional subject did not achieve suppression during gestation. While there were no HIV transmissions, 17.2% (5/29) of women were inadequately suppressed at delivery, which is of concern.

Plasma concentrations have been widely correlated retrospectively to the rate and maintenance of viral suppression in non-pregnant adults; however, no papers known to us have attempted to demonstrate a PK-PD relationship in pregnancy.

No difference was found in the days to undetectable viral load if a patient’s first antepartum plasma concentration was above or below the clinically relevant threshold. When patients were stratified by the mean of their antepartum concentrations, data showed suggested that patients with mean antepartum plasma NFV concentrations and CRs below the known therapeutic
thresholds appeared to achieve an undetectable pVL more quickly. This trend is inconsistent with data published in non-pregnant adults should be considered with caution as the sample distribution into the two stratifications was uneven and there was a small sample size. Further research is required to establish a concentration response relationship in pregnancy.

The inability to establish a concentration-response relationship might have been partially affected by what is called the white-coat syndrome. Patients may have been adherent to therapy in the days immediately prior to the collection of the blood sample (resulting in a therapeutic drug level), but were non-compliant between clinical visits causing suppression to be slow or non-existent (95). It is possible that the complex social dynamics of these women (recent immigrants with large families, active use of drugs of dependency etc) may have exacerbated this affect. It is also important to consider that all twelve patients with a plasma concentration mean below the clinically relevant threshold achieved and maintained a undetectable viral load; a distinct threshold may need to be established for different time points in pregnancy.

Patients who did not have optimal viral suppression in the third trimester had poor immune function compared to those who maintained suppression. Mean and median NFV concentrations at 32-37wks did not differ statistically between those with optimal viral suppression and those that did not, suggesting that other factors may play a significant role in this population. When the viral resistance and patient-reported profiles of these five women were considered, mutations against the NRTI 3TC was found in the virus of three women, with further mutations against NFV in two. The association of viral suppression and resistance in pregnancy requires further study.
USE of RANDOMED TIMED DRUG LEVELS IN PREGNANCY

Some clinicians are recommending increased dosing of PIs during later pregnancy (36), despite a lack of information and the generally held belief that drug doses at the lower end of the therapeutic range should be prescribed to minimize fetal risk, as toxicities increase with increased dosing. The use of random timed drug levels for TDM could ultimately provide personalized therapy to pregnant women by providing the information necessary to make timely and appropriate dose adjustments similar to what is being explored in non-pregnant adult populations (78).

TDM of ART in pregnancy, however, requires more investigations before it can be of significant clinical benefit. While this study has attempted to define the necessary concentration-effect relationship (132), the virologic response to differing levels of NFV remains unclear, especially when compounded by intermittent adherence or viral isolates with resistance mutations. This study has also suggests marked intrapatient variability; changing dosing based on a single plasma concentration may result in inappropriate interventions.

Future clinical use of TDM in pregnancy will also require significant knowledge of previous treatments and concomitant medications, as well as expert interpretation of levels to give full benefit, all within the short time frame of the second half of pregnancy, following HAART initiation (133).
LIMITATIONS

SAMPLE SIZE
This study did not reach sufficient enrolment to power comparisons between the antepartum time points or the Kaplan Meier stratifications. It is possible that stronger correlations might be made with a larger study population.

ADHERENCE
The accuracy of patient reported adherence is limited, especially due to the physician-patient power dynamic. It is generally believed that pregnant women are highly adherent to therapy because they desire preventing their infant from becoming infected; it is possible, however, that the emphasis placed on the benefits of treatment in pregnancy may bias subjects towards reporting excellent adherence.

While our adherence questionnaire also attempted to capture aspects of adherence besides count of dosages taken, it is likely that it did not capture to a great enough extent, the other components that are important to clinical outcomes.

VIRAL RESISTANCE PROFILES
Relevant resistance profiles were not available on the majority of subjects enrolled in the study, and so the number and type of baseline PI and NRTI mutations were not included in the analysis. As drug potency lessens in the presence of these mutations, related clinical outcomes deteriorate, affecting the study outcomes.
NRTI BACKBONE

The contribution of the NTRI backbone to virologic response is not easily studied due to the intracellular nature of the active moieties of these drugs. While they may affect suppression, it is unlikely that they will be measurable in the clinical setting.

USE OF CONCENTRATION RATIOS

Random timed blood samples are advantageous in studying pregnant women because they require no specific time for blood draw; however, there are several disadvantages to using CRs for analysis. Reference curves are required for every drug, and even perhaps for each regimen, and populations used to construct the curve should reflect the average patient population to which the curve is applied. CRs also assume that ratios are constant over the course of the dosing interval (95).

FUTURE DIRECTIONS

Future investigations should focus on determining what TDM measure is best suited to establishing a concentration-response relationship in pregnancy, as Cmin or an IQ value may be more appropriate than random timed samples. Similarly, it will be important to further understand how drug levels change over the course of pregnancy to inform clinicians how measures at different gestational time points relate to clinical outcomes.

Drug levels and incidence of adverse outcomes should also be examined to establish upper-limit thresholds to prevent unnecessary toxicity to mother and fetus. Finally, long-term maternal clinical outcomes associated with plasma concentrations should be investigated, primarily the acquisition of PI resistance mutations due to sub therapeutic levels at the end of pregnancy.
Further in the future, clinical trials might determine if dose adjustments can increase plasma concentrations and increase the rate of viral suppression and breakthroughs, while remaining safe in pregnancy.

To date, the major success of HAART in pregnancy has been preventing vertical transmission. While these results are truly remarkable, there is growing evidence of the harms caused by intermittent therapy. If these women are receiving imperfect treatment, the long-term health outcomes could be devastating. Low drug levels may permit the generation of viral sub-populations with significant resistance. Alternatively, high drug levels may be associated with toxicities the scientific community is just beginning to explore, including exacerbated osteoporosis, more radical lipid distribution and altered fetal development. These factors may also be compounded over the course of multiple pregnancies.

The information garnered from this study suggests that further PK studies of PIs in pregnancy may be necessary to ensure that their use as the core of safe and effective drug regimens applied to prevent vertical transmission are associated with minimal risk of perinatal infection and positive long-term maternal outcomes.
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APPENDIX 1: ACTG SELF-REPORTING ADHERENCE QUESTIONNAIRE

A. When was the last time the subject missed any medications?

Within the past week – go to question B
1-2 weeks ago – go to C
2-4 weeks ago – go to C
1-3 months ago – go to C
>3 months ago – go to C
Never – go to C

B. If the subject had missed dose(s) within the past week, determine how many doses were missed:

Yesterday
2 days ago
3 days ago
4 days ago
Other

C. How closely did the subject follow the specific schedule?

All the time
Most of the time
About half of the time
Some of the time
Never

D. Did the subject follow special dosing instructions? (eg. take with food, take on empty stomach)

No
Yes – skip to question F

E. What were the main reasons for non-adherence?

Forgot
away from home
finds schedule difficult
too busy
ran out of pills
felt sick
avoid side effects
other
APPENDIX 2: HEIRARCHY FOR REPORTING HIV EXPOSURE CATEGORY

For the purposes of national HIV surveillance reporting, the Public Health Agency of Canada (PHAC) requires that only one exposure category is assigned to each reported positive HIV test result or AIDS diagnosis. As a person may report several risk factors, a hierarchy was established to determine the activities or situations that are considered to have the highest risk of HIV transmission. A positive test result or AIDS diagnosis would be assigned the reported risk factor that appears highest on the list below.

- **MSM**: Men who report having had sex with men; this includes men who report either homosexual or bisexual contact (i.e. some will also report having had sex with women as well). It is important to note here that this exposure category refers to sexual behaviour and not a person’s self-identified sexual identity.

- **MSM/IDU**: Men who have had sex with men and have injected drugs.

- **IDU**: People who inject drugs, also called injecting drug users. The acronym IDU is also often applied to the behaviour of injecting drug use, or what is also commonly referred to as injection drug use.

- **Blood/Blood Products**: 
  - **Recipient of Blood**: Received transfusion of whole blood or blood components, such as packed red cells, plasma, platelets or cryoprecipitate.
  - **Recipient of Clotting Factor**: Received pooled concentrates of clotting factor VIII or IX for treatment of hemophilia/coagulation disorder.

- **Heterosexual Contact/Endemic**: 
  - **Origin from a Pattern II Country**: People who were born in a country in which the predominant means of HIV transmission is heterosexual contact;
- **Sexual Contact with a Person at Risk**: People who report heterosexual contact with a person who is either HIV-infected or who is at increased risk for HIV infection. A person at increased risk for HIV infection would be considered in this case to include someone who is an injecting drug user, a bisexual man, a person born in a country in which the predominant means of HIV transmission is heterosexual contact, a person with hemophilia/ coagulation disorder, or a person with suspected HIV infection or AIDS.

- **NIR-HET**: If heterosexual contact is the only risk factor reported and nothing is known about the HIV-related risk factor(s) associated with the partner, the case would be classified as No Identified Risk-Heterosexual (NIR-HET).

- **Occupational Exposure**: Exposure to HIV-contaminated blood or body fluids, or concentrated virus in an occupational setting.

- **Other**: Used to classify a person whose mode of HIV transmission is known but who cannot be classified into any of the major exposure categories listed.

- **NIR (No Identified Risk)**: Where the history of exposure to HIV through any of the other categories is unknown, or there is no reported history. This exposure category may include:
  - people who are currently being followed up by their local health department;
  - people whose exposure history is incomplete because they have died;
  - people whose exposure history is incomplete because they declined to be interviewed or were lost to follow-up; and
  - people who cannot identify any mode of transmission.

- **Exposure Category Not Reported**: In certain provinces, it is not possible to report information regarding exposure category. In these situations, people are classified as Exposure Category Not Reported. This category is used only for positive HIV test reports.
• **Perinatal Transmission:** The transmission of HIV from an HIV-infected mother to her child either
  
  o during pregnancy,
  
  o during labour,
  
  o at birth, or
  
  o after birth through breastfeeding.
APPENDIX 3: CONCOMITANT MEDICATIONS OF INTEREST

Drugs identified as having drug-drug interactions with nelfinavir by Thompson MICROMEDEX database are listed below.

• ALFUZOSIN
• AMBRISENTAN
• AMIODARONE
• AMLODIPINE BESYLATE /ATORVASTATIN CALCIUM
• AMPRENAVIR
• APREPITANT
• ASPIRIN/PRAVASTATIN SODIUM
• ASTEMIZOLE
• ATORVASTATIN
• AZITHROMYCIN
• CARBAMAZEPINE
• CASPOFUNGIN
• CERIVASTATIN
• CISAPRIDE
• CYCLOSPORINE
• DARIFENACIN
• DASATINIB
• DELAVIRDINE
• DIDANOSINE
• DIHYDROERGOTAMINE
• DIHYDROERGOTAMINE/HEPARIN
• ELETRIPTAN

• INDINAVIR
• IXABEPILONE
• LAMIVUDINE/ZIDOVUDINE
• LAPATINIB
• LOPINAVIR/ritonavir
• LOVASTATIN
• LOVASTATIN/NIACIN
• MARAVIROC
• METHADONE
• METHYLERGONOVINE
• MIDAZOLAM
• NIFEDIPINE
• NILOTINIB
• NORETHINDRONE
• PARICALCITOL
• PHENOBARBITAL
• PHENYTOIN
• PIMOXIDE
• PRAVASTATIN
• QUINIDINE
• RANOLAZINE
• RIFABUTIN
• RIFAMPIN
• EPLERENONE
• ERGOLOID MESYLATES
• ERGONOVINE
• ERGOTAMINE
• ERLOTINIB
• ESZOPICLONE
• ETONOGESTREL
• ETRAVIRINE
• EZETIMIBE/SIMVASTATIN
• FELODIPINE
• FENTANYL
• FENTANYL/DROPERIDOL
• FLUTICASONE
• FLUTICASONE PROPIONATE /SALMETEROL XINAFOATE
• FOSAMPRENAVIR
• FOSAPREPIANT
• FOSPHENYTOIN
• RIFAPENTINE
• RITONAVIR
• ROSUVASTATIN
• SALMETEROL
• SAQUINAVIR
• SILDENAFIL
• SIMVASTATIN
• SIMVASTATIN/NIACIN
• SOLIFENACIN
• SUNITINIB
• TACROLIMUS
• TADALAFIL
• TEMSIROLIMUS
• TERFENADINE
• TRAZODONE
• TRIAZOLAM
• VARDENAFIL
• VORICONAZOLE
APPENDIX 4: UBC RESEARCH ETHICS BOARD CERTIFICATES OF APPROVAL

The original full board approval from the UBC Clinical Research Ethics Board and all amendment approvals for significant changes or additions are included.
# Certificate of Full Board Approval

**Clinical Research Ethics Board Official Notification**

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<th>Principal Investigator</th>
<th>Department</th>
<th>Number</th>
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<td>Money, D.</td>
<td>Obstetrics/Gynaecology</td>
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**Institutions Where Research Will Be Carried Out**

Children's & Women's Health Centre

**Co-Investigators**

Alexander, Christopher, Medicine; Alimenti, Ariane, Paediatrics; Burdge, David, Medicine; Cote, Helene, Biochemistry & Molec Biology; Forbes, Jack, Paediatrics; Khoo, Dominique, Pharmaceutical Sci; Montaner, Julio, Medicine; Stone, Julie, Obstetrics/Gynaecology

**Sponsoring Agencies**

Canadian Foundation for AIDS Research

**Title:**

Effect of Antiretroviral Therapy on Maternal Blood Cell Mitochondrial DNA Levels During Pregnancy in HIV-Infected Women

**Approval Date:**

23 November 2004

**Term (Years):**

1

**Documents Included in This Approval:**

- Protocol dated 13 October 2004 v.2; Consent Form Study Subjects dated 10 September 2004 v.1; Consent Form Control Subjects dated 10 September 2004 v.1; Consent Form Study Infants dated 10 September 2004 v.1; Subject Data Forms dated 10 September 2004 v.1; Control Data Forms dated 10 September 2004 v.1

**Certification:**

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

**The CREB approval for this study expires one year from the approval date.**

---

*Approval of the Clinical Research Ethics Board by one of:*

- Dr. P. Loewen, Chair
- Dr. A. Gagnon, Associate Chair
- Dr. J. McCormack, Associate Chair
**Certificate of Expedited Approval: Amendment**

Clinical Research Ethics Board Official Notification

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**INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT**

Children's & Women's Health Centre

**CO-INVESTIGATORS:**

- Alimenti, Ariane, Paediatrics; Burdge, David, Medicine; Cote, Helene, Biochemistry & Molec Biology; Forbes, John, Paediatrics; Khoo, Dominique, Pharmaceutical Sciences; Montaner, Julio, Medicine; Phillips, Elizabeth, Medicine; Stone, Julie, Obstetrics/Gynaecology

**SPONSORING AGENCIES**

Canadian Institutes of Health Research

**TITLE:**

Mitochondrial DNA Damage in Infants Exposed to HIV Antiretroviral In Utero

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The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

The CREB approval period for this amendment expires on the one year anniversary date of the CREB approval for the entire study.

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Approval of the Clinical Research Ethics Board by one of:

Dr. Gail Bellward, Chair

Dr. James McCormack, Associate Chair
Certificate of Expedited Approval: Amendment
Clinical Research Ethics Board Official Notification

<table>
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Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair
Certificate of Expedited Approval: Amendment
Clinical Research Ethics Board Official Notification

Principal Investigator: Money, D.M.
Department: 

Institution(s) Where Research Will Be Carried Out:
Children's & Women's Health Centre

Co-investigators:
Alimenti, Ariane, Paediatrics; Burdige, David, Medicine; Cote, Helene, Biochemistry & Molec Biology; Forbes, John, Paediatrics; Khoo, Dominique, Pharmaceutical Sciences; Lansdorp, Peter, Medicine; Montaner, Julio, Medicine; Phillips, Elizabeth, Medicine; Stone, Julie, Obstetrics/Gynaecology

Sponsoring Agencies:
Canadian Foundation for AIDS Research

Title:
Effect of Antiretroviral Therapy on Maternal Blood Cell Mitochondrial DNA Levels During Pregnancy in HIV-Infected Women

Approval Date: 05-10-31
Term (Years): 1

Amendment:
Protocol Amendment 02 dd 18 May 2006; Subject Consent Form Version 3 dd 18 May 2006; Control Consent Form Version 6 dd 18 May 2006; Infant Consent Form Version 3 dd 18 May 2006; Addition of Co-Investigator

Amendment Approved: 31 May 2006

Certification:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
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Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bollward, Chair
Dr. James McCormack, Associate Chair
Dr. John Russell, Associate Chair
Dr. Caron Strahlendorf, Associate Chair