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The Pharmacogenetics of Efavirenz and its Side Effects

by

Susan C. Hanson

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

Nursing

in the

GRADUATE DIVISION

of the

Copyright 2013
by
Susan C. Hanson

Dedication

To my beloved Grandmother Clara Marsh Pelton;
Though not here to witness this achievement, the belief you instilled in me that I could accomplish anything I put my mind to gave me the strength and resolve to never stop
To my husband Leif Kirschenbaum,
my soul mate, playmate, and confidant; with you by my side this journey was less daunting
To my parents Grayson and Vera Mae Hanson,
my siblings and their spouses Dean and Debbie Hanson
David and Jill Hanson, Donald and Tammy Hanson and Yuri and Tricia Hofmann;
your ceaseless support and belief in me carried me through the many challenges I faced in pursuing this doctorate
To my nieces and nephews Hope, Echo, Eli, Eva Mae, Tess, Eric, Grayson, James, and Olivia; how you rock your generation has kept me in forward motion
and
To my friend Michelle Miller Clark;
your energy, encouragement, and inquisitive nature has always inspired me to think more, feel more, do more, and be more

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Abstract

Though efavirenz is an effective antiretroviral therapy for the treatment of HIV, its use is associated with pronounced interindividual variability in plasma concentration (exposure) and toxicity, increasing the risk of adverse events or therapy failure. Both genetic and non-genetic factors contribute to efavirenz exposure variability. This dissertation research contributes to our understanding of this phenomenon by modeling the influence of genetic and non-genetic factors on efavirenz exposure *and* describing a broad spectrum of symptoms and side effects and their association with genotypes that influence efavirenz exposure. A total of 182 SNPs and 45 haplotypes in 9 genes were analyzed with previously identified non-genetic factors in relationship to efavirenz drug exposure in 111 women on efavirenz who had undergone 24-hour blood sampling following a witnessed dose under routine conditions. Area-under-the-time curves (AUC) were calculated. We observed three alleles from two genes associated with an increase in efavirenz exposure: CYP2B6 (rs3745274); CYP2B6 (rs28399499), ABCB1 Haplotype A1 (rs27779562 and rs4148745). Of all the non-genetic factors assessed only orange juice consumption and increases in alanine aminotransferase remained statistically significant when genetic factors were included in the final multivariate model. CYP2B6 rs3745274 and rs28399499 can be combined into the CYP2B6 Metabolizer diplotype. Metabolizer diplotype was associated with an increase in efavirenz exposure for both slow and intermediate metabolizers. Risk alleles associated with increased efavirenz exposure were evaluated for associations with a broad spectrum of symptoms and side effects.

Variation in CYP2B6 rs28399499 was associated with a perceived change in the amount of fat in the buttocks and change in buttock fat. Variability in CYP2B6 rs3745274 was associated with report of dietary changes to influence body shape. SNPs in the ADME pathway are associated not only with efavirenz exposure but also side effects. Pharmacogenetic testing may improve efavirenz outcomes and reduce symptoms and side effects associated with efavirenz exposure variability.

Key Words: ADME pharmacogenetics, adverse events, antiretroviral therapy, ATP-binding cassette (ABC) B1 haplotype, body habitus changes, cytochrome P450 (CYP) 2B6 single nucleotide polymorphisms (SNPs), CYP2B6 metabolizer diplotype, drug exposure, efavirenz, gene variations, HIV, plasma concentration, side effects and symptoms.

Table of Contents

Title Page

Copyright ii

Dedication & Acknowledgements iii

Abstract iv

List of Tables vi

List of Figures vii

Chapter 1 Introduction 1

Chapter 2 Efavirenz Pharmacogenetics Literature Review 24

Chapter 3 Study Methods 66

Chapter 4 Efavirenz Pharmacogenetics Substudy 100

Chapter 5 Efavirenz Side Effects and Symptoms Genetic Associations 127

Chapter 6 Conclusion 179

Appendices 197

List of Tables

Chapter 1

Table 1-1 Antiretroviral drugs 21

Table 1-2 Adverse effects associated with different antiretroviral drug classes 23

Table 1-3 Antiretroviral pharmacokinetic parameter sex differences 23

Chapter 2

Table 2-1 Efavirenz Chemical Description and Structures 64

Chapter 3

Table 3-1 WIHS Intensive Pharmacokinetic Substudy Antiretrovirals and their classification 97

Table 3-2 WIHS Intensive Pharmacokinetic Substudy Continuous Variables 97

Table 3-3 WIHS Intensive Pharmacokinetic Substudy Categorical Variables 98

Table 3-4 Efavirenz Pharmacogenetics Substudy Candidate Genes and SNPs 99

Chapter 4

Table 4-1 Analysis between each SNP/haplotype and EFV exposure (AUC/dose) controlling for non-genetic predictors 125

Table 4-2 Factors Associated With Short-Term Efavirenz Exposure Among 111 Subjects From the Women's Interagency HIV Study 126

Chapter 5

Table 5-1 WIHS Self-Reported Ethnicity 150

Table 5-2 Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy 151

Table 5-3 Genetic association with demographic and general clinical characteristics 160

Table 5-4 Genetic association with therapeutic regime 162

Table 5-5 Genetic association with general side effects 164

Table 5-6 Genetic association with gastrointestinal-related symptoms 166

Table 5-7 Genetic association with neuropathic and pain-related symptoms 167

Table 5-8 Genetic association with Neurological, psychological, and fatigue-related symptoms 169

Table 5-9 Genetic association with body habitus 171

List of Figures

Figure 1-1. The life cycle of Human Immunodeficiency Virus 22

Figure 2.1 Efavirenz Metabolism 65

Chapter 1

Introduction

Introduction

First recognized in 1981, human immunodeficiency virus (HIV) infection, a retroviral infection that can lead to acquired immunodeficiency syndrome [AIDS], is now pandemic (Sharp & Hahn, 2011). A complex disease, there are many considerations in treating HIV-infected patients. Initially, research efforts were focused on HIV-associated mortality and morbidity. However, as therapeutic treatment options began to emerge and HIV infection became an incurable but chronic and manageable disease, other issues began to emerge (Moreno, 2010).

Interindividual response to antiretroviral therapy is now widely recognized as an important issue and ranges from inability to achieve the desired therapeutic effect (i.e., efficacy) to minor side effects to adverse events that can be life threatening (i.e., safety). Pharmacogenetics[1], drug interactions, sex and racial/ethnic differences, and the impact of coexisting conditions are important factors currently being examined. The goal of such research is to minimize side effects and adverse events while optimizing response to therapy and outcomes.

HIV Treatment Options

Antiretroviral therapy for treatment of HIV infection has been available since 1986. To date, over 25 antiretroviral drugs, Table 1-1, and a host of immunomodulatory and adjunctive agents have provided an array of therapeutic options available to treat

[1] Pharmacogenetic research focuses on *host* genetic factors that may influence drug safety and efficacy (i.e., side effects, adverse events, and drug resistance or failure) in the absorption, distribution, metabolism, and elimination pathway. The overall goal of pharmacogenetics is to identify patients by genotype who are at risk of adverse events, individuals who may not derive the intended benefit, and/or those who may gain a particular benefit from a treatment modality (Fox, Boffito, and Winston, 2006).

HIV. The increasing number of antiretroviral agents makes individualizing treatment more feasible but conversely poses challenges due to the complexity of drug profiles and diversity of patient populations (comorbidities, age, treatment history, concomitant medications, drug resistance) that may complicate treatment decisions (Este & Cihlar, 2010; Hawkins, 2010). The primary goal of antiretroviral therapy is the maximal and durable suppression of plasma viremia (i.e., <50 ribonucleic acid [RNA] copies/ml). The selection of drugs are guided by viral genome resistance mutations[2] and desire to preserve and/or restore cluster of definition 4 (CD4) + T-cell count (CD4 count) conferring an overall clinical benefit to the patient (Este & Cihlar, 2010). Though there is no cure for HIV infection, these therapies are understood to retard the progression of HIV and prolong life (Flexner, 2006; Fox et al., 2006; Quirk & Powderly, 2004).

Antiretroviral drugs are generally classified by the retroviral target molecule that the drug inhibits in the HIV life cycle, Figure 1-1. Individual drug classes as they pertain to this dissertation are discussed in subsequent chapters. In brief, the HIV life cycle begins upon binding to a cell surface CD4 receptor (a glycoprotein found primarily on the surface of helper T cells) and one of two co-receptors, an alpha chemokine (C-X-C motif) receptor (CXCR4) or C-C chemokine receptor type 5 (CCR5) on the T-lymphocyte cell surface. Binding to the CD4 receptor and co-receptor CXCR4 or CCR5 must occur for the virus to enter the cell. The HIV enzyme reverse transcriptase converts the single-stranded HIV RNA genome to double-stranded HIV deoxyribonucleic acid (DNA). HIV DNA enters the cell nucleus where another enzyme, integrase, inserts the HIV DNA in

[2] As a consequence of viral mutations, resistance may emerge in the viral proteins targeted by antiretroviral agents (Clavel and Hance, 2004).

the cell's DNA. The integrated HIV DNA is termed provirus and may remain inactive for several years. When the provirus becomes activated, the RNA polymerase enzyme creates copies of the HIV genomic material. Messenger RNA (mRNA) functions as a blueprint to create long chains of HIV proteins. Protease, the HIV enzyme, cleaves these long polypeptide chains into smaller proteins that are assembled into a new virus particle. The new virus exudes in virion buds from the host cell which uses host cell glycoproteins to encapsulate the virus and which serves as the ligand the virus uses to bind to new CD4 and co-receptor-bearing cells to propagate the infection (University of New Mexico [UNM], 2008). Current therapeutic treatments to obstruct the HIV life cycle include: the nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors which function similarly to prevent HIV replication, protease inhibitors which inhibit the assembly of new virions, integrase inhibitors which disrupt the integration of HIV into the cell nucleus, and entry inhibitors and C-C chemokine receptor type 5 antagonists, fusion inhibitors which function similarly to block entry of HIV into the host cell (Santoro & Perno, 2013).

Antiretroviral Treatment Guidelines

Historically, criteria for initiating antiretroviral therapy have been fairly consistent across guidelines: patients with AIDS[3] and those with low CD4 cell counts (<200 cells/mm^3) must be started on antiretroviral therapy. The timing of treatment initiation, however, for asymptomatic individuals or patients in a chronic phase of

[3] CD4 criteria for the initiation of antiretroviral therapy is as follows: <200 treat irrespective of clinical stage, 200-350 consider treatment and initiate before CD4 count drops below 200 Cells/mm^3, >350 do not initiate treatment (United States Department of Health and Human Services [USDHHS], 2009).

infection was less directed. Recent guidelines suggest a more aggressive criterion, that treatment be initiated for all individuals with a CD4 cell count of <350 cells/mm^3. Additionally, treatment initiation is recommended for patients who are pregnant, have HIV-1-associated nephropathy, and those co-infected with hepatitis B virus irrespective of the CD4 count. The optimal timing for patients who fall outside these categories remains less clear. Though studies report high mortality rates in HIV-infected patients even when CD4 counts are relatively high, expert panels remain divided as to whether or not treatment should be initiated in patients with CD4 counts of >500 cells/mm^3 (USDHHS, 2009; Moreno, 2010). Promising benefits of decreased mortality and morbidity were recently observed in multiple cohort studies with treatment initiated when CD4 cell counts are between 350 and 500 cells/mm^3 and, in some cases, >500 cells/mm^3 (Moreno, 2010). Future guideline alterations will presumably be guided by these data and subsequent study findings.

Despite the promising results of antiretroviral studies, evidence of virologic resistance, frequency of adverse reactions, and reports of therapy discontinuation often erodes the optimism placed in antiretroviral treatment regimes. Large prospective cohort studies suggest that up to 50% of antiretroviral therapy recipients will not achieve the desired goal (Bartlett, 2006).

Though the Department of Health and Human Services has outlined antiretroviral treatment failure and resistance parameters in their published guidelines (i.e., virologic failure, immunologic failure, clinical progression), many clinicians hold different opinions on how suboptimal response to treatment is defined and determined

(i.e., virologic load versus CD4 cell count). There is however a general consensus that suboptimal antiretroviral therapeutic results are attributed to drug-drug interactions, coexisting comorbid conditions, viral genetic influences[4], host genetic influences, and antiretroviral therapy non-adherence (Quirk & Powderly, 2004).

Significance of Adverse Events

What is known about treatment adherence is that approximately one in four people do not adhere well to prescribed drug therapy (Simpson, 2006). The most common reason for non-adherence, switching, and/or discontinuing therapy reported with virtually all antiretroviral drugs are adverse events (O'Brien, Clark, Besch, Myers, & Kissinger, 2003; Prosperi et al., 2012). Though randomized controlled clinical trials are the gold standard for evaluating drug efficacy, they may underestimate adverse events due to study participants desire to remain in the trial and receive support from trial staff. Additionally, the short duration of trials likely results in not observing events that take time to develop and which result in decreased adherence (Hawkins, 2010). Over time, data on adverse events to antiretroviral drugs have been recorded in numerous trials, in post-marketing analyses, and in anecdotal reports. These findings can be

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[4] Viral genetics are dynamic due to the rapid growth of viruses and change due to mutation or recombination. Because viruses grow rapidly, there is a greater chance of mutations occurring over a short period of time (Hunt, 2010). HIV infection is characterized by high levels of viral production and rapid turnover. Reverse transcription of viral RNA into DNA is prone to error, producing on average one mutation for each viral genome transcribed. Viral quasispecies develop that confer a selective advantage to the virus such as a decrease in susceptibility to an antiretroviral agent. The quasispecies follow a Darwinian selection process rapidly reproducing causing high levels of resistance by accumulation of drug-resistant mutations (Clavel and Hance, 2004).

usefully divided into short term symptoms (i.e., fever, rash, fatigue, malaise, nausea, vomiting, diarrhea, impaired liver function, dyspnea, anemia, central nervous system anomalies, jaundice and scleral icterus, lymphadenopathy, hypersensitivity reaction [a cluster of these symptoms]) and long term events (i.e., lactic acidosis, hepatoxicity, pancreatitis, dyslipidemia, peripheral neuropathy, body habitus changes) and characterized between clinical and laboratory abnormalities specific to each antiretroviral class (Hawkins, 2010; Phillips, 2000). Though there is significant overlap of adverse events associated with different classes of antiretrovirals, there are also notable differences. With protease inhibitors, gastrointestinal-related adverse events such as nausea, vomiting, and diarrhea are commonly reported and are a significant reason for discontinuation. For nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors, these classes have comparatively few associated gastrointestinal adverse effects. Conversely, hypersensitivity reaction more notably occurs with drugs in the nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors classes (Hawkins, 2010). Table 1-2 summarizes the most commonly documented adverse events for each antiretroviral class.

Antiretroviral Pharmacogenetics

Pharmacogenetic research focuses on *host* genetic factors in the absorption, distribution, metabolism, and elimination (ADME) pathway that may influence drug safety (adverse events) and efficacy (drug resistance or failure). The overall goal of pharmacogenetics is to identify patients by genotype who are at risk of adverse events, individuals who may not derive the intended benefit, and/or those who may gain a

particular benefit from a treatment modality. Studies of gene variations and their influence on pharmacotherapy are currently being conducted with HIV- infected individuals treated with antiretroviral therapy to identify patients at risk for adverse events and those in whom therapy is suboptimal (Fox et al., 2006).

ADME Pharmacogenetic Study Methods

Variations in the genes that encode for proteins involved in the ADME of drugs are known to lead to different plasma concentrations resulting in a lack of efficacy or toxic effects (Benet , Krotz, & Sheiner, 1996). Nearly all HIV studies assessing antiretroviral response now involve pharmacogenetic research methods. Two methods are typically employed to perform genetic analyses: genome-wide analyses[5] and candidate gene studies. The majority of pharmacogenetic studies employ a direct, hypothesis-driven candidate gene approach whereby the plausible association between a gene variation of probable impact on drug metabolism and/or toxicity is examined (Tozzi, 2010; Walgren, Meucci, & McLeod, 2005). The focus is on genes suspected to be involved in the variance of a particular trait or measure based on what is known about the protein product it encodes for and its function vis-à-vis a trait of interest. Though genome-wide analyses bring new possibilities to this area of research, candidate gene analyses continue to be a method of choice for many researchers.

Barriers to Clinical Utility of Pharmacogenetic Research

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[5] Genome-wide analyses looks for the difference in the frequency of genetic variations between unrelated affected and unaffected individuals which serve as controls (Kruglyak, 1999). The entire genome is interrogated and uncovers causal genes as well as genes that are typically neither necessary nor sufficient for trait occurrence that modify risk for a trait (Walgren, et al., 2005).

Though a significant body of antiretroviral pharmacogenetic research exists, there remain barriers to the clinical utility of this literature and its contribution to our understanding of the host of variables that influence drug response variability. These include: inadequate representation of women in study populations, incomplete gene variation coverage, single studies that have not been reproduced, few studies that have comprehensively assessed nongenetic and genetic factors and their association with drug exposure (i.e., drug dose and corresponding drug concentration) and drug effects (i.e., efficacy adverse events), and guidelines that translate study results into clinical decisions (Zanger, 2010). A former barrier was that ethnic/racial groups were not adequately represented in ADME gene variation studies. However, known ADME gene variations established to influence antiretroviral drug exposure are now well characterized across ethnic/racial groups. Nevertheless, additional research is needed to improve our understanding of the influence of genetic and nongenetic factors on the efficacy and safety of each HIV drug across ethnic/racial groups in both men and women.

Sex Disparity in Pharmacogenetic Research

Though men account for a greater number of new HIV infections (73%) as compared to women (27%), globally the percentage of women living with HIV remains at approximately 50%. However, the rate of women infected is increasing in several countries. In the United States, the rate of transmission in women from 2004 through 2007 increased by 8% (Este & Cihlar, 2010; USDHHS, 2007). And yet, women continue to be under-represented in antiretroviral research. Many clinical trials evaluating the

safety and efficacy of antiretroviral treatment regimes have focused primarily on male cohorts. For many disease states and corresponding therapeutic treatment regimes, sex disparity in research remains a challenge to generalizing study findings. Although it is reasonable to generalize some study results, women do differ in many respects. Differences in antiretroviral-related adverse events and effectiveness of antiretroviral treatment regimes may not be the same in men and women. Though several studies suggest that sex influences the frequency, presentation, and severity of antiretroviral therapy-related adverse events, the majority of clinical trials are not powered to determine such differences (Clark, 2006). However, a few studies have shown sex differences in antiretroviral pharmacokinetic parameters that may influence safety and efficacy, Table 1-3. Because all of the mechanisms are not fully understood, differences between men and women continue to be explored.

Sex differences in pharmacokinetics. Physiological differences between men and women (i.e., body-water space, muscle mass, organ blood flow, organ function menopause, pregnancy, menstruation) as well as pharmacokinetics (i.e., bioavailability, volume of distribution, metabolism, excretion) are hypothesized as reasons for sex-related differences (Zopf et al., 2008). Between 1995 and 2000 in reviewing new drug applications, the FDA found that 11 drugs showed a >40% difference in pharmacokinetics between men and women (Anderson, 2005; Chen et al, 2000).

In general, women weigh less than men however, few drugs are dose corrected for body weight. In FDA bioequivalence studies, not adjusting for weight in drug dosing resulted in 20%-88% higher plasma area under the concentration time curves (AUCs) in

women as compared with men. Women also have a higher body fat percentage than men which affects the volume of distribution of some drugs. A larger volume of distribution may cause decreased maximum concentration (Cmax) (e.g., the larger the volume of distribution the larger the dose required to attain a desired concentration), increased half-life (e.g., increase in fat sequestration, a tissue not involved in body clearance), and increased duration of effect when the same dose of a drug is given to a woman as compared with a man (Anderson, 2005; Ginsberg, Hattis, Russ, & Sonawane, 2005). Renal clearance of unchanged drug is also decreased in women, the glomerular filtration rate is approximately 10% lower in women as compared to men after correcting for body size (Anderson, 2005; Chen et al., 2000). Differences in hepatic enzyme expression by sex have been observed in both animal model (Clark, 2006) and human studies (Gandhi, Aweeka, and Greenblatt, 2004). These differences may account for drug concentration variability whereby higher antiretroviral concentrations could be associated with a higher risk for adverse events and lower concentrations associated with lower efficacy, and the potential for antiretroviral resistance (Clark, 2006). In HIV-infected populations, knowledge of sex differences associated with responses to treatment is somewhat limited and further investigation is warranted.

Food and Drug Administration Pharmacogenetic Research Guidance

It is recognized by the FDA that gene variations can influence the exposure-response relationship of drugs. In response to the burgeoning pharmacogenetic research in all therapeutic areas, the FDA drafted guidelines for clinical pharmacogenomics to assist investigators involved in drug development in evaluating

how variations in the human genome may affect clinical pharmacology and clinical responses of drugs (USDHHS, 2005). Though the guidelines are not directed toward compounds already approved, they can be meaningfully applied. The FDA notes that variation in drug response results from the interaction of multiple factors or covariates, including genetic, demographic, and environmental factors. Understanding specific covariate and gene-covariate interactions on variability in drug response may be useful in understanding the relative impact of genetic versus nongenetic factors on the dosing efficacy and safety of each drug. The FDA acknowledges that across the drug research continuum, there is a need for genomic data to elucidate the molecular basis for lack of efficacy or adverse events, identify individuals who are more at risk for drug-induced adverse events and identify those who may benefit from a genotype/covariate-modified dose or dosing interval. The intent of genetic findings is to make drug therapy more effective and safe. Even if no genomic effect is found, the FDA further acknowledges the information may streamline future research by confirming that certain suspected pathways are not likely to contribute significantly to interindividual variability in pharmacokinetics, pharmacodynamics, efficacy or safety (USDHHS, 2005).

Emerging ADME Antiretroviral Pharmacogenetic Research

In the antiretroviral pharmacogenetic literature, the number of studies of gene variations and interindividual drug exposure variability are increasing and will be examined in the literature review as it relates to this dissertation. Aside from the continued exploration of the ADME pathway for gene variations that may influence drug exposure, the relationship between gene variations known to influence drug exposure

and adverse events has become an important research focus. Pharmacogenetic studies examining hypersensitivity reaction syndrome, hepatotoxicity, peripheral neuropathy, hyperbilirubinemia, dyslipidemia, pancreatitis, renal toxicity, and central nervous system-related side effects have been undertaken (Boffito, Winston, & Owen, 2005; Tozzi, 2010). Pharmacogenetic markers in treatment discontinuation and dose reduction to reduce adverse events have also become areas of pharmacogenetic research interest. Studies have also emerged examining a range of nongenetic factors (e.g., sex, comorbidity, concomitant therapy, recreational drug use, smoking, food interactions) and their contribution to drug response variability. The next step is to explore genetic and nongenetic factors together to identify the factors that influence drug exposure. Multivariate modeling to fit predictive models is an important component of that next step. A goal of pharmacogenetic studies extending beyond the discernment of drug exposure variability is to quantify genetic and nongenetic factors into meaningful models that contribute to the development of drug and dose optimizing algorithms to individualize therapy.

Dissertation Research Focus

The purpose of the research undertaken in this dissertation is to understand the role of gene variations in the drug exposure variability and adverse events of a common antiretroviral drug, efavirenz. A candidate gene approach was employed and focused on genes in the ADME pathway to determine the association between gene variations and drug exposure and adverse events in a sample of HIV-infected participants from the

Women's Interagency HIV Study (WIHS). This study is uniquely positioned to examine the following aims:

1. To identify genotypes that influence variance in drug concentration (defined by area-under-the-plasma concentration-time curve divided by target dose) of efavirenz, the most commonly used non-nucleoside reverse transcriptase inhibitor.
2. To describe the prevalence by genotype of adverse events, signs, and symptoms in a subgroup of women taking efavirenz.

Description of Dissertation Chapters

The chapters that follow in this dissertation include a literature review/theoretical framework, a methodology chapter, two pharmacogenetic analyses, and a conclusion.

The literature review/theoretical framework (Chapter 2) provides a review of ADME antiretroviral pharmacogenetics that focuses the non-nucleoside reverse transcriptase inhibitor, efavirenz.

The methodology chapter (Chapter 3) describes the methods used to conduct this cross-sectional cohort study using stored DNA for genetic analysis and previously collected data from the Women's Interagency HIV Study (WIHS) Pharmacokinetic (PK) substudy participants.

The study featured in Chapter 4 describes the findings identifying genotypes that influence variance in drug concentration (defined by area-under-the-plasma concentration-time curve divided by target dose) of the non-nucleoside reverse

transcriptase inhibitor, efavirenz, among WIHS women enrolled in the Intensive Pharmacokinetics Substudy. The findings presented in Chapter 4 represent a subset of those recently published in the *Journal of Acquired Immune Deficiency Syndrome* April 2009 and titled Nonucleoside reverse transcriptase inhibitor pharmacokinetics in a large unselected cohort of HIV-infected women (Gandhi, et al., 2009), and the *Journal of Infectious Diseases* titled A single-nucleotide polymorphism leads to >3fold increases in efavirenz concentrations in plasma and hair among HIV-infected women (Gandhi et al, 2012).

The study featured in Chapter 5 describes the a broad spectrum of symptoms and side effects and their association with genotypes that influence efavirenz exposure.

The conclusion (Chapter 6) discusses the results, study strengths and limitations, conclusions, application of the study findings, and future research directions.

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Table 1-1. Antiretroviral drugs

Brand Name	Generic Name	FDA Year	Drug Class	*Comments
Nucleoside Reverse Transcriptase Inhibitor (NRTI)				
Atripla	emtricitabine/tenofovir/efavirenz	2006	2NRTI/ 1NNRTI	
Combivir	zidovudine/lamuvidine	1997	NRTI	
Emtriva	Emtricitabine	2003	NRTI	
Epivir	Lamivudine	1995	NRTI	
Epzicom	abacavir/lamivudine	2004	NRTI	
Hivid*	Zalcitabine	1992	NRTI	*manufacturer discontinued in 2006
Retrovir	Zidovudine	1987	NRTI	
Trizivir	zidovudine/lamivudine/abacavir	2000	NRTI	
Truvada	tenofovir/emtricitabine	1997	NRTI	
Videx/Videx EC	Didanosine	1991	NRTI	
Viread	Tenofovir	2001	NRTI	
Zerit	Stavudine	1994	NRTI	
Ziagen	Abacavir	1998	NRTI	
Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)				
Intelence	etravirine	2008	NNRTI	
Rescriptor	delavirdine	1998	NNRTI	
Sustiva	efavirenz	1998	NNRTI	
Viramune	nevirapine	1996	NNRTI	
Protease Inhibitor (PI)				
Agenerase*	amprenavir	1999	PI	*manufacturer discontinued in 2007
Aptivus	tipranavir	2005	PI	
Crixivan	indinavir	1996	PI	
Fortovase*	saquinivir	1997	PI	*manufacturer discontinued in 2006
Invirase	saquinavir	1995	PI	
Kaletra, Aluvia	lopinavir/ritonavir	2000	PI	
Lexiva	fosamprenavir	2003	PI	
Norvir	ritonavir	1996	PI	
Prezista	darunavir	2006	PI	
Reyataz	atazanavir	2003	PI	
Viracept	nelfinavir	1997	PI	
Integrase Inhibitors (II)				
Isentress	raltegravir	2007	II	
C-C Chemokine Receptor Type 5 Antagonists (CCR5A)				
Selzentry	maraviroc	2007	CCR5A	
Fusion Inhibitors (FI)				
Fuzeon	enfuvirtide	2003	FI	

(USDHHS, 2009)

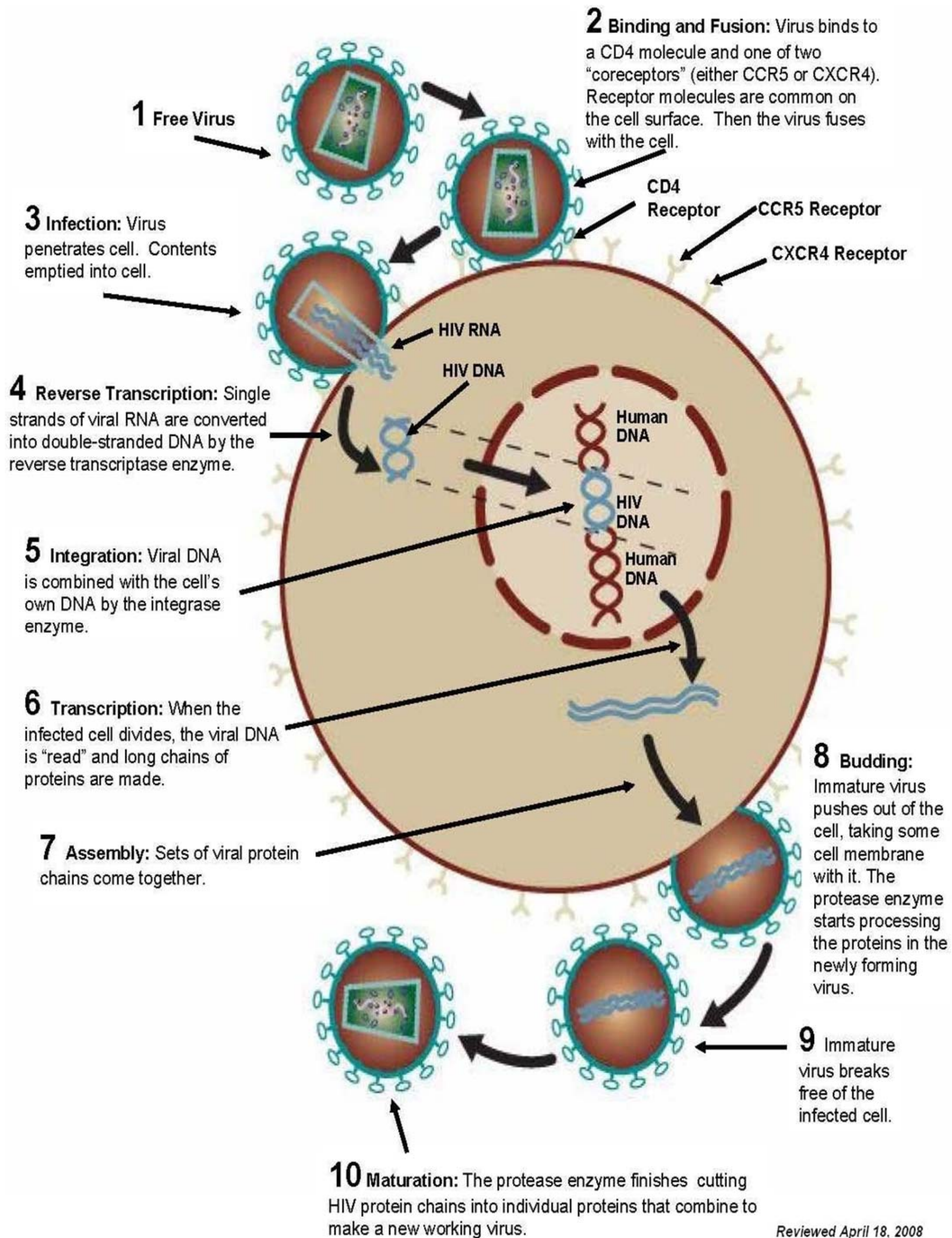

Figure 1-1. The life cycle of Human Immunodeficiency Virus (HIV). Antiretroviral drugs are generally classified by the retrovirus target molecule that the drug inhibits (UNM, 2008).

Table 1-2. Adverse effects associated with different antiretroviral drug classes

Drug Class	Adverse effects
Nucleoside Reverse Transcriptase Inhibitor	Anemia, nausea, rash, myopathy, dyslipidemia, lipoatrophy, pancreatitis, lactic acidosis, hepatic steatosis, hypersensitivity reaction, hepatotoxicity, renal insufficiency, bone loss
Non-nucleoside Reverse Transcriptase Inhibitor	Central nervous system adverse effects, rash hepatotoxicity, lipoatrophy, teratogenicity, hypertriglyceridemia, rash, hypersensitivity reaction
Protease Inhibitors	Nausea, diarrhea, rash, dyslipidemia, insulin resistance, hepatotoxicity, jaundice, scleral icterus, nephrolithiasis, heart disease
Entry inhibitors	Injection site reactions, pneumonia, hypersensitivity reaction, cough, fever, respiratory tract infections, hepatotoxicity
Integrase inhibitors	Headache, insomnia, dizziness, fatigue

Note. Modified from Hawkins, 2009

Table 1-3. Antiretroviral pharmacokinetic parameter sex differences

Drug	Drug class	Sex difference (women compared with men)
Atazanavir	Protease inhibitor	Mean concentrations were 20% higher in women
Indinavir	Protease inhibitor	Concentration minimum was 22% less in women
Lopinavir	Protease inhibitor	Mean concentrations were 20% higher in women
Saquinavir	Protease inhibitor	Higher concentrations and decreased clearance in women
Efavirenz	Non-nucleoside reverse transcriptase inhibitor	Variable results showing both increase and decrease concentration in women
Nevirapine	Non-nucleoside reverse transcriptase inhibitor	Higher concentrations in women and decreased clearance

Note. Modified from Clark, 2006.

Chapter 2

Efavirenz Pharmacogenetics Literature Review

Pharmacogenetic research focuses primarily on understanding the relationship between gene variations in genes participating in the ADME pathway of pharmacotherapy and interindividual treatment response variability. Early pharmacogenetic research showed that for some drugs with a narrow therapeutic range,[6] interindividual variations in therapeutic response often included a genetic basis (e.g., warfarin CYP2C9 metabolizing enzyme gene variation) (Evans & Relling, 1999). Pharmacogenetic research has expanded to include drugs where interindividual response to therapy is notable and adverse events prevalent (Fox, Boffito, & Winston, 2006; Quirk & Powderly, 2004). Gene variations are believed to account for up to 95% of the variability seen in patients, however, the amount of variability explained depends on the drug (Calmy, Hirschel, Cooper, & Carr, 2007; Deeks, 2000; Kalow, Tang, & Endrenyi, 1998; Ross, Anand, Joseph, & Pare, 2012; Shankarkumar, Shankarkumar & Ghosh 2011). In human immunodeficiency (HIV) therapeutics, pharmacogenetics is pursued due to the prevalence of toxicity, the long-term nature of treatment, and the complexity of multidrug therapy that could benefit from predictive tools to identify the combination of compounds most likely to be tolerated and effective among different individuals (Lubomirov et al., 2011; Shankarkumar et al., 2011). Up to 50% of patients on antiretroviral therapy discontinue their drug treatment regimes due to poor treatment tolerance (i.e., side effects, adverse drug reactions, therapy failure) (Calmy et al., 2007; Deeks, 2000; Kalow et al., 1998; Shankarkumar et al., 2011). Recent

[6] Narrow therapeutic index (NTI) drugs are defined as those drugs where small differences in dose or blood concentration may lead to dose and blood concentration dependent serious therapeutic failures or adverse drug reactions (Burns, 1999).

pharmacogenetic research suggests that higher treatment discontinuation rates correlate with genetic variations in the ADME pathway (Lubomirov et al., 2011). Though pharmacogenetic research has demonstrated convincing associations with laboratory abnormalities, additional research is needed to confirm how these data translate into signs and symptoms that lead to clinical action such as treatment discontinuation (Lubomirov et al., 2011). The extent to which pharmacogenetics can be used as a predictive tool continues to be investigated.

Disease Impact and Therapeutic Options

Of all the infectious diseases in history, HIV has posed the greatest threat to humans (Shankarkumar et al., 2011). Though the incidence of HIV infection is believed to have peaked in the late 1990's, the latest reports based on statistics from 2011 estimate, approximately 2.5 million (95% confidence interval [CI]: 2.2 – 2.8 million) people worldwide became newly infected, 34 million (95% CI: 31.4-35. million) people worldwide were living with HIV, and 1.7 million (95% CI: 1.5 – 1.9 million) lost their lives to acquired immunodeficiency syndrome (AIDS) (i.e., a severe immunological disorder cause by HIV), related causes. The 2011 estimated incidence of HIV infection in North America is 51,000 (95% CI: 19,000 – 120,000) and the prevalence is estimated to be 1.4 million (95% CI: 1.1-2.0 Million). Since the first cases were reported in 1981, over 25 million people worldwide have died of AIDS (Joint United Nations Programme on HIV/AIDS [UNAIDS] 2012).

Though there is no cure for HIV, antiretroviral therapy delays the progression of HIV and prolongs life (Flexner, 2006). More than 25 FDA-approved antiretroviral drugs

are now available as treatment options. These compounds target HIV reverse transcriptase, protease, or viral entry receptors. Lifelong administration of these drugs requires continual monitoring of drug safety and efficacy. Though AIDS-related mortality is reduced by these drugs, interindividual response to antiretroviral therapy is widely recognized and is attributed to age, sex, therapy adherence, drug interactions, food interactions, coexisting conditions, and genetic influences (Quirk & Powderly, 2004; Mahungu, Johnson, & Owen, 2009; Shankarkumar et al., 2011; Shi, Bleavins, & de la Iglesia, 2001).

Numerous associations between gene variations and many antiretroviral drug classes are reported resulting in a rapidly growing body of literature. The purpose of this review is to describe and discuss the most commonly prescribed non-nucleoside reverse transcriptase inhibitor, efavirenz, associated adverse events, related drug discontinuation, and ADME pharmacogenetics. Sex disparity in this area of research will be highlighted and the scope of this review will be limited to the adult patient. HIV drug resistance and genotype resistance testing[7], another important area of research, will not be included in this review.

[7] HIV is susceptible to ongoing mutation due to the lack of proof reading activity in HIV reverse transcriptase enzyme. This results in the occurrence of daily random point mutations. With antiretroviral drug exposure, positive selection of mutant strains occur which are able to escape the selective drug pressure resulting in the emergence of drug resistant mutants. These mutants gradually replace the wild-type virus. Genotypic assays detect known mutations associated with drug resistance and phenotypic assays test the response of a patient's HIV reaction to each drug (Sen, Tripahty, Paranjape, 2006).

Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors were the third class of antiretroviral drugs to be developed and have acquired a definitive position as part of the first-line treatment of HIV infections. Non-nucleoside reverse transcriptase inhibitor compounds are targeted at a specific 'pocket' binding site within the HIV-1 reverse transcriptase. By binding to the reverse transcriptase, a disruption of the reverse transcriptase enzyme's catalytic site occurs and viral RNA is prevented from converting to viral DNA that would infect healthy cells (De Clercq, 1998). Guideline recommendations indicate efavirenz as the first-choice non-nucleoside reverse transcriptase inhibitor in antiretroviral therapy-naïve patients (United States Department of Health and Human Services [USDHHS], 2011).

Use of Efavirenz in HIV-Pharmacotherapeutics

The primary focus of antiretroviral therapy is to achieve a durable suppression of viral replication below the level of detection (i.e., <50 RNA copies/ml) in conjunction with immune reconstitution as measured by an increase of CD4+ cells all while minimizing adverse events (Este & Cihlar, 2009). In seeking to achieve this, pharmacotherapy treatment decisions are based on virologic efficacy, toxicity, pill burden, dosing frequency, drug-drug interaction potential, resistance testing results, pregnancy, and comorbid conditions. Efavirenz, Table 2-1, is the most potent of the non-nucleoside reverse transcriptase inhibitor and has a favorable pharmacokinetic profile (i.e., efficient absorption, long half-life) (Gerber, 2000). It is the most commonly prescribed antiretroviral drug in combination therapy and is widely used in initial,

subsequent, and salvage antiretroviral regimens. The usual adult dose for HIV infection is 600 mg administered orally once daily (Manfredi, Calza, & Chiodo, 2004).

Efavirenz Pharmacology

The site of action of efavirenz is inside the HIV-infected cells (Almond, Hoggard, Edirisinghe, Khoo, & Back, 2005). Its activity is primarily mediated by noncompetitive inhibition of HIV-1 reverse transcriptase. Efavirenz binds to reverse transcriptase, blocking the RNA-dependent and DNA-dependent DNA polymerase activities inducing conformational changes that cause a disruption of the enzyme's catalytic site. HIV-2 reverse transcriptase and human cellular DNA polymerases alpha, beta, gamma, and delta are not inhibited by efavirenz (Adkins & Noble, 1998). Studies in both healthy volunteers and infected patients have established efavirenz ADME parameters.

Efavirenz absorption. Efavirenz is highly lipophilic, reported to be nearly insoluble in water (Cristofoletti et al., 2013). Following oral administration, peak plasma concentrations are achieved in approximately 2-5 hours. Plasma concentration measures of efavirenz are dose related. In studies, dose-dependent increases in maximum concentration (Cmax) and area-under-the-concentration-time curve (AUC), a bioavailability measurement based on plots of plasma concentrations sampled at specific intervals, were seen up to 1600 mg. However, at higher doses, the dose-dependent increases became less proportional which suggests diminished absorption at higher doses. This is attributed to the limited aqueous solubility of efavirenz and dose-related delayed gastric emptying (Adkins & Noble, 1998; Balani, Kauffman, deLuna, & Lin, 1999; Cristofoletti et al., 2013). The amount of unchanged drug that reaches

systemic circulation following oral administration (bioavailability) is 40% - 45% without food. However, when taken with food, significant changes in bioavailability parameters can occur. Co-administration with a high fat meal (54% calories from fat) and a reduced fat meal (4% calories from fat) resulted in a mean increase in efavirenz Cmax of 39% and 51%, respectively, and mean AUC increase of 22% and 17%, respectively, compared to fasting conditions. It is suggested that the increased efavirenz concentrations observed when co-administered with food may lead to an increase of adverse events. It is therefore recommended that efavirenz be taken on an empty stomach (Adkins & Noble, 1998; Cristofoletti et al., 2013).

Efavirenz distribution. Efavirenz traverses the intestinal epithelial membrane via passive diffusion and is concentration gradient driven (Gerber, 2000). It is widely and rapidly distributed with a volume of distribution is approximately 280 - 500L, which is consistent with its high lipophilicity (i.e., drugs that have an affinity for lipids have higher volumes of distribution as compared to drugs with an affinity for plasma). It is predominantly protein bound (approximately 99.5% - 99.75%) and is primarily bound to albumin (Cristofoletti et al., 2012; Smith, DiCenzo, & Morse, 2001). Once in the intestinal epithelial cells, lipophilic drugs can be transported back to the luminal surface via P-glycoprotein, a multidrug-resistance-transport protein and/or metabolized by the intestinal cytochrome (CYP) P450 3A isoenzyme (Gerber, 2000). Though many antiretroviral drugs are known substrates of P-glycoprotein, the relationship between efavirenz and P-glycoprotein is not well understood. Contradictory findings regarding

an association between efavirenz plasma concentrations and P-glycoprotein expression have been reported (Fellay et al., 2002; Winzer et al., 2005).

Efavirenz metabolism and elimination. Efavirenz is primarily metabolized by the cytochrome (CYP) P450 system[8] to hydroxylated metabolites with subsequent glucuronidation of these hydroxylated metabolites. Efavirenz is metabolized into three primary metabolites, Figure 2.1; two are hydroxylated metabolites (8-hydroxy-EFV [8-OH-EFV] and 7-hydroxy-EFV [7-OH-EFV]) and the third is a glucuroconjugated product (N-glucuronide –EFV [N-gln-EFV]). The main metabolic pathway is 8-hydroxylation (approximately 92%) mediated via CYP2B6 activity (di Iulio et al., 2009; Ward et al., 2003). Recent i*n vitro* studies suggest that 7-hydroxylation is a secondary pathway (<8%), mediated mainly via CYP2A6 activity and to a lesser extent CYP2B6 activity (di Iulio et al., 2009; Desta et al., 2007). Though N-gln-EFV has been identified in human plasma and urine, the pathway has not been well characterized (di Iulio et al., 2009; Mutlib, et al., 1999). Only recently UDP glucuronosyltransferase (UGT) 2B7 was identified as potentially being the main enzyme mediating efavirenz N-glucuronidation (Kwara, Lartey, Sagoe, Kenu, & Court, 2009).

The product label implicates CYP3A4 metabolism involvement. However, evidence in support of this claim is limited and contradictory whereby one study showed

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[8] Cytochrome (CYP) 450 represent the most common family of Phase I drug metabolizing enzymes. The term "cytochrome P450" emerged following the observance of the wavelength of light (450 nm) that is absorbed by these isoenzymes. The CYP nomenclature is attributed to Nebert et al who in 1987 recommended the classification identifying the root as the letters CYP, the family by the number following the root (i.e., the subfamily by a capitol letter), and ending with a number identifying the gene (i.e., CYP3A4, CYP=root, 3=family, A=subfamily, 4=isoenzyme/gene) (Nebert et al., 1987).

no correlation between efavirenz systemic exposure and hepatic CYP3A4 activity in humans and another study observed a correlation between CYP3A activity and efavirenz 8-hydroxyylation. Of note, the authors of the latter study noted their findings may not be due to actual CYP3A4 involvement but derived from the significant correlation between CYP3A and CYP2B6 activity in the human livers tested in this study (Spearman r = 0.72; p = 0.0234) (Mouly et al., 2002; Ward et al., 2003). CYP1A2 and CYP3A5 have also been noted to form 8-hydroxyefavirenz from efavirenz but the contribution of these isoforms to efavirenz metabolism appears minor (Ward et al., 2003). Though other CYP450 enzymes are implicated in the metabolism of efavirenz, evidence supports that the main metabolic pathway, 8-hydroxylation, is mediated primarily by CYP2B6 (di Iulio et al., 2011; Heil et al., 2012; Ward, et al., 2003).

Efavirenz appears to autoinduce (i.e., induces and enhances the enzymes that moderate its own metabolism) the CYP 450 system. Multiple doses of 200 - 400 mg per day over 10 days resulted in lower than predicted drug accumulation (22% -42% lower) and a shortened terminal half-life of 40 - 55 hours as compared to a single dose half-life of 52 -76 hours (Adkins & Noble, 1998; Cristofoletti et al, 2013)

Efavirenz has a terminal half-life (time required to eliminate half the administered dose of a drug) of approximately 52 hours after a single dose and 40-50 hours after multiple doses (Maggioli, 2009; Toutain & Bousquet-Melou, 2004). Elimination studies using mass balance/excretion and radiolabel drug technique observed approximately 14%-34% of the radiolabel was recovered in the urine and 16%-

61% was recovered in the feces. Nearly all of the urinary elimination of the radiolabeled drug was in the form of metabolites (Adkins, & Noble, 1998; Maggioli, 2009).

Substantial variation in the metabolism and elimination of efavirenz is demonstrated by an intraindividual variation of 16% - 23% and an interindividual variation of 55% - 84% in efavirenz pharmacokinetics. Efavirenz clearance appears to be reduced in blacks as compared to whites resulting in higher drug concentrations (Csajka, et al., 2003; King & Alberg, 2009; Stahle, Moberg, Svensson, & Sonnerborg, 2004). The effect of sex on efavirenz metabolism is somewhat inconclusive. Some studies reported an increase in efavirenz drug exposure in female patients as compared with male patients whereas other studies showed no difference (King & Alberg, 2008).

Efavirenz FDA Approval, Supporting Research, and Adverse Events

Efavirenz was approved by the FDA in September 1998 as the fourteenth antiretroviral drug to become available in the United States. The approval was based on two clinical trials that demonstrated prolonged suppression of HIV RNA, Study 006 and ACTG Protocol 364 (USDHHS, 2010). In these studies, the most common adverse reactions (>5%, moderate-severe) were rash, dizziness, nausea, headache, fatigue, insomnia, and vomiting. The overall discontinuation rate due to adverse events was 8% in Study 006 and 3% in ACTG Protocol 364. Sex disparity in both trials was notable with 83% of the patients in Study 006 and 88% in ACTG Protocol 364 being male. These findings are therefore at risk for being poorly generalizable to the female patient population (USDHHS, 2010).

Post FDA-approval efavirenz experience resulted in the identification of additional adverse reactions (i.e., fat maldistribution, central and peripheral nervous system disorders, hepatic failure, psychiatric disorders, hypercholesterolemia, hypertriglyceridemia, musculoskeletal disorders, flushing, palpitations) (USDHHS, 2010). Central nervous system (CNS) side effects (i.e., dizziness, insomnia, impaired concentration, somnolence, abnormal dreams, hallucinations) are reported in 40%-70% of patients who receive efavirenz with CNS adverse effect reported to be more frequent in patients with higher efavirenz drug exposure. However, these symptoms reportedly resolve after 2 - 4 weeks of treatment with a CNS-related side effect discontinuation occurrence of 2%-5% (USDHHS, 2010; 2003; King & Alberg, 2009). Another frequently reported efavirenz adverse event is rash. Post-marketing research and database analyses have shown that rash occurs in approximately 15%-27% of patients taking efavirenz but does not usually require discontinuation (USDHHS, 2010).

A study was conducted in 2003 to identify the key components of non-nucleoside reverse transcriptase inhibitor tolerability and efficacy. Though the intent was to compare two different non-nucleoside reverse transcriptase inhibitors (i.e., efavirenz and nevirapine), discontinuation and tolerability issues observed for efavirenz were significant findings. The overall frequency of efavirenz discontinuation was 11.5% and events reported to result in discontinuation were: CNS disturbances, persisting metabolic abnormalities, gynecomastia (i.e., a form of fat maldistribution), and pancreatic abnormalities (Manfredi et al., 2004). Of note, 62.5% of the study participants were men (Manfredi et al., 2004). This higher representation of men

challenges the generalizability of these findings to the female patient population and sex-related differences were not examined.

Despite advances in therapeutic treatment regimes, adverse events have continued to affect patient's quality of life and willingness to adhere to their treatment regime (Este & Cihlar, 2009). HIV pharmacogenetics is pursued because of the prevalence of toxicity, the long-term nature of therapy, and complexity of multidrug treatment regimes that could benefit from predictive tools that identify drug combinations most likely to be effective and well tolerated (Lubomirov, Csajka, & Telenti, 2007).

Underpinnings of Antiretroviral ADME Pharmacogenetic Research

Early antiretroviral ADME pharmacogenetic research focused primarily on pharmacokinetic variability (differences in drug exposure) and the correlation with gene variations in the ADME pathway. Interpatient pharmacokinetic variability had not been fully elucidated by factors hypothesized to influence antiretroviral drug exposure (i.e., drug-drug interactions, drug-food interactions, sex, comorbid conditions, pregnancy) (Cressey & Lallemant, 2007). The hypothesis that gene variations could play a role in influencing pharmacokinetic variability was supported by prior drug membrane transporter protein and metabolizing enzyme studies which showed a correlation with single nucleotide polymorphisms (SNPs), transporter expression variability, and metabolizing enzyme variability associated with a poor or rapid metabolizer phenotype (Dahl, Johansson, Berilsson, Ingelman-Sundberg, & Sjoqvist, 1995; de Morais et al., 1994; Cressey & Lallemant, 2007; Hoffmeyer et al., 2000). Reports of high interpatient

non-nucleoside reverse transcriptase inhibitor pharmacokinetic variability and ethnic groups having significantly different antiretroviral drug pharmacokinetics and clinical response further supported research that included a genetic component (Cressey & Lallemant, 2007). The impact of SNPs in both drug transporter proteins and metabolizing enzyme genes have remained the focus of antiretroviral pharmacogenetics (Cressey & Lallemont, 2007). However, this research has evolved into studies aiming to elucidate the relationship between genetic markers, drug efficacy, differences in drug exposure, *and* adverse events and associated treatment discontinuation. The next phase of research will aim to model all of the variables, including genetic markers to enhance individualization strategies aim of to increase efficacy, safety, and tolerability (Rotger et al., 2007).

Metabolizing Enzyme Pharmacogenetics

Efavirenz is converted to inactive metabolites via the CYP 450 enzyme system being primarily metabolized by CYP2B6 enzyme activity (Ward, et al., 2003). The CYP2B6 gene is highly polymorphic and harbors several functional single nucleotide polymorphisms (SNPs) identified in different populations (Klein et al., 2005; Ma, Brazeau, Forrest, & Morse, 2007; Tong, et al., 2006; Wang et al., 2006). Of these functional SNPs, the CYP2B6 c.516G>T (rs3745274) variation is the most extensively studied and associated with differences in drug exposure (Fox, et al., 2006; Ma et al., 2007). A significant decrease in efavirenz clearance and corresponding increase in drug concentration observed in patients with the CYP2B6 rs3745274 TT genotype has been replicated in numerous ethnically diverse studies (Haas et al., 2004, Haas et al., 2005;

Ma et al, 2007; Rotger et al., 2005; Tsuchiya et al., 2004). In one of the largest early studies conducted (N=167), a 3-fold higher efavirenz exposure in patients with the CYP2B6 rs3745274 TT genotype as compared with individuals homozygous for the common GG genotype was observed. There was a significant trend for a rare allele dose dependent increase in plasma levels (p=<0.001) (i.e., GG, GT, TT respectively) (Rotger et al., 2005). In these early studies women were underrepresented and accounted for approximately 18% to 24% of the patients studied (Haas et al., 2004, 2005; Ma et al., 2007; Rotger et al., 2007). The smallest study (n=35) included only one female (Tsuchiya et al., 2004). Differences in sex were not assessed limiting the generalizability of these findings.

Additional CYP2B6 research established that a less frequent variation c.983C>T (rs28399499) was also predictive of increased efavirenz exposure (Wyen et al., 2008). Interestingly, a subsequent study reported that the SNP combination (i.e., diplotype) CYP2B6 rs3745274 TT genotype and CYP2B6 rs28399499 CC genotype was associated with an increase in efavirenz exposure (Ribaudo et al., 2010). The frequency of the CYP2B6 rs3845274 T/rs28399499 C haplotype was observed to be higher in individuals of African ancestry as compared with white individuals (Haas et al., 2009). These findings prompted additional studies that led to the assignment of the slow, intermediate, and extensive metabolizer phenotype, which is identified by the rs3745274/rs28399499 genotype combinations and reported associations with steady-state efavirenz pharmacokinetics. Slow metabolizer denotes 2 variant alleles (i.e., CYP2B6 rs3745274 TT, CYP2B6 rs28399499 CC, CYP2B6 rs3745274 GT with rs28399499

TC). Intermediate metabolizer denotes a single variant allele at either rs3745274 or rs28399499 but not both. Extensive metabolizer denotes no variant allele at either rs3745274 or rs28399499 (Haas et al., 2009; Rotger et al., 2007; Stahle, Moberg, Svensson, & Sonnerborg, 2004; Wang et al., 2006). The representation of women ranged from 24% to 73% which lends to the utility of this phenotype in analyzing study populations that include both men and women (Haas et al., 2009, Rotger et al,. 2007; Stahle et al., 2004; Wang et al., 2006).

A relationship between CYP2B6 and CYP2A6 was recently discovered whereby very high efavirenz drug concentration levels were noted in the presence of impaired CYP2B6 function and CYP2A6 gene variations resulting in reduced enzyme activity (Arab-Alameddine et al., 2009; di Iulio, Fayet et al., 2009). It is theorized that because efavirenz is metabolized into three primary metabolites: 8-hydrox-EFV, 7-hydroxy-EFV, and N-glucuronide-EFV, when CYP2B6 function is impaired, the influence of the main isoenzyme responsible for 7-hydroxylation, CYP2A6, may increase. It is hypothesized that in patients with limited CYP2B6 function, genetic variations in CYP2A6 may contribute to very high and yet unexplained variability in efavirenz drug exposure (di Iulio et al., 2009). Though women only represented 27% of the study participants, sex differences were assessed. Sex was one of several factors (e.g., body weight, age, black ethnicity, height) observed to influence efavirenz clearance. However, multivariate analyses revealed that body weight accounted for the effect of height, age, and sex and was the only factor, besides black ethnicity, shown to influence efavirenz clearance (Arab-Alameddine et al., 2009). These findings are limited by an incomplete

understanding of functional variations in other genes involved in efavirenz metabolism and additional studies are needed to elucidate metabolism accessory pathways (di Iulio et al., 2009).

Though CYP3A4/CYP3A5 has been implicated in efavirenz metabolism, pharmacogenetic research is somewhat limited. In a study that included CYP3A4/CYP3A5 gene variations with CYPB6 gene variations known to be associated with increase efavirenz exposure, two SNPs (CYP3A4 c.-392 A>G [rs2740574] and CYP3A5 c.6986 A>G [rs776746] were associated with plasma efavirenz exposure by univariate analysis. However, under recursive partitioning (a form of multivariate analysis) the degree of influence these SNPs had on efavirenz exposure was noted to be small (Haas et al., 2004). Another study included CYP3A4 rs2740574, CYP3A4 c.671-202C>T (rs4646437) diminished function alleles and CYP3A5 3* (rs776746) ,6* (rs10264272),7* (rs41303343), 10* (rs41279854), 11* (rs72552791) loss of function alleles in the candidate gene panel to assess the influence of gene variation on efavirenz clearance. The CYP3A4 diminished function alleles were significantly associated with a decrease in efavirenz clearance and the influence of the CYP3A5 loss of function alleles on clearance was small but significant. Though only 27% of the study participants were women, it was reported that no differences were observed between men and women in this study (Arab-Alameddine et al., 2009). Additional research is needed to confirm these findings.

Phase II enzymes involved in the ADME pathway are less well characterized for efavirenz. Uridine 5'diphospho-glucuronosyltransferases (UDP glucuronosyltransferases

[UGTs]) are implicated as putative candidate genes for further research (Lubomirov et al., 2007). Though UGT1A1 is implicated in the metabolism of efavirenz by the glucuronidation of efavirenz metabolites, the role of gene variations in UGT1A1 on efavirenz metabolism remains unclear. A recent study assessing kinetic parameters observed limited efavirenz pharmacokinetic UGT1A1 interaction (Ji, Lee, Lim, Kim, & Lee, 2012). Recent reports suggest that UGT2B7 is the main enzyme responsible for mediating the glucuronidation of efavirenz and its three hydroxyl metabolites (i.e., 8-ydroxyefavirenz, 7-hydroxyefavirenz and 8,14-dihydroxyefavirenz) (Bae, Jeong, Lee, & Liu, 2011; Belanger et al., 2009). A recent study assessed plasma and intracellular efavirenz and 8-hydroxyefavirenz in patients which included associations with UGT2B7 gene variations. Efavirenz pharmacokinetics were documented at week 4 and week 16 of therapy. It was observed that in patients with the UGT2B7*2 c.-327G>A AA (rs7662029) genotype that there was no significant effect on plasma efavirenz pharmacokinetics. However, at week 16, carriers of the UGT2B7*2 rs7662029 rare A allele displayed significantly higher plasma 8-hydroxyefavirenz level (P=0.01) and a significantly lower efavirenz metabolic ratio (*p*=0.02) as compared to the GG genotype group. Sex differences were analyzed and though no differences were noted in plasma efavirenz, females showed significantly higher plasma concentrations of 8-hydroxyefavirenz compared with males at both time points in the study. Additionally, efavirenz metabolic ratio was affected by sex at week 16 but not at week 4 (Habetwold et al., 2011). The time-dependent UGT2B7 genotype association is proposed to be related to CYP2B6 autoinduction thereby modulating the rate of 8-hydroxyefavirenz

formation (Habetwold et al., 2011). These novel findings would benefit from additional research in an ethnically diverse patient population as this study only included patients in Ethiopia and the race/ethnicity was not reported.

Drug Membrane Transporter Protein Pharmacogenetics

Because many drugs are known to be transported to the luminal surface via P-glycoprotein, numerous antiretroviral pharmacogenetic studies have focused attention on the adenosine triphosphate (ATP)-binding cassette, subfamily B (ABCB1), also known as the multidrug transporter 1 gene, the product of which is P-glycoprotein. However, only one study which included efavirenz, in addition to other compounds, observed an association between antiretroviral drug concentration and the gene variation ABCB1 c.3435 C>T (rs1045642). It was reported that an association between the ABCB1 rs1045642 TT genotype and lower efavirenz drug concentration was seen as compared with the CT and CC genotypes (Fellay et al., 2002). However, these findings have remained controversial because subsequent studies produced contrasting results reporting no relationship between ABCB1 rs1045642 and response to therapy (Cressey & Lallemont, 2007; Haas et al., 2004, 2005; Tsuchiya et al., 2004; Winzer et al., 2005).

Other known drug transporter genes, the products of which are hypothesized to play a role in the transportation of various antiretrovirals have been studied. ABCC1, also known as multidrug resistant protein 1, and ABCC2, also known as multidrug resistant protein 2, were included in several efavirenz studies. However, no relationship between ABCB1 and ABCC2 expression and efavirenz drug levels were observed (Fellay et al., 2002).

Though efavirenz is 99% protein bound and mainly binds to albumin (a blood transport protein), one study group suggested that efavirenz also binds to the plasma carrier orosomucoid, a small acute-phase glycoprotein and known carrier of other antiretroviral drugs (Lubomirov et al., 2007). However, this observation has not been replicated and pharmacogenetic research conducted to date has shown no orosomucoid gene variation influence on efavirenz pharmacokinetics (Colombo et al., 2006). Though albumin gene research has identified gene variations that alter albumin concentration and function, research determining albumin gene variation and binding affinity of efavirenz has not yet been undertaken (Madison et al., 1991; Minchiotti, Galiano, Kragh-Hansen, & Peters, 2008).

Haplotype Analyses

SNP haplotype analyses[9] have expanded the exploration of ADME gene variations. Haplotype structure has been investigated in several cohorts to identify associations with efavirenz plasma concentrations. In a Chilean cohort, linkage disequilibrium (LD) analysis of 13 CYP2B6 polymorphisms showed a significant degree of LD. Pairwise tagging SNP[10] analysis identified 3 SNPs CYP2B6 (i.e., c.172-468T>G [rs10403955], c.823-197T>C [rs2279345], and c.1294+53C>T [rs8192719]) that were representative of the 11 SNPs associated with plasma efavirenz concentrations. The

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[9] SNP haplotypes are a combination of SNPs that are statistically associated. Strong correlations between SNPs in a region provide enough information to predict much of the information about the remainder of the common SNPs in that region (Crawford & Nickerson, 2005).

[10] A tag SNP is an informative marker that acts as a proxy for other SNPs with which they are highly correlated. In pairwise analysis a linkage disequilibrium threshold is set to determine the best SNP correlation (De Bakker, Graham, Altshuler, Henderson, & Haiman, 2006).

number of alleles associated with high efavirenz concentration were analyzed. Carrying four to six risk alleles was associated with significantly higher median efavirenz plasma concentrations, which was greater than the minimal toxic concentration of 4μg/ml (Carr, la Port, Primohamed, Owen, & Cortes, 2010). Haplotype analysis of the three SNPs showed that this haplotype was more frequent in Chilean patients (0.34) than in a Caucasian population (0.27) and in a West African (Yoruba) population (0.04) (Carr et al., 2010). Of note, one of the three SNPs (CYP2B6 c.172-468T>G [rs10403955] tagged CYP2B6 c.516G>T 9 (rs37452740) which has a well established association with high efavirenz plasma concentrations. This may in part explain the contribution of rs10403955 to the 3 SNP haplotype. However, it is less clear what contribution the other two SNPs have within this haplotype. It is possible that their contribution is indirect by tagging other functional variants within the CYP2B6 gene (Carr et al., 2010). The number of men and women were not identified in this study so no meaningful sex-related conclusions can be drawn.

In another cohort, LD analysis of 13 CYP2B6 polymorphisms associated with efavirenz plasma concentrations that exceeded the therapeutic range showed a significant degree of correlation. Further analysis identified 3 SNPs (i.e., CYP2B6 c.516G>T [rs3745274], CYP2B6 c.785A>G [rs2279343], CYP2B6 g.21563C>T [rs8192719]) that were representative of the 13 SNPs. This haplotype was significantly associated with higher efavirenz plasma concentrations (p=0.0179) (Sukasem et al., 2012). Again, the association of CYP2B6 rs3745274 and high efavirenz plasma concentrations is observed and the impact of the other two SNPs is less clear. Though women were

highly represented in this study (>50% of the study population), the study size was small and sex-related differences were not reported.

In a recent study, haplotype analyses of genes encoding for proposed efavirenz metabolizing enzymes were performed (i.e., CYP2A6, CYP2B6, CYP2D6, and CYP3A5). Increased plasma concentrations of efavirenz were present in individuals with the 2 loss of function alleles in CYP2B6 *6/*6 (i.e., c.516G>T [rs3745274] and c.785A>G [rs2279343]) diplotype, or *6/*18 (i.e., c.516G>T [rs3745274] and c.983T>C [rs28399499]) diplotype compared with the CYP2B6 *1/*1 reference diplotype (62% increase [95% CI, 44.0-80.1). Though the study was small (N=54) and only included 15 women (28%) sex differences were assessed but were not found to be statistically significant (Heil et al., 2012). However, the sample size may have been insufficient to identify sex differences.

Though these studies indicate that a genetic model that includes multiple CYP2B6 SNPs (haplotype) are associated with high efavirenz plasma concentrations, these observations warrant further research (Carr et al., 2010). Haplotype structure and allele frequencies require characterization in larger ethnically diverse studies in addition to confirming reduced dose safety by haplotype (Carr et al., 2010; Gatanaga et al., 2007; Sukasem et al., 2012).

Multi-Gene Interaction Studies

Thus far, the single gene single locus CYP2B6 rs3745274 has been consistently predictive of higher efavirenz plasma concentrations. However, a study undertaken to determine the predictability of multiple genes and efavirenz exposure and toxicity

failure (defined as any severe or life threatening toxic side effect not managed by dose reduction, discontinuation or in-class substitution) present interesting findings that suggest efavirenz treatment response is a complex phenotype influenced by multiple genes (Motsinger et al., 2006). In this study, multifactor dimensionality reduction[11] was used to identify potential interactions. In white patients, efavirenz exposure was best predicted by a gene-gene interaction between CYP2B6 c.516 G>T (rs3745274) and ABCB1 c.2677 T>G (rs2032582). Higher efavirenz plasma concentrations were associated with this model to 82% accuracy (p<0.001) as opposed to the single locus model involving CYP2B6 rs3745274 with 73% accuracy (p=0.001) (Motsinger et al., 2006). Patients with the CYP2B6 rs3745274 TT genotypes demonstrated no drug exposure dependence on any of the ABCB1 rs2032582 genotypes however white patients with the CYP2B6 rs3745274 GT genotype demonstrated a significant variation of drug exposure value with the ABCB1 rs2032582 genotypes (Motsinger et al., 2006). The single CYP2B6 rs3745274 TT model was the best model among blacks (69% accuracy; p=0.001). Additional research is needed to confirm these findings and examine multi-gene interaction and it's predictability. Though there was a cross representation of race/ethnicities in this study, women were underrepresented (17%) and differences in sex were not assessed (Motsinger et al., 2006).

[11] Multifactor dimensionality reduction is a nonparametric strategy to detect gene-gene and gene-environment interactions in categorical, dichotomous, and independent variables. It performs an exhaustive search of all possible combinations creating optimal sets of models which can be reduced to a best/final model (Ritchie & Motsinger, 2005).

Drug Toxicity (Adverse Events) Pharmacogenetics

Building on the efavirenz ADME pharmacogenetic study findings, a growing interest in the genetic determinants of adverse events has developed. The research goal being to determine how pharmacogenetic-dependent variations in drug exposure translate into adverse events and whether or not there are idiosyncratic adverse events not drug exposure-dependent that can perhaps be identified by gene variations.

The impact of the gene variation CYP2B6 c.516G>T (rs3745274) on efavirenz exposure and association with adverse events is the most widely evaluated. The most common adverse event assessed: central nervous system (CNS) disorders (i.e., dizziness, insomnia, impaired concentration, somnolence, abnormal dreams, and hallucinations) (King & Alberg, 2008). A 2004 study showed that the CYP2B6 rs3745274 TT genotype was associated with adverse CNS symptoms at 1 week but not significantly at week 24. Prior findings were reconfirmed whereby efavirenz drug exposure was observed to be approximately three-fold higher in CYP2B6 rs3745274 TT genotypes as compared to GG genotypes. Drug exposure in patients with the GT genotype was intermediate. The TT genotype was found to be more common in African-Americans (20%) than European-Americans (3%). Eighteen percent of the patients in this study were female (Haas et al., 2004) which vastly underrepresents women and renders these findings poorly generalizable to the female patient population. A subsequent study showed that in patients with persistent CNS disorders (with a median duration of efavirenz treatment of 21 months), the presence of the CYP2B6 rs3745274 TT genotype was two to three times more frequent. Twenty-four percent of the patients in this

study were women (Rotger et al., 2005) which again underscores the challenge of translating these data in a meaningful way to female patients. A recent study in 2010 observed that the previously discussed SNP combination associated with high efavirenz exposure CYP2B6 rs3745274 T and CYP2B6 c.983T (rs28399499) genotype, showed an association with an increase in CNS event reporting in white patients treated with efavirenz but not black patients (Ribaudo et al., 2010). Only 19% of the patients in this study were female and sex difference were not examined. Of note, all studies to date have consistently found the CYP2B6 rs3745274 TT genotype to be associated with higher efavirenz exposure and now CNS-associated adverse events (King & Alberg, 2008).

Though efavirenz drug transporter research has yielded contradictory findings, the multi-gene interaction study previously discussed included an adverse event component. Adverse events were captured as toxicity related failures defined as any severe or life-threatening toxic side effect that could not be managed by dose reductions, temporary drug discontinuation, or within-class substitutions. A SNP combination ABCB1 c.2677 G>T (2032582) and ABCB1 c.3435 C>T (rs1045642) was found to be predictive of toxicity failure in patients treated with efavirenz (71% accuracy; $p<0.001$) (Motsinger et al., 2006).

These studies offer an important initial contribution and additional studies should be performed to explore adverse events that have not yet been thoroughly examined.

Pharmacogenetic Markers in Drug Discontinuation and Dose Reduction.

Treatment discontinuation and dose reduction have become areas of pharmacogenetic research interest. A 2011 study evaluating the association of gene variations with treatment discontinuation included a cohort of patients taking efavirenz. It was observed that a higher risk of discontinuation (71%) was associated with patients in whom the following risk alleles were present: CYP2B6 rs3745274 T, CYP2B6 rs35303484 G, CYP2B6 rs35979566 A, CYP2B6 rs28399499 C, CYP2A6 rs28399433 G, and CYP3A4 rs4646437 T. Though the number of women in the study were not reported, sex showed an independent statistically significant effect on efavirenz discontinuation with women showing a higher risk (Hazard Ratio = 3.08, 95% CI: 1.62-5.85, P= 0.001) (Lubomirov et al., 2011).

Determining the feasibility of pharmacogenetic-guided dose reduction as it relates to high efavirenz exposure and adverse events has now been undertaken. In a 2007 study, dose reduction was instituted in patients with very high drug exposure noted to be 6*/*6 carriers (CYP2B6 c.415 A>G [rs12721655] AA, c.499 C>G [rs3826711] CC, c. 516 G>T [rs3745274] TT, c. 777 C>A [reference sequence identifier not available] CC, c.785 A>G [rs2279343] GG, c.983 T>C [rs28399499] TT, c.1375 A>G [rs number not available] AA, c.1459 C>T [rs3211371] CC), and 6*/26* carriers which has the same genotype pattern with the exception of c.499 C>G (rs3826711) being CG. With the dose reduction there was a significant reduction in CNS-related symptoms and in most patients the efavirenz concentration decreased proportionally with the dose-reduction ratio. The resultant lower efavirenz dose plasma concentrations in all the patients that

were CYP2B6 6*/6* and 6*/26* carriers were shown to be therapeutic (i.e., concentrations maintained above the minimum target concentration of 1000 ng/ml) (Gatanaga et al., 2007). These findings support the possibility of pharmacogenetic-guided dose reduction in relieving CNS-related symptoms. However, additional research is warranted given the small size of the study and lack of blinding in the trial.

Ethnicity and Ancestry Markers

Though early antiretroviral pharmacogenetic research did not represent racial/ethnic groups well, subsequent research has included ethnically diverse patient populations. This has contributed to a better understanding of gene variation frequency in different racial/ethnic populations and the possibility of identifying different genetic risk factors for variability in pharmacokinetics and pharmacodynamics. However, these studies used self-report to differentiate racial/ethnic groups, which does not take into account an individual's ancestral proportions (Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008). A more advanced method of differentiating ancestry is the use of genetic markers. Studies conducted to identify panels of ancestry informative markers (AIMs), have yielded markers that provide reliable ancestry estimates. AIMs now provide a mechanism whereby gene variation frequencies between populations from different geographical regions can be identified (Halder, et al., 2008). AIMs are now used in research to estimate ancestry and used as a tool to minimize bias due to population stratification that could not be gleaned from self-report questionnaires (Halder et al., 2008). AIMS should be included in most population-based genetic studies since differences in population genetic structure can confound study findings (Kosoy,

Nassir, Tian, White, Butler et al, 2009). This approach is critical for both admixture mapping and adjusting for population genetic structure in association testing.

Conclusion

Patients treated with antiretroviral drugs require life long therapy and face the challenge of adverse events that affect their quality of life and willingness to adhere to therapy. Genetic factors appear to contribute to interpatient drug variability and may have predictive value in determining which patients will be susceptible to sub-optimal drug exposure and adverse events. Several metabolism and drug transporter genetic variants have been identified which influence drug pharmacokinetics. However, despite the many advances in antiretroviral pharmacogenetic studies, the clinical application of this research is in its infancy. This is primarily due to very few clearly established relationships between genotype and patient outcomes (Ma et al., 2007). It is predicted that research will eventually lead to the development of complex models that incorporate the variables that most strongly contribute to patient outcomes.

Pharmacogenetic research has only begun to explore the inclusion of other factors that may influence drug exposure in conjunction with gene variations (i.e., drug co-administration, age, sex, alcohol use, liver function, kidney function, body weight and body mass index). Though current research has lessened this gap, additional studies are needed which incorporate a multifactorial approach (that includes genotypic data). Missing from most research in general is the incorporation real-world factors into the research model (i.e., diet, non-prescription drug use, and recreational drug use).

Antiretroviral pharmacogenetics may provide a component of treatment individualization alongside race/ethnicity and sex (Ma et al., 2007). Though great strides have been made in determining differences based on race/ethnicity, the sex disparity highlighted throughout this review warrants additional research focus. Historically, women have been under-represented in antiretroviral research. Several studies suggest pharmacokinetic and response differences between men and women which have prompted more detailed investigations in women to determine if dosing adjustment by sex is warranted (Ma et al., 2007).

Pharmacogenetic-guided drug dosing is used in other therapeutic areas (e.g., to assess common genetic variants in patients such as CYP2C9 to guide anticoagulation therapy and CYP2D6/CYP2C19 to guide psychiatric drug therapy). Arrival at this juncture was preceded by studies that carefully elucidated the pharmacogenetic component of drug response variability. It is anticipated that with additional research, pharmacogenetics will contribute to a predictive tool that will individualize antiretroviral therapy with the goal of optimal safety and efficacy.

Only recently have researchers begun to examine other factors that may influence drug exposure in conjunction with gene variations such as drug co-administration, age, sex, alcohol use, body weight, and body mass index (BMI). Though this research gap is now closing, research is needed to develop algorithms which incorporate a multifactorial approach to estimate the optimal drug and dose in antiretroviral therapy.

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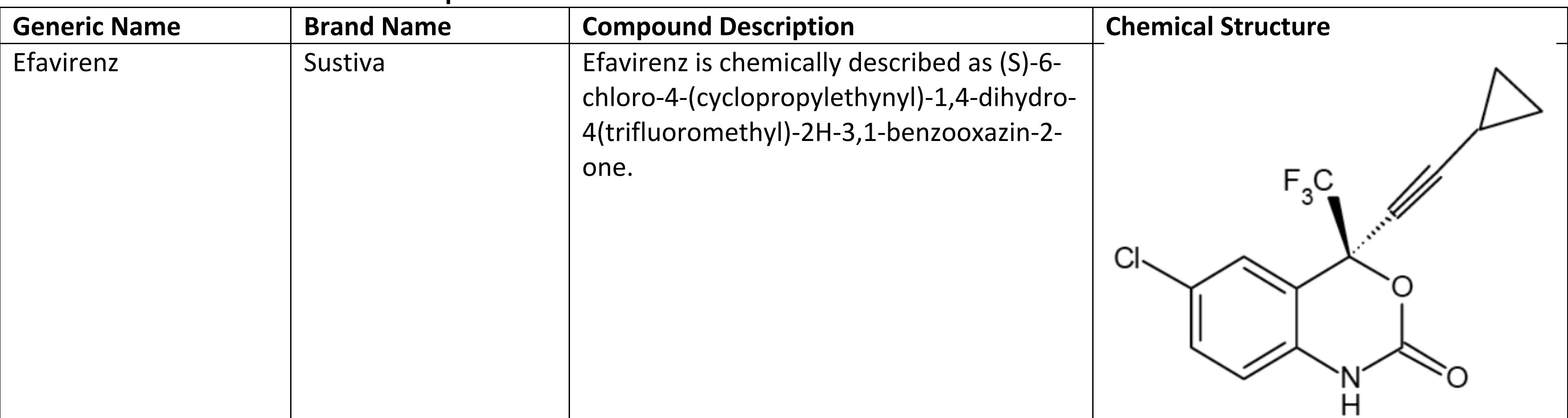
Tables and Figures

Table 2-1. Efavirenz Chemical Description and Structures

Generic Name	Brand Name	Compound Description	Chemical Structure
Efavirenz	Sustiva	Efavirenz is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4(trifluoromethyl)-2H-3,1-benzooxazin-2-one.	

(DHHS, 2010)

Figure 2-1. Efavirenz Metabolism

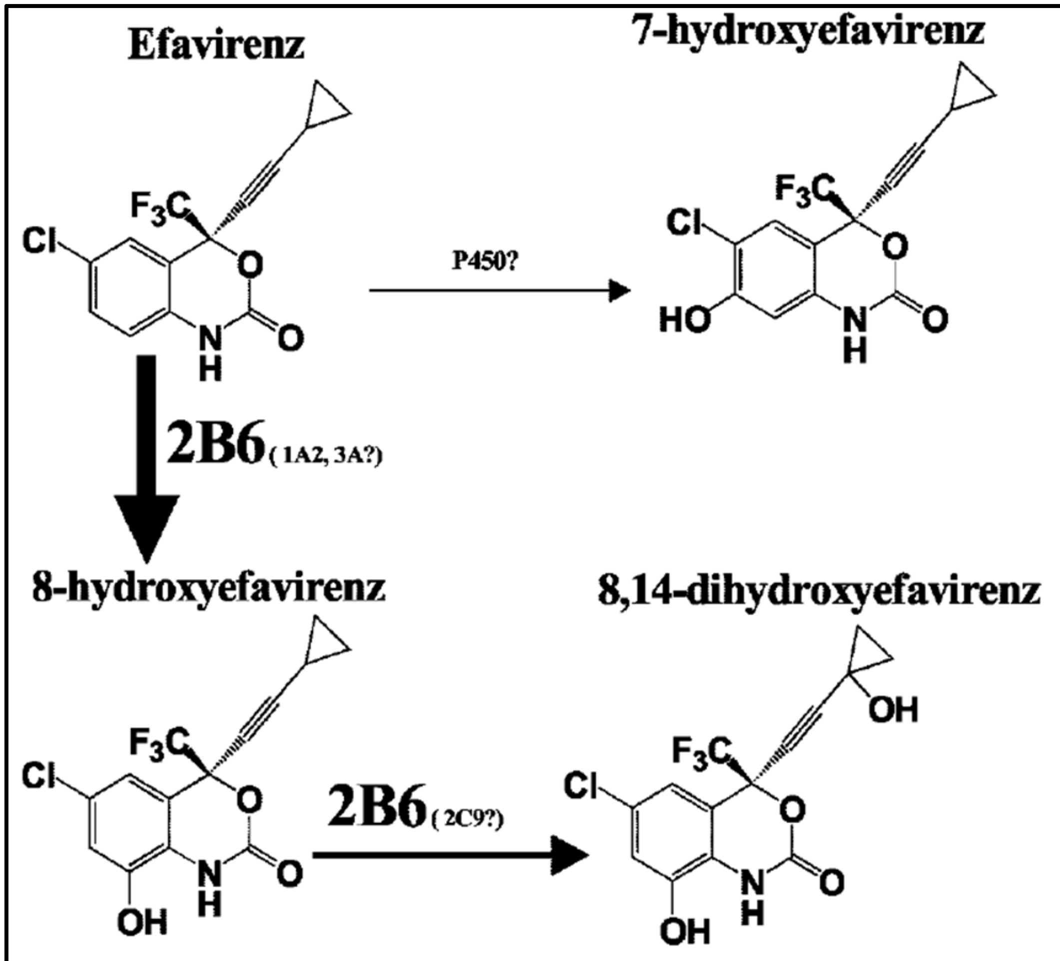


Figure from Ward et al., (2003). Efavirenz is metabolized to the inactive primary metabolites 8-hydroxyefavirenz, 7-hydroxyevafirenz, and 8,14 dihydroxyefavirenz via the cytochrome P450 2B6 (CYP2B6) pathway. Though other CYP450 enzymes are implicated in the metabolism of efavirenz, the main metabolic pathway is 8-hydroxylation, mediated primarily by CYP2B6 (di Iulio et al., 2009; Heil et al., 2012).

Chapter 3

Pharmacogenetic Study Methodology

Introduction

The impact of pharmacogenetics in antiretroviral therapy is now widely recognized. Beyond early examples of differential drug metabolism and transport, research has shifted toward patients' response to antiretroviral therapy: including its impact on survival, adverse event and side effect profiles. Antiretroviral therapy is considered especially suitable for pharmacogenetic research as both exposure and treatment response can be quantified and certain adverse effects can be assessed with validated measures (Ma, Brazeau, Forrest, & Morse, 2007).

This pharmacogenetic study was undertaken to understand the role of genetic variation in antiretroviral drug exposure variability and adverse events. The overall goal was to identify genetic variations linked to adverse events that may influence successful use of antiretroviral medication in the treatment of patients infected with the Human Immunodeficiency Virus (HIV). The Women's Interagency HIV Study (WIHS) provided a unique collaborative multidisciplinary opportunity to pursue pharmacogenetic research. The WIHS Intensive Pharmacokinetic Substudy provide the foundation for the current pharmacogenetics study. This chapter will summarize the methods employed in this research project.

Description of the Research Setting

The Women's Interagency HIV Study

The WIHS, established in 1993, is a prospective cohort study of women with or at risk for HIV designed to investigate factors associated with HIV disease progression. The women recruited for this study are followed at six regional sites in the United States

(i.e., San Francisco, Los Angeles, Chicago, Washington DC, Bronx, Brooklyn) with a data-coordinating center in Baltimore, Maryland. A complete description of the WHIS can be found in the WIHS Manual of Operations available on request (Johns Hopkins Bloomberg School of Public Health [JHSPH], 2013).

In brief, 2,625 women (2,056 HIV positive and 569 HIV negative) were enrolled in 1994 and 1995. Included in this cohort were women previously diagnosed with clinical AIDS or women with low CD4+ counts. An additional 1,143 women (254 HIV positive antiretroviral naïve; 484 HIV positive antiretroviral treated; 406 HIV negative) were enrolled in 2001 and 2002. Follow-up appointments are conducted biannually and include interview administered questionnaires on sociodemographics, medical, alcohol, tobacco, and other drug use. An extensive medication inventory is conducted utilizing visual aids that are updated regularly to include newly available therapies. Physical examinations are performed as well as fat maldistribution exams (i.e., body measures, bioelectric impedance analysis). Blood is collected for analysis to assess virologic, immunologic, fasting fat maldistribution markers, and liver/renal function. Outcomes (i.e., serioconversion, AIDS diagnoses, malignancies, mortality, tuberculosis, cardiovascular diagnoses, liver biopsies, hysterectomies) are assessed at each study visit. Data collection methods include self-report, medical record abstraction, established disease registry searches (cancer, TB), and cross-referencing the National Death Index (JHSPH, 2013).

The WIHS research agenda includes substudies to assess measures of adherence, pharmacokinetics, adverse events, drug response variability, and genetic influences.

Women's Interagency HIV Study Intensive Pharmacokinetic Substudy

Enrollment for the Intensive Pharmacokinetic Substudy was initiated in April 2003. All participants in the WIHS study were offered enrollment (Gandhi et al., 2009). From five of the six WIHS clinical sites (i.e., San Francisco, Chicago, Washington D, Bronx, and Brooklyn), 502 participants taking one or more of the Intensive Pharmacokinetic Substudy target antiretroviral medications, Table 3-1, were enrolled over two years (Gandhi et al., 2009). Five antiretroviral drugs were included in the study however, only the Intensive Pharmacokinetic Substudy data for the non-nucleoside reverse transcriptase inhibitor, efavirenz, will be examined as this drug is the focus of the current pharmacogenetics study and the only Intensive Pharmacokinetic Substudy data published to date.

The aims of the Intensive Pharmacokinetic Substudy were:

1. Assess exposure to antiretroviral treatment among HIV-positive women including indices of adherence, clearance involving the processes of drug metabolism and elimination and bioavailability;
2. Identify the factors that significantly influence variation in bioavailability and clearance of antiretroviral drugs among WIHS women, including use of other drugs and medications, ethnicity, body mass, liver and renal function, diet, symptoms (e.g., diarrhea, fever), smoking, and concurrent infections such as Hepatitis C.

The only eligibility criteria for participation were use of a target antiretroviral for at least 6 months prior to the study and patient informed consent. The parameter used

to define drug exposure was area-under-the-plasma concentration-time curve (AUC) divided by target antiretroviral dose witnessed at the initiation of pharmacokinetic sampling (AUC/dose) (Gandhi et al., 2009). This study was considered observational with each participant bringing in her usual dose of the target antiretroviral drug for witnessed consumption during the study.

Intensive Pharmacokinetic Substudy Methods

The Intensive Pharmacokinetic Substudy protocol summary, questionnaires, and procedures are outlined in Appendix A. The data collection forms for this study may be accessed via the WIHS website: https://statepiaps.jhsph.edu/wihs/index-forms.htm. The following sections focus on the key components of the Intensive Pharmacokinetic Substudy that form the foundation for data collected and utilized in the present pharmacogenetic project study analyses. During the Intensive Pharmacokinetic Substudy, every effort was made to replicate each study participant's usual environmental conditions (i.e., cigarette smoking, consuming their typical diet, regular medication regime) to facilitate the "real-world" factors hypothesized to contribute to drug exposure and efficacy (Gandhi et al., 2009).

Study Day Procedures. Each study participant on their designated study day was administered a series of questionnaires which included: current antiretroviral regimen, adherence, concomitant medications, recent or current symptoms and illness, current menstrual, contraceptive and obstetric events, substance use patterns, and diet. Weights and urine pregnancy tests were also performed. Placement of an intravenous saline lock catheter was done to facilitate blood draws. The first time point of

pharmacokinetic sampling (time point '0') was drawn prior to the ingestion of the antiretroviral drug under study. The participants brought and took their own antiretroviral medications and concomitant medications and were observed when the antiretroviral was ingested and the time point was documented (Gandhi et al., 2009).

Timed Blood Draws. Timers were set to correspond with the appropriate blood draws and the times were recorded with each sample. Efavirenz is typically dosed at 600 mg once daily therefore blood draws were performed over a 24-hour period (e.g., 0, 0.5, 1, 2, 2.5, 3, 4, 8, 12, 15, 18, and 24 hours) after a dose was witnessed (Gandhi et al., 2009).

Antiretroviral Blood Specimen Documentation. Specimen forms corresponding to efavirenz were used to catalog the date and time each blood sample was drawn along with the patient weight and verification of a pregnancy test and/or verification of pregnancy status. If a pregnancy test was needed, it was performed in a local lab. Patient weight was used to calculate body mass index (BMI) and lean body mass as this method is typically used to predict drug dosages (Green & Duffull, 2004). All blood samples were processed, batched, and then shipped to a laboratory specializing in pharmacokinetic measurement (Gandhi et al., 2009).

Antiretroviral Drug Exposure. The outcome variable for the Intensive Pharmacokinetic Substudy was total drug exposure. Procedures for measuring antiretroviral blood levels have been previously described in the literature (Egge-Jacbosen, Unger, Niemann, 2004; Gandhi et al., 2009). Blood samples were kept on ice until they were processed for storage at -80°C prior to analysis. Plasma samples (0.1

ml) were prepared for injection by adding A-86093 (Abbott Laboratories, Abbott Park, IL) as an internal standard, adding acentonitrile (0.4 ml) to precipitate the protein, mixing, centrifuging, transferring the supernatant to an autosampler vial, and diluting as necessary. Plasma was analyzed for antiretrovirals by standard techniques of liquid chromatography/tandem mass spectrometry. Efavirenz was analyzed with a base-deactivated saline Hypersil C18 (4.6 X 50 mm, 5 µm) analytical column and a 3 mm X 2mm octadeclysilane guard column (Thermo Eectron Corp, Waltham, MA). Efavirenz data analysis was performed with MassLynx 3.5 software (Micromass Manchester, UK) (Gandhi et al., 2009).

AUC for study participants taking doses disparate from the standard efavirenz unit dose was calculated using the trapezoidal rule[12] and exposure metrics were calculated using traditional equations programmed in Stata/SE version 9.2 (Gandhi et al., 2009).

Efavirenz data analyses were performed with MassLynx 3.5 software (Micromass Manchester, UK). The absolute recovery of efavirenz from plasma 99.8%. Intraday and interday precision for efavirenz was <11.7% and accuracies ranged from -6.0% to 14.8% (Egge-Jacobsen et al., 2004; Gandhi et al., 2009).

Dietary Assessment. Dietary information was collected via telephone interview and documented prior to the Intensive Pharmacokinetic Substudy. This was done to

[12] The trapezoidal rule is a method used to approximate the area under a curve. The area is first divided into equal intervals. Then, the area under the curve is approximated by assuming a linear drug concentration between each sampling point. The sum of these approximations gives the final numerical result of the area under the curve (Huang & Pang, 2012).

prepare for the meals consumed for the time period the patient was in the study to maintain dietary consistency. The data captured with this form included dietary patterns of both meals and snacks, allergies, coffee, tea, and herbal infusions, consumption of orange juice or grapefruit juice, meal and snack frequency, fast food consumption, and alcohol intake (Gandhi et al., 2009).

Fat Intake. The percentage of fat in the usual diet in the preceding 30 days was ascertained using a validated 17-item Block dietary questionnaire (Block, Hartman, & Naughton, 1990). Percentage fat calculations were performed via the on-line Berkeley Nutrition Services fat screener tool and were categorized as <30%, 30%-35%, 36-40%, or >40% usual fat intake (Block et al., 1990, Gandhi et al., 2008).

Antiretroviral Adherence. The degree of adherence to efavirenz was assessed. Doses missed the day preceding the study, 2 days before that, 3 days before, and 2 weeks before the study date were documented. Using visual cues (response cards), participants were queried as to how much drug they had taken in the past month measured in percentage and the last time a dose was missed, measured in various time increments. To determine the period of time patients were compliant with prescribed antiretroviral therapy (Gandhi et al., 2009).

Smoking, Alcohol, and Recreational Drug Use. The frequency and amount of smoking and alcohol consumption and recent substance abuse was collected. Information on substances included: cocaine, crack, heroin, methadone, methamphetamines, other (amphetamines, narcotics, hallucinogens), and marijuana was captured relative to frequency (Gandhi et al., 2009).

Additional Data. Patient weight was documented and used to calculate BMI, lean body (a method is typically employed to predict drug dosages), ideal body weight, adjusted body weight, and predicted normal weight using standard equations (Gandhi et al., 2009; Green & Duffull, 2004). WIHS core data was accessed to include measures of height, fat free mass, as measured by bioelectrical impedance analysis, renal function (Cockcroft-Gault and Modification of Diet in Renal Disease [MDRD] creatinine clearance calculations), hepatic function (aspartate transaminase [AST], alanine transaminase [ALT], and gamma-glutamyl transferase [GGT]) and other laboratory values over time. Recent illness, obstetrics and gynecology (OB-GYN) information, menstrual history, and hepatitis B and C coinfection status were accessed along with concurrent medications information to be analyzed as a covariate (i.e., concomitant metabolism pathway (cytochrome [CYP] P450) competing medications) (Gandhi et al., 2009).

Intensive Pharmacokinetic Substudy Data Analyses and Findings

Data collected during the Intensive Pharmacokinetic Substudy and WIHS core data were used to model the factors that influence efavirenz drug exposure. Virologic response and side effects were also assessed in relation to drug exposure (Gandhi et al., 2009).

The dose-adjusted parameter used to define drug exposure, area-under-the concentration-time curve (AUC) divided by dose (AUC/dose), was log transformed to reduce skewness of the data (Gandhi et al., 2009). Since pharmacokinetic measures are typically skewed, the accepted statistical distribution for AUC is a log-normal distribution which is derived by log transforming the data (Senn, 2008). The log-

transformed data was then analyzed in relation to the continuous and categorical variables delineated in Tables 3-2 and 3-3 as categorized by the Intensive Pharmacokinetic Substudy group (Gandhi et al., 2009).

To model the relationship between the log-transformed AUC data and the variables of interest, linear regressions by univariate analysis were performed. Manual forward stepwise selection was used to construct multivariable models by adding variables in increasing order of p-value obtained during univariate analysis. Only p-values less than 0.10 were incorporated into the model. To avoid excessive deletion of observations with missing values on unselected predictors, candidate models were run separately. Missing data points were interpolated by straight line fits using data points before and after the missing data point. Obvious collinear covariates were not included in the same models. All multivariate models included ideal body weight, race, and age as variables. All statistical analyses were performed using the Stata/SE 9.2 statistical package (Gandhi et al., 2009).

The Intensive Pharmacokinetic Substudy findings were published by Gandhi et al in the Journal of Acquired Immune Deficiency Syndrome, April 2009. Increased efavirenz exposure was associated with hepatic transaminase levels, albumin levels, and orange juice consumption whereas being amenorrhea $\geq$12 months, concomitant use of tenofovir, Ideal body weight, and African American Race decreased efavirenz exposure. (Gandhi et al., 2009).

Pharmacogenetics Study Project

The pharmacogenetic study project employs a cross-sectional design using data from the Intensive Pharmacokinetic Substudy, the WIHS database, and stored DNA for genetic analysis. The project involves a two-tiered analysis plan that examines gene variations in selected candidate genes to examine the role of gene variation on antiretroviral drug exposure and adverse event response to antiretroviral therapy. The analysis objectives are:

1. To model the genetic and previously characterized Intensive Pharmacokinetic Substudy factors to efavirenz exposure.
2. To describe the prevalence by genotype of adverse events, signs and symptoms in a subgroup of Intensive Pharmacokinetic Substudy women taking efavirenz

Candidate Gene Selection and Common Disease-Common Variant Theory in Pharmacogenetics

A candidate gene approach focusing on genes participating in the ADME pathway of antiretroviral drugs was selected to determine the association between gene variants, antiretroviral drug exposure, and adverse event response. Pharmacologic outcomes are traits that are complex and though much remains to be discovered as to the genetic contribution to pharmacologic variability and outcomes, appropriate candidate genes can be identified based on what is known (i.e., pathophysiology of the underlying disease, drug ADME properties, physiologic pathways that correspond with

side effects). The 'common disease-common variant' theory was applied in the selection of genetic variants for examination in relation to pharmacokinetic measures.

The 'common disease-common variant' theory first proposed in 2001 asserts that common, complex diseases or traits are caused by one or several common susceptibility alleles (variations) at each disease susceptibility locus (Dorris, 2002; Peng & Kimmel, 2006; Pritchard & Cox, 2002; Reich & Lander, 2001; Smith & Lusis, 2002). Common variants are defined as having a minor allele frequency of 0.05 or greater. It is assumed that in the genome there are genes that harbor variations that could contribute to susceptibility for a disease. At each gene locus there is the possibility for allelic variation. Predictions follow a simple model with the two equivalence classes of alleles: 'normal' (N) and 'susceptibility' (S) whereby S alleles lead to increased disease risk or trait variance (Pritchard & Cox, 2002). Two relevant measures are: total frequency of S alleles and the degree of allelic identity within the S class (Pritchard & Cox, 2002). These measures depend on evolutionary mechanisms (i.e., mutation, selection, and random drift). Mutation rate of disease alleles is an important parameter in determining allelic architecture (Pritchard & Cox, 2002). With the expansion of the human population, mutation rates from N to S alleles (and a lower reverse mutation rate) and impact on allele frequency distribution can be postulated and estimated (Pritchard & Cox, 2002). It should follow that the allelic spectrum at all loci would gradually increase over time (Smith & Lusis, 2002). However, because the effective numbers of alleles of common diseases increase more slowly than rare diseases, even with population expansion common diseases have a more simple spectra and common

alleles remain important markers of disease (Peng & Kimmel, 2006; Smith & Lusis, 2002). Though the 'common disease-common variant' theory does not provide the complete answer to the nature of the genetic architecture of human populations (Pritchard & Cox, 2002), it provides a framework from which to consider marker selection in genetic association studies.

An objective of pharmacogenetic research is to identify the genetic contribution to an individual's drug response. Applying the 'common disease, common variant' theory to pharmacogenetic research assumes that gene variations underlying a trait of interest existed within the founding population. Susceptibility alleles may have been (and continue to be) present perhaps because of selective neutrality (Dorris, 2002) and the frequency distribution adequately representative. Though there are a few examples whereby a single gene exerts a dominant effect on treatment efficacy requiring patient identification prior to treatment initiation, less is known about the genetic basis of drug response variability as it relates to adverse events (Singer, Grossman, Avidan, Beckmann, & Pe'er, 2005). The observable expression (phenotype) is generally classified as multifactorial resulting from the interaction of a number of different genetic and environmental factors (Singer et al., 2005). Until data is better understood to improve the chances of matching the causal alleles frequency, marker selection is restricted to a modest but growing set of validated gene variations (Singer et al., 2005).

The candidate gene approach is in essence a focused strategy that investigates the validity of an educated guess. The components of the candidate gene selection process are: pathway selection, reviewing the body of evidence built from the literature,

and accessing public databases of common gene variations (Marion & Belmont, 2011). The strength of this study lies in the selection of candidate genes known to influence antiretroviral ADME.

Candidate Gene and Candidate SNP Selection

Assessing candidate genes for this pharmacogenetic study involved the review of antiretroviral ADME pathways, ADME proteins, their encoding genes, and gene variations (i.e., single nucleotide polymorphisms [SNPs]) reported in the literature and cataloged in public databases. Nine genes implicated in efavirenz ADME were selected.

Cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6). CYP2B6 is a member of the multigene cytochrome P450 (CYP450) family of hemethiolate monooxygenases that catalyze the oxidative biotransformation of numerous drugs. Localized primarily to hepatic endoplasmic reticulum, it is also found to a lesser degree in extrahepatic tissues including intestine, kidney, and lung. CYP2B6 is known to metabolize the non-nucleoside reverse transcriptase inhibitor, efavirenz (Lang et al., 2004). The CYP2B6 gene is highly polymorphic and several functional SNPs occur in different populations. Several genetic association studies demonstrated decreased protein expression resulting in high plasma drug concentration making this an ideal candidate gene for further study (Haas et al., 2005; Rotger et al., 2007; Wyen et al., 2008).

Cytochrome P450 family 2, subfamily C, polypeptide 19 (CYP2C19). CYP2C19 is also a member of the CYP450 family. This protein is localized to the hepatic endoplasmic reticulum metabolizes a wide variety of drugs. CYP2C19 is an important

factor in the metabolism of some non-nucleoside reverse transcriptase inhibitors. However, genetic association studies are limited to a single study showing an association with a CYP2C19 polymorphism in patients taking efavirenz (Haas et al., 2005). There is a need to replicate these findings.

Cytochrome P450 family, 2 subfamily D, polypeptide 6 (CYP2D6). The CYP2D6 protein localizes to the endoplasmic reticulum and is known to metabolize approximately 20% of all drugs. This gene is highly polymorphic and a single study analyzing several CYP2D6 polymorphisms showed a trend toward higher drug plasma concentrations in patients treated with efavirenz (Fellay et al., 2002). Though these findings were not statistically significant, research to confirm these results is warranted.

Cytochrome P450 family 3, subfamily A, polypeptide 4 (CYP3A4). CYP3A4 catalyzes the largest number of pharmacologic agents, metabolizing approximately 50% of all drugs. Present in the largest quantity of all CYPs in the liver, this protein localizes to the endoplasmic reticulum. (Siest et al., 2004). However, the functional relevance of gene variations remains unproven. CYP3A4 is a major metabolizer for several non-nucleoside reverse transcriptase inhibitors. Though CYP3A4 gene variations are known to modulate enzyme activity, antiretroviral research has produced varying results none of which yielded statistically significant findings. Haas et al reported a trend to higher AUC whereas Saitoh et al reported a trend to lower AUC for the same polymorphism. (Haas et al., 2005; Saitoh et al., 2005). These contradictory findings warrant further investigation.

Cytochrome P450 family, 3, subfamily A, polypeptide 5 (CYP3A5). Analysis of CYP3A5 enzyme activity uncovered overlapping substrate specificity with CYP3A4 (Kuehl et al., 2001). The 502-amino acid CYP3A5 protein shares 85% sequence similarity with CYP3A4. Polymorphisms of CYP3A5 exhibit severely reduced enzyme activity. Antiretroviral pharmacogenetic studies showed a trend toward higher plasma drug concentrations in patients taking efavirenz (Haas et al., 2004).

Adenosine triphosphate (ATP)-binding cassette, subfamily B, member 1 (ABCB1). ATP-binding cassette (ABC) proteins transport endogenous and exogenous compounds out of the cell. The protein encoded by ABCB1, also known as multi-drug resistance 1, is called P-glycoprotein is expressed in barrier and excretory tissues such as the intestines, liver, kidney, blood-brain barrier, and placenta. Located on the plasma membrane, P-glycoprotein transports a wide range of compounds. Interindividual variability of P-glycoprotein expression and function is associated with changes in transport function which is hypothesized to modulate the pharmacokinetics of P-glycoprotein substrates (Ambudkar et al., 1999; Colombo et al., 2005). Pharmacogenetic studies of efavirenz are inconsistent. One published study showed a relationship between a SNP and efavirenz exposure whereas subsequent studies report no relationship (Cressey & Lallemont, 2007, Haas et al., 2004, 2005; Tsuchiya et al., 2003; Winzer et al., 2003).

Adenosine triphosphate (ATP)-binding cassette subfamily C, member 2 (ABCC2). ATP-binding cassette (ABC) proteins transport various molecules across extra- and intra-cellular membranes. The protein encoded by ABCC2, also known as multi-

drug resistance protein 2, is expressed in the apical part of hepatocytes and functions in biliary transport. ABCC2 plays an important role in transporting a wide range of compounds, especially conjugates of lipophilic substances with glutathione, glucuronate, and sulfate which are known as phase II products of biotransformation. Additionally, ABBC2 can also co-transport uncharged compounds with glutathione and can therefore modulate the pharmacokinetics of many drugs. ABCC2 gene variations leading to the absence of functional ABCC2 protein from the apical membrane have been associated with hyperbilirubinemia, a side effect known to occur in HIV patients treated with antiretroviral therapy (Fellay et al., 2002; Jedlitschy et al., 2006). ABCC2 mediates membrane transport of some antiretroviral compounds; however, information as to ABCC2 substrate specificity for some antiretroviral drugs is lacking (Colombo et al., 2005; Strazielle & Ghersi-Egea, 2005). A single small study reported no relationship between ABCC2 and efavirenz exposure (Fellay et al., 2002) therefore further study is warranted.

Solute carrier family 22 member 6 (SLC22A6). The SLC22A6 is a member of the family of human organic anion transporters. The SLC22A6 protein is an integral membrane protein localized on the basolateral membrane of the proximal renal tubule. The transporter functions as a facilitative organic anion exchanger, carrying its substrates into renal cells where they are secreted across the apical membrane into the tubule lumen. Though expression of this transporter is highest in human kidneys, it is detectable at lower levels in the human brain and skeletal muscle (Fujita et al., 2005). *In vitro* studies demonstrated that this transporter has a diverse substrate specificity

transporting a wide range of drugs including several antiretrovirals (Strazielle & Ghersi-Egea, 2005). Transporter specificity for some antiretrovirals is poorly studied and/or remains to be determined. This has generated interest in the possibility that variants in SLC22A6 may be partly responsible for antiretroviral drug response variability. However, only one pharmacogenetic study (i.e., nucleoside reverse transcriptase inhibitor, tenofovir) has been reported. No association was observed between SLC22A6 variations and pharmacokinetic parameters or clinical effects (Kiser et al., 2008). Given the paucity of information, additional research is warranted.

Uridine Diphosphate Glucuronyltransferase (UDP)-glycosyltransferase 1 family polypeptide A1 (UGT1A1). UDP-glycoglucuronosyltransferase UGT1A1 encodes for a protein which functions as an enzyme in the glucoronidation pathway catalyzing the addition of the glycosyl group from a nucleotide sugar to a small hydrophobic molecule critical in the elimination process. Glucuronidation represents a major pathway that renders compounds more water-soluble (Ritter, Yeatman, Ferreira, & Owens, 1992). One study observed a reduction in bilirubin (an endogenous substrate for UGT1A1) with a UGT1A1 polymorphism in patients receiving efavirenz (Rotger et al., 2005). The findings of this study are consistent with the induction of UGT1A1 (Rotger et al., 2005). These limited but important findings warrant additional study.

Single Nucleotide Polymorphisms

Two processes were involved in the selection of SNPs for this study. First, a literature review was conducted to identify ADME SNPs that were functional (effect protein coding, splicing regulation, transcriptional regulation, or post-translation and

likely associated with pathological outcomes) and/or reported repeatedly (i.e., consistently replicated findings) (Carlton, Ireland, Useche, and Faham, 2006). Second, a web-based SNP application (Snagger) was used to select tagging SNPs to capture neighboring regions in high linkage disequilibrium (LD) across coding and noncoding regions of the genes implicated in ADME (Gandhi et al., 2012). Snagger was selected for its capability in tagSNP selection across multiple ethnic groups (Edlund et al., 2008). Tagging SNPs were required to be common (defined as a minor allele frequency [MAF] $\geq$.05 in public databases [e.g., HapMap]). A custom array was designed to interrogate the set of literature-driven SNPs selected for study. Overall, among 9 candidate genes, 230 SNPs were analyzed (Gandhi et al., 2012).

DNA Samples and Genotype Data

Genomic DNA was isolated from cell pellets accessed from the WIHS repository that had been stored from participants recruited into the Intensive Pharmacokinetic Substudy. Samples were genotyped using a combination of the GoldenGate genotyping platform (Illumina, San Diego, CA) and TaqMan Allelic discrimination assay (Applied Biosystems, Foster City, CA). Genotyping was performed blind to participants' outcomes and positive (i.e., one nuclear family to verify Mendelian inheritance, a duplicate sample in each microplate) and negative controls (no template controls) were included. For each SNP, signal intensity plots and resulting genotype calls were visually inspected by two blinded reviewers. Disagreements were discussed until consensus was established. Genotype data that failed visual inspection or for which positive and negative controls provided conflicting results were excluded from downstream analysis. Quality control

measures were instituted to ensure robust analyses. SNPs with call rates of <93.5% or Hardy-Weinberg p-values <0.001 were excluded from further analysis. SNPs with allele frequencies of <5% were also excluded from analysis (Gandhi et al., 2012). Of the 230 SNPs analyzed, 182 SNPs passed all quality control filters (ABCB1: 63 of 70 SNPs; ABCC2: 20 of 28 SNPS; CYP2B6: 23 of 38 SNPs; CYP2C19: 24 of 28 SNPs; CYP2D6: 5 of 7 SNPs; CYP3A4/CYP3A5: 21 of 30 SNPs; SLC22A6: 5 of 8 SNPs; and UGT1A1: 22 of 26 SNPs) Table 3-4.

Statistical Analyses

The statistical analyses common to both studies are discussed below and followed by study-specific statistical analyses. The allele and genotype frequencies for each SNP were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square exact test. Haploview 4.1 was used to compute measures of linkage disequilibrium (LD) (i.e., D', r^2 [shared variance]) from study participants' genotypes. LD-based haplotype block definition was based on the D'[13] confidence interval (Gabriel et al., 2002). Haplotype analyses were conducted to localize the association signal within each gene and determine if haplotypes improved the strength of the association for SNPs in the same haploblock. PHASE version 2.1 (Stephens, Smith, & Donnelly, 2001) was used to construct haplotypes. The procedure to construct haplotypes was repeated five times to improve the stability of haplotype inference.

[13] Normalized measure of allelic association with defined LD confidence interval (CI) parameters to determine either strong LD (no historical recombination: one-sided upper 95% CI >0.98 and lower bound above 0.7) or weak LD (strong evidence of historical recombination: D' <0.9) (Gabriel et al., 2002).

Across the five iterations, only haplotypes inferred with probability estimates of $\geq$0.85 and a frequency estimate of $\geq$20% were retained for analysis (Gandhi et al., 2012).

To minimize bias due to population stratification and estimate ancestry, ancestry informative markers (AIMs) were genotyped in the cohort (Gandhi et al., 2012). Informative AIM panels now provide ancestral population models which estimate the genetic structure of various populations and are useful for inferring ancestral proportions in study populations (Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008). Principal component[14] (PC) analysis was used to estimate the homogeneity in ancestry among the study participants. This analysis method is used to evaluate factors or variables, assess their patterns, and determine the number of groups that can be extracted, what differentiates them, and the relationship between groups (Price et al., 2006; Reich, Price & Patterson, 2008). Scatter plots of orthogonal PCs were visually inspected to distinguish the major racial/ethnic groups in the sample (i.e., European, African, Asian) i.e., PC 1 versus PC2, PC2 versus PC3). The first three PCs accounted for the majority of the variability self-report race and ethnicity in the sample and were retained as covariates in multivariate models that included genetic predictors (Gandhi et al., 2012).

All statistical analyses were conducted using Stata (version 11.2, College Station, TX) and SAS (version 9.2, SAS Institute, Cary, NC).

[14] An analysis method used to evaluate factors or variables, assess their patterns, and determine the number of groups that can be extracted, what differentiates them, and the relationship between groups (Price et al., 2006).

Intensive pharmacokinetic substudy pharmacogenetic analyses. The purpose of the pharmacogenetic study presented in chapter 4, was to test for an association(s) between ADME gene variations and efavirenz exposure when modeled with nongenetic factors previously characterized in the Intensive Pharmacokinetic Substudy. The exposure outcome, plasma area under the concentration time curves (AUC) over dose, was analyzed in relation to nongenetic and genetic factors that may influence efavirenz pharmacokinetics (Gandhi et al., 2012).

For each SNP, four genetic models were assessed: unstructured, additive, dominant, and recessive. An unstructured model makes no assumptions about how the exposure outcome for heterozygotes compare between the 3 genotypic classes (i.e., 1 heterozygote and 2 homozygotes). An additive genetic model assumes that each additional copy of the rare allele increases the exposure outcome risk by the same amount. A dominant model treats the rare homozygote genotypes and heterozygotes as a single category. This combined category is compared against the common homozygotes and assumes that heterozygotes have the same exposure outcome as the rare homozygotes. A recessive model compares common allele homozygotes and heterozygotes against rare allele homozygotes (Lunetta, 2008). The genetic model that best fit the data was selected for each SNP by examining the p-value (Gandhi et al., 2012).

Linear regression was performed to first assess the relationship of SNPs and haplotypes and log-transformed AUCs/dose when single genetic predictors were added to the nongenetic factor model previously identified in the WIHS Intensive

pharmacokinetic study (i.e., age, ALT, albumin, ideal body weight, oranges or orange juice consumption, amenorrhea, tenofovir, and African American race). Both forward and backward stepwise approaches were used to construct multivariate models. To the nongenetic factor model, SNPs were added using a forward stepwise approach. Each SNP was tested, controlling for nongenetic factors, until no remaining SNP candidates met the *a priori* significance threshold $p<0.001$. For the final model, backward elimination of the non-genetic factors was performed. Each nongenetic factors was eliminated one by one until all remaining predictors had a p-value of <0.05 (Gandhi et al., 2012).

Analyses of symptoms and side effects by genotype in the intensive pharmacokinetic pharmacogenetics substudy. The purpose of the analysis summarized in Chapter 5 was to describe the prevalence by genotype of symptoms and side effects in the women enrolled in the Intensive Pharmacokinetic substudy. To be included in this analysis, a criterion was imposed that required at least 61 study participant responses (50%) for each respective variable. The missing data which excluded variables from analysis are accounted for by the difference in general WIHS data collection instruments used at each biannual visit. For patients enrolled in the Intensive Pharmacokinetic substudy, aside from the substudy data collection forms, the general WIHS data collection instruments that corresponded with the closest biannual visit were used. Data collected at each biannual visit differs depending on the visit number.

To examine the distribution of the continuous, ordinal, and categorical variables of interest and summarize the sample characteristics, univariate analyses were

performed. To explore the association between the individual SNP genotypes, metabolizer phenotype, and haplotype and each variable of interest bivariate analyses were performed. For continuous variables, analysis of variance or student's t-test were employed to examine for difference in the groups. For count variables, chi square test or Fisher's exact test were employed to examine for difference in the groups. For ordinal variables, Wilcoxon-Mann Whitney or Kruskal-Wallis tests were employed to examine for difference in the groups.

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Tables

Table 3-1. WIHS Intensive Pharmacokinetic Substudy Antiretrovirals and their classification

Brand Name	Generic Name	Pharmacologic Classification
Reyataz®	Atazanavir	Antiretroviral, Protease Inhibitor
Sustiva®	Efavirenz	Antiretroviral, Non-nucleoside Reverse Transcriptase Inhibitor
Kaletra®	Lopinavir/ritonavir	Antiretroviral , Protease Inhibitors
Viracept®	Nelfinavir	Antiretroviral, Protease Inhibitor
Viramune®	Nevirapine	Antiretroviral, Non-nucleoside Reverse Transcriptase Inhibitor

Note. Antiretrovirals are generally classified by the retrovirus target molecule that the drug inhibits.

Table 3-2. WIHS Intensive Pharmacokinetic Substudy Continuous Variables

Table 3-2. WIHS Intensive Pharmacokinetic Substudy Continuous Variables
Age at time of study entry
Hepatitis C RNA levels in hepatitis C-infected subjects
Creatinine clearance (as described in Table 3-3)
Body mass index (BMI)
Fat free mass measurements[a]
Serum aspartate aminotransferase (AST) levels
Alanine aminotransferase (ALT),
Gamma glutamyl-transferase (GGT) levels

[a] Because measures of lean body mass are typically used to predict drug dosages, ideal body weight, lean body weight, adjusted body weight, and predicted normal weight were estimated from height and weight parameters using standard equations and assessed for their independent relationships to the outcome (Gandhi et al., 2009).

Table 3-3. WIHS Intensive Pharmacokinetic Substudy Categorical Variables

Variable (Categorical)	How Categorized
Race/ethnicity	African American versus other; including Caucasian, Hispanic, Native American, and Asian
Education	Did not complete High School (HS); completed HS; some college; completed college; more than college
Smoker	Yes/No
Drug use (multiple categories)	Yes/No
Alcohol use	Light (<3 drinks/week); moderate (3-13 drinks/week); severe (≥14 drinks/week)
Adherence	≤35%; 36-65%; 66-80%; 81-94%; ≥95%
Hepatitis C positive	Yes/No
Chronic Hepatitis B	Positive surface antigen, Yes/No
Low Platelet Count	Platelets <150/ml versus ≥150/ml
Menstruating at time of study enrollment	Yes/No
Amenorrhea for ≥12 months (self report)	Yes/No
Pregnancy	Yes/No
Renal insufficiency creatinine clearance (calculated using Cockcroft-Gault equation)	CrCl <60mil/min versus ≥60 ml/min
Renal insufficiency glomerular filtration rate Modification of Diet in Renal Disease (MDRD) equation	GFR <80ml/min versus ≥80 ml/min
Percent fat consumption in past 30 days	<30%; 30-35%; 36-40%; >40%
Persistent diarrhea within 30 days	3 or more soft stools a day within 30 days, Yes/No
Oranges or orange juice consumption in past 5 days	Yes/No
Concomitant medications known to be CYP3A4 inhibitors	Comparative list, Yes/No
Concomitant medications known to be CYP3A4 inducers	Comparative list, Yes/No
Use of tenofovir	Yes/No

Note. Categorized in the Intensive Pharmacokinetic study by the primary investigator (Gandhi et al., 2009).

Table 3-4. Efavirenz Pharmacogenetics Substudy Candidate Genes and SNPs

Gene	Chromosome	Protein	tag-SNPs	Literature-driven SNPs	Failed QC[a]
Metabolization Genes					
CYP2B6	19	Cytochrome P450, family 2, subfamily B, polypeptide 6	27	11	15
CYP2C19	10	Cytochrome P450, family 2, subfamily C, polypeptide 19	28	1	4
CYP2D6	22	Cytochrome P450, family 2, subfamily D, polypeptide 6	2	3	2
CYP3A4/ CYP3A5[b]	7	Cytochrome P450, family 3, subfamily A, polypeptide 4 and polypeptide 5	21	9	9
Transporter Genes					
ABCB1	7	ATP-binding cassette, subfamily B, (MDR/TAP), member 1	60	10	7
ABCC2	7	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	21	7	8
SLC22A6	2	Solute carrier family 22 (organic anion transporter), member 6	6	2	3
Elimination Genes					
UGT1A1	11	UDP glycosyltransferase 1 family polypeptide A1	25	1	4

[a]Quality Control filters. [b]These two genes are adjacent and form a cluster.

Chapter 4

Efavirenz Pharmacogenetic Substudy

Introduction

Though efavirenz was the 14th antiretroviral drug to become available in the United States, it is the non-nucleotide reverse transcriptase inhibitor most widely used in initial, subsequent, and/or salvage antiretroviral regimens (Manfredi, Calza, & Chiodo, 2004; World Heath Organization [WHO], 2006). Efavirenz is well studied and substantial interindividual variability in the pharmacokinetics of this drug is known. The coefficient variation in apparent clearance[15] of efavirenz ranges from 40%-50% (Cabrera et al., 2009; Kappelhoff et al., 2005). The variability in efavirenz plasma concentrations in response to the adult fixed dose of 600 mg is up to 120% (Kwara, Ramachandran, & Swaminathan, 2010; Marzolini et al., 2001). Additionally, several studies have shown a relationship between increased efavirenz plasma concentrations and adverse events (Kappelhoff et al., 2005; Marzolini et al., 2001; van Luin et al., 2009). The most common adverse events in both the study setting and real-world reporting are dermatological effects (rash) and central nervous system (CNS) symptoms (e.g., headache, dizziness, insomnia, and fatigue) (Perez-Molina, 2002; Smith et al., 2007). Primarily attributed to adverse events, real-world efavirenz discontinuation rates are reportedly as high as 50% (Calmy, Hirschel, Cooper, & Carr, 2007; Deeks, 2000; Kalow, Tang, &Endrenyi, 1998). However, despite these findings adults are often uniformly dosed (e.g., 600 mg daily) (Gandhi et al, 2012; Kappelhoff et al., 2005).

[15] A pharmacokinetic parameter used to describe the efficiency of elimination of a drug from the body. It is defined as the volume of blood from which drug can be completely removed per unit of time. Apparent (oral) clearance is estimated when intravenous drug administration is not feasible (Craig & Stizel, 2004).

Growing evidence suggests that variations in drug metabolism and/or transporter genes influence responses to efavirenz (Motsinger et al., 2006). Both systems may work in synergy whereby transporter mediated drug influx and efflux facilitates metabolism by modulating the drug load to and from the metabolizing enzyme system (Nolan, Phillips, Mallal, 2006). Efavirenz is largely metabolized by the cytochrome p450 (CYP) 2B6 enzyme and it has been observed that CYP2B6 activity (measured in human liver microsomal preparations) varies 20- to 80-fold for this substrate (Desta et al., 2007; Ekins et al., 1998; Michaud et al., 2012). Several single nucleotide polymorphisms (SNPs) influence drug exposure (Haas et al., 2004; Rotger et al., 2005; Tsuchiya et al., 2004). Fairly recently, P-glycoprotein, the ATP-binding cassette (ABC) efflux transporter encoded by the multidrug resistance transporter ABCB1 gene became recognized as a determinant in the pharmacokinetics of some antiretrovirals (Weiss et al., 2009). Although data is limited, SNPs in the ABCB1 gene have been linked with variations in efavirenz levels (Fellay et al., 2002). As pharmacogenetic studies continue to examine the ADME pathway, inclusion of the SNPs encompassing the majority of variability in functional genes for ADME is important. Additionally, studies suggest that efavirenz pharmacokinetics and treatment response are complex traits influenced by multiple factors (i.e., treatment adherence, comorbid conditions, concomitant medications, illicit drug use, diet, body mass, renal, and/or hepatic function)(Gandhi et al., 2012; Motsinger et al., 2006). Therefore, analyses that include both genetic and nongenetic risk factors are required to better understand the independent contribution of each factor in efavirenz treatment response variability.

The WIHS study research group previously undertook the Intensive Pharmacokinetic Substudy to determine the nongenetic factors that contribute to efavirenz exposure (defined by area-under-the-concentration-time curve [AUC] divided by target dose) (Gandhi et al., 2009). An association with increased efavirenz exposure and hepatic transaminase levels, albumin levels, and orange juice consumption was observed. Conversely, a decrease in efavirenz exposure was associated with concomitant tenofovir use, increased weight, African-American ethnicity, and amenorrhea (Gandhi et al., 2009). The purpose of the current study was to examine the contribution of ADME gene polymorphisms to efavirenz exposure when modeled with WIHS Intensive Pharmacokinetic Substudy nongenetic factors (Gandhi et al., 2012). To capture the majority of genetic variability in ADME genes implicated in efavirenz exposure, literature-based SNPs and tagging SNPs were selected and examined in patients enrolled in the WIHS Intensive Pharmacokinetic Substudy (Gandhi et al., 2009).

Methods

The study population, protocol, laboratory procedures, gene selection, SNP selection, blood collection and genotyping have been previously described in Chapter 3. A brief summary follows.

Study population. The WIHS Intensive Pharmacokinetic Substudy included 121 participants receiving efavirenz-based therapy that underwent 24-hour blood sampling following a witnessed dose under conditions of routine use at steady-state. From these 121 participants, genomic DNA could be isolated from cell pellets and successfully

genotyped for 111 WIHS Intensive Pharmacokinetic Substudy participants. (Gandhi et al., 2012).

Efavirenz exposure. Blood samples were analyzed for efavirenz by standard techniques of liquid chromatography/tandem mass spectrometry (LC/MS/MS). The absolute recovery of efavirenz from plasma was 99.8%, intraday and interday precision were each <11.7%, and accuracies range from -6.0% to 14.8%. The plasma efavirenz assay was validated according to the current FDA guidelines for bioanalytical method validation. All quality control samples were within 15% of their respective nominal value (Gandhi et al., 2012).

Exposure was assessed by calculation of the AUC divided by dose. Plasma AUC was calculated using the trapezoidal rule from 24-hour intensive pharmacokinetic studies. Because pharmacokinetic measures are typically skewed, the accepted statistical distribution for AUC is a log-normal distribution derived by log transforming the data (Senn, 2008). AUCs were log-transformed for subsequent statistical modeling (Gandhi et al., 2012).

Gene selection, SNP selection, and genotyping. A literature search was conducted to identify genes and SNPs implicated in efavirenz ADME. A custom SNP genotyping array was designed to interrogate each candidate gene using literature-driven SNPs and tagging SNPs that were selected to capture neighboring regions in high linkage disequilibrium (LD) across coding and noncoding regions of ADME implicated genes (Gandhi et al., 2012). The web-based SNP application Snagger was used to select tagging SNPs for its capability in tagSNP selection across multiple ethnic groups (Edlund

et al., 2008). This was particularly relevant for the WIHS participants as multiple ethnic groups were well represented (i.e., African American, Hispanic, Asian, Pacific Islander, Caucasian). Tagging SNPs were required to be common (defined as a minor allele frequency of $\geq$.05) in public databases (e.g., HapMap) (Gandhi et al, 2012).

Two hundred thirty-four SNPs were selected to evaluate 9 genes (adenosine triphosphate-binding cassette protein [ABC] B1 [ABCB1], ABCC2, cytochrome P450 [CYP] 2B6 [CYP2B6], CYP2C19, CYP2D6, CYP3A4, CYP3A5, solute carrier-like protein [SLC] A6 [SLC22A6], and uridyl diphosphate glucuronosyltransferase-1 family, polypeptide A1 [UGT1A1]) (Gandhi et al., 2012).

Genotyping was performed blinded to the study outcomes, and positive and negative controls were included. Samples were genotyped using a combination of the GoldenGate genotyping platform (Illumina, san Diego, CA) and the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). GoldenGate genotype data were processed according to standard protocols (GenomeStudio, Illumina). Signal-intensity profiles and resulting genotype calls for each SNP were visually inspected by 2 blinded reviewers, with disagreements discussed until consensus was established (Gandhi et al., 2012).

To ensure robust analyses, SNP quality control filtering was performed. SNPs with call rates of <93.5% (n=8), Hardy-Weinberg P values of <0.001 (n=9), or allele frequencies of <5% (n=35) were excluded from analysis. This resulted in 182 SNPs across 9 candidate genes passing these quality control parameters to be included in the analyses (Gandhi et al., 2012).

SNP allele and genotype frequencies were determined by gene counting and Hardy Weinberg equilibrium was assessed by the X^2 exact test. Measures of LD (i.e., D' [normalized measure of allelic association] and r^2 [variance]) were computed from the participants' genotypes with Haploview 4.1. LD-based haplotype block definition was based on the D' confidence interval (Gabriel et al., 2002; Gandhi et al., 2012).

SNP haplotypes were constructed using the program PHASE, version 2.1, a method which uses a coalescent theory of assigning haplotype predictions based on the distribution of haplotypes in natural populations (Stephens, Smith, & Donnelly, 2001). Haplotype analyses were conducted for SNPs in the same haploblock to localize the association signal within each gene and to determine whether haplotypes improved the strength of the association. (Gandhi et al., 2012; Stephens et al., 2001). To improve the stability of haplotype inference, the haplotype procedure was repeated 5 times. Only haplotypes that were inferred with probability estimates of $\geq$0.85 across the 5 iterations and estimated to occur at a frequency of $\geq$20% were retained for downstream analysis (Gandhi et al., 2012).

A SNP genotype combination (i.e., diplotype) composed of rs3745274 and rs28399499 was also included for analysis. Entitled the CYP2B6 "Metabolizer" diplotype, the alleles are defined by their associated function as "slow", "intermediate", and "fast" metabolizer. The slow metabolizer diplotype is composed of rare alleles (i.e., rs3745274 TT and rs28399499 CC or rs3745274 GT and rs28399499 TC). The intermediate metabolizer diplotype is composed of a rare allele at either rs3745274 or rs28399499 but not both. The extensive metabolizer diplotype is composed of only common alleles

at both rs3745274 and rs28399499. (Haas et al., 2009; Ribaudo et al., 2010; Rotger et al., 2007; Stahle, Moberg, Svensson, & Sonnerborg, 2004; Wang et al., 2006).

As a tool to minimize population stratification bias and estimate ancestry, ancestry informative markers (AIMs) were used (Gandhi et al., 2012; Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008). Informative AIM panels provide ancestral population models, which estimate the genetic structure of various populations and are useful for inferring ancestral proportions in study populations (Halder et al., 2008). Both AIMs and self report race/ethnicity were examined in this study. Cluster and principal component (PC) analysis was used to verify the homogeneity in ancestry among the study participants (Gandhi et al., 2012; Price et al., 2006). The number PCs were sought that would delineate the major racial/ethnic subgroups in this study sample (European, African, Asian) via inspection of scatter plots of orthogonal (independent) PCs (i.e., PC1 vs. PC2, and PC2 vs. PC3). To adjust for potential confounding due to population structure (i.e., race/ethnicity), the first 3 PCs were selected for inclusion in all multiple regression models. AIMS and their PCs were available for 97 of the 111 study participants (Gandhi et al., 2012).

Statistical Analyses

All analyses were conducted using Stata (version 11.2, College Station, TX) and SAS (version 9.2, SAS Institute, Cary, NC). Descriptive statistics were used to summarize the characteristics of the study participants. Four genetic models were assessed for each SNP: unstructured, additive, dominant, and recessive. An unstructured model makes no assumptions about how the exposure outcome for heterozygotes compare

between the 3 genotypic classes (i.e., 1 heterozygote 2 homozygotes). An additive genetic model assumes that each additional copy of the rare allele increases the exposure outcome risk by the same amount. A dominant model treats the heterozygote and the rare homozygote genotypes as a single category. A recessive model compares common allele homozygotes and heterozygotes against rare allele homozygotes (Lunetta, 2008).

Linear regression was performed to first assess the relationship of SNPs and haplotypes and log-transformed AUCs (i.e., efavirenz exposure/dose) adding each as a single predictor to the nongenetic predictor model previously identified in the WIHS intensive pharmacokinetic study (i.e., alanine aminotransferase, albumin, oranges/orange juice consumption, amenorrhea, tenofovir, ideal body weight, and African American race) (Gandhi et al., 2009; 2012). A multivariate model was then constructed by adding genetic predictors in a stepwise manner, keeping genetic predictors with a significance of $P<0.001$. Then nongenetic factors were eliminated one by one until all remaining predictors had a P value of <0.05.
Only those demographic and clinical characteristics identified by Gandhi et al. (2009) were retained for the evaluation of genetic predictors (Gandhi et al., 2009; 2012).

Results

Genomic DNA could be isolated and successfully genotyped for only 111 of the 121 participants originally recruited into the WIHS Intensive Pharmacokinetic Substudy. Of the 111 participants in this study, 8% were white, 13% were Hispanic, 78% were

African American, and 1% were of other races/ethnicities. The median age of this study group was 43.1 years (range, 20.6-60.4 years) (Gandhi et al., 2012).

The mean efavirenz AUC among the study participants was 78μg x h/ml (range, 11-519 μg x h/ml). The dose of efavirenz taken by the majority of the study participants was 600 mg per day. Only 4 out of the 111 were taking alternative doses (Gandhi et al., 2012). The exposure outcome, efavirenz AUC, was modeled as a function of the following nongenetic predictor variables: race, age, hepatitis B or C status, ovulatory cycle phase or menopausal status, pregnancy status, smoking, alcohol/substance use, percentage of fat in the typical diet, diarrhea, other concurrent symptoms or infections, concomitant medications with potential drug-drug interactions, calculated creatinine clearance, body mass index and fat free mass, and hepatic function tests (Gandhi et al., 2009; 2012). In the previous WIHS Intensive Pharmacokinetic Substudy analysis, variables identified as independently influencing exposure were: age, alanine aminotransferase (ALT) level, albumin, ideal body weight, orange or orange juice consumption in the preceding 5 days, amenorrhea for ≥12 months, tenofovir use, and race (Gandhi et al., 2009; 2012).

Using linear regression, a total of 182 SNPs and 45 haplotypes were analyzed in relationship to efavirenz drug exposure. In the first round of models of single genetic predictors, 1 of 75 in ABCB1, 2 of 23 in ABCC2, 16 of 27 in CYP2B6, and 9 of 32 genetic predictors in UGT1A1 were associated with exposure with a *P* value of <0.001 when combined with nongenetic predictors (age, alanine aminotransferase [ALT], albumin,

ideal body weight, orange/orange juice consumption in the past 5 days, amenorrhea for ≥12 months, tenofovir use, race) as shown in Table 4-1 (Gandhi et al., 2012).

The final multivariate model of factors associated with efavirenz exposure (AUC) was constructed using forward selection for the genetic predictors, Table 4-2. Of note, the only nongenetic predictors that remained independently associated with exposure were increases in the ALT level and consumption of oranges and/or orange juice in the prior 5 days. The model explained 53% of the interpatient variability. Because the effects of genetic factors on efavirenz exposure were similar prior to backward elimination of nongenetic factors, (within 3%), only the simple model is presented (Gandhi et al, 2012).

In the final multivariate model of 2 SNPs (CYP2B6 (c.516G>T , rs3745274; c.983T>C, rs28399499) and 1 haplotype (ABCB1 c.3085-72C>G, rs7779562 and c.2927+377C>A, rs4148745) were associated with efavirenz exposure. Individuals homozygous for the CYP2B6 rs3745274 rare T allele ("TT" genotype) displayed 3.50-fold (2.7 -4.5 [95% CI], p=1.4 x 10^{-18}) increases in AUC as compared to carriers of the common "G" allele ("TG" and GG" genotypes). Individuals homozygous or heterozygous for the CYP2B6 rs28399499 rare C allele ("CC" or "CT" genotype) showed 1.96-fold (1.54-2.5 [95% CI], p=2.2 x 10^{-10}) increases in AUC as compared to carriers of the minor "T" allele ("TT" genotype). A haplotype composed of the common alleles from 2 SNPs ("C" at rs7779562 and "G" at rs4148745) in transporter ABCB1 was associated with AUC increases of 1.60-fold (1.24-2.1 [95% CI], p=0.0004). There was insufficient data to examine the direction of the association between elevated ALT levels and exposure.

However, the final models excluding ALT levels did not significantly alter the fixed predictor effects (orange juice consumption and genetic predictors) on efavirenz exposure (Gandhi et al., 2012).

An association with the CYP2B6 Metabolizer diplotype was observed. Compared to individuals designated as fast metabolizers, intermediate and slow metabolizers displayed elevated efavirenz exposure (Odds Ratio =1.63, 95% CI: 1.43, 1.87, p=1.3 E-10). However, this relationship was not linear across the intermediate and slow metabolizer groups, with the intermediate group displaying a 1.36-fold (1.11-1.67 [95% CI], p=0.003) increase in AUC as compared to fast metabolizers and slow metabolizers displaying a 3.0-fold (2.3-4.0 [95% CI], *p*=1E-11) increase in AUC as compared with fast metabolizers.

Discussion

HIV-infected individuals are required to maintain lifelong therapy and the availability of effective antiretroviral therapy has successfully affected the disease prognosis. However, there is marked intra- and interindividual variation in plasma drug levels, efficacy, and susceptibility to adverse events (Nolan et al., 2006; Michaud et al., 2012). Treatment discontinuation, adverse events, and adherence continue to pose challenges for clinicians in selecting an optimal treatment strategy. Personalizing care for patients should augment treatment selection, optimize dosing strategies, and improve outcomes (Wang, McLeod, & Weinshilboum, 2011). HIV medicine is one of the few fields that already employs pharmacogenetic methodologies (Gandhi et al., 2012) in

managing the effectiveness of therapy altered by viral sensitivity to antiretroviral drugs (drug-resistant mutations (Michaud et al., 2012).

There has been a need for adequate cohorts and studies to be developed to provide a clear definition of a clinical phenotype (Nolan et al., 2006) and explore the many contributing factors and their degree of influence. Pharmacokinetics and treatment response are complex traits influenced by multiple factors; therefore, analyses that include both genetic and nongenetic determinants are expected to play a crucial role in elucidating the full suite of factors that influence treatment response variability. New genotyping technology such as those applied in the current study, allows for a systematic exploration of genes implicated in the ADME of antiretroviral drugs, enhancing analysis capabilities (Lubomirov, Csajka, & Telenti, 2007). The WIHS Intensive Pharmacokinetic Substudy pharmacogenetic cohort opportunely examines the contribution of ADME gene polymorphisms to efavirenz exposure when modeled with nongenetic factors (Gandhi et al., 2012).

A comprehensive examination of 182 SNPs and 45 haplotypes in 9 efavirenz ADME implicated genes modeled with nongenetic factors was performed in a heterogeneous population of HIV-infected patients (Gandhi et al., 2012). Orange juice consumption, 2 CYP2B6 SNPs (c.516 G>T , rs3745274; c.983C>T, rs28399499), and an ABCB1 haplotype (c.3085-72C>G, rs7779562 and c.2927+377C>A, rs4148745) were associated with an elevation in AUC. Both CYP2B6 SNPs were previously noted in the literature to influence plasma drug levels (Ribaudo et al., 2010). Citric components in oranges or orange juice inhibition of p-glycoprotein (encoded by the ABCB1 gene),

transport, or downregulation of enteric CYP3A4 may lead to increased bioavailability and an increase in efavirenz exposure (Gandhi et al., 2012). However, the most important finding of this study was the tripling of efavirenz exposure as measured via AUC associated with the CYP2B6 rs3745274 rare T allele (Gandhi et al., 2012).

Although the CYP2B6 Metabolizer diplotype has been repeatedly observed to influence efavirenz exposure (Haas et al., 2009; Ribaudo et al., 2010), we did not observe that this combination of SNPs added any additional information beyond the component SNPs in the final model. This may be due to differences in study design, participant variability and analysis methods, variability in patient population sampling, and sample size. In one study, though women were highly represented (73%), the study was small (n=34) and only included healthy HIV-negative African American individuals. Study methods also differed with regard to plasma samples for pharmacokinetic analyses obtained over a 13 day period of time and parameters presented as median values and interquartile ranges (Haas et al., 2009). Another study, though large (n=831), underrepresented women (19%) and only included treatment naïve study participants. Pharmacokinetic plasma sampling and analysis methods also differed from our study with plasma sampling occurring at weeks 1, 4, 12, and 24 without specifying the sampling times and efavirenz concentration time curve percentiles were generated by pharmacokinetic model simulations (Ribaudo et al., 2010). However, it is possible that genetic associations identified with single drug doses, as in our study, may differ at steady state concentration (Haas et al., 2009).

Our multivariate model of genetic and nongenetic factors explains 53% of interindividual variability in log AUC/dose of efavirenz. While this estimate suggests that the majority of the variance in log AUC/dose of efavirenz is explained by our model, there may be additional factors not assessed in this study which may not only contribute to interindividual efavirenz exposure variability but may be clinically useful tools to improve exposure outcomes. Interim studies have identified additional genes in the efavirenz ADME pathway that may contribute to efavirenz exposure variability (i.e., CYP2A6 as an accessory pathway when CYP2B6 is down regulated, UGT2B7 as a primary enzyme mediating efavirenz N-glucuronidation (Arab-Alamadine et al., 2009; Kwara, Lartey, Sagoe, Kenu, & Court, 2009).

A less stringent criterion for inclusion in the final multivariate model (i.e., p-value >0.001) would have led to the identification of additional genetic predictor variables. Alternatively, a larger sample size may have resulted in a subset of these associations meeting the significance threshold criterion. All of the predictor variables in Table 4-1 were candidates for inclusion in the final multivariate model however, only three met the conservative threshold of p<0.001. Several UGT1A1 SNPs and haplotypes neared this statistical significance criterion. Though UGT1A1 has been implicated in the glucuronidation of efavirenz metabolites, the relationship is not well studied. A recent study observed limited efavirenz UGT1A1 interaction (Ji, Lee, Lim, Kim, & Lee, 2012) however, the impact of UGT1A1 gene variations was not examined. The paucity in the literature and our findings support the inclusion of UGT1A1 in future studies.

Two ABCC2 SNPs also neared statistical significance. Though ABCC2 has been hypothesized to play a role in the transportation of several antiretroviral drugs, efavirenz research is limited. A single study reported that no relationship between ABCC2 expression and efavirenz drug levels was observed (Fellay et al., 2002). However, this small study (i.e., n=69) only analyzed two ABCC2 SNPs (i.e., rs227369, 1346C>G [reference sequence identifier not available]) and it is likely that the study was not adequately powered to detect a potential association at this locus. The paucity of literature on the relationship between ABCC2 and efavirenz exposure prior to our study, coupled with our own findings, suggests that additional research to validate this association is warranted.

Efavirenz is the most widely used non-nucleoside reverse transcriptase inhibitor and numerous studies have examined the relationship between host traits and plasma efavirenz levels (Arab-Alameddine et al., 2009; di Iulio et al., 2009; Haas et al., 2004; Haas et al., 2005; Ribaudo et al., 2010; Rotger et al., 2005). CYP2B6 SNP c.516 G>T , rs3745274 has been repeatedly associated with increased efavirenz exposure and higher rates of discontinuation (Maimbo, Kiyotani Mushirodo, Masimirembwa, & Nakamura, 2012; Wyen et al., 2011). Though genotype-based efavirenz dose adjustment has been shown to be feasible, it is not routinely performed (Gandhi et al., 2012; Gatanaga et al., 2007). Large collaborative efforts across biostatisticians, epidemiologist, pharmacologists, and clinicians are needed to provide robust evidence to support individualized treatment to improve drug efficacy and safety (Ross, Anad, Joseph, & Pare, 2012).

Interpreting a single measure of antiretroviral concentration poses limitations and does not reflect typical patterns of medication use, variations in diet, concomitant medications, illicit substance use, comorbid states, and adherence (Gandhi et al., 2012). However, exposure is more robustly estimated by AUC from intensive pharmacokinetic sampling which was the method employed in this study. Additionally, the relationship between genetic and nongenetic traits was examined and may be useful in the development of protocols to individualize and optimize efavirenz dosing in the clinical setting (Gandhi et al., 2012).

The strength of this study lies in the comprehensive assessment of efavirenz implicated ADME genes and the inclusion of real-world factors that contribute to drug exposure and efficacy. However, this study is not without limitations. The sample size is relatively small and poses limitations on the depth and interpretation of the analyses. To detect a pharmacogenetic effect with uncommon alleles a large sample size would be optimal. The sample size of this cohort limits both SNP and haplotype analyses as additional signals for SNPs and haplotypes that are rare could have been missed. Additionally the adopted threshold of 0.001 was relatively conservative. A less conservative threshold would have permitted interpretation of SNPs close to this threshold but because they did not meet the artificial threshold set in this study they were not assessed. With a larger sample size multilocus interactions and haplotype stratification could have been performed to enrich the growing body of efavirenz pharmacogenetics.

Given the high rates of efavirenz discontinuation in the clinical settings, identifying all of the factors that lead to durable effects on exposure is important (Gandhi et al., 2012). The next step is to examine adverse events, and signs, and symptoms by genotype in patients taking efavirenz to enrich the findings of this study and contribute to the development of individualized treatment strategies.

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Tables and Figures.

Table 4-1. Analysis between each SNP/haplotype and EFV exposure (AUC/dose) controlling for non-genetic predictors

Gene	Name	Nucleotide position	Genetic change	Fold-effect on EFV AUC/dose (±95%CI), p-value
ABCB1	**rs1002205**	**86979110**	**G>C**	0.48 (0.29, 0.81) p=0.0059
ABCC2	**rs2180989**	**101527731**	**T>G**	0.73 (0.58, 0.91) p=0.0054
	rs2073337	**101557416**	**A>G**	0.80 (0.68, 0.95) p=0.0092
CYP2B6	**rs2054675**	**46187595**	**T>C**	2.70 (1.98, 3.80) p=1.2E-8
	rs16974799	**46195917**	**T>C**	2.30 (1.67, 3.10) p=5.96E-7
	rs4803417	**46199860**	**A>C**	0.68 (0.57, 0.80) p=1.02E-5
	rs3745274	**46204681**	**516G>T**	2.30 (1.74, 3.10) p=1.02E-7
	rs8113200	**46206203**	**A>G**	0.70 (0.60, 0.82) p=1.18E-5
	rs2279345	**46207542**	**T>C**	0.69 (0.59, 0.81) p=5.53E-6
	rs2306606	**46208022**	**T>C**	1.48 (1.28, 1.70) p=2.38E-7
	rs28399499	**46210061**	**983T>C**	1.50 (1.13, 1.98) p=0.0056
	rs8192719	**46210613**	**T>C**	2.70 (1.98, 3.80) p=1.2E-8
	rs7255374	**46212191**	**T>C**	2.30 (1.71, 3.20) p=4.72E-7
	rs11666982	**46223208**	**T>G**	0.66 (0.48, 0.89) p=0.0073
	rs7255146	**46223966**	**A>C**	2.50 (1.74, 3.70) p=3.69E-6
	Hap1A2			0.68 (0.57, 0.80) p=1.02E-5
	Hap1A4			2.70 (1.98, 3.80) p=1.2E-8
	HapB9			0.68 (0.58, 0.79) p=2.08E-6
	HapB14			2.50 (1.82, 3.30) p=4.40E-8
	Metabolizer[a]			1.63 (1.43, 1.87) p=13E-11
UGT1A1	**rs7572563**	**234281975**	**A>G**	1.71 (1.15, 2.60) p=0.0093
	rs7564935	**234309925**	**C>A**	0.68 (0.51, 0.90) p=0.0072
	rs3755319	**234332321**	**G>T**	1.70 (1.16, 2.50) p=0.0069
	rs4148328	**234342398**	**C>T**	1.36 (1.13, 1.65) p=0.0015
	rs10199882	**234350003**	**T>C**	0.69 (0.53, 0.91) p=0.0080
	rs6746002	**234354160**	**T>C**	2.60 (1.41, 4.60) p=0.0023
	HapA7			1.88 (1.23, 2.90) p=0.0040
	HapB1			1.69 (1.16, 2.50) p=0.0069
	HapB2			0.62 (0.46, 0.84) p=0.0026

Note. Non-genetic predictors controlled for in each model: age, alanine aminotransferase (ALT), albumin, ideal body weight, orange/orange juice consumption in the prior 5 days, amenorrhea for $\geq$12 months, tenofovir use, race.

[a] When the metabolizer genotypes are considered together and statistically tested non-linear effect is observed producing a quadratic p-value of 0.022.

Table 4-2. Factors Associated With Short-Term Efavirenz Exposure Among 111 Subjects From the Women's Interagency HIV Study

Factor	Fold-Effect on AUC (95% CI)	*P*	Distribution of Factor in Sample
Oranges or orange juice in preceding 5 d	1.26 (1.05-1.50)	0.012	consumed by 76 (68.5%)
For every doubling of ALT level	1.23 (1.11-1.36)	0.0001	Median ALT level 23 IU/L (range, 8-117 IU/L)
CYP2B6 516 G>T (rs3745274)	...		53 (47.7%), 0 doses
0 or 1 dose of minor allele (GG, GT)	1.00	1.4×10^{-18}	44 (39.6%),1 dose
2 doses of minor allele (TT)	3.5 (2.7-4.5)		14 (12.6%), 2 doses
	...		95 (85.6%), 0 doses
CYP2B6 983 T>C (rs28399499)			
0 doses of minor allele (TT)	1.00	2.2×10^{-10}	15 (13.5%), 1 dose
1 or 2 doses of minor allele (TC, CC)	1.96 (1.54-2.5)		1 (0.9%), 2 doses
	...		14 (12.6%), 0 doses
ABCB1 haplotype (2 SNPs: rs7779562 and rs4148745)			
0 doses of the haplotype	1.00		48 (43.2%), 1 dose
1 or 2 doses of the haplotype	1.60 (1.24-2.1)	0.0004	49 (44.1%), 2 doses

Note. ALT, alanine aminotransferase; AUC, area under the concentration time curve; CI, confidence interval (Gandhi et al., 2012).

Chapter 5

Efavirenz Symptoms and Side Effects Genetic Associations

Introduction

Patients treated with antiretroviral therapy require lifelong treatment and face the challenge of treatment-related symptoms and side effects that may affect their quality of life and adherence to therapy. Genetic factors contribute to variations in drug concentration and may have predictive value in determining which patients will be susceptible to therapy discontinuation due to these symptoms and side effects (Fox, Boffito, &Winston, 2006; Gandhi et al., 2012).

The focus of efavirenz exposure and adverse events moderated by pharmacogenetics has primarily centered on the central nervous system (CNS) (Haas et al., 2004; King & Alberg, 2008; Ribaudo et al., 2010; Rotger et al., 2005). This is an important area of study as up to 40% of patients receiving efavirenz experience CNS-related adverse events (Gazzard, 1999; Marzolini et al., 2001). Because of the severity or persistence of these adverse events, approximately 4% of patients discontinue efavirenz (Marzolini et al., 2001; Nelson & Silleni, 1999). Studies have repeatedly demonstrated an association between efavirenz plasma concentration and CNS toxicity including dizziness, abnormal dreams, and insomnia (Gallego et al., 2004; Marzolini et al., 2001; Nunez et al., 2001). Pharmacokinetic focused candidate gene studies have consistently found the CYP2B6 c.516G>T (rs3745274) T allele to be associated with higher efavirenz exposure and more recently with CNS-associated adverse events (Haas et al., 2004; King & Alberg, 2008; Ribaudo et al., 2010; Rotger et al., 2005). These studies, though important, have left other less prevalent but potentially important symptoms and side effects relatively unexplored.

A recent study investigating adverse events in patients who discontinued first-line therapy, which included efavirenz, reported that the most represented adverse events were gastrointestinal (28.5%), hematological (13.2%), metabolic (lipid and glucose abnormalities and fat redistribution) (11.3%), hypersensitivity (9.3%) and CNS (6.0%) (Prosperi et al., 2012). When considering the drugs included in this study individually, a higher probability of discontinuation for adverse events was observed for efavirenz (Prosperi et al, 2012). Though research is limited, a few non-CNS adverse events (rash and hepatic abnormalities) are associated with an increase in efavirenz exposure (Kappelhoff et al., 2005). Some of these adverse events could be due to pharmacogenetics however our understanding of the contribution to non-CNS adverse events remains incomplete.

The Pharmacogenetic Substudy, first presented in Chapter 4, reported three alleles from two genes associated with an increase in efavirenz exposure: CYP2B6 c.516G>T (rs3745274); CYP2B6 c.983T>C (rs28399499), ABCB1 Haplotype A1. Individuals homozygous for the CYP2B6 rs3745274 T allele displayed a greater than 3-fold increase in AUC compared with individuals with the TG or GG genotypes. Individuals homozygous or heterozygous for the CYP2B6 rs28399499 C allele (i.e., CC or CT genotype) displayed a nearly 2-fold increase in AUC compared to individuals with the TT genotype. The ABCB1 haplotype A1 (i.e., composed of the common alleles from two SNPs: rs7779562 C and rs418745 G) was associated with a 1.60-fold increase in AUC in individuals with either one or two doses of the haplotype (Gandhi et al., 2012). These findings validated previously published research and provided a platform to explore the

relationship between gene variations known to impact efavirenz pharmacokinetics and their potential relationship with non-CNS adverse events.

The purpose of this study was to describe the association of a broad spectrum of symptoms and side effects with genotype in study participants included in the WIHS Intensive Pharmacokinetic Substudy.

Methods

The overall methods for this analysis are outlined in Chapter 3. The two SNPs and one haplotype associated with efavirenz increase in AUC and described in Chapter 4, were evaluated for their associations with adverse effects (i.e., CYP2B6 rs3745274 assuming a recessive model, CYP2B6 rs28399499 assuming a dominant model, ABCB1 Haplotype A1 [common alleles from two SNPs: rs27779562 C and rs4148745 G] assuming a dominant model) (Gandhi et al., 2012; Lunetta, 2008). A combined genotype (diplotype) of rs3745274 and rs28399499, entitled the CYP2B6 "Metabolizer" diplotype and delineated as "slow", "intermediate", and "fast" metabolizers was also included for analysis. Slow metabolizer is composed of rare alleles (i.e., rs3745274 TT and rs28399499 CC or rs3745274 GT with rs28399499 TC); the intermediate metabolizer denotes a single rare allele at either rs3745274 or rs28399499 but not both; and fast metabolizer is composed of only common alleles at both rs3745274 or rs28399499 (Haas et al., 2009). This haplotype was included because the effect of rs3745274 and rs28399499 combined was observed in studies to be predictive of efavirenz exposure (Haas et al., 2009; Solus et al, 2004; Wang et al., 2006).

All variables were selected from data collected during the WIHS Intensive Pharmacokinetic Substudy and from WIHS core data. The main categories of variables assessed were: demographic and general clinical; general side effects; gastrointestinal-related side effects; neuropathic and pain-related; neurological, psychological, and fatigue-related; and body habitus.

Inclusion Criteria

To be included in this analysis, an inclusion criterion was imposed requiring at least 61 study participant responses (50%) for each respective variable. This criteria was imposed to avoid making unreliable inferences based on small sample sizes. Eighty-nine variables did not meet this criterion and were excluded from the analyses. Though data for only 111 study participants were included in the pharmacogenetic substudy analyses, 10 additional participants from the original WIHS Intensive Pharmacokinetic Substudy were successfully genotyped and included in this descriptive study.

Statistical Analyses

Univariate analyses were performed to examine the distribution of the continuous, ordinal, and categorical variables of interest and summarize the sample characteristics. Bivariate analyses were performed to explore the association between the individual SNP genotypes, metabolizer phenotype, and haplotype and each variable of interest. All analyses were conducted using Stata (version 12.1, College Station, TX).

Results

Characteristics of the Study Population. The mean age of the study participants was 43 $\pm$ 8 years. Participants self-reported as Caucasian Non-Hispanic (10), Caucasian

Hispanic (4), African American Non-Hispanic (88), African American Hispanic (5), other Hispanic (13), and Native American/Alaskan (1). Genotype frequencies by self-reported ethnic group are presented in Table 5-1. For rs3745274 and rs2839959, the observed minor allele frequencies were in relative agreement with published data. Table 5-2 lists the distribution of variables investigated in this analysis from the WIHS Intensive Pharmacokinetic Substudy.

Genetic associations of ABCB1 haplotype A1. There was no evidence of association with demographic and general clinical characteristics, therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms , observed for the ABCB1 haplotype A1, as shown in Tables 5-3 through 5-9.

Genetic associations of CYP2B6 rs3745274. Individuals homozygous for the CYP2B6 rs3745274 T rare allele (i.e., TT genotype) were less likely to be smoking currently as compared to individuals that carried the common G allele (i.e., GG or GT genotypes) (p=0.03), Table 5-3. CYP2B6 rs3745274 TT homozygotes were more likely to change their diet to influence their body shape as compared to G allele carriers (p=0.02), Table 5-9. No evidence of association with therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms, was observed for CYP2B6 rs3745274 as shown in Tables 5-4 through 5-8.

Genetic associations of CYP2B6 rs28399499. Associations with CYP2B6 rs28399499 and body habitus were observed (Table 5-9). Carriers of the rare C allele (i.e., CC or TC genotype) were less likely to report a change in the amount of fat in their buttocks (p=0.02). Additionally, carriers of the rare C allele were less likely to report

mild, moderate, and severe changes in buttock fat (p=0.04), Table 5-9. No evidence of association with demographic and general clinical characteristics, therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms was observed for CYP2B6 rs28399499, as shown in Tables 5-4 through 5-8.

Genetic associations of CYP2B6 Metabolizer Diplotype. Individuals designated as CYP2B6 “slow” metabolizers were less likely to be smoking currently (p=0.04) as compared to “intermediate” and “fast” metabolizers, Table 5-3. Individuals designated as CYP2B6 “intermediate” metabolizers were more likely to report a change in the amount of fat in legs (p=0.04) as compared to “slow” or “fast” metabolizers, Table 5-9. No evidence of association with therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms, was observed for the CYP2B6 Metabolizer haplotype, as shown in Tables 5-4 through 5-8.

Discussion

Efavirenz is mainly metabolized by the CYP2B6 enzyme and certain CYP2B6 SNPs are useful predictors of the pharmacokinetics of this drug (Gandhi et al., 2012; Haas et al., 2004; Zanger & Klein, 2013). Higher plasma efavirenz levels and higher discontinuation rates have consistently been linked to these SNPs (Gandhi et al., 2012; Lubomirov et al., 2011; Wyen et al., 2008; 2011). However, aside from CNS-associated adverse events, less prevalent but potentially important symptoms and side effects have been relatively unexplored. Limiting the scope this analysis to risk alleles associated with increased efavirenz exposure provided a focused approach and reduced the probability of a Type I error.

In our study, individuals homozygous for the CYP2B6 rs3745274 T rare allele (i.e., TT genotype) and individuals designated as CYP2B6 "slow" metabolizers were less likely to be currently smoking compared to individuals with the GT/TT genotype and "intermediate/"fast" metabolizers, respectively. The CYP2B6 enzyme has been reported to metabolize nicotine, although to a lesser extent than CYP2A6, which mediates approximately 80% of nicotine metabolism. Few studies have examined the SNPs that affect CYP2B6 enzyme activity and nicotine metabolism and the study findings were contradictory. In one study, the CYP2B6 rs3745274 T rare allele was found to be associated with increased rates of nicotine metabolism (Ring et al., 2007). Another study found no association between CYP2B6 rs3745274 and nicotine metabolism (Lee et al., 2007).

CYP2B6 and CYP2A6 co-regulation is important to consider given the apparent overlapping, albeit oppositely weighted, metabolism pathways of nicotine (i.e., primary metabolism by CYP2A6, secondary metabolism by CYP2B6) and efavirenz (i.e., primary metabolism by CYP2B6, secondary metabolism by CYP2A6). The complementary relationship between CYP2B6 and CYP2A6 and the effect on efavirenz drug concentration was only recently discovered. Very high efavirenz drug concentration levels were noted in the presence of impaired CYP2B6 function and CYP2A6 gene variations with known reduced enzyme activity (Arab-Alameddine et al., 2009; di Iulio et al., 2009). It was theorized that because efavirenz is metabolized into three primary metabolites (i.e., 8-hydrox-EFV, 7-hydroxy-EFV, N-glucuronide-EFV), when CYP2B6 function is impaired the relevance of CYP2A6, the main isoenzyme responsible for 7-

hydroxylation, increases (di Iulio et al., 2009). A subsequent efavirenz metabolism characterization study confirmed that CYP2A6-mediated efavirenz 7-hydroxylation accounts for approximately 23% of efavirenz metabolism (Ogburn et al., 2010).

Analogously, a complementary relationship was recently observed in nicotine metabolism, though opposite in direction to the efavirenz findings. Among individuals with decreased CYP2A6 function, the CYP2B6 rare allele carriers (i.e., poor metabolizers), had a higher nicotine clearance than those with the CYP2B6 common genotype. The authors concluded that the magnitude of the CYP2B6 effect on nicotine metabolism depended on the decreased or normal CYP2A6 activity as inferred by genotype in the individual (Ring et al., 2007). However, in addition to highlighting the complimentary relationship between CYP2B6 and CYP2A6, these two studies also underscore that the same variants in CYP2B6 appear to display opposite effects towards different substrate drugs (i.e., decreased clearance and concomitant high efavirenz concentration, and increased clearance and low nicotine concentration) (Arab-Alameddine et al., 2009; Ring et al., 2007).

Nicotine is responsible for the reinforcing effects of cigarette smoking and smokers regulate their cigarette use to maintain desirable plasma nicotine concentrations (Benowitz, 1988; McMorrow & Foxx, 1983). It is thought that faster nicotine metabolizers are more prone to develop a smoking dependency to maintain a desired nicotine range (i.e., to offset the faster clearance of nicotine) (Audrain-McGovern et al., 2007; Ray, Tyndale, & Lerman, 2009). Studies have observed increased nicotine clearance in smokers versus nonsmokers, potentially related to cigarette

smoke-mediated CYP2B6 induction (Kyerematen, Damiano, Dvorchik, & Vessell, 1982; Kyerematen, Morgan, Chattopadhyay, deBethizy, & Vesell, 1990; Schoedel, Sellers, Palmour & Tyndale, 2002). Associations between CYP2A6 genotype and smoking status were evaluated in several studies (Munafo, Clark, Johnstone, Murphy, & Walton, 2004). Reduced or null activity CYP2A6 alleles are significantly more prevalent among nonsmokers of Caucasian, Japanese, and African-American descent (Ray et al., 2009). However, because CYP2A6 was not studied in our cohort, we cannot draw conclusions as to the interaction between CYP2B6, CYP2A6, nicotine metabolism, efavirenz exposure and side effects.

A recent study reported a statistically significant difference in efavirenz concentrations observed between smokers and nonsmokers (p=0.02) with lower drug concentrations in smokers versus nonsmokers (Cortes et al., 2013). Though CYP2B6 induction and the CYP2B6 rs3745274 T allele are associated with increased rates of nicotine metabolism (Ring et al., 2007), nicotine levels were not available for analysis in the current study.

Preliminary evidence of an association between pharmacogenetic SNPs and body habitus was observed in our study. Individuals with one or two doses of the CYP2B6 rs28399499 rare C allele were less likely to report a change in buttock fat had occurred and less likely to indicate that the change was mild, moderate and/or severe. Intermediate metabolizers were also more likely to report a change in the amount of fat in their legs.

A spectrum of body habitus changes, often termed lipodystrophy or fat maldistribution, reportedly occurs in 20%-80% of patients receiving antiretroviral therapy (Safrin, & Grunfeld, 1999; Sattler, 2003; Wanke, 1999). Epidemiological studies have observed that women are at increased risk of developing HIV therapy-associated body habitus changes (Milinkovic & Martinez, 2005). Sex differences in adipose tissue distribution is known (Bjorntorp, 1991) and differences in HIV therapy-associated body habitus changes between men and women has been suggested (Gali et al., 2003). However, most body habitus studies have underrepresented women and sex differences in the effect of antiretroviral therapy on body habitus changes is poorly understood (Tien et al., 2007).

Though the characteristic HIV therapy-associated body-shape changes are largely attributed to other antiretroviral classes, body habitus changes are a known side effect of efavirenz primarily in the form of lipoatrophy (Haubrich et al., 2009). Body habitus-related pharmacogenetic studies have thus far focused on the expression and modulation of genes that play a key role in determining the adipocyte phenotype and genes involved in lipid metabolism and cell cycle control (Eposito et al., 2012). This is the first study to describe CYP2B6 efavirenz exposure-associated SNPs, CYP2B6 metabolizer phenotype, ABCB1 haplotype and body habitus changes. Our findings support what has been reported with regard to antiretroviral exposure and body habitus. Several studies of both protease inhibitors and nucleotide reverse transcriptase inhibitors and one study that included efavirenz observed no association with high plasma concentration and body habitus changes (Autar et al., 2007; Gonzales

et al., 2003; Hofstede, Koopmans, Burger, 2007). Our pharmacogenetic analysis demonstrates that SNP genotypes associated with higher AUC are associated with less body habitus change. Other body habitus self-report responses, highlighted in yellow in Table 5-9, trended in a similar direction, lending support to the statistically significant findings. The U-shaped distribution of the association between amount of fat in the legs and metabolizer diplotype findings should be interpreted with caution. Though it is possible, that individuals with the intermediate metabolizer diplotype are more likely to experience a change in the amount of fat in legs as compared to the two other groups, the small sample size may have provided a suboptimal sampling of intermediate diplotype carriers. In addition, the fact that this U-shaped distribution has not been reported for other phenotypes examined in relation to the Metabolizer diplotype suggests that this observation may be due to chance (i.e., false positive). This finding warrants replication in an independent sample.

Of note, combination therapy is attributed to increasing the risk of developing lipodystrophy (Satler, 2003). Though 93% of the patients in this pharmacogenetic study were on combination therapy, insufficient sample size precluded pursing multivariate analyses to assess the relationship among age, combination therapy, genotype, and body habitus changes. Of note, the target antiretrovirals used in combination with efavirenz in this study population are not metabolized via CYP2B6 (i.e., CYP3A4 predominantly).

There are many obstacles in designing and conducting body habitus studies and comparatively assessing previously reported results. To date there is no universally

accepted definition of this phenomenon. There is also an absence of standardized case definitions, variability in the way fat distribution is clinically assessed, and variation in the collection methods of self-report changes (Eposito et al., 2012). Several national and international research groups have attempted to develop standardized and objective definitions for body habitus changes in patients with HIV. However, additional reproducibility testing is needed (Satler, 2003).

An important consideration in discussing the findings of our study are selection events that occur with efavirenz as a first-line therapeutic agent. Adverse events leading to efavirenz discontinuation are most likely to occur in the first five months of therapy (Shubber et al., 2013). The time frame of our study occurred well after the period of time when therapy is switched to minimize adverse events and maximize therapy efficacy. Two self-selection events may have skewed the study population under consideration: abnormally high drug concentrations leading to toxicity causing patients to discontinue efavirenz therapy or abnormally low drug concentrations leading to subtherapeutic effects causing patients to discontinue therapy prior to this study. Thus, the generalizability of these preliminary findings warrant replication in an independent cohort(s).

Limitations and Future Research Direction

This study has limitations that should be considered when interpreting the findings. A larger sample size would allow a more precise assessment of associations and the opportunity to perform multiple regression analysis to estimate the independent contributions of independent predictors of body habitus changes. An

additional limitation was the lack of objective measures with regard to body habitus changes. Future studies should feature the inclusion of objective measures of body fat (i.e., anthropometry, bioimpedance analysis, dual X-absorptiometry, magnetic resonance imaging, computed tomography, ultrasonography) (Milinkovic & Martinez, 2005). In this study body habitus was assessed by self-report and there are disadvantages to the use of self-report alone i.e., validity issues such as subjective interpretation (Schwarz, 2007). For example, how individuals differ in their definition and determination of mild, moderate, or severe in assessing a particular phenomenon. Optimally, self-report and clinical assessment would be supplemented by objective measures. Though consensus has not yet been achieved regarding the definition of body habitus changes, a model studied to develop an objective, sensitive, specific, and broadly applicable case definition showed an all inclusive model (i.e., self report, clinical assessment, laboratory, body measures) had the highest sensitivity 79% (95% confidence interval 70-85) and specificity 80% (95% confidence interval 71-87) (Carr et al., 2003).

Our findings regarding smoking leave unanswered questions that warrant further study. For example, did the findings in our study emerge due to a CYP2B6/CYP2A6 efavirenz metabolism interaction? Can the CYP2B6 rs3745274 predict smoking status in patients taking efavirenz? These questions cannot be answered without a larger sample size than is available currently.

Conclusions

This is the first study to report on CYP2B6 efavirenz exposure-associated SNPs, CYP2B6 metabolizer phenotype and body fat changes. This analysis demonstrated that SNP genotypes associated with higher AUC were associated with less body habitus changes. The findings also showed that study participants homozygous for the CYP2B6 rs3745274 T allele *and* slow metabolizer phenotype were less likely to be currently smoking. Further research is needed to confirm these findings.

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Table 5-1. WIHS Self-Reported Ethnicity

	Caucasian Non-Hispanic	Caucasian Hispanic	African American	African American Hispanic	Other Hispanic	Native American/ Alaskan
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
rs3745274						
GG	4 (40)	1 (25)	39 (44)	3 (75)	9 (69)	0 (0)
GT	5 (50)	2 (50)	35 (40)	1 (25)	4 (31)	1 (100)
TT	1 (10)	1 (25)	14 (16)	1 (25)	0 (0)	0 (0)
WIHS MAF (T)	0.35	0.50	0.36	0.30	0.15	0.50
Reference[a]	0.27	—	0.28	—	—	—
rs28399499						
TT	9 (100)	2 (100)	69 (83)	44 (100)	11 (85)	1 (100)
CT	0 (0)	0 (0)	13 (16)	0 (0)	2 (15)	0 (0)
CC	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
WIHS MAF (C)	0.00	0.00	0.09	0.00	0.08	0.00
Reference[a]	0.00	—	0.08	—	—	—
ABCB1_ HAPA1						
Zero doses	0 (0)	1 (50)	12 (14)	1 (20)	0 (0)	0 (0)
1 dose	0 (0)	0 (0)	39 (47)	2 (40)	8 (62)	0 (0)
2 doses	9 (100)	1 (50)	32 (39)	2 (40)	5 (38)	1 (100)
CYP2B6 Metabolizer[b]						
Slow	4 (40)	1 (25)	28 (34)	3 (75)	7 (54)	0 (0)
Intermediate	5 (50)	2 (50)	40 (48)	1 (25)	6 (46)	1 (100)
Fast	1 (10)	1 (25)	15 (18)	1 (25)	0 (0)	0 (0)

[a]Estimates of rare (minor) allele frequency (MAF) from public databases was only sought when greater than 10 observations in a racial or ethnic subgroup were available. CEU (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection) was used as reference for WIHS Caucasian Non-Hispanic; ASW (African ancestry in Southwest USA) was used as reference for African American); other Hispanic reference was not provided as this is not a well defined category.
[b]Comparison of WIHS data with published data shows relative agreement for CYP2B6 Metabolizer categories. e.g., 0.21 slow, 0.50 intermediate, 0.29 fast in the African American population (Haas, Gebrestadik, Mayo, Menon, Acosta et al, 2009).

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

Demographic and General Clinical Characteristics	
	Mean (SD)
Age	43 (8)
Race Distribution	**N (%)**
Caucasian Non-Hispanic	10 (8)
Caucasian Hispanic	4 (3)
African American Non-Hispanic	88 (73)
African American Hispanic	5 (4)
Other Hispanic	13 (11)
Native American/Alaskan	1 (1)
Drinking Group	
Abstainer	72 (60)
Light (<3 drinks/week)	37 (31)
Moderate (3-13 drinks/wk)	9 (7)
Heavier (>=14 drinks/wk)	3 (3)
Smoking Status	
Currently smoking	73 (60)
Not currently smoking	48 (40)
Smoking History	
Never smoked	27 (22)
Smoked	94 (78)
Marital Status	
Married/partnered	26 (22)
Not married or partnered	92 (78)
Education	
Some high school	40 (33)
Completed High school/some college/completed college	81 (67)
Low Red Blood Cell Count/anemia: intensity since last visit	
Not bad at all	94 (78)
Not bad	11 (9)
Bad	5 (4)
Very bad	1 (1)
Terrible	2(2)
Missing	8 (7)
Told CD4 count less than 200 (14%) since last visit	
Yes	19 (16)
No/never heard of it	102 (84)
Therapeutic Regime	
	N (%)
Planned medication break for 2 or more days in the past 3 months	
Yes	3 (2)
No	112 (93)
Took an unplanned medication break for 1 or more days in the past 3 months	
Yes	32 (28)
No	83 (72)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

How often took ARV in last 6 months	
100% of the time	63 (53)
95-99% of the time	33 (28)
75-94% of the time	19 (16)
<75% of the time	5 (4)
How closely medication schedule followed	
Never	7 (6)
Some of the time	9 (8)
About half of the time	4 (3)
Most of the time	39 (66)
All of the time	61 (51)
How often special instructions followed	
Some of the time	6 (8)
Most of the time	26 (37)
All of the time	39 (55)
Participant currently taking combination therapy	
Yes	113 (93)
No	8 (7)

General Side Effects

	N (%)
Fever: intensity since last visit	
Not bad at all	91 (80)
Not bad	12 (11)
Bad	6 (5)
Very bad	2 (2)
Terrible	3 (3)
Fever over 100F for more than 1 month since last visit	
Yes	6 (5)
No	115 (95)
Drenching night sweats since last visit	
Yes	23 (19)
No	98 (81)
	N (%)
Chills: intensity since last visit	
Not bad at all	89 (78)
Not bad	14 (12)
Bad	6 (5)
Very bad	2 (2)
Terrible	3 (3)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

Rash: intensity since last visit	
Not bad at all	93 (82)
Not bad	13 (11)
Bad	5 (4)
Very bad	2 (2)
Terrible	1 (1)
Dry mouth: intensity since last visit	
Not bad at all	81 (71)
Not bad	15 (13)
Bad	10 (9)
Very bad	4 (4)
Terrible	4 (4)
Gastrointestinal-Related Symptoms	
	N (%)
Lack of appetite: intensity since last visit	
Not bad at all	85 (75)
Not bad	12 (11)
Bad	10 (9)
Very bad	5 (4)
Terrible	2 (2)
Nausea and/or vomiting: Intensity since last visit	
Not bad at all	87 (77)
Not bad	12 (11)
Bad	11 (10)
Very bad	3 (3)
Diarrhea: intensity since last visit	
Not bad at all	90 (79)
Not bad	11 (10)
Bad	9 (8)
Very bad	4 (4)
	N (%)
Constipation: intensity since last visit	
Not bad at all	93 (82)
Not bad	16 (14)
Bad	3 (3)
Very bad	1 (1)
Terrible	1 (1)
Neuropathic and Pain-Related Symptoms	
	Mean (SD)
Pain	71 (26)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

	N (%)
Headache: intensity since last visit	
Not bad at all	54 (47)
Not bad	40 (35)
Bad	11 (10)
Very bad	5 (5)
Terrible	4 (4)
Numbness, tingling, burning in arms/legs/hands for more than 2 weeks since last visit	
Yes	14 (12)
No	107 (88)
Pain/tingling in feet or hands: intensity since last visit	
Not bad at all	86 (75)
Not bad	12 (11)
Bad	10 (9)
Very bad	3 (3)
Terrible	3 (3)
Muscle aches or pains: intensity since last visit	
Not bad at all	68 (60)
Not bad	15 (13)
Bad	15 (13)
Very bad	9 (8)
Terrible	7 (6)
Abdominal pains or cramps: intensity since last visit	
Not bad at all	81 (71)
Not bad	16 (14)
Bad	8 (7)
Very bad	7 (6)
Terrible	2 (2)
Neurological, Psychological, and Fatigue-Related Symptoms	
	Mean (SD)
Emotional well-being	71 (27)
Overall depression score	14 (12)
Energy/Fatigue function	56 (26)
	N (%)
Drowsiness/tiredness: intensity since last visit	
Not bad at all	54 (47)
Not bad	24 (21)
Bad	22 (19)
Very bad	11 (10)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

Terrible	3 (3)
Dizziness or lack of concentration: intensity since last visit	
Not bad at al	89 (79)
Not bad	10 (9)
Bad	11 (10)
Very bad	3 (3)
Major memory/concentration problems for more than 2 weeks since last visit	
Yes	3 (2)
No	118 (98)
Confusion, getting lost, difficulty with mental tasks since last visit	
Yes	3 (2)
No	118 (98)
Body Habitus	
	N (%)
Unintentional weight loss 10 lbs or more >1 month since last visit	
Yes	21 (17)
No	100 (83)
Changes in shape of body, increase/decrease in fat since last visit	
Yes	62 (51)
No	59 (49)
Shift of body fats: intensity since last visit	
Not bad at all	89 (78)
Not bad	11 (10)
Bad	9 (8)
Very bad	4 (4)
Terrible	1 (1)
	N (%)
Change in the shape of face	
Yes	25 (40)
No	37 (60)
Face increase or decrease	
Decrease	14 (23)
No change	37 (60)
Increase	11 (18)
Severity of face change	
None	37 (60)
Mild	13 (21)
Moderate	7 (11)
Severe	5 (8)
Change in the amount of fat in cheeks	
Yes	26 (42)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

No	36 (58)
Fat in cheeks increase or decrease	
Decrease	14 (23)
No change	36 (58)
Increase	12 (19)
Severity of cheek change	
None	36 (52)
Mild	17 (25)
Moderate	7 (10)
Severe	9 (13)
Change in the size of neck	
Yes	18 (29)
No	44 (71)
Size of neck change increase or decrease	
Decrease	9 (15)
No change	44 (71)
Increase	9 (15)
Severity of neck change	
None	44 (71)
Mild	11 (18)
Moderate	5 (8)
Severe	2 (3)
Change in the amount of fat in upper back	
Yes	21 (34)
No	41 (66)
	N (%)
Fat in upper back increase or decrease	
Decrease	7 (11)
No change	41 (66)
Increase	14 (23)
Severity of upper back change	
None	41 (66)
Mild	11 (18)
Moderate	7 (11)
Severe	3 (5)
Change in size of one/both breasts	
Yes	29 (24)
No	33 (27)
Breast size increase or decrease	
Decrease	12 (19)
No change	33 (53)
Increase	17 (27)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

Severity of breast change	
None	33 (53)
Mild	19 (31)
Moderate	7 (11)
Severe	3 (5)
Change in amount of fat in arms	
Yes	30 (48)
No	32 (52)
Amount of fat in arms increase or decrease	
Decrease	16 (26)
No change	32 (52)
Increase	14 (23)
Severity of arm change	
None	32 (52)
Mild	18 (29)
Moderate	9 (15)
Severe	3 (5)
Change in the size of belly/abdominal fat	
Yes	47 (76)
No	15 (24)
	N (%)
Belly/abdominal fat increase or decrease	
Decrease	17 (27)
No change	15 (24)
Increase	30 (48)
Severity of belly change	
None	15 (24)
Mild	23 (37)
Moderate	16 (26)
Severe	8 (13)
Change of Waist size	
Yes	46 (74)
No	16 (26)
Waist increase or decrease	
Decrease	20 (32)
No change	16 (26)
Increase	26 (42)
Severity of waist change	
None	16 (26)
Mild	24 (39)
Moderate	15 (24)
Severe	7 (11)

Table 5-2. Distribution of Variables in the WIHS Intensive Pharmacokinetic Substudy

	N (%)
Change in the amount of fat in buttocks	
Yes	43 (69)
No	19 (31)
Amount of fat in buttocks increase or decrease	
Decrease	24 (39)
No change	19 (31)
Increase	19 (31)
Severity of buttocks change	
None	19 (31)
Mild	22 (35)
Moderate	12 (19)
Severe	9 (15)
Change in the amount of fat in legs	
Yes	38 (61)
No	24 (39)
Amount of fat in legs increase or decrease	
Decrease	25 (40)
No change	24 (39)
Increase	13 (21)
	N (%)
Severity of leg change	
None	24 (39)
Mild	21 (34)
Moderate	10 (16)
Severe	7 (11)
Changed diet to influence body shape	
Yes	22 (18)
No	99 (82)
Changed HIV medications to influence body shape	
Yes	26 (23)
no	89 (77)
Changed exercise habits to influence body shape	
Yes	17 (14)
No	104 (86)
Taken nutritional supplements to influence body shape	
Yes	34 (30)
No	81 (70)
Taken growth hormones/steroids to influence body shape	
Yes	2 (2)
No	113 (98)

Note. These data represent the variables that met the inclusion criteria for analysis of at least 50% reporting (i.e., responses from 61 study participants for each respective variable).

Table 5-3. Genetic association with demographic and general clinical characteristics

	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Factors / **Genotype Mean (SD)**	**GG/GT Mean (SD)**	**TT Mean (SD)**	**TT Mean (SD)**	**CT/CC Mean (SD)**	**Fast Mean (SD)**	**Int. Mean (SD)**	**Slow Mean (SD)**	**Zero doses Mean (SD)**	**1 or 2 doses Mean (SD)**
Age (Mean/SD)	43(8)	43(8)	41(9)	43(8)	42(7)	44(1)	42(1)	41(8)	43(8)
P Value	0.17		0.36		0.19			0.23	
Factors / **Genotype *N (%)***	**GG/GT *N (%)***	**TT *N (%)***	**TT *N (%)***	**CT/CC *N (%)***	**Slow *N (%)***	**Int. *N (%)***	**Fast *N (%)***	**Zero doses *N (%)***	**1 or 2 doses *N (%)***
Race Distribution									
Caucasian Non-Hispanic	9 (90)	1 (10)	9 (90)	0 (0)	4 (40)	5 (50)	1 (10)	0 (0)	9 (90)
Caucasian Hispanic	3 (75)	1 (25)	2 (50)	0 (0)	1 (25)	2 (50)	1 (25)	1 (25)	1 (25)
African American Non-Hispanic	74 (84)	14 (16)	69 (78)	14 (16)	28 (32)	40 (45)	15 (17)	12 (14)	71 (81)
African American Hispanic	4 (80)	1 (20)	5 (100)	0 (0)	3 (60)	1 (20)	1 (20)	1 (20)	4 (80)
Other Hispanic	13 (100)	0 (0)	11 (85)	2 (15)	7 (54)	6 (46)	0 (0)	0 (0)	13 (100)
Native American/Alaskan	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
P Value	0.49		0.75		0.65			0.21	
Drinking Group									
Abstainer	61 (59)	11 (65)	57 (59)	11 (69)	26 (60)	33 (60)	11 (61)	11 (79)	58 (59)
Light (<3 drinks/week)	32 (31)	5 (29)	29 (30)	5 (31)	12 (28)	16 (29)	6 (33)	3 (21)	30 (30)
Moderate (3-13 drinks/week)	9 (9)	0 (0)	9 (9)	0 (0)	4 (9)	5 (9)	0 (0)	0 (0)	9 (9)
Heavier (>=14 drinks/week)	2 (2)	1 (6)	2 (2)	0 (0)	1 (2)	1 (2)	1 (6)	0 (0)	2 (2)
P Value	0.65		0.38		0.98			0.18	
Factors / **Genotype *N (%)***	**GG/GT *N (%)***	**TT *N (%)***	**TT *N (%)***	**CT/CC *N (%)***	**Fast *N (%)***	**Int. *N (%)***	**Slow *N (%)***	**Zero doses *N (%)***	**1 or 2 doses *N (%)***
Smoking Status									
Currently smoking	67 (64)	6 (35)	38 (39)	8 (50)	27 (63)	37 (67)	6 (33)	9 (64)	58 (59)
Not currently smoking	37 (36)	11 (65)	59 (61)	8 (50)	16 (37)	18 (33)	12 (67)	5 (36)	41 (41)
P Value	0.03		0.42		0.04			0.78	

Table 5-3. Genetic association with demographic and general clinical characteristics

	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Smoking History									
Never smoked	21 (20)	6 (35)	20 (21)	6 (38)	8 (19)	10 (18)	7 (39)	3 (21)	23 (23)
Smoked	83 (80)	11 (65)	77 (79)	10 (63)	35 (81)	45 (82)	11 (61)	11 (79)	76 (77)
P Value	0.20		0.19		0.17			1.00	
Marital Status									
Married/partnered	24 (24)	2 (12)	22 (23)	3 (19)	10 (24)	11 (21)	3 (17)	3 (21)	23 (24)
Not married or partnered	77 (76)	15 (88)	72 (77)	13 (81)	32 (76)	42 (79)	15 (83)	11 (79)	73 (76)
P Value	0.35		1.00		0.86			1.00	
Education									
Some high school	37 (36)	3 (18)	32 (33)	7 (44)	16 (37)	20 (36)	3 (17)	6 (43)	32 (32)
Completed High school/some college/completed college	67 (64)	14 (82)	65 (67)	9 (56)	27 (63)	35 (64)	15 (83)	8 (57)	67 (68)
P Value	0.17		0.40		0.26			0.55	
Genotype *N (%)* **Factors**	**GG/GT *N (%)***	**TT *N (%)***	**TT *N (%)***	**CT/CC *N (%)***	**Fast *N (%)***	**Int. *N (%)***	**Slow *N (%)***	**Zero doses *N (%)***	**1 or 2 doses *N (%)***
Low Red Blood Cell Count/anemia: intensity since last visit									
Not bad at all	80 (82)	14 (88)	73 (81)	13 (87)	28 (74)	48 (91)	15 (88)	11 (79)	75 (82)
Not bad	10 (10)	1 (6)	10 (11)	1 (7)	7 (18)	2 (4)	1 (6)	3 (21)	8 (9)
Bad	4 (4)	1 (6)	4 (4)	1 (7)	1 (3)	2 (4)	1 (6)	0 (0)	5 (5)
Very bad	1 (1)	0 (0)	1 (1)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	1 (1)
Terrible	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	0 (0)	2 (2)
P Value	0.74		0.73		0.30			0.98	
Told CD4 count less than 200 (14%) since last visit									
Yes	16 (15)	3 (18)	17 (18)	0 (0)	11 (26)	5 (9)	3 (17)	1 (7)	16 (16)
No/never heard of it	88 (85)	14 (82)	80 (82)	16 (100)	32 (74)	50 (91)	15 (83)	13 (93)	83 (84)
P Value	0.73		0.12		0.08			0.69	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-4. Genetic association with therapeutic regime

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
Planned medication break for 2 or more days in the past 3 months									
Yes	2 (2)	1 (6)	2 (2)	1 (6)	0 (0)	2 (4)	1 (6)	0 (0)	3 (3)
No	97 (98)	15 (94)	89 (98)	15 (94)	39 (100)	52 (96)	16 (94)	14 (100)	90 (97)
P Value	0.37		0.38		0.27			1.00	
Unplanned medication break for 1 or more days in the past 3 months									
Yes	2 (2)	1 (6)	24 (26)	4 (25)	10 (26)	16 (30)	4 (24)	4 (29)	24 (26)
No	97 (98)	15 (94)	67 (74)	12 (75)	29 (74)	38 (70)	13 (76)	10 (71)	69 (74)
P Value	1.00		1.00		0.87			0.75	
How often took ARV in last 6 months									
100% of the time	54 (52)	9 (53)	52 (54)	7 (44)	27 (63)	26 (48)	9 (50)	10 (71)	49 (50)
95-99% of the time	28 (27)	5 (29)	26 (27)	6 (38)	7 (16)	17 (31)	6 (33)	3 (21)	30 (31)
75-94% of the time	17 (17)	2 (12)	15 (16)	2 (13)	7 (16)	9 (17)	2 (11)	1 (7)	15 (15)
<75% of the time	4 (4)	1 (6)	3 (3)	1 (6)	2 (5)	2 (4)	1 (6)	0 (0)	4 (4)
P Value	0.93		0.56		0.63			0.15	
Currently taking combination therapy									
Yes	97 (93)	16 (94)	90 (93)	15 (94)	39 (91)	53 (96)	17 (94)	13 (93)	92 (93)
No	7 (7)	1 (6)	7 (7)	1 (6)	4 (9)	2 (4)	1 (6)	1 (7)	7 (7)
P Value	1.00		1.00		0.58			1.00	
How closely medication schedule followed									
Never	7 (7)	0 (0)	6 (6)	0 (0)	2 (5)	5 (9)	0 (0)	0 (0)	6 (6)
Some of the time	8 (8)	1 (6)	5 (5)	3 (19)	1 (2)	7 (13)	1 (6)	3 (21)	5 (5)
About half of the time	3 (3)	1 (6)	4 (4)	0 (0)	1 (2)	2 (4)	1 (6)	0 (0)	4 (4)
Most of the time	33 (32)	6 (35)	31 (32)	5 (31)	15 (35)	15 (28)	7 (39)	4 (29)	31 (32)
All of the time	52 (50)	9 (53)	50 (52)	8 (50)	24 (56)	25 (46)	9 (50)	7 (50)	52 (53)
P Value	0.69		0.85		0.37			0.76	
How often special instructions followed									
Some of the time	6 (10)	0 (0)	1 (2)	3 (43)	0 (0)	4 (15)	0 (0)	2 (25)	3 (5)
Most of the time	23 (37)	3 (33)	24 (41)	2 (29)	13 (42)	9 (33)	3 (30)	3 (38)	22 (38)
All of the time	33 (53)	6 (67)	34 (58)	2 (29)	18 (58)	14 (52)	7 (70)	3 (38)	33 (57)

Table 5-4. Genetic association with therapeutic regime

Factors / Genotype *N (%)*	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
P Value	0.64		0.05		0.50			0.21	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be available for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-5. Genetic association with general side effects

Factors / Genotype *N (%)*	CYP2B6 rs3745274 c.516G>T GG/GT *N (%)*	CYP2B6 rs3745274 c.516G>T TT *N (%)*	CYP2B6 rs28399499 c.983T>C TT *N (%)*	CYP2B6 rs28399499 c.983T>C CT/CC *N (%)*	CYP2B6 Metabolizer Fast *N (%)*	CYP2B6 Metabolizer Int. *N (%)*	CYP2B6 Metabolizer Slow *N (%)*	ABCB1_ HAPA1 Zero doses *N (%)*	ABCB1_ HAPA1 1 or 2 doses *N (%)*
Fever: intensity since last visit									
Not bad at all	79 (81)	12 (75)	45 (49)	5 (33)	31 (79)	44 (83)	13 (76)	12 (86)	72 (78)
Not bad	9 (9)	3 (19)	30 (33)	8 (53)	5 (13)	3 (6)	3 (18)	1 (7)	11 (12)
Bad	6 (6)	0 (0)	9 (10)	1 (7)	2 (5)	3 (6)	0 (0)	1 (7)	4 (4)
Very bad	2 (2)	0 (0)	5 (5)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	2 (2)
Terrible	2 (2)	1 (6)	2 (2)	1 (7)	1 (3)	1 (2)	1 (6)	0 (0)	3 (3)
P Value	0.75		0.58		0.94			0.64	
Fever over 100F for more than 1 month since last visit									
Yes	6 (6)	0 (0)	6 (6)	0 (0)	3 (7)	3 (5)	0 (0)	1 (7)	5 (5)
No	98 (94)	17 (100)	91 (94)	16 (100)	40 (93)	52 (95)	18 (100)	13 (93)	94 (95)
P Value	0.59		0.59		0.74			0.55	
Drenching night sweats since last visit									
Yes	21 (20)	2 (12)	20 (21)	2 (13)	10 (23)	10 (18)	2 (11)	3 (21)	19 (19)
No	83 (80)	15 (88)	77 (79)	14 (88)	33 (77)	45 (82)	16 (89)	11 (79)	80 (81)
P Value	0.52		0.73		0.62			1.00	
Chills: intensity since last visit									
Not bad at al	74 (76)	15 (94)	69 (76)	14 (93)	29 (74)	40 (75)	16 (94)	11 (79)	71 (77)
Not bad	13 (13)	1 (6)	12 (13)	1 (7)	5 (13)	7 (13)	1 (6)	3 (21)	11 (12)
Bad	6 (6)	0 (0)	5 (5)	0 (0)	2 (5)	4 (8)	0 (0)	0 (0)	5 (5)
Very bad	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	0 (0)	2 (2)
Terrible	3 (3)	0 (0)	3 (3)	0 (0)	2 (5)	1 (2)	0 (0)	0 (0)	3 (3)
P Value	0.22		0.25		0.42			0.82	
Rash: intensity since last visit									
Not bad at all	80 (82)	13 (81)	72 (79)	14 (93)	32 (82)	43 (81)	14 (82)	12 (86)	74 (80)
Not bad	11 (11)	2 (13)	12 (13)	0 (0)	4 (10)	7 (13)	2 (12)	0 (0)	12 (13)
Bad	4 (4)	1 (6)	5 (5)	0 (0)	2 (5)	2 (4)	1 (6)	0 (0)	5 (5)
Very bad	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	1 (7)	1 (1)
Terrible	1 (1)	0 (0)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	1 (7)	0 (0)
P Value	1.00		0.42		0.99			0.87	

Table 5-5. Genetic association with general side effects

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
Dry mouth: intensity since last visit									
Not bad at all	69 (70)	12 (75)	62 (68)	13 (87)	25 (64)	39 (74)	13 (76)	8 (57)	67 (73)
Not bad	14 (14)	1 (6)	14 (15)	0 (0)	8 (21)	6 (11)	1 (6)	2 (14)	11 (12)
Bad	8 (8)	2 (13)	9 (10)	1 (7)	2 (5)	6 (11)	2 (12)	3 (21)	7 (8)
Very bad	4 (4)	0 (0)	2 (2)	1 (7)	2 (5)	1 (2)	0 (0)	0 (0)	4 (4)
Terrible	3 (3)	1 (6)	4 (4)	0 (0)	2 (5)	1 (2)	1 (6)	1 (7)	3 (3)
P Value	0.84		0.30		0.71			0.33	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-6. Genetic association with gastrointestinal-related symptoms

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
Lack of appetite: intensity since last visit									
Not bad at all	74 (76)	11 (69)	66 (73)	12 (80)	30 (77)	39 (74)	12 (71)	11 (79)	67 (73)
Not bad	9 (9)	3 (19)	9 (10)	3 (20)	3 (8)	5 (9)	3 (18)	2 (14)	10 (11)
Bad	9 (9)	1 (6)	10 (11)	0 (0)	3 (8)	6 (11)	1 (6)	0 (0)	10 (11)
Very bad	4 (4)	1 (6)	4 (4)	0 (0)	2 (5)	2 (4)	1 (6)	1 (7)	3 (3)
Terrible	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	0 (0)	2 (2)
P Value	0.66		0.49		0.95			0.68	
Nausea and/or vomiting: Intensity since last visit									
Not bad at all	75 (77)	12 (75)	67 (74)	13 (87)	30 (77)	39 (75)	13 (76)	11 (79)	69 (76)
Not bad	10 (10)	2 (13)	11 (12)	0 (0)	6 (15)	4 (8)	2 (12)	1 (7)	10 (11)
Bad	10 (10)	1 (6)	10 (11)	1 (7)	3 (8)	7 (13)	1 (6)	1 (7)	10 (11)
Very bad	2 (2)	1 (6)	2 (2)	1 (7)	0 (0)	2 (4)	1 (6)	1 (7)	2 (2)
P Value	0.86		0.53		0.94			0.92	
Diarrhea: intensity since last visit									
Not bad at all	77 (79)	13 (81)	72 (79)	12 (80)	33 (85)	40 (75)	14 (82)	11 (79)	72 (78)
Not bad	9 (9)	2 (13)	9 (10)	1 (7)	2 (5)	6 (11)	2 (12)	0 (0)	10 (11)
Bad	8 (8)	1 (6)	7 (8)	1 (7)	3 (8)	4 (8)	1 (6)	2 (14)	7 (8)
Very bad	4 (4)	0 (0)	3 (3)	1 (7)	1 (3)	3 (6)	0 (0)	1 (7)	3 (3)
P Value	0.83		1.00		0.73			0.89	
Constipation: intensity since last visit									
Not bad at all	79 (81)	14 (88)	74 (81)	13 (87)	29 (74)	47 (89)	15 (88)	9 (64)	77 (84)
Not bad	15 (15)	1 (6)	13 (14)	2 (13)	8 (21)	4 (8)	1 (6)	4 (29)	12 (13)
Bad	2 (2)	1 (6)	2 (2)	0 (0)	1 (3)	1 (2)	1 (6)	1 (7)	1 (1)
Very bad	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
Terrible	1 (1)	0 (0)	1 (1)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	1 (1)
P Value	0.68		0.71		0.49			0.24	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-7. Genetic association with neuropathic and pain-related symptoms

Factor	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Genotype Mean (SD)	**GG/GT Mean (SD)**	**TT Mean (SD)**	**TT Mean (SD)**	**CT/CC Mean (SD)**	**Fast Mean (SD)**	**Int. Mean (SD)**	**Slow Mean (SD)**	**Zero doses Mean (SD)**	**1 or 2 doses Mean (SD)**
Pain	71(26)	59(71)	12(6.7)	70(27)	80(23)	67(6)	71(5)	74(7)	79(25)
P Value	0.45		0.13		0.82			0.16	
Factor / **Genotype *N (%)***	**GG/GT *N (%)***	**TT *N (%)***	**TT *N (%)***	**CT/CC *N (%)***	**Fast *N (%)***	**Int. *N (%)***	**Slow *N (%)***	**Zero doses *N (%)***	**1 or 2 doses *N (%)***
Headache: intensity since last visit									
Not bad at all	49 (50)	5 (31)	45 (49)	5 (33)	19 (49)	28 (53)	5 (29)	6 (43)	44 (48)
Not bad	33 (34)	7 (44)	30 (33)	8 (53)	14 (36)	16 (30)	8 (47)	6 (43)	32 (35)
Bad	9 (9)	2 (13)	9 (10)	1 (7)	3 (8)	6 (11)	2 (12)	1 (7)	9 (10)
Very bad	5 (5)	0 (0)	5 (5)	0 (0)	2 (5)	3 (6)	0 (0)	0 (0)	5 (5)
Terrible	2 (2)	2 (13)	2 (2)	1 (7)	1 (3)	0 (0)	2 (12)	1 (7)	2 (2)
P Value	0.18		0.47		0.32			0.85	
Numbness, tingling, burning in arms/legs/hands for more than 2 weeks since last visit									
Yes	13 (13)	1 (6)	12 (12)	2 (13)	5 (12)	8 (15)	1 (6)	2 (14)	12 (12)
No	91 (88)	16 (94)	85 (88)	14 (88)	38 (88)	47 (85)	17 (94)	12 (86)	87 (88)
P Value	0.68		1.00		0.69			0.68	
Factor / **Genotype *N (%)***	**GG/GT *N (%)***	**TT *N (%)***	**TT *N (%)***	**CT/CC *N (%)***	**Fast *N (%)***	**Int. *N (%)***	**Slow *N (%)***	**Zero doses *N (%)***	**1 or 2 doses *N (%)***
Pain/tingling in feet or hands: intensity since last visit									
Not bad at all	74 (76)	12 (75)	67 (74)	13 (87)	26 (67)	42 (79)	13 (76)	11 (79)	69 (74)
Not bad	11 (11)	1 (6)	12 (13)	0 (0)	5 (13)	6 (11)	1 (6)	0 (0)	12 (13)
Bad	8 (8)	2 (13)	7 (8)	1 (7)	6 (15)	2 (4)	2 (12)	2 (14)	6 (6)
Very bad	2 (2)	1 (6)	2 (2)	1 (7)	0 (0)	2 (4)	1 (6)	0 (0)	3 (3)
Terrible	3 (3)	0 (0)	3 (3)	0 (0)	2 (5)	1 (2)	0 (0)	1 (7)	3 (3)
P Value	0.92		0.48		0.56			0.95	

Table 5-7. Genetic association with neuropathic and pain-related symptoms

	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Muscle aches or pains: intensity since last visit									
Not bad at all	59 (60)	9 (56)	54 (59)	10 (67)	21 (54)	35 (66)	10 (59)	9 (64)	54 (59)
Not bad	13 (13)	2 (13)	13 (14)	2 (13)	7 (18)	5 (9)	2 (12)	1 (7)	14 (15)
Bad	13 (13)	2 (13)	12 (13)	2 (13)	8 (21)	4 (8)	2 (12)	3 (21)	11 (12)
Very bad	8 (8)	1 (6)	6 (7)	1 (7)	1 (3)	6 (11)	1 (6)	0 (0)	8 (9)
Terrible	5 (5)	2 (13)	6 (7)	0 (0)	2 (5)	3 (6)	2 (12)	1 (7)	5 (5)
P Value	0.68		0.56		0.78			0.77	
Abdominal pains or cramps: intensity since last visit									
Not bad at all	69 (70)	12 (75)	63 (69)	12 (80)	23 (59)	41 (77)	13 (76)	8 (57)	66 (72)
Not bad	14 (14)	2 (13)	12 (13)	3 (20)	7 (18)	7 (13)	2 (12)	4 (29)	11 (12)
Bad	7 (7)	1 (6)	8 (9)	0 (0)	5 (13)	2 (4)	1 (6)	2 (14)	6 (7)
Very bad	6 (6)	1 (6)	6 (7)	0 (0)	3 (8)	2 (4)	1 (6)	0 (0)	7 (8)
Terrible	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	0 (0)	2 (2)
P Value	0.75		0.37		0.25			0.53	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-8. Genetic association with Neurological, psychological, and fatigue-related symptoms

Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Genotype Mean (SD)	GG/GT Mean (SD)	TT Mean (SD)	TT Mean (SD)	CT/CC Mean (SD)	Fast Mean (SD)	Int. Mean (SD)	Slow Mean (SD)	Zero doses Mean (SD)	1 or 2 doses Mean (SD)
Emotional well being	71(27)	66(27)	66(26)	65(27)	65(26)	62(6)	67(5)	66(20)	66(28)
P Value	0.49		0.50		0.75			0.49	
Overall depression score	14(12)	14(12)	17(14)	14(12)	17(12)	14(2)	14(2)	18(9)	14(12)
P Value	0.19		0.17		0.84			0.16	
Energy/Fatigue function	56.3(26)	56.8(24)	54.2(27)	55(27)	63(21)	50(6)	60(4)	49(17)	57(27)
P Value	0.38		0.18		0.36			0.18	
Factors Genotype *N (%)*	**GG/GT** *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
Drowsiness/tiredness: intensity since last visit									
Not bad at all	45 (46)	9 (56)	41 (45)	9 (60)	17 (44)	26 (49)	9 (56)	4 (29)	45 (49)
Not bad	23 (23)	1 (6)	20 (22)	4 (27)	9 (23)	12 (23)	2 (13)	3 (21)	21 (23)
Bad	18 (18)	4 (25)	18 (20)	2 (13)	8 (21)	8 (15)	3 (19)	6 (43)	15 (16)
Very bad	10 (10)	1 (6)	10 (11)	0 (0)	4 (10)	6 (11)	1 (6)	0 (0)	10 (11)
Terrible	2 (2)	1 (6)	2 (2)	0 (0)	1 (3)	1 (2)	1 (6)	1 (7)	1 (1)
P Value	0.81		0.16		0.88			0.17	
Factors Genotype *N (%)*	**GG/GT** *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
Dizziness or lack of concentration: intensity since last visit									
Not bad at all	77 (79)	12 (80)	69 (76)	13 (87)	33 (87)	39 (74)	13 (76)	10 (71)	72 (78)
Not bad	7 (7)	3 (20)	8 (9)	2 (13)	2 (5)	4 (8)	4 (24)	3 (21)	7 (8)
Bad	11 (11)	0 (0)	11 (12)	0 (0)	1 (3)	10 (19)	0 (0)	0 (0)	11 (12)
Very bad	3 (3)	0 (0)	3 (3)	0 (0)	2 (5)	0 (0)	0 (0)	1 (7)	2 (2)
P Value	1.00		0.43		0.70			0.10	
Major memory/concentration problems for more than 2 weeks since last visit									
Yes	3 (3)	0 (0)	3 (3)	0 (0)	1 (2)	2 (4)	0 (0)	0 (0)	3 (3)
No	101 (97)	17 (100)	94 (97)	16 (100)	42 (98)	53 (96)	18 (100)	14 (100)	96 (97)
P Value	1.00		1.00		1.00			1.00	

Table 5-8. Genetic association with Neurological, psychological, and fatigue-related symptoms

	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Confusion, getting lost, difficulty with mental tasks since last visit									
Yes	2 (2)	1 (6)	1 (1)	1 (6)	0 (0)	2 (4)	1 (6)	0 (0)	2 (2)
No	102 (98)	16 (94)	96 (99)	15 (94)	43 (100)	53 (96)	17 (94)	14 (100)	97 (98)
P Value	0.36		0.26		0.38			1.00	

Note. The criteria set for data to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each related question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Table 5-9. Genetic association with body habitus

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
Unintentional weight loss 10 lbs or more lasted more than 1 month since last visit									
Yes	16 (15)	5 (29)	19 (20)	2 (13)	6 (14)	9 (16)	5 (28)	3 (21)	18 (18)
No	88 (85)	12 (71)	78 (80)	14 (88)	37 (86)	46 (84)	13 (72)	11 (79)	81 (82)
P Value	0.17		0.73		0.40			0.72	
Changes in shape of body, increase/decrease in fat since last visit									
Yes	52 (50)	10 (59)	52 (54)	7 (44)	16 (37)	33 (60)	11 (61)	8 (57)	51 (52)
No	52 (50)	7 (41)	45 (46)	9 (56)	27 (63)	22 (40)	7 (39)	6 (43)	48 (48)
P Value	0.60		0.59		0.06			0.78	
Shift of body fats: intensity since last visit									
Not bad at all	76 (78)	13 (81)	70 (77)	12 (80)	27 (69)	44 (83)	14 (82)	9 (64)	73 (79)
Not bad	9 (9)	2 (13)	10 (11)	0 (0)	7 (18)	2 (4)	2 (12)	1 (7)	9 (10)
Bad	9 (9)	0 (0)	6 (7)	3 (20)	3 (8)	5 (9)	0 (0)	4 (29)	5 (5)
Very bad	3 (3)	1 (6)	4 (4)	0 (0)	2 (5)	1 (2)	1 (6)	0 (0)	4 (4)
Terrible	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
P Value	0.78		0.90		0.57			0.35	
Change in the shape of face									
Yes	23 (44)	2 (20)	24 (46)	1 (14)	9 (56)	14 (42)	2 (18)	1 (13)	24 (47)
No	29 (56)	8 (80)	28 (54)	6 (86)	7 (44)	19 (58)	9 (82)	7 (88)	27 (53)
P Value	0.18		0.22		0.17			0.12	
Face increase or decrease									
Decrease	12 (23)	2 (20)	13 (25)	1 (14)	5 (31)	7 (21)	2 (18)	1 (6)	13 (25)
No change	29 (56)	8 (80)	28 (54)	6 (86)	7 (44)	19 (58)	9 (82)	7 (44)	27 (53)
Increase	11 (21)	0 (0)	11 (21)	0 (0)	4 (25)	7 (21)	0 (0)	8 (50)	11 (22)
P Value	0.49		0.75		0.79			0.79	

Table 5-9. Genetic association with body habitus

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
Severity of face change									
None	29 (56)	8 (80)	28 (54)	6 (86)	7 (44)	19 (58)	9 (82)	7 (88)	27 (53)
Mild	13 (25)	0 (0)	13 (25)	0 (0)	5 (31)	8 (24)	0 (0)	1 (13)	12 (24)
Moderate	6 (12)	1 (10)	7 (13)	0 (0)	2 (13)	4 (12)	1 (9)	0 (0)	7 (14)
Severe	4 (8)	1 (10)	4 (8)	1 (14)	2 (13)	2 (6)	1 (9)	0 (0)	5 (10)
P Value	0.35		0.26		0.34			0.09	
Change in the amount of fat in cheeks									
Yes	23 (44)	3 (30)	25 (48)	1 (14)	9 (56)	14 (42)	3 (27)	2 (25)	24 (47)
No	29 (56)	7 (70)	27 (52)	6 (86)	7 (44)	19 (58)	8 (73)	6 (75)	27 (53)
P Value	0.50		0.12		0.36			0.45	
Fat in cheeks increase or decrease									
Decrease	12 (23)	2 (20)	13 (25)	1 (14)	5 (31)	7 (21)	2 (18)	1 (13)	13 (25)
No change	29 (56)	7 (70)	27 (52)	6 (86)	7 (44)	19 (58)	8 (73)	6 (75)	27 (53)
Increase	11 (21)	1 (10)	12 (23)	0 (0)	4 (25)	7 (21)	1 (9)	1 (13)	11 (22)
P Value	0.76		0.70		0.93			0.88	
Severity of cheek change									
None	29 (56)	7 (41)	27 (52)	6 (86)	7 (44)	19 (58)	8 (73)	6 (75)	27 (53)
Mild	16 (31)	1 (6)	17 (33)	0 (0)	6 (38)	10 (30)	1 (9)	2 (25)	15 (29)
Moderate	3 (6)	4 (24)	4 (8)	0 (0)	1 (6)	2 (6)	1 (9)	0 (0)	4 (8)
Severe	4 (8)	5 (29)	4 (8)	1 (14)	2 (13)	2 (6)	1 (9)	0 (0)	5 (10)
P Value	0.63		0.23		0.51			0.24	

Table 5-9. Genetic association with body habitus

Genotype *N (%)*	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Factors	**GG/GT** ***N (%)***	**TT** ***N (%)***	**TT** ***N (%)***	**CT/CC** ***N (%)***	**Fast** ***N (%)***	**Int.** ***N (%)***	**Slow** ***N (%)***	**Zero doses** ***N (%)***	**1 or 2 doses** ***N (%)***
Change in the size of neck									
Yes	17 (33)	1 (10)	15 (29)	2 (29)	8 (50)	9 (27)	1 (5)	2 (25)	15 (29)
No	35 (67)	9 (90)	37 (71)	5 (71)	8 (50)	24 (73)	19 (95)	6 (75)	36 (71)
P Value	0.26		1.00		0.08			1.00	
Size of neck change increase or decrease									
Decrease	8 (15)	1 (10)	9 (17)	0 (0)	5 (31)	3 (9)	1 (9)	1 (13)	8 (16)
No change	35 (67)	9 (90)	37 (71)	5 (71)	8 (50)	24 (73)	10 (91)	6 (75)	36 (71)
Increase	9 (17)	0 (0)	6 (12)	2 (29)	3 (19)	6 (18)	0 (0)	1 (13)	7 (14)
P Value	0.61		0.21		0.52			0.94	
Severity of neck change									
None	35 (67)	9 (90)	37 (71)	5 (71)	8 (50)	24 (73)	19 (95)	6 (75)	36 (71)
Mild	11 (21)	0 (0)	10 (19)	1 (14)	5 (31)	6 (18)	0 (0)	1 (13)	10 (20)
Moderate	4 (8)	1 (10)	3 (6)	1 (14)	1 (6)	3 (9)	1 (5)	1 (13)	3 (6)
Severe	2 (4)	0 (0)	2 (4)	0 (0)	2 (13)	0 (0)	0 (0)	0 (0)	2 (4)
P Value	0.30		0.98		0.19			0.86	
Change in the amount of fat in upper back									
Yes	20 (38)	1 (10)	18 (35)	2 (29)	8 (50)	12 (36)	1 (5)	3 (38)	17 (33)
No	32 (62)	9 (90)	34 (65)	5 (71)	8 (50)	21 (64)	19 (95)	5 (63)	34 (67)
P Value	0.30		0.11		0.23			0.71	
Fat in upper back increase or decrease									
Decrease	6 (12)	1 (10)	7 (13)	0 (0)	4 (25)	2 (6)	1 (9)	1 (13)	6 (12)
No change	32 (62)	9 (90)	34 (65)	5 (71)	8 (50)	21 (64)	10 (91)	5 (63)	34 (67)
Increase	14 (27)	0 (0)	11 (21)	2 (29)	4 (25)	10 (30)	0 (0)	2 (25)	11 (22)
P Value	0.26		0.47		0.28			0.91	

Table 5-9. Genetic association with body habitus

Genotype *N (%)*	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Factors	**GG/GT** *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
Severity of upper back change									
None	32 (62)	9 (90)	34 (65)	5 (71)	8 (50)	21 (64)	19 (95)	5 (63)	34 (67)
Mild	11 (21)	0 (0)	10 (19)	1 (14)	5 (31)	6 (18)	0 (0)	2 (25)	9 (18)
Moderate	6 (12)	1 (10)	5 (10)	1 (14)	1 (6)	5 (15)	1 (5)	1 (13)	5 (10)
Severe	3 (6)	0 (0)	3 (6)	0 (0)	2 (13)	1 (3)	0 (0)	0 (0)	3 (6)
P Value	0.18		0.78		0.22			0.93	
Change in size of one/both breasts									
Yes	27 (52)	2 (20)	24 (46)	3 (43)	10 (63)	15 (45)	3 (27)	3 (38)	24 (47)
No	25 (48)	8 (80)	28 (54)	4 (57)	6 (38)	18 (55)	8 (73)	5 (63)	27 (53)
P Value	0.08		1.00		0.23			0.72	
Breast size increase or decrease									
Decrease	11 (21)	1 (10)	12 (23)	0 (0)	6 (38)	5 (15)	1 (9)	1 (13)	11 (22)
No change	25 (48)	8 (80)	28 (54)	4 (57)	6 (38)	18 (55)	8 (73)	5 (63)	27 (53)
Increase	16 (31)	1 (10)	12 (23)	3 (43)	4 (25)	10 (30)	2 (18)	2 (25)	13 (25)
P Value	0.66		0.16		0.51			0.77	
Severity of breast change									
None	25 (48)	8 (80)	28 (54)	4 (57)	6 (38)	18 (55)	8 (73)	5 (71)	27 (53)
Mild	18 (35)	1 (10)	16 (31)	2 (29)	6 (38)	11 (33)	2 (18)	1 (14)	17 (33)
Moderate	6 (12)	1 (10)	6 (12)	0 (0)	2 (13)	4 (12)	1 (9)	1 (14)	5 (10)
Severe	3 (6)	0 (0)	2 (4)	1 (14)	2 (13)	0 (0)	0 (0)	0 (0)	2 (4)
P Value	0.12		0.94		0.22			0.92	
Change in amount of fat in arms									
Yes	27 (52)	3 (30)	27 (52)	1 (14)	10 (63)	17 (52)	3 (27)	3 (38)	25 (49)
No	25 (48)	7 (70)	25 (48)	6 (86)	6 (38)	16 (48)	8 (73)	5 (63)	26 (51)
P Value	0.30		0.11		0.23			0.71	
Amount of fat in arms increase or decrease									
Decrease	13 (25)	3 (30)	15 (29)	1 (14)	5 (31)	8 (24)	3 (16)	2 (25)	14 (27)
No change	25 (48)	7 (70)	25 (48)	6 (86)	6 (38)	16 (48)	8 (42)	5 (63)	26 (51)
Increase	14 (27)	0 (0)	12 (23)	0 (0)	5 (31)	9 (27)	8 (42)	1 (13)	11 (22)

Table 5-9. Genetic association with body habitus

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
P Value	0.24		0.82		0.54			0.83	
Severity of arm change									
None	25 (48)	7 (70)	25 (48)	6 (86)	6 (38)	16 (48)	8 (73)	5 (63)	26 (51)
Mild	17 (33)	1 (10)	17 (33)	1 (14)	6 (38)	11 (33)	1 (9)	3 (38)	15 (29)
Moderate	7 (13)	2 (20)	7 (13)	0 (0)	2 (13)	5 (15)	2 (18)	0 (0)	7 (14)
Severe	3 (6)	0 (0)	3 (6)	0 (0)	2 (13)	1 (3)	0 (0)	0 (0)	3 (6)
P Value	0.36		0.08		0.33			0.40	
Change in the size of belly/abdominal fat									
Yes	40 (77)	7 (70)	40 (77)	5 (71)	14 (88)	25 (76)	8 (73)	5 (63)	40 (78)
No	12 (23)	3 (30)	12 (23)	2 (29)	2 (13)	8 (24)	3 (27)	3 (38)	11 (22)
P Value	0.70		0.67		0.64			0.38	
Belly/abdominal fat increase or decrease									
Decrease	13 (25)	4 (40)	16 (31)	1 (14)	6 (38)	7 (21)	4 (36)	1 (13)	16 (31)
No change	12 (23)	3 (30)	12 (23)	2 (29)	2 (13)	8 (24)	3 (27)	3 (38)	11 (22)
Increase	27 (52)	3 (30)	24 (46)	4 (57)	8 (50)	18 (55)	4 (36)	4 (50)	24 (47)
P Value	0.24		0.48		0.56			0.59	
Severity of belly change									
None	12 (23)	3 (30)	12 (23)	2 (29)	2 (13)	8 (24)	3 (27)	3 (38)	11 (22)
Mild	17 (33)	6 (60)	18 (35)	4 (57)	5 (31)	11 (33)	7 (64)	3 (38)	19 (37)
Moderate	15 (29)	1 (10)	14 (27)	1 (14)	5 (31)	10 (30)	1 (9)	1 (13)	14 (27)
Severe	8 (15)	0 (0)	8 (15)	0 (0)	4 (25)	4 (12)	0 (0)	1 (13)	7 (14)
P Value	0.11		0.25		0.09			0.36	

Table 5-9. Genetic association with body habitus

Genotype *N (%)* / Factors	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
Change of waist size									
Yes	39 (75)	7 (70)	40 (77)	3 (43)	13 (81)	25 (76)	8 (73)	5 (63)	38 (75)
No	13 (25)	3 (30)	12 (23)	4 (57)	3 (19)	8 (24)	3 (27)	3 (38)	13 (25)
P Value	0.71		0.08		0.92			0.67	
Waist increase or decrease									
Decrease	15 (29)	5 (50)	20 (38)	0 (0)	5 (31)	10 (30)	5 (45)	2 (25)	18 (35)
No change	13 (25)	3 (30)	12 (23)	4 (57)	3 (19)	8 (24)	3 (27)	3 (38)	13 (25)
Increase	24 (46)	2 (20)	20 (38)	3 (43)	8 (50)	15 (45)	3 (27)	3 (38)	20 (39)
P Value	0.14		0.26		0.54			0.82	
Severity of waist change									
None	13 (25)	3 (30)	12 (23)	4 (57)	3 (19)	8 (24)	3 (27)	3 (38)	13 (25)
Mild	18 (35)	6 (60)	21 (40)	1 (14)	5 (31)	12 (36)	7 (64)	3 (38)	19 (37)
Moderate	14 (27)	1 (10)	12 (23)	2 (29)	4 (25)	10 (30)	1 (9)	2 (25)	12 (24)
Severe	7 (13)	0 (0)	7 (13)	0 (0)	4 (25)	3 (9)	0 (0)	0 (0)	7 (14)
P Value	0.18		0.18		0.20			0.36	
Change in the amount of fat in buttocks									
Yes	36 (69)	7 (70)	39 (75)	2 (29)	11 (69)	24 (73)	7 (64)	4 (50)	37 (73)
No	16 (31)	3 (30)	13 (25)	5 (71)	5 (31)	9 (27)	4 (36)	4 (50)	14 (27)
P Value	1.00		0.02		0.87			0.23	
Amount of fat in buttocks increase or decrease									
Decrease	19 (37)	5 (50)	22 (42)	2 (29)	6 (38)	12 (36)	5 (45)	3 (38)	21 (41)
No change	16 (31)	3 (30)	13 (25)	5 (71)	5 (31)	9 (27)	4 (36)	4 (50)	14 (27)
Increase	17 (33)	2 (20)	17 (33)	0 (0)	5 (31)	12 (36)	2 (18)	1 (13)	16 (31)
P Value	0.39		0.68		0.69			0.70	
Severity of buttocks change									
None	16 (31)	3 (30)	13 (25)	5 (71)	5 (31)	9 (27)	4 (40)	4 (50)	14 (27)
Mild	19 (37)	3 (30)	21 (40)	1 (14)	7 (44)	12 (36)	2 (20)	2 (25)	20 (39)
Moderate	8 (15)	4 (40)	10 (19)	1 (14)	1 (6)	6 (18)	4 (40)	2 (25)	9 (18)
Severe	9 (17)	0 (0)	8 (15)	0 (0)	3 (19)	6 (18)	0 (0)	0 (0)	8 (16)

Table 5-9. Genetic association with body habitus

	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
Genotype *N (%)* **Factors**	**GG/GT** *N (%)*	**TT** *N (%)*	**TT** *N (%)*	**CT/CC** *N (%)*	**Fast** *N (%)*	**Int.** *N (%)*	**Slow** *N (%)*	**Zero doses** *N (%)*	**1 or 2 doses** *N (%)*
P Value	0.94		0.04		0.79			0.26	
Change in the amount of fat in legs									
Yes	34 (65)	4 (40)	33 (63)	3 (43)	8 (50)	25 (76)	4 (36)	3 (38)	33 (65)
No	18 (35)	6 (60)	19 (37)	4 (57)	8 (50)	8 (24)	7 (64)	5 (63)	18 (35)
P Value	0.17		0.42		0.04			0.24	
Amount of fat in legs increase or decrease									
Decrease	21 (40)	4 (40)	23 (44)	2 (29)	6 (38)	14 (42)	4 (36)	2 (25)	23 (45)
No change	18 (35)	6 (60)	19 (37)	4 (57)	8 (50)	8 (24)	7 (64)	5 (63)	18 (35)
Increase	13 (25)	0 (0)	10 (19)	1 (14)	2 (13)	11 (33)	0 (0)	1 (13)	10 (20)
P Value	0.47		0.67		0.72			0.58	
Severity of leg change									
None	18 (35)	6 (60)	19 (37)	4 (57)	8 (50)	8 (24)	7 (64)	5 (63)	18 (35)
Mild	19 (37)	2 (20)	18 (35)	3 (43)	5 (31)	13 (39)	2 (18)	3 (38)	18 (35)
Moderate	8 (15)	2 (20)	8 (15)	0 (0)	1 (6)	7 (21)	2 (18)	0 (0)	8 (16)
Severe	7 (13)	0 (0)	7 (13)	0 (0)	2 (13)	5 (15)	0 (0)	0 (0)	7 (14)
P Value	0.19		0.16		0.07			0.08	
Changed diet to influence body shape									
Yes	15 (14)	7 (41)	20 (21)	1 (6)	8 (19)	7 (13)	7 (39)	4 (29)	17 (17)
No	89 (86)	10 (59)	77 (79)	15 (94)	35 (81)	48 (87)	11 (61)	10 (71)	82 (83)
P Value	0.015		0.29		0.06			0.29	
Changed HIV medications to influence body shape									
Yes	23 (23)	3 (19)	21 (23)	5 (31)	10 (26)	13 (24)	2 (13)	4 (29)	22 (24)
No	76 (77)	13 (81)	70 (77)	11 (69)	29 (74)	41 (76)	14 (88)	10 (71)	71 (76)
P Value	1.00		0.53		0.90			0.74	
Changed exercise habits to influence body shape									
Yes	13 (13)	4 (24)	16 (16)	1 (6)	6 (14)	7 (13)	4 (22)	2 (14)	15 (15)
No	91 (88)	13 (76)	81 (84)	15 (94)	37 (86)	48 (87)	14 (78)	12 (86)	84 (85)
P Value	0.25		0.46		0.63			1.00	

Table 5-9. Genetic association with body habitus

Factors / Genotype *N (%)*	CYP2B6 rs3745274 c.516G>T		CYP2B6 rs28399499 c.983T>C		CYP2B6 Metabolizer			ABCB1_ HAPA1	
	GG/GT *N (%)*	TT *N (%)*	TT *N (%)*	CT/CC *N (%)*	Fast *N (%)*	Int. *N (%)*	Slow *N (%)*	Zero doses *N (%)*	1 or 2 doses *N (%)*
Taken nutritional supplements to influence body shape									
Yes	32 (32)	2 (13)	31 (34)	2 (13)	14 (36)	17 (31)	2 (12)	4 (29)	29 (31)
No	67 (68)	14 (88)	60 (66)	14 (88)	25 (64)	37 (69)	15 (88)	10 (71)	64 (69)
P Value	0.14		0.14		0.16			1.00	
Taken growth hormones/steroids to influence body shape									
Yes	2 (2)	0 (0)	2 (2)	0 (0)	1 (3)	1 (2)	0 (0)	0 (0)	2 (2)
No	97 (98)	16 (100)	89 (98)	16 (100)	38 (97)	53 (98)	17 (100)	14 (100)	91 (98)
P Value	1.00		1.00		1.00			1.00	

Note. The criteria set for variables to be included in these analyses required that at least 50% of data be present for each variable i.e., a response from 61 study participants for each respective question. Statistical methods employed: Fisher's Exact test for dichotomous variables, Kruskal-Wallis for ordinal variables, t-test and ANOVA for continuous variables.

Chapter 6
Conclusion

Introduction

Of all the infectious diseases in history, HIV infection has posed the greatest threat to humans (Shankarkumar, Shankarkumar, & Ghosh, 2011). Though there is no cure, antiretroviral therapy delays the progression of HIV, prolonging life (Flexner, 2006). Advances in antiretroviral therapy have substantially improved survival transforming HIV from a terminal illness to a chronic disease (Kwong, 2013; Owen & Khoo, 2008). Safe and effective use of antiretroviral drugs can differ significantly from one drug to another according to their respective pharmacokinetic and pharmacodynamic properties (Dellamonica, Perri, & Garraffo, 2012). Therefore, lifelong administration of antiretroviral therapy requires continual drug safety and efficacy monitoring to assure optimal but nontoxic drug exposure.

Efavirenz has been a component of HIV treatment for nearly 15 years and is the preferred NNRTI used in combination therapy with other antiretroviral drugs (Cristofoletti et al., 2012; Maggioli, 2009). Though efavirenz is the most commonly used NNRTI, its use is impacted by pronounced interindividual variability in plasma concentration (exposure) and toxicity, the clinical relevance of which translates to an increased risk of virologic failure or adverse events (Csajka et al., 2003; Kappelhoff et al., 2005; Marzolini et al., 2001; Molto et al., 2007; Sanchez et al., 2011).

Nongenetic Influence on Efavirenz Exposure

Efavirenz exposure variability is affected by both nongenetic and genetic factors (i.e., age, sex, body mass, patient therapy adherence, drug interactions, food

interactions, coexisting conditions, renal and/or hepatic function, host genetics) (Burger et al., 2005; Gandhi et al., 2009; Stohr et al., 2008). Few studies have been optimally designed to comprehensively assess non-genetic factors in parallel with genetic factors and limited by the underrepresentation of various patient populations (i.e., women, certain ethnic/racial groups). Though gene variations associated with efavirenz exposure variability are now well characterized across ethnic/racial groups, women have continued to be underrepresented, until now. A feature of our study was the representation of an ethnically/racially diverse cohort of HIV-infected women. Factors that influence exposure in "real-world" populations were taken into account and a more comprehensive set of factors were assessed than in previous studies (Gandhi et al., 2009). Age, race/ethnicity, education, dietary patterns of both meals and snacks (i.e., allergies, coffee, tea, herbal infusion, orange juice, grapefruit juice, fast food consumption), fat intake, concomitant medications, antiretroviral adherence, smoking, alcohol use, and recreational drug use, weight, height, fat free mass (via bioimpedence), platelet count, renal function (Cockcroft-Gault and Modification of Diet in Renal Disease [MDRD] creatinine clearance calculations), and hepatic function (aspartate transaminase [AST], alanine transaminase [ALT], and gamma-glutamyl transferase [GGT]), recent illnesses, persistent diarrhea, menstrual history, pregnancy, and hepatitis B and C co-infection comprised the nongenetic factors in our study (Gandhi et al., 2009).

Non-genetic associations. Of all the nongenetic factors assessed only orange juice consumption and increases in alanine aminotransferase (ALT) remained statistically significant when genetic factors were included in the final model (Gandhi et

al., 2012). Efavirenz AUC increase 1.23-fold (95% CI: 1.11-1.36, p=0.0001) per ALT doubling and 1.26-fold (95% CI: 1.05-1.50, p=0.012) with orange/orange juice consumption (Gandhi et al., 2012).

Non-genetic Factor Clinical Implications

The clinical implications of the nongenetic findings of this study translate to patient education regarding the consumption of orange juice and careful observation of liver enzymes. Efavirenz dose reduction in patients reluctant to forgo drinking orange juice or exhibit ALT elevations that exceed acceptable parameters should be considered.

Efavirenz ADME Genetic Associations

Overall, 182 SNPs among 9 candidate genes (ABCB1: 63 of 70 SNPs, ABCC2: 20 of 28 SNPs, CYP2B6: 23 of 38 SNPs, CYP2C19: 24 of 28 SNPs, CYP2D6: 5 of 7 SNPs, CY3A5/CYP3A4: 21 of 30 SNPs, SCL22A6: 5 of 8 SNPs, and UGT1A1: 22 of 26 SNPs) passed quality control filters and these SNPs and 45 haplotypes were analyzed in our efavirenz exposure study (Gandhi et al., 2012). Risk alleles associated with increased efavirenz exposure were then retained for analyses with a broad spectrum of symptoms and side effects in patients taking efavirenz.

Some of the gene variations included in our studies have been previously linked with variability in efavirenz exposure. However, a systematic evaluation of SNPs which capture the majority of genetic variability in key genes implicated in the efavirenz ADME pathway had not been previously undertaken (Gandhi et al., 2012). To our knowledge, this also is the first study to explore a broad spectrum of symptoms and side effects and their relationship with efavirenz exposure-associated gene variations. Though gene

variations associated with an increase in efavirenz concentration are linked to central nervous system (CNS)-related adverse events, little is known about genetic associations with other symptoms and side effects known to be associated with this drug.

Genetic associations of ABCB1 Haplotype A1. We observed an increase in efavirenz exposure associated with the ABCB1 Haplotype A1 composed of the common alleles for ABCB1 c.3085-72C>G (rs7779562) and c.2927+377C>A (rs4148745). The ABCB1 haplotype A1 was associated with a 1.60-fold (95% CI: 1.24-2.1, p=0.0004) AUC increase (Gandhi, et al., 2012). However, there was no evidence of association observed for the ABCB1 Haplotype A1 with demographic and general clinical characteristics, therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms.

Genetic associations of CYP2B6 rs3745374. We observed an increase in efavirenz exposure associated with CYP2B6 c.516G<T (rs3745274). Individuals homozygous for the CYP2B6 rs3745274 T allele displayed a 3.50-fold (95% CI: 2.7 -4.5 , $p=1.4 \times 10^{-18}$) increase in AUC compared with individuals with the TG or GG genotypes (Gandhi et al., 2012). We also observed that Individuals homozygous for the CYP2B6 rs3745274 T rare allele (i.e., TT genotype) were less likely to be smoking currently as compared to individuals that carried the common G allele (i.e., GG or GT genotypes) (Fisher's Exact test, p=0.03). CYP2B6 rs3745274 TT homozygotes were more likely to change their diet to influence their body shape as compared to G allele carriers (Fisher's Exact test, p=0.02). No evidence of association was observed for CYP2B6 rs3745274

with therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms.

Genetic associations of CYP2B6 rs28399499. We observed an increase in efavirenz exposure associated with CYP2B6 c.983C>T (rs28399499). Individuals homozygous or heterozygous for the CYP2B6 rs28399499 C allele (i.e., CC or CT genotype) displayed a 1.96-fold (95% CI: 1.54-2.5, p=2.2 x 10^{-10}) increase in AUC compared to individuals with the TT genotype (Gandhi et al., 2012). Associations with CYP2B6 rs28399499 and body habitus were also observed. Individuals with the rare C allele (i.e., CC or TC genotype) were less likely to report a change in the amount of fat in their buttocks (Fisher's Exact test, p=0.02). Additionally, individuals with the rare C allele were less likely to report mild, moderate, and severe changes in buttock fat (Kruskal-Wallis, C^2[1, N=59], 4.16, p=0.04). There was no evidence of association observed for CYP2B6 rs28399499 with demographic and general clinical characteristics, therapeutic regime, general side effects, gastrointestinal symptoms, and neuropathic and pain-related symptoms.

Genetic associations of CYP2B6 Metabolizer Diplotype. An association with the combined genotype of rs3745274 and rs28399499, entitled the CYP2B6 "Metabolizer" diplotype, was also observed. The CYP2B6 Metabolizer diplotype is delineated as "slow" (i.e., rs3745274 TT with rs28399499 CC or rs3745274 GT with rs28399499 TC), "intermediate" (i.e., a single rare allele at either rs3745274 or rs28399499 but not both), and "fast" (i.e., only common alleles at both rs3745274 or rs28399499) (Haas et al., 2009). Intermediate metabolizers displayed a 1.36-fold (95% CI: 1.11-1.67, p=0.003)

increase in AUC and slow metabolizers displayed a 3.00-fold (95% CI: 2.3-4.0 , *p*=1E-11) increase as compared to fast metabolizers. Individuals designated as CYP2B6 “slow” metabolizers were less likely to be smoking currently (Fisher’s Exact test, *p*=0.04) as compared to “intermediate” and “fast” metabolizers. Individuals designated as CYP2B6 “intermediate” metabolizers were more likely to report a change in the amount of fat in legs (Fisher’s Exact test *p*=0.04) as compared to “slow” or “fast” metabolizers.

Genetic Factor Clinical implications

The immediate clinical implications of the observed genetic associations with efavirenz exposure is that genetic testing prior to initiation of a patient’s therapeutic regime could be used to tailor the dose. In fact, our findings suggests that the development of a dose-optimization algorithm can be broadened to include both nongenetic and genetic factors. A barrier to the implementation of research such as this is the lack of clear, curated, peer-reviewed guidelines that synthesize study results into actionable prescribing decisions (Relling & Klein, 2011). The multivariate model of factors described in Chapter 4, explains 53% of interindividual variability in efavirenz exposure. This model can be used to develop dose-optimization algorithms to individualize therapy (i.e., regression equations). Until comprehensive multifactorial algorithms are developed, the findings reported herein may have a delayed clinical benefit.

The associations between genotype and symptoms and side effects have clinical implications both for patients about to initiate efavirenz therapy and in patients who have been on long term therapy. The prevalence of body habitus changes attributed to

efavirenz, in the form of lipoatrophy, is reportedly as high as 32% (Haubrich et al., 2009). Genotyping patients before therapy initiation could be done to provide a risk assessment for the development body habitus changes. For patients that exhibit body habitus changes whereby such changes affect their quality of life, genotyping and efavirenz levels could also be assessed. This may facilitate discussion and decisions regarding treatment such as change in HIV regimen, cosmetic options, and adjunctive therapy (i.e., leptin, human growth hormone, gemfibrozil), currently being studied and not yet FDA approved. Patients may benefit from the use of a genetic biomarker to inform and personalize their therapy.

Research Implications

The studies have several research implications. Though the research methods used in the efavirenz exposure study are not new, estimating drug concentration using intensive pharmacokinetic sampling in patients taking efavirenz had not been done previously. This allowed for more robust estimates of drug concentration than that of single plasma level and nonlinear mixed effects modeling utilized in prior studies (Gandhi et al., 2012). Our study also identified and quantitated previously unknown factors that modify efavirenz exposure in the “real world” setting (Gandhi et al., 2009). These study components should be considered in the development of future study designs.

Though the combined effect of rs3745274 and rs28399499 is predictive of efavirenz exposure (Rotger et al., 2005, 2007; Wang et al., 2006), in the final model of the efavirenz exposure study described in Chapter 4, this SNP combination, also termed

the Metabolizer diplotype, added any no additional information beyond the component SNPs. This observation may be due to differences in study design, analysis methods, patient populations, and sample size. These differences should be considered in the development of future studies.

The stringent criterion for inclusion in the final multivariate model (i.e., p-value of >0.001) may have resulted in missing additional genetic predictor variables. SNPs in both UGT1A1 and ABCC2 approached this *a priori* significance threshold and a less stringent criterion could have resulted in their inclusion. Though UGT1A1 is implicated in the glucuronidation of efavirenz metabolites and ABCC2 is hypothesized to play a role in the transportation of several antiretroviral drugs, association with efavirenz exposure is not well studied (Fellay et al., 2002; Ji, Lee, Lim, Kim, & Lee, 2012). The paucity in the literature for both UGT1A1 and ABCC2 suggest inclusion in future studies. The stringent criterion for retention of genetic risk factors for efavirenz exposure also excluded these potential genetic risk factors from the symptoms and side effects analyses. Because both are implicated in the ADME of efavirenz, future studies examining the genetic predictors of efavirenz-related symptoms and side effects should include UGT1A1 and ABCC2.

To our knowledge, the side effects and symptoms study described in Chapter 5 is the first to describe the association of CYP2B6 SNPs and body habitus changes. Until now, related genetic epidemiology studies have focused on adipogenic differentiation genes (i.e., adiponectin, lipoprotein lipase, leptin, tissue necrosis factor alpha) and master regulators of adipogenesis (i.e., peroxisome proliferator-activated receptor

gamma, CCAAT/enhancer-binding proteins alpha) (Gallego-Escuredo et al., 2010). Though important, this has left a gap in knowledge. The paucity in the literature on ADME pharmacogenetics and body habitus must be addressed.

Recent evidence suggests complex interactions between the different antiretroviral drug classes influence differences in body habitus changes. Lipoatrophy was recently observed to be more pronounced in patients taking efavirenz in combination with a protease inhibitor and less pronounced in combination with a nucleoside analog reverse transcriptase inhibitor (Haubrich et al, 2009). In our study, 93% of the patients were on combination therapy. A larger sample may provide greater statistical power to investigate more complex models, which may shed light on relationships between variables that our study was not powered to assess.

The limitations of our study emphasize the challenges involved in studying body habitus changes and the need for well-designed studies that include: self-report measures, clinical assessment, and biomarkers of body habitus. Furthermore, consensus of the definition of body habitus changes, standardization of case definitions, consistency in the way fat distribution is clinically assessed and documented, and conformity in the collection of self-report would enhance both the design and interpretation of future studies. However, clinically meaningful, objective, and standardized definitions for body habitus changes must first be developed. Validation of these definitions via prospective longitudinal studies with appropriate controls and quantifiable body fat measurements need also be performed.

Though our study did not demonstrate an association with any other symptom or side effect included for analysis, the modest sample size and lack of precision of the measures employed may have resulted in false negatives test results. More pronounced symptoms and side effects are most likely to occur in the first two to four weeks of treatment, after which they diminish significantly (Clifford et al, 2005) or a patient's therapy is switched. Patients included in our study had long surpassed this time frame resulting in a possible selection event. There is however, some evidence that mild to moderate side effects may persist during long-term treatment (Fumaz et al, 2005), which our study suggest can be detected. However, more subtle effects may have been missed in our study.

Future Research

Future research should focus on studies exploring accessory pathways of metabolism to identify new gene variations that may influence variability in efavirenz exposure. Additional research that is warranted includes confirmation studies for gene variations recently linked with efavirenz exposure variability, genetic and nongenetic factor studies that include the most recent gene variation findings, comparison of traditional and pharmacogenetic influenced dosing methods, and eventual evaluation of pharmacogenetic algorithms as they are developed. Randomized controlled trials are warranted to determine whether the use of pharmacogenetic-guided dosing using both genetic and nongenetic factors decreases the risk of adverse events while maintaining therapeutic drug levels. A study examining the feasibility of pharmacogenetic-guided dose reduction has already been undertaken successfully (Gatanaga et al., 2007). These

findings however, require replication. Additional research should assess the impact of pharmacogenetic-guided dosing on quality of life, hospitalizations, cost effectiveness, therapy discontinuation, and morbidity and mortality.

Our symptoms and side effects study and body habitus findings, being novel, should be replicated to determine if the findings are reproducible. Future research studies should feature a combination of questionnaires (self-report), clinical assessment, and biomarkers of body habitus could provide novel insight into other genetic risk factors associated with changes in body habitus from antiretroviral therapy. Exploration of accessory pathways of metabolism may identify new gene variations to include in body habitus change studies. Additionally, studying patients early in their therapy before adjustments or switches may enhance a study designed to explore the relationship between symptoms and side effects and efavirenz-associated ADME gene variations.

Conclusion

With improvements in survival rates of patients infected with HIV due to antiretroviral therapy, other challenges in treating this patient population have emerged. Use of efavirenz, the most commonly prescribed NNRTI, is impacted by interindividual exposure and toxicity. In recent years there has been significant progress in our understanding of the complex mixture of genetic and nongenetic factors that influence efavirenz ADME. This dissertation research contributes to the burgeoning body of knowledge of efavirenz pharmacogenetics in two ways: modeling the influence of genetic and non-genetic influences on efavirenz exposure *and* describing a broad

spectrum of symptoms and side effects and their association with genotypes that influence efavirenz exposure. This dissertation research offers compelling results that support the continued exploration of efavirenz pharmacogenetics.

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Appendices

Intensive Pharmacokinetic Substudy Overview

Participant Eligibility and Enrollment

WIHS participants at the five clinical sites were subject to the follow criteria:

1. The participant was currently taking one or more of the target antiretroviral medications under study (Table 3-1) and;
2. The participant was not to have undergone intensive pharmacokinetic sampling for the same medication at a previous pharmacokinetic visit.

If the participant was taking more than one of the target medications, pharmacokinetic sampling for each agent was conducted at a single pharmacokinetic visit. Pharmacokinetic testing for efavirenz, occurred during a 24-hour visit. Each participant who qualified for the Intensive Pharmacokinetic Substudy was enrolled at core WIHS visit 18, 19, 20, or 21 as outlined in the study procedure section.

Study Procedure

1. CORE VISIT PROTOCOL (ENROLLMENT)

   a. at the core WIHS visit (18, 19, 20, or 21), an eligibility form for the Intensive Pharmacokinetic Substudy study was completed, as shown in Appendix A Table A-1 for Intensive Pharmacokinetic Substudy form list.

   b. eligible participants were shown the Intensive Pharmacokinetic Substudy flyer and offered enrollment in the substudy. The flyer was read to the participant and it was explained to the potential participant that the substudy would involve a scheduled visit to the General Clinical Research Center (GCRC) or similar clinical center within one month of the core visit. This visit would be 24 hours long and sleeping arrangement and all meals would be provided. An intravenous catheter would be placed in the participants arm to draw blood every 30 minutes to four hours during that time and a reimbursement of $150.00 would be provided.

   c. Eligible participants interested in enrolling (or had further questions about the study) were instructed to circle "1" under question B4 on form PK01 (Eligible, okay to call to schedule appointment). The potential participant was instructed that a staff member would be contacting her to schedule her appointment for the GCRC.

2. SCHEDULING THE INTENSIVE PHARMACOKINETIC SUBSTUDY VISIT

   Intensive Pharmacokinetic Substudy visits were scheduled to take place within one month of the core visit and no later than six weeks after the core visit. The staff member scheduling the visit conducted a quick dietary assessment (on the phone) using the PK-*diet* form. The purpose was to document basic dietary patterns prior to presentation at the GCRC to enable the dieticians to simulate the participants usual meals during the Intensive Pharmacokinetic Substudy stay. For those sites where the GCRC was not utilized, meal planning was not always possible.

3. STUDY GROUP DESIGNATION

Qualifying participants were divided into groups with corresponding procedures based on their drug treatment regime.

a. participants taking efavirenz but NOT taking any other target antiretroviral. Participants required a 24-hour GCRC visit. Staff reminded the participants to bring their antiretroviral medications. Admission time was scheduled to correspond with the time of day the participant usually took the medication. If dosed at two separate times during the day, two separate Intensive Pharmacokinetic Substudy visits were scheduled.

b. Participants who were taking efavirenz ALSO taking one or more of the other three target antiretroviral medications. Participants required a 24-hour GCRC visit. Admission time was scheduled to correspond with the time of day the participant usually took the medication. If dosed at two separate times during the day, two separate Intensive Pharmacokinetic Substudy visits were scheduled.

For both groups: arrangements were made for participants with poor venous access to have a peripherally inserted central catheter line placed by local services on the day prior to or the day of the Intensive Pharmacokinetic Substudy visit. Transportation to the GCRC was arranged per the GCRC protocol.

4. INTENSIVE PHARMACOKINETIC SUBSTUDY VISIT PROCEDURES

Participants presenting to the GCRC for admission who reported discontinuation of the target medication under study (outlined in the eligibility section) were disqualified from the study. Meals and snacks were provided as routinely designated by the study participant. Participants were allowed to consume other concurrent medications, herbal supplements, vitamins etc.

Visit protocols of participants were as follows:

a. It was verified that the participant brought her medications to the Intensive Pharmacokinetic Substudy visit. Participants who did not bring their medications were rescheduled.

b. Informed consent was obtained for the study form PKNOTI.

c. Form PK02 Current Antiretroviral Medication Use and PKO5a, b, and c Weight and Specimen Collection form were completed.

d. Weight was measured and recorded in pounds (rounded to the nearest 1.0 pound) on form PKO5.

e. Urine pregnancy tests were performed with results recorded on form PKO5. If the test was not done, this too was recorded on the form.

f. 24-hour stay participants medication dosing was witnessed and documented by GCRC staff.

g. A saline lock venous catheter was placed.

h. The morning of the study, 5 ml of blood was drawn and labeled time point '0'

i. Participants were instructed to take their medications which was observed and documented.

j. Blood draws were performed for the groups as follows:

efavirenz with no other antiretroviral: 1, 2, 4, 6, 8, 12, 15, 18, 21, and 24 hours after medication dosage.

Efavirenz with other antiretrovirals: 30, 60, 120, 150, 180, 240, 300, 360, 480, 720, 900, 1080, 1260, and 1440 minutes after drug dosage. Each blood draw date and time were recorded on form PK05 that corresponds with each group and individual patient in that group.

k. Between blood draws, the remaining forms outlined in Appendix A Table A-1 were administered.

l. After all blood draws and forms were completed, the saline lock catheter was removed and the participants discharged.

NOTE: If a blood draw time point was missed during the Intensive Pharmacokinetic Substudy visit it was documented on form PK05 using a standard WIHS notation for missing data.

5. INTENSIVE PHARMACOKINETIC SUBSTUDY LABORATORY ANALYSIS METHODS

Procedures for measuring antiretroviral blood levels have been previously published (Egge-Jacobsen et al., 2004). Plasma samples (0.1 ml) were prepared for injection by adding A-86093 (Abbott Laboratories, Abbott Park, IL) as an internal standard, adding acentonitrile (0.4 ml) to precipitate the protein, mixing, centrifuging transferring the supernatant to an auto sampler vial, and diluting if necessary. Plasma was analyzed for the treatment groups by standard techniques of liquid chromatography/tandem mass spectrometry. Data analysis was performed with MassLynx 3.5 software (Micromass Manchester, UK) (Egge-Jacobsen et al., 2004).

Table A-1. PK study factors measured that may influence drug exposure

(PK NOTI) Participation Notification
(PK 01) Eligibility
(PK-diet) Dietary Assessment (administered via telephone interview prior to PK study visit
(PK 02) Current Antiretroviral Medication Use
(PK 02a) Antiretroviral Adherence (One form for each antiretroviral participant taking)
(PK 03) Recent illnesses, Concurrent Medications and OB/Gyn History
(PK 04) Recent Substance Abuse
(PK 05) Weight and specimen Collection
(PK 06) Dosing of Antiretroviral Medications
(PK 07) Plasma Separation and Freezing
(PK 08) Dietary Fat Percentage Questionnaire
(F22 MED) Medical and Health History
(F22 HX) Follow-Up Health History
(F26) Psychosocial Measures

(JHSPH, 2013) *Note.* All data collection forms used in this study may be accessed via the following URL: https://statepiaps.jhsph.edu/wihs/index-forms.htm

WOMEN'S INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PKNOTI: PARTICIPATION NOTIFICATION

INSTRUCTIONS:

The purpose of this form is to track in the Data Management System when a participant enrolls into the Intensive PK Study. This form should be completed for each participant that is eligible and has consented to enroll in the Intensive PK Study.

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |___| - |___|___| - |___|___|___|___| - |___|

A2. FORM VERSION: 0 4 / 0 1 / 0 3 (M / D / Y)

A3. FORM COMPLETED BY: ___ ___ ___

A4. DOES PARTICIPANT CONSENT TO ENROLL INTO THE INTENSIVE PK STUDY?

YES 1
NO................................2 **(END)**

A5. WIHS CORE VISIT NUMBER AT WHICH PARTICIPANT WAS ENROLLED INTO THE INTENSIVE PK STUDY: ___ ___

A6. DATE OF INTENSIVE PK STUDY ENROLLMENT VISIT: ___ ___ / ___ ___/ ___ ___ (M / D / Y)

A7. TARGET MEDICATIONS(S) TAKEN BY PARTICIANT AT ENROLLMENT **(CIRCLE YES FOR ALL THAT APPLY).**

	YES	NO
a. SUSTIVA (EFAVIRENZ)...................................	1	2
b. VIRAMUNE (NEVIRAPINE)	1	2
c. KALETRA (LOPINAVIR/RITONAVIR)...........	1	2
d. VIRACEPT (NELFINAVIR)	1	2

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK01: ELIGIBILITY FORM

THIS FORM SHOULD BE COMPLETED AT THE WIHS CORE VISIT FOR ALL WIHS PARTICIPANTS (EXCEPT FOR THOSE ENROLLED AT THE LA WIHS SITE).

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__| - |__|

A2. WIHS STUDY VISIT #: ___ ___

A3. FORM VERSION: 04/01/03

A4. DATE OF COMPLETION: ___ ___ / ___ ___ / ___ ___ (M D Y)

A5. INTERVIEWER'S INITIALS: ___ ___ ___

SECTION B: ELIGIBILITY

B1. IS PARTICIPANT HIV-POSITIVE?

YES.................. 1
NO..................... 2 **PARTICIPANT IS INELIGIBLE FOR PK SUBSTUDY. SKIP TO B3.**

B2. CHECK EACH ANTIRETROVIRAL MEDIATION BELOW THAT PARTICIPANT IS CURRENTLY TAKING. IF THE PARTICIPANT IS NOT ON ANY OF THESE MEDICATIONS, CHECK 'NONE OF THE ABOVE.'

_____ *Kaletra* (lopinavir/ritonavir) **(ELIGIBLE)**
_____ *Viracept* (nelfinavir) **(ELIGIBLE)**
_____ *Sustiva* (efavirenz) **(ELIGIBLE)**
_____ *Viramune* (nevirapine) **(ELIGIBLE)**
_____ NONE OF THE ABOVE **(INELIGIBLE)**

B3. OUTCOME:

ELIGIBLE, OKAY TO CALL TO SCHEDULE APPOINTMENT 1
ELIGIBLE, DECLINED TO PARTICIPATE.. 2 **(END)**
INELIGIBLE .... 3 **(END)**

B4. CIRCLE THE GROUP TO WHICH PARTICIPANT BELONGS BASED ON ANTIRETROVIRALS CHECKED IN B2. GROUP

Participant is on *Kaletra, Viracept* and/or *Viramune* and NOT on *Sustiva* ....A
Participant is on *Sustiva* and NOT on *Kaletra, Viracept* and/or *Viramune* ....B
Participant is on *Sustiva* and ALSO on *Kaletra, Viracept* and/or *Viramune* C

IF PARTICIPANT IS ELIGIBLE AND HAS CONSENTED TO ENROLL IN THE INTENSIVE PK STUDY, REFER HER FOR GCRC VISIT. AT GCRC VISIT, COMPLETE AND DATA ENTER FORM *PKNOTI*.

DO NOT DATA ENTER FORM *PK01*.

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK02: CURRENT ANTIRETROVIRAL MEDICATION USE

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ____ ___
M D Y

A5. INTERVIEWER'S INITIALS: ___ ___ ___

SECTION B: ANTIRETROVIRAL MEDICATION USE

B1. **INTRODUCTION TO PARTICIPANT:** Now, I am going to ask you a series of questions about antiretroviral medications (HIV drugs) you may have taken since your last core WIHS visit when you were scheduled for this "Intensive PK Substudy" visit.

PROMPT: HAND PARTICIPANT ANTIRETROVIRAL PHOTO MEDICATION CARDS. GO THROUGH THE CARDS WITH THE PARTICIPANT, SAYING THE NAME OF EACH DRUG ALOUD AND ASKING HER TO TELL YOU "YES" OR "NO' WHETHER SHE HAS TAKEN THIS DRUG SINCE HER LAST CORE WIHS VISIT. CHECK EACH DRUG THE PARTICIPANT REPORTS HAVING TAKEN.

B2. Since your last core WIHS visit, have you taken. . .

Nucleoside/Nucleotide RTIs	**Protease Inhibitors**
204 __ Epivir (3TC, lamivudine)	219 __ Agnerase (amprenavir)
218 __ Ziagen (abacavir)	212 __ Crixivan (indinavir)
092 __ Retrovir (AZT, zidovudine, ZDV)	**217 __ Kaletra (lopinavir/ritonavir)**
	216 __ Viracept (nelfinavir)
227 __ Combivir (AZT + 3TC)	211 __ Norvir (ritonavir)
159 __ Zerit (d4T, stavudine)	210 __ Invirase or Fortovase (saquinavir)
094 __ Hivid (dideoxycytidine, Zalcitabine, ddC)	243 __ Atazanavir (BMS-232632)
147 __ Videx (dideoxyinosine, Didanosine, ddI)	238 __ Tipranvir (PNU-140690)
240 __ Trizivir (abacavir + AZT + 3TC)	
234 __ Viread (Tenofovir)	**Entry Inhibitors**
239 __ Coviracil (emtricitabine, FTC)	233 __ Fuzeon (T-20, enfuviratide)
Non-Nucleoside RTIs	__Other antiretroviral
194 __ Rescriptor (delavirdine)	
220 __ Sustiva (efavirenz)	Drug code: |__|__|__|
191 __ Viramune (nevirapine)	Specify: ________________________

PLEASE FILL OUT A PK ADHERENCE FORM (FORM *PK02A*) FOR EACH TARGET MEDICATION (Sustiva, Viramune, Kaletra, Viracept) CHECKED ABOVE.

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK02A: ANTIRETROVIRAL ADHERENCE

SECTION A: GENERAL INFORMATION

PLEASE FILL OUT ONE *PK02A* FORM FOR EACH TARGET ANTIRETROVIRAL MEDICATION (Sustiva, Viramune, Kaletra, Viracept) REPORTED ON *PK02*, QUESTION B2.

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF COMPLETION: ___ ___ / ___ ___ / ____ ___
M D Y

A5. INTERVIEWER'S INITIALS: ___ ___ ___

SECTION B: ADHERENCE INFORMATION

CHECK THE SPECIFIC DRUG FOR WHICH INFORMATION WILL BE CAPTURED ON THIS FORM.

Non-nucleoside RTIs
220 __ Sustiva (efavirenz)
191 __ Viramune (nevirapine)

Protease inhibitors
217 __ Kaletra (lopinavir/ritonavir)
216 __ Viracept (nelfinavir)

PROMPT: PLEASE NAME THE ACTUAL ANTIRETROVIRAL MEDICATION FOR WHICH WE ARE ASESSSING ADHERENCE EVERYTIME THE PROMPT (DRUG) IS WRITTEN.

B1. You said you have taken (DRUG) since your WIHS core study visit.

What was the date you began taking (DRUG)? I just need the month and year. If this is a medication that you had stopped using for some time, but started again since your WIHS core study visit, please give me the most recent date that you began taking this medication.

___ ___ / ___ ___ ___ ___
MONTH/YEAR

PROMPT: IF THIS IS THE FIRST ADHERENCE FORM (PK02A) YOU ARE COMPLETING FOR THIS PARTICIPANT, READ INTRODUCTION 1, OTHERWISE SKIP TO INTRODUCTION 2.

INTRODUCTION 1:
This section of the questionnaire asks about how you are currently taking (DRUG). Most people with HIV have many pills to take at different times during the day. Many people find it hard to always remember their pills. I need to understand how people with HIV are really doing with their medication doses. Please tell me what you are actually doing. Don't worry about telling me that you don't take all your doses. I need to know what is really happening, not what you think I want to hear.

INTRODUCTION 2:
Now I'm going to ask you about how you are currently taking (DRUG).

WIHS ID #

B2. According to your doctor, how many times a day (doses a day) are you *supposed* to take (DRUG)?

- Once per day 1
- Twice per day 2
- Three times per day 3

B3. How many doses of (DRUG) did you **miss yesterday** (DAY)?

- None 0
- One 1
- Two 2
- Three 3
- DON'T KNOW .... <-8>

B4. How many doses of (DRUG) did you **miss the day before yesterday** (DAY)?

- None 0
- One 1
- Two 2
- Three 3
- DON'T KNOW .... <-8>

B5. How many doses of (DRUG) did you **miss the day before that** (3 days ago) (DAY)?

- None 0
- One 1
- Two 2
- Three 3
- DON'T KNOW .... <-8>

B6. SHOW PARTICIPANT RESPONSE CARD PK02A-6.
How many doses of (DRUG) have you **missed in the last 2 weeks?**

- None 0
- One 1
- Two 2
- 3-5 3
- 6-10 4
- 11-20 5
- 21-40 6
- More than 40 7
- All of them 8
- DON'T KNOW .... <-8>

WIHS ID #

B7. SHOW PARTICIPANT RESPONSE CARD PK02A-7.
Please point on the line shown on the response card to your **best guess** about **how much** (DRUG) **you have taken in the past month.** We would be surprised if this was 100% for most people.

0% means you have **taken no** (DRUG).
50% means you have **taken half of** your (DRUG).
100% means you have **taken every single dose of** (DRUG).

0-5%	1	51-55%	11
6-10%	2	56-60%	12
11-15%	3	61-65%	13
16-20%	4	66-70%	14
21-25%	5	71-75%	15
26-30%	6	76-80%	16
31-35%	7	81-85%	17
36-40%	8	86-90%	18
41-45%	9	91-95%	19
46-50%	10	96-100%	20

B8. SHOW PARTICIPANT RESPONSE CARD PK02A-8.
When was the last time **you missed a dose** of (DRUG)?

Today	1
Yesterday	2
Earlier this week	3
Last week	4
Less than a month ago	5
More than a month ago	6
Never	7
DON'T KNOW	<-8>

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK03: RECENT ILLNESSES, CONCURRENT MEDICATIONS AND OB/GYN HISTORY

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___
M D Y

A5. INTERVIEWER'S INITIALS: ___ ___ ___

INTRODUCTION TO PARTICIPANT:

Now, I am going to ask you some questions about your recent health history. I will be asking you a series of questions about symptoms or infections you may have had recently and about medicines you may have taken.

I would like to emphasize that all your answers are confidential, and the responses you provide will in no way affect your clinical care.

SECTION B: MEDICAL SYMPTOMS AND INFECTIONS

B1. First, I'm going to ask you about some medical symptoms. In the last 30 days, have you had any of the following symptoms?

	YES	NO	
a. Fever greater than 100ºF or 38ºC	1	2	
b. Burning or pain when urinating	1	2	
c. Diarrhea with 3 or more soft/liquid stools per day	1	2	
d. Cough producing green or yellow spit	1	2	
e. Runny or congested nose or sore throat	1	2	
f. Sinus infection or sinusitis	1	2	
g. Sores/ulcers inside mouth or on lips	1	2	
h. Sores/ulcers on or around vagina or buttocks	1	2	
i. Any other medical symptoms	1	2	**(B2)**

Specify: ____

WIHS ID #

B2. In the last 30 days, have you had any of the following infections?

	YES	NO	
a. Pneumonia/lung infection	1	2	
b. Skin infection	1	2	
c. Brain infection/abnormal brain scan	1	2	
d. Gonorrhea or Chlamydia	1	2	
e. Any other type of infection	1	2	**(C1)**

Specify: ____

SECTION C: VACCINATIONS

C1. In the last 30 days, have you had any vaccinations?

YES 1
NO 2

SECTION D: MEDICATIONS

The previous questions asked about events in the last 30 days. In this next series of questions, I will be asking you about medications or substances you may have taken in the last **five** days.

D1. In the last five days, did you take any medicine for stomach acid or heartburn?

YES 1
NO .... 2 **(D2)**

HAND PARTICIPANT PK MEDICATION CARD A: STOMACH MEDICATIONS.

Did this include any of the following:

	YES	NO
a. Prilosec (Omeprazole)	1	2
b. Prevacid (Lansoprazole)	1	2
c. Tagamet (Cimetidine)	1	2

D2. In the last five days, did you take any medicine for seizures?

YES 1
NO .... 2 **(D3)**

WIHS ID #

HAND PARTICIPANT PK MEDICATION CARD B: SEIZURE MEDICATIONS.

Did this include any of the following?

		YES	NO
a.	Tegretol (Carbamazepine)	1	2
b.	Dilantin (Phenytoin)	1	2
c.	Felbatol (Felbamate)	1	2
d.	Mysoline (Primidone)	1	2
e.	Topamax (Topiramate)	1	2
f.	Mebaral (mephobarbital)	1	2
g.	Phenobarbital (Luminal, Solfoton)	1	2

D3. In the last five days, did you take any medicine for blood pressure or for your heart?

YES 1
NO .... 2 **(D4)**

HAND PARTICIPANT PK MEDICATION CARD C: HEART AND BLOOD PRESSURE MEDICATIONS.

Did this include any of the following:

		YES	NO
a.	Amiodarone (Cordarone, Pacerone)	1	2
b.	Quinidine (Cardioquin, Quin-Tab, Quinadure, Quinaglute, Quinidex)	1	2
c.	Verapamil (Calan, Verelan, Covera, Isoptin)	1	2
d.	Diltiazem (Cardizem, Cartia, Dilacor, Tiamate, Tiazac)	1	2
e.	Nicardipine (Cardene)	1	2
f.	Nifedipine (Procardia, Adalat)	1	2
g.	Felodipine (Plendil)	1	2

D4. In the last five days, did you take any medicine for cholesterol?

YES 1
NO .... 2 **(D5)**

WIHS ID #

HAND PARTICIPANT PK MEDICATION CARD D: CHOLESTEROL MEDICATIONS.

Did this include any of the following:

	YES	NO
a. Mevacor (Lovastatin)	1	2
b. Lipitor (atrovastatin)	1	2
c. Zocor (Simvastatin)	1	2

D5. In the last five days, did you take medicine for anxiety, depression or your nerves?

YES 1
NO .... 2 **(D6)**

HAND PARTICIPANT PK MEDICATION CARD E: PSYCH MEDICATIONS.

Did this include any of the following?

	YES	NO
a. Zyprexa (Olanzapine)	1	2
b. Serzone (Nefazodone)	1	2
c. Luvox (Fluvoxamine)	1	2
d. Zoloft (Sertraline)	1	2
e. Celexa (Citalpram)	1	2
f. St. John's Wort (Amber, Goat weed, Hardhay, Klamath weed, Tipton weed)	1	2

D6. HAND PARTICIPANT PK MEDICATION CARD F: PREDNISONE & TAMOXIFEN.

In the last five days, did you take a medicine to prevent breast cancer called Tamoxifen?

YES 1
NO 2

D7. In the last five days, did you take Prednisone?

YES 1
NO 2

D8. In the last five days, did you use a Duragesic (Fentanyl) patch for pain?

YES 1
NO 2

WIHS ID #

D9. In the last five days, did you take any antibiotics or antifungals or any other medicines to fight bacterial or fungal infections?

YES 1
NO .... 2 **(D10)**

HAND PARTICIPANT PK MEDICATION CARD G: ANTIBIOTICS.

Did this include any of the following:

	YES	NO
a. Erythromycin (e-mycin, Ilosone, Eryc, Erythromycin ethyl succinate or EES)	1	2
b. Clarithromycin (Biaxin)	1	2
c. Azithromycin (Zithromax)	1	2
d. Dirithromycin (Dynabac)	1	2
e. Norfloxacin (Noroxin)	1	2
f. Ciprofloxacin (Cipro)	1	2
g. Fluconazole (Diflucan)	1	2
h. Itraconazole (Sporanox)	1	2
i. Ketoconazole (Nizoral)	1	2
j. Rifampin (Rifadin)	1	2
k. Rifabutin (Mycobutin)	1	2

D10. In the last five days, have you had:

	YES	NO
a. Grapefruits or grapefruit juice	1	2
b. Oranges or orange juice	1	2
c. Red wine	1	2

SECTION E: RECENT OB/GYN HISTORY

E1. Are you currently pregnant?

YES .... 1 **(E5)**
NO 2

E2. Have you been through menopause?

YES .... 1 **(END FORM)**
NO 2
DON'T KNOW ....-8

WIHS ID #

E3. Are you currently menstruating?

YES 1
NO .... 2 **(E5)**

E4. When was the first day of your current period? Please try to remember as best you can.

___ ___ / ___ ___ / ___ ___ **(END FORM)**
M D Y

E5. When was the first day of your most recent period? Please try to remember as best you can.

___ ___ / ___ ___ / ___ ___
M D Y

E6. How many days did that period last? Please try to remember as best as you can.

|__|__|
#DAYS

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK04: RECENT SUBSTANCE USE

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___
M D Y

A5. INTERVIEWER'S INITIALS: ___ ___ ___

INTRODUCTION TO PARTICIPANT:

Now, I am going to ask you some personal questions about recent cigarette, alcohol and drug use. I would like to emphasize that ***all your answers are strictly confidential***, and the responses you provide will in no way affect your clinical care.

SECTION B: CIGARETTE AND ALCOHOL USE

B1. Since your last core WIHS visit, have you smoked cigarettes?

YES...1
NO..2 **(B3)**

B2. How many cigarettes, on the average, do you smoke each day?

|__|__|__| NUMBER

PACKS………………….1
CIGARETTES…………..2

B3. How *often* did you have a drink containing alcohol since your last core WIHS visit? By a drink, I mean one can, bottle or glass of beer, a glass of wine, a shot of liquor, a mixed drink, or any other kind of alcoholic beverage. **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day...1
Nearly every day..2
3-4 days a week..3
1-2 days a week..4
1-2 times a month 5
None since my last core visit ....6 **(B5)**
I never drink..7 **(B5)**

WIHS ID#

B4. Since your last core WIHS visit, on the days that you did drink alcoholic beverages, how many drinks did you *usually* have altogether?

1-2 drinks 1
3-4 drinks 2
5-6 drinks 3
7 or more drinks 4

B5. On average, how often have you used marijuana or hash since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use marijuana or hash 7

B6. Since your last core WIHS visit, have you used any crack or cocaine or heroin?

YES... 1
NO... 2 **(B10)**

B7. On average, how often have you used crack (ready rock or freebase cocaine) since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use crack 7

WIHS ID#

B8. On average, how often have you used cocaine since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use cocaine... 7

B9. On average, how often have you used heroin since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use heroin... 7

B10. Since your last core WIHS visit, have you used any amphetamines (speed, uppers) or hallucinogens (LSD, acid)?

YES... 1
NO... 2 **(B13)**

B11. On average, how often have you used speed since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use speed.. 7

WIHS ID#

B12. On average, how often have you used LSD since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use LSD 7

B13. Since your last core WIHS visit, have you used any "club or rave drugs" (like **Ecstas**y – XTC, MDMA, X, E; **GHB -** Gamma-hydroxy butyrate, G, Georgia Home Boy, Scoop; **Rohypnol –** Roofies, ropes, ropies, roches; or **Ketamine –** Special K, K, Vitamin K, Super K)?

YES.. 1
NO.. 2 **(END FORM)**

B14. On average, how often have you used Ecstasy (XTC, MDMA, X, E) since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use ecstasy 7

B15. On average, how often have you used GHB (Gamma-hydroxy butyrate, G, Georgia Home Boy, Scoop) since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use GHB 7

B16. On average, how often have you used Rohypnol (roofies, ropes, ropies, roches) since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use Rohypnol 7

B17. On average, how often have you used Ketamine (Special K, K, Vitamin K, Super K) since your last core WIHS visit? **SHOW PARTICIPANT RESPONSE CARD PK04 AND CIRCLE ONE ANSWER.**

At least once a day 1
Nearly every day 2
3-4 days a week.. 3
1-2 days a week.. 4
1-2 times a month 5
None since my last core visit 6
I never use Ketamine 7

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK05C: WEIGHT/SPECIMEN COLLECTION FORM
FOR GROUP C PARTICIPANTS

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF COLLECTION: ___ ___ / ___ ___ / ___ ___
M D Y

A5. COLLECTOR'S INITIALS: ___ ___ ___

A6. NAME OF TARGET MEDICATION TAKEN IN ADDITION TO *SUSTIVA* **(CIRCLE YES OR NO FOR EACH)**:

	YES	NO
a. NEVIRAPINE *(VIRAMUNE)*	1	2
b. NELFINAVIR *(VIRACEPT)*.........................	1	2
c. LOPINAVIR/RITONAVIR (*KALETRA)*......	1	2

SECTION B: WEIGHT

B1. WEIGHT OF PARTICIPANT IN POUNDS (LBS): (ROUND TO NEAREST 1.0 POUND) |___|___|___|
WEIGHT (LBS)

SECTION C: URINE PREGNANCY TEST

C1. URINE PREGNANCY TEST
PREGNANT 1
NOT PREGNANT.. 2
NOT DONE 3
SPECIFY REASON: ____________________________

SECTION D: TIMES OF BLOOD COLLECTIONS FOR PK SAMPLING

D1. TIME PARTICIPANT TOOK NON-SUSTIVA TARGET MEDICATION(S) THE MORNING OR EVENING BEFORE THE PK VISIT (AS CONFIRMED BY TELEPHONE INTERVIEW): |___|___| : |___|___| AM....... 1 PM 2

WIHS ID #

COLLECT FIRST PK SAMPLE ('0' TIMEPOINT) AND RECORD THE TIME OF SAMPLING IN D2. INSTRUCT PARTICIPANT TO TAKE THE TARGET ANTIRETROVIRAL MEDICATION(S) LISTED IN A6, HER SUSTIVA, AND ANY OTHER MEDICATION THAT SHE USUALLY TAKES AT THE SAME TIME. PARTICIPANT SHOULD BE INSTRUCTED TO TAKE HER ANTIRETROVIRAL MEDICATIONS WITH FOOD IF THAT IS HER USUAL ROUTINE AT HOME. RECORD THE TIME OF HAART DOSING IN D3 AND RECORD WHETHER THE PARTICIPANT TOOK HER MEDICATIONS WITH OR WITHOUT FOOD IN D4. SET ELECTRONIC TIMER.

D2. TIME OF BLOOD DRAW FOR FIRST PK SAMPLE ('0' TIMEPOINT): |___|___| : |___|___| AM...........1 PM 2

D3. TIME PARTICIPANT TOOK SUSTIVA AND OTHER TARGET MEDICATION(S): |___|___| : |___|___| AM...........1 ....PM 2

D4. DID PARTICIPANT TAKE HER TARGET MEDICATION(S) WITH OR WITHOUT FOOD?

WITH FOOD 1
WITHOUT FOOD 2

	SAMPLE NUMBER	PK TIMEPOINT	TIME OF BLOOD DRAW
D5.	2	30 minutes (1/2 hr)	|___|___| : |___|___| AM ....1 PM...............2
D6.	3	60 minutes (1 hr)	|___|___| : |___|___| AM ....1 PM...............2
D7.	4	120 minutes (2 hr)	|___|___| : |___|___| AM ....1 PM...............2
D8.	5	150 minutes (2.5 hr)	|___|___| : |___|___| AM ....1 PM...............2
D9.	6	180 minutes (3 hr)	|___|___| : |___|___| AM ....1 PM...............2
D10.	7	240 minutes (4 hr)	|___|___| : |___|___| AM ....1 PM...............2
D11.	8	300 minutes (5 hr)	|___|___| : |___|___| AM ....1 PM...............2
D12.	9	360 minutes (6 hr)	|___|___| : |___|___| AM ....1 PM...............2
D13.	10	480 minutes (8 hr)	|___|___| : |___|___| AM ....1 PM...............2
D14.	11	720 minutes (12 hr)	|___|___| : |___|___| AM ....1 PM...............2
D15.	12	900 minutes (15 hr)	|___|___| : |___|___| AM ....1 PM...............2

WIHS ID #

D16.	13	1080 minutes (18 hr)	|___|___| : |___|___| AM...............1 PM...............2
D17.	14	1260 minutes (21 hr)	|___|___| : |___|___| AM...............1 PM...............2
D18.	15	1440 minutes (24 hr)	|___|___| : |___|___| AM...............1 PM...............2

PROMPT: URINE PREGNANCY TESTS SHOULD BE PERFORMED IN THE LOCAL LAB. PK SAMPLES WILL BE PROCESSED, BATCHED AND THEN SHIPPED WITHIN ONE MONTH TO DR. LESLIE BENET'S LAB.

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK05B: WEIGHT/SPECIMEN COLLECTION FORM
FOR GROUP B PARTICIPANTS

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF COLLECTION: ___ ___ / ___ ___ / ___ ___
M D Y

A5. COLLECTOR'S INITIALS: ___ ___ ___

SECTION B: WEIGHT

B1. WEIGHT OF PARTICIPANT IN POUNDS (LBS): (ROUND TO NEAREST 1.0 POUND) |___|___|___|
WEIGHT (LBS)

SECTION C: URINE PREGNANCY TEST

C1. URINE PREGNANCY TEST
PREGNANT 1
NOT PREGNANT 2
NOT DONE 3
SPECIFY REASON: ________________________________

SECTION D: TIMES OF BLOOD COLLECTIONS FOR PK SAMPLING

IN THE EVENING, COLLECT FIRST PK SAMPLE ('0' TIMEPOINT) AND RECORD THE TIME OF SAMPLING IN D1. INSTRUCT PARTICIPANT TO TAKE HER *SUSTIVA* AND ANY OTHER MEDICATIONS THAT SHE USUALLY TAKES AT THE SAME TIME. PARTICIPANT SHOULD BE INSTRUCTED TO TAKE HER *SUSTIVA* WITH FOOD IF THAT IS HER USUAL ROUTINE AT HOME. RECORD THE TIME OF *SUSTIVA* DOSING IN D2 AND RECORD WHETHER PARTICIPANT TOOK *SUSTIVA* WITH OR WITHOUT FOOD IN D3. SET ELECTRONIC TIMER.

D1. TIME OF BLOOD DRAW FOR FIRST PK SAMPLE ('0' TIMEPOINT): |___|___| : |___|___| AM...........1 PM 2

D2. TIME PARTICIPANT TOOK *SUSTIVA*: |___|___| : |___|___| AM...........1 PM 2

D3. DID PARTICIPANT TAKE HER *SUSTIVA* WITH OR WITHOUT FOOD?
WITH FOOD 1
WITHOUT FOOD 2

WIHS ID #

	SAMPLE NUMBER	PK TIMEPOINT	TIME OF BLOOD DRAW
D4.	2	60 minutes (1 hr)	|___|___| : |___|___| AM...............1 PM...............2
D5.	3	120 minutes (2 hr)	|___|___| : |___|___| AM...............1 PM...............2
D6.	4	240 minutes (4 hr)	|___|___| : |___|___| AM...............1 PM...............2
D7.	5	360 minutes (6 hr)	|___|___| : |___|___| AM...............1 PM...............2
D8.	6	480 minutes (8 hr)	|___|___| : |___|___| AM...............1 PM...............2
D9.	7	720 minutes (12 hr)	|___|___| : |___|___| AM...............1 PM...............2
D10.	8	900 minutes (15 hr)	|___|___| : |___|___| AM...............1 PM...............2
D11.	9	1080 minutes (18 hr)	|___|___| : |___|___| AM...............1 PM...............2
D12.	10	1260 minutes (21 hr)	|___|___| : |___|___| AM...............1 PM...............2
D13.	11	1440 minutes (24 hr)	|___|___| : |___|___| AM...............1 PM...............2

PROMPT: URINE PREGNANCY TESTS SHOULD BE PERFORMED IN THE LOCAL LAB. PK SAMPLES WILL BE PROCESSED, BATCHED AND THEN SHIPPED WITHIN ONE MONTH TO DR. LESLIE BENET'S LAB.

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK06: DOSING OF ANTIRETROVIRAL MEDICATIONS

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE |__| - |__|__| - |__|__|__|__|__| - |__|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE: 04/01/03

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

A5. INTERVIEWER'S INITIALS: ___ ___ ___

FOR EACH OBSERVED ANTIRETROVIRAL MEDICATION THE PARTICIPANT TAKES AT THIS PK VISIT, PLEASE CHECK THE MEDICATION AND RECORD THE DOSAGE TAKEN IN MG.

		Nucleoside/Nucleotide RTIs	**Dosage**
204	__	Epivir (3TC, lamivudine)	|__|__|__|__|__|.|__|__| mg
218	__	Ziagen (abacavir)	|__|__|__|__|__|.|__|__| mg
092	__	Retrovir (AZT, zidovudine, ZDV)	|__|__|__|__|__|.|__|__| mg
227	__	Combivir (AZT + 3TC)	|__|__|__|__|__|.|__|__| mg
159	__	Zerit (d4T, stavudine)	|__|__|__|__|__|.|__|__| mg
094	__	Hivid (dideoxycytidine, Zalcitabine, ddC)	|__|__|__|__|__|.|__|__| mg
147	__	Videx (dideoxyinosine, Didanosine, ddI)	|__|__|__|__|__|.|__|__| mg
240	__	Trizivir (abacavir + AZT + 3TC)	|__|__|__|__|__|.|__|__| mg
234	__	Viread (Tenofovir)	|__|__|__|__|__|.|__|__| mg
239	__	Coviracil (emtricitabine, FTC)	|__|__|__|__|__|.|__|__| mg
		Non-Nucleoside RTIs	
194	__	Rescriptor (delavirdine)	|__|__|__|__|__|.|__|__| mg
220	__	Sustiva (efavirenz)	|__|__|__|__|__|.|__|__| mg
191	__	Viramune (nevirapine)	|__|__|__|__|__|.|__|__| mg

WIHS ID #

Protease Inhibitors

219 __ Agenerase (amprenavir) .... |__|__|__|__|__|.|__|__| mg

212 __ Crixivan (indinavir)... |__|__|__|__|__|.|__|__| mg

217 __ Kaletra (lopinavir/ritonavir) .... |__|__|__|__|__|.|__|__| mg

216 __ Viracept (nelfinavir).. |__|__|__|__|__|.|__|__| mg

211 __ Norvir (ritonavir)... |__|__|__|__|__|.|__|__| mg

210 __ Invirase or Fortovase (saquinavir)................... |__|__|__|__|__|.|__|__| mg

243 __ Atazanavir (BMS-232632) .... |__|__|__|__|__|.|__|__| mg

239 __ Tipranvir (PNU-140690) .... |__|__|__|__|__|.|__|__| mg

Entry Inhibitors

233 __ Fuzeon (T-20, enfuviratide) .... |__|__|__|__|__|.|__|__| mg

Other

207 __ Hydroxyurea (Droxia, Hydria)......................... |__|__|__|__|__|.|__|__| mg

|__|__|__| __ Other antiviral (from Drug List 1)................... |__|__|__|__|__|.|__|__| mg

Name of Drug: ____

WOMENS INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
PK07: PLASMA SEPARATION AND FREEZING FORM

ID LABEL HERE ---> |__| - |__|__| - |__|__|__|__|__| - |__| VISIT #: ___ ___ ___ FORM COMPLETED BY: ___ ___ ___

VERSION DATE **4/01/03**

ANY MISSING INFORMATION MUST BE EXPLAINED ON THIS FORM

EDTA TUBES

B1. TIMEPOINT OF EDTA TUBE ______ MINUTES

B2. DATE EDTA TUBE DRAWN: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

B3. DATE EDTA TUBE RECEIVED IN LAB: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

B4. TIME EDTA TUBE RECEIVED IN LAB: |___|___| : |___|___| AM............1 PM............2

B5. DATE EDTA TUBES CENTRIFUGED IN LAB: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

a. TIME: |___|___| : |___|___| AM............1 PM............2

B6. PLASMA SEPARATION DATE: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

a. TIME: |___|___| : |___|___| AM............1 PM............2

B7. PLASMA FROZEN DATE: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

a. TIME: |___|___| : |___|___| AM............1 PM............2

B8. TOTAL VOLUME OF PLASMA FROZEN: |___|___| . |___| ml

DO NOT DATA ENTER FORM PK07.

WOMEN'S INTERAGENCY HIV STUDY
INTENSIVE PK STUDY
FORM PK08: DIETARY FAT PERCENTAGE QUESTIONNAIRE

A1. PARTICIPANT ID: |___|-|___|___|-|___|___|___|___|-|___|

A2. LAST WIHS CORE VISIT #: ___ ___

A3. VERSION DATE 05/14/03

A4. DATE OF COMPLETION: ___ ___ / ___ ___ / ___ ___
M D Y

A5. INTERVIEWER'S INITIALS ___ ___ ___

HAND PARTICIPANT RESPONSE CARD PK08
Think about your eating habits over the last year or so. About how often do you eat each of the following foods? Remember to include breakfast, lunch, dinner, snacks and eating out.

		1 TIME PER MONTH OR LESS	2-3 TIMES PER MONTH	1-2 TIMES PER WEEK	3-4 TIMES PER WEEK	MORE THAN 5 TIMES/WEEK
B1.	Hamburgers, ground beef, meat burritos, tacos	1	2	3	4	5
B2.	Beef or pork, such as steaks, roasts, ribs, or in sandwiches	1	2	3	4	5
B3.	Fried chicken	1	2	3	4	5
B4.	Hot dogs, Polish or Italian sausage	1	2	3	4	5
B5.	Cold cuts, lunch meats, ham (not low-fat)	1	2	3	4	5
B6.	Bacon or breakfast sausage	1	2	3	4	5
B7.	Salad dressings (not low-fat)	1	2	3	4	5
B8.	Margarine, butter or mayo on bread or potatoes	1	2	3	4	5
B9.	Margarine, butter or oil in cooking	1	2	3	4	5
B10.	Eggs (not Egg Beaters or just egg whites)	1	2	3	4	5
B11.	Pizza	1	2	3	4	5
B12.	Cheese, cheese spread (not low-fat)	1	2	3	4	5
B13.	Whole milk	1	2	3	4	5
B14.	French fries or fried potatoes	1	2	3	4	5
B15.	Corn chips, potato chips, popcorn, crackers	1	2	3	4	5
B16.	Doughnuts, pastries, cake, cookies (not low-fat)	1	2	3	4	5
B17.	Ice cream (not sherbet or non-fat)	1	2	3	4	5

INSTRUCTIONS FOR COMPLETION OF QUESTION C1:

1. **GO TO BERKELEY NUTRITION SERVICES, ON-LINE FAT SCREENER FORM: http://www.nutritionquest.com/fat_screener.html**
2. **ENTER THE RESPONSES INDICATED BY THE PARTICIPANT FOR QUESTIONS B1 – B17 INTO THE FORM AND CLICK "SUBMIT QUESTIONNAIRE."**
3. **THE FIRST PARAGRAPH OF THE "FAT SCREENER RESULTS" WILL CONTAIN AN ESTIMATE OF THE PARTICIPANT'S FAT INTAKE.**
4. **CIRCLE THE PARTICPANT'S ESTIMATED FAT INTAKE BELOW IN C1.**
5. **DATA ENTER FORM INTO APOLLO.**

C1. PERCENT FAT IN DIET:

<30% 0
30-35% 1
36-40% 2
>40% 3

WOMEN'S INTERAGENCY HIV STUDY

MEDICAL AND HEALTH HISTORY

FORM 22 MED

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE

|__|- |__|__| - |__|__|__|__| - |__|

A2. WIHS STUDY VISIT #: ___ ___

A2a. WIHS Core Visit 1
3 Month VRS Visit 2

A3. FORM VERSION: 0 4 / 0 1 / 0 3 (M / D / Y)

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

A5. INTERVIEWER'S INITIALS: ___ ___ ___

A6. DATE OF LAST STUDY VISIT (FROM VISIT CONTROL SHEET) ___ ___ / ___ ___ / ___ ___ (M / D / Y)

A7. TIME MODULE BEGAN: |__|__| : |__|__|

AM 1
PM 2

INTRODUCTION TO PARTICIPANT:

Now, I am going to ask you a series of questions about medicines you may have had or taken since your study visit on __ __ / __ __ / __ __.
(M / D / Y)

Also, if at any point in the interview you wish to stop, let me know.

Finally, I need to re-emphasize that all your answers are confidential, and the responses you provide will in no way affect your clinical care.

WIHS ID#

SECTION B. ANTIRETROVIRAL HISTORY

B1. Since your (MONTH) study visit, have you had a vaccine injection against HIV or participated in a vaccine trial? A vaccine against HIV can include vaccines, which prevent infection with HIV, or therapeutic vaccines (those which prevent progression of the infection).

YES..1
NO..2

START F22MEDS3

B2. Now I'm going to ask about any antiretroviral medications you may have taken since your (MONTH) study visit. In addition to all your prescribed medications, please include any antiretroviral medications you have taken as part of a research study, including those in which you may have been blinded to the study medication.

PROMPT: HAND PARTICIPANT ANTIVIRAL PHOTO MEDICATION CARDS. GO THROUGH THE CARDS WITH THE PARTICIPANT, SAYING THE NAME OF EACH DRUG ALOUD AND ASKING HER TO TELL YOU "YES" OR "NO" WHETHER SHE HAS TAKEN THIS DRUG SINCE HER LAST VISIT.

CHECK THE DRUG(S) THE PARTICIPANT HAS TAKEN. FOR DRUGS NOT ON THE LIST, RECORD THE NAME UNDER "OTHER" AS STATED BY THE PARTICIPANT AND FILL IN THE CORRESPONDING THREE-DIGIT DRUG CODE FROM DRUG LIST 1.

A. Since your (MONTH) study visit, have you taken…

Nucleoside/Nucleotide RTIs

204 __ Epivir (lamivudine, 3-TC)
218 __ Ziagen (abacavir, 1592U89)
092 __ Retrovir (AZT, zidovudine, ZDV)
227 __ Combivir (AZT + 3TC)
159 __ Zerit (stavudine, d4T)
094 __ Hivid (dideoxycytidine, zalcitabine, ddC)
147 __ Videx / Videx EC (dideoxyinosine, didanosine, ddI)
240 __ Trizivir (abacavir + AZT + 3TC)
234 __ Viread (tenofovir, bis-POC-PMPA)
239 __ Coviracil (emtricitabine, FTC)

Non-Nucleoside RTIs

194 __ Rescriptor (delavirdine, U-90)
220 __ Sustiva (efavirenz, DMP266)
191 __ Viramune (nevirapine)

Protease Inhibitors

219 __ Agenerase (amprenavir, 141W94)
212 __ Crixivan (indinavir)
217 __ Kaletra (lopinavir/ritonavir, ABT-378/r)
216 __ Viracept (nelfinavir)
211 __ Norvir (ritonavir)
210 __ Invirase or Fortovase (saquinavir)
243 __ Atazanavir (BMS-232632)
238 __ Tipranavir (PNU-140690)

Entry Inhibitors

233 __ Fuzeon (T-20, enfuviratide)

Other

207 __ Droxia or Hydrea (hydroxyurea)
__ Other anti-viral(s) (from Drug List 1)

Name of Drug:	Drug Code: |__|__|__|
Name of Drug:	Drug Code: |__|__|__|

END F22MEDS3

PLEASE COMPLETE DRUG FORM 1 FOR EACH MEDICATION MARKED ABOVE IN QUESTION B2A.

B. If the participant has not taken ANY antiviral medication since her (MONTH) study visit, check here: __ **GO TO QB8**

B3. Sometimes patients stop taking all of their antiretroviral medications for planned or prescribed periods of time to try to boost their immune systems. These therapy breaks, also called structured treatment interruptions, are

very different from any therapy breaks that were not planned or prescribed, such as forgetting to take your medications, running out of pills, or simply taking a break because you felt like you needed one. In the next series of questions, I want to know only about any breaks in your antiretroviral medications that you have taken in the past three months that were planned by you or prescribed by your provider.

a. In the past 3 months, was there a planned or prescribed period of time when you stopped taking all of your antiretroviral medications for at least two consecutive days?

YES..1
NO..2 **GO TO QB4**

b. How many planned breaks in your antiretroviral therapy did you take over the past 3 months?

|___|___|
BREAKS

c. Were these planned breaks prescribed by your doctor or health care provider?

YES..1
NO..2 **GO TO QB3e**

d. What was the main reason that your doctor or health care provider told you to interrupt your treatment?

CIRCLE ONLY ONE ANSWER

To strengthen your immunity to HIV 1
Because your viral load was going up or your CD4 count was falling.........2
Because you were having side effects..3
Because you were pregnant...4
Because you had another illness 5
Other reason 6

____
(SPECIFY)

e. When was the last time that you interrupted or took a planned break in all of your antiretroviral medications for at least two days? I just need to know the month. INTERVIEWER FILL IN YEAR.

____ ____ / ____ ____
M Y

f. During this last therapy interruption in (MONTH), how long did you go without taking any of your antiretroviral medications? You can tell me in days or weeks, whatever is easiest for you.

|___|___| DAYS 1
WEEKS 2

B4. Now I am going to ask you about any therapy breaks you may have taken that were unplanned.

a. In the past 3 months, was there a time when you took any unplanned breaks in all of your prescribed antiretroviral therapy for at least one full day?

YES..1
NO..2 **GO TO QB5**

WIHS ID#

b. How many unplanned breaks in your antiretroviral therapy did you take over the past 3 months?

|___|___|
BREAKS

c. When was the last time that you skipped or took an unplanned break in all your antiretroviral medications for at least one full day? I just need to know the month. INTERVIEWER FILL IN YEAR.

____ ____ / ____ ____
M Y

d. During this last unplanned therapy break in (MONTH), how long did you go without taking any of your antiretroviral medications? You can tell me in days or weeks, whatever is easiest for you.

|___|___| DAYS 1
WEEKS 2

For the remaining questions, I want you to focus on how you have taken your medications over the past six months.

B5. a. In general, over the past 6 months, how often did you take your antiretrovirals as prescribed?

100% of the time..1 **GO TO QB6**
95-99% of the time 2
75-94% of the time 3
< 75% of the time 4
I haven't taken any of my prescribed medications......5

b. **PROMPT: HAND PARTICIPANT RESPONSE CARD D1.**

People skip or miss taking their medications for various reasons. Here is a list of possible reasons why you may miss taking your medications. Since your (MONTH) study visit, how often have you missed taking your anti-retroviral medications because you:

	Never	Rarely	Sometimes	Often
Simply forgot?	0	1	2	3
Had a change in daily routine (e.g., vacation, holiday, non-workday)?	0	1	2	3
Fell asleep or slept through dose time?	0	1	2	3
Had too many pills to take?	0	1	2	3
Ran out of pills?	0	1	2	3
Did not feel like taking any pills?	0	1	2	3
Did not want others to notice you taking medications?	0	1	2	3
Were on drugs or drank too much?	0	1	2	3
Wanted to avoid side effects?	0	1	2	3
Felt like the drug was toxic or harmful?	0	1	2	3
Felt too sick to take medications?	0	1	2	3
Felt too depressed to take medications?	0	1	2	3
Had difficulty following special instructions (e.g., take with meals or on empty stomach)?	0	1	2	3
Other reason	0	1	2	3

Specify reason:

WIHS ID#

B6. **PROMPT: HAND PARTICIPANT RESPONSE CARD D2.**

a. Most anti-HIV medications need to be taken on a schedule, such as "every 12 hours" or "every 8 hours." In general, how closely do you follow your specific schedule?

Never..1
Some of the time....................................2
About half of the time............................3
Most of the time.....................................4
All of the time..5

b. Do any of your anti-HIV medications have special instructions such as "take with food" or "take on an empty stomach" or "take with plenty of fluids?"

Yes...1
No..2 **GO TO QB7**

PROMPT: HAND PARTICIPANT RESPONSE CARD D2.

c. In general, how often do you follow these special instructions?

Never..1
Some of the time....................................2
About half of the time............................3
Most of the time.....................................4
All of the time..5

B7. IS PARTICIPANT CURRENTLY TAKING COMBINATION THERAPY?

YES..1
NO..2 **GO TO QC1**

HAND PARTICIPANT RESPONSE CARD 12.
I am going to read to you some things that people taking combination drug treatments believe about transmission of HIV. Please tell me if you strongly agree, agree, if you are uncertain or if you disagree or strongly disagree.

	STRONGLY AGREE	AGREE	UNCERTAIN	DISAGREE	STRONGLY DISAGREE
a. Since starting combination drug treatments, I worry less about passing HIV to other people during sex..........	1	2	3	4	5
b. I worry less about always using condoms since I started combination drug treatments..........	1	2	3	4	5
c. I think that it is less likely that I could infect other people during sex now that I am on combination drug treatments..........	1	2	3	4	5
d. I would be less worried about a new partner's HIV serostatus now that I am on combination drug treatments..........	1	2	3	4	5

GO TO QC1

WIHS ID#

B8. **PROMPT: HAND PARTICIPANT RESPONSE CARD D3.**

What is your main reason for not taking any antiviral medications or treatments?

CIRCLE ONE ANSWER ONLY.

I am HIV negative.. 1
My CD4+ was too high / viral load was too low 2
I feel too healthy 3
I am taking alternative medications 4
I don't want side effects... 5
They are too hard to swallow... 6
My doctor did not prescribe them...................................... 7
I can't afford them/have no insurance coverage 8
I am concerned about resistance 9
I'm having a baby 10
Personal decision to wait 11
They didn't work for my friends.. 12
Any other reason.. 13

Specify reason:

WIHS ID#

SECTION C. OI MEDICATION HISTORY

START F22MEDS4

C1. **PROMPT: HAND PARTICIPANT RESPONSE CARD D4. READ THE NAME OF EACH MEDICATION ALOUD. ASK THE PARTICIPANT IF SHE IS TAKING THIS MEDICATION. IF SHE ANSWERS YES, CHECK NEXT TO THE DRUG NAME.**

A. Since your (MONTH) visit, have you taken the following inhaled medication?

114 __ Pentamidine (aerosolized)

i. If the participant has not taken ANY medication in C1A since her (MONTH) study visit, check here: __ **GO TO QC1B**

B. Since your (MONTH) visit, have you taken any of the following injected or infused drugs?

091 __ Foscarnet (Foscavir)
125 __ Ganciclovir (DHPG, Cytovene)
232 __ Nandralone (Deca-Durabolin)
157 __ Medication to increase white blood cell count (G-CSF, GM-CSF, Neupogen)
117 __ Medication to increase red blood cell count (Erythropoietin, Epogen, Procrit, EPO)
090 __ Interferon
124 __ Amphotericin B (Ampho B)
242 __ Pegylated interferon (PEGASYS, PEG-Intron A, Peg Interferon alpha-2a)

i. If the participant has not taken ANY medication in C1B since her (MONTH) study visit, check here: __ **GO TO QC1C**

C. Since your (MONTH) visit, have you used any of the following pills, liquids or creams?

112 __ Bactrim (Septra, TMP/SMX)
184 __ Biaxin (Clarithromycin)
153 __ Cipro (Ciprofloxacin)
113 __ Dapsone
116 __ Diflucan (Fluconazole)
213 __ Famvir (Famcyclovir)
138 __ INH (Isoniazid)
154 __ Lamprene (Clofazimine)
190 __ Mepron (Atovaquone)
540 __ Methadone
229 __ Monistat (Miconazole)
137 __ Myambutol (Ethambutol)
145 __ Mycelex or Lotrimin (Clotrimazole)
127 __ Nizoral (Ketoconazole)
144 __ Nystatin (Mycostatin)
228 __ Oxandrin (Oxandralone)
702 __ Prednisone (Deltasone)
182 __ PZA (Pyrazinamide)
235 __ Rebetron (Ribavirin & Alpha Interferon)
093 __ Rifabutin (Mycobutin)
139 __ Rifadin (Rifampin)
169 __ Sporanox (Itraconazole)
230 __ Terazol (Terconazole)
198 __ Valtrex (Valacyclovir)
152 __ Zithromax (Azithromycin)
146 __ Zovirax (Acyclovir)

i. If the participant has not taken ANY medication in C1C since her (MONTH) study visit, check here: __ **GO TO QD1**

END F22MEDS4

PLEASE COMPLETE DRUG FORM 2 FOR EACH MEDICATION MARKED ABOVE IN QUESTION C1.

WIHS ID#

SECTION D. HEPATITIS MEDICATION HISTORY

START F22MEDS9

D1. **PROMPT: HAND PARTICIPANT RESPONSE CARD D4a. READ THE NAME OF EACH MEDICATION ALOUD. ASK THE PARTICIPANT IF SHE HAS TAKEN THIS MEDICATION FOR HEPATITIS. IF SHE ANSWERS YES, CHECK THE DRUG NAME.**

A. Since your (MONTH) study visit, have you taken (MEDICATION) for Hepatitis (B or C)?

090 __ Interferon (Intron A)
242 __ Pegylated interferon (PEGASYS or Peg interferon alfa 2a)
(PEG-Intron or Peg interferon alfa 2b)
058 __ Ribavirin (Rebetol)
235 __ Rebetron (Ribavirin and interferon alfa 2b)
204 __ Epivir (lamivudine, 3-TC)
234 __ Tenofovir (Viread, bis-POC-PMPA)
224 __ Adefovir (Preveon, bis-POM PMPA, GS 840)
239 __ Coviracil (Emtricitabine, FTC)
708 __ Infergen (Interferon alfacon-1)
213 __ Famvir (Famciclovir)

B. If the participant has not taken ANY medication in D1A, check here: __ **GO TO QE1**

END F22MEDS9

PLEASE COMPLETE DRUG FORM 3 FOR EACH MEDICATION MARKED ABOVE IN QUESTION D1A.

SECTION E. OTHER PRESCRIPTION MEDICATION USE

E1. Since your (MONTH) study visit, have you received any of the following vaccinations?

	YES	NO	DON'T KNOW
a. Hepatitis A	1	2	<-8>
b. Hepatitis B	1	2	<-8>
c. Pneumovax	1	2	<-8>
d. Varicella (chicken pox)	1	2	<-8>
e. Tetanus	1	2	<-8>
f. Smallpox	1	2	<-8>

WIHS ID#

E2. Since your (MONTH) study visit, have you taken any medication for blood pressure or your heart?

YES 1
NO ....2 **(E3)**

a. How many blood pressure or heart drugs are you taking now? |__|__|

HAND PARTICIPANT RESPONSE CARD D4b: HEART AND BLOOD PRESSURE MEDICATIONS

	Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this in the past 5 days?	
		YES	NO	YES	NO
b.	Amiodarone (Cordarone, Pacerone)	1	2 **(c)**	1	2
c.	Quinidine (Cardioquin, Quin-Tab, Quinadure, Quinaglute, Quinidex)	1	2 **(d)**	1	2
d.	Verapamil (Calan, Verelan, Covera, Isoptin)	1	2 **(e)**	1	2
e.	Diltiazem (Cardizem, Cartia, Dilacor, Tiamate, Tiazac)	1	2 **(f)**	1	2
f.	Nicardipine (Cardene)	1	2 **(g)**	1	2
g.	Nifedipine (Procardia, Adalat)	1	2 **(h)**	1	2
h.	Felodipine (Plendil)	1	2 **(E3)**	1	2

WIHS ID#

E3. Since your (MONTH) study visit, have you taken any medication to lower your cholesterol, triglyceride, or blood lipid level?

YES 1
NO ....2 **(E4)**

HAND PARTICIPANT RESPONSE CARD D4c: CHOLESTEROL MEDICATIONS

Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this in the past 5 days?	
	YES	NO	YES	NO
a. Lescol (Fluvastatin)	1	2 **(b)**	1	2
b. Lipitor (Atorvastatin)	1	2 **(c)**	1	2
c. Mevacor (Lovastatin)	1	2 **(d)**	1	2
d. Pravachol (Pravastatin)	1	2 **(e)**	1	2
e. Zocor (Simvastatin)	1	2 **(f)**	1	2
f. Lopid (Gemfibrozil)	1	2 **(g)**	1	2
g. TriCor (Fenofibrate)	1	2 **(h)**	1	2
h. Colestid (Colestipol)	1	2 **(i)**	1	2
i. Questran (Cholestyramine)	1	2 **(j)**	1	2
j. Welchol (Colesevelam)	1	2 **(k)**	1	2
k. Niaspan (Niacin)	1	2 **(E4)**	1	2

WIHS ID#

E4. Since your (MONTH) study visit, have you taken any medication to lower your blood sugar?

YES 1
NO ....2 **(E5)**

HAND PARTICIPANT RESPONSE CARD D4d: BLOOD SUGAR MEDICATIONS

Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this in the past 5 days?	
	YES	NO	YES	NO
a. Insulin (injection)	1	2 **(b)**	1	2
b. Acarbose (Precose)	1	2 **(c)**	1	2
c. Chlorpropamide (Diabinese)	1	2 **(d)**	1	2
d. Glimepiride (Amaryl)	1	2 **(e)**	1	2
e. Glipizide (Glucotrol)	1	2 **(f)**	1	2
f. Glyburide (Micronase, Diabeta)	1	2 **(g)**	1	2
g. Metformin (Glucophage)	1	2 **(h)**	1	2
h. Miglitiol (Glyset)	1	2 **(i)**	1	2
i. Orlistat (Xenical)	1	2 **(j)**	1	2
j. Pioglitazone (Actos)	1	2 **(k)**	1	2
k. Repaglinide (Prandin)	1	2 **(l)**	1	2
l. Rosiglitazone (Avandia)	1	2 **(m)**	1	2
m. Starlix (Nateglinide)	1	2 **(E5)**	1	2

WIHS ID#

E5. Since your (MONTH) study visit, have you taken any medication to prevent or treat osteoporosis?

YES 1
NO ....2 **(E6)**

HAND PARTICIPANT RESPONSE CARD D4e: OSTEOPOROSIS MEDICATIONS

Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this in the past 5 days?	
	YES	NO	YES	NO
a. Vitamin D supplements	1	2 **(b)**	1	2
b. Calcium supplements	1	2 **(c)**	1	2
c. Estrogen Replacement Therapy	1	2 **(d)**	1	2
d. Fosimax (Alendronate)	1	2 **(e)**	1	2
e. Evista (Raloxifene)	1	2 **(f)**	1	2
f. Forteo (teriparatide)	1	2 **(E6)**	1	2

E6. Since your (MONTH) study visit, have you taken any medication for seizures?

YES 1
NO ....2 **(E7)**

HAND PARTICIPANT RESPONSE CARD D4f: SEIZURE MEDICATIONS

Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this in the past 5 days?	
	YES	NO	YES	NO
a. Tegretol (Carbamazepine)	1	2 **(b)**	1	2
b. Dilantin (Phenytoin)	1	2 **(c)**	1	2
c. Felbatol (Felbamate)	1	2 **(d)**	1	2
d. Mysoline (Primidone)	1	2 **(e)**	1	2
e. Topamax (Topiramate)	1	2 **(f)**	1	2
f. Mebaral (mephobarbital)	1	2 **(g)**	1	2
g. Phenobarbital (Luminal, Solfoton)	1	2 **(E7)**	1	2

WIHS ID#

E7. Since your (MONTH) study visit, have you taken any medication for psychological conditions or depression?

YES 1
NO ....2 **(E8)**

HAND PARTICIPANT RESPONSE CARD D4g: PSYCH MEDICATIONS

Did these include any of the following:	i) Have you taken this in the past 6 months?		ii) Have you taken this the past 5 days?	
	YES	NO	YES	NO
a. Zyprexa (Olanzapine)	1	2 **(b)**	1	2
b. Serzone (Nefezodone)	1	2 **(c)**	1	2
c. Luvox (Fluvoxamine)	1	2 **(d)**	1	2
d. Zoloft (Sertraline)	1	2 **(e)**	1	2
e. Celexa (Citalpram)	1	2 **(f)**	1	2
f. Depakote	1	2 **(E8)**	1	2

E8. **PROMPT: HAND PARTICIPANT RESPONSE CARD D4h**

Since your (MONTH) visit, have you taken any of the following hormone replacement therapies (hormones, estrogen, progesterone) for more than one month? These therapies could have been taken in the form of a pill, cream, or patch worn on the skin?

ESTROGEN:
Premarin, Estrace, Estratab, Menest, Ogen, Cenestin, Estraderm, Climera

PROGESTERONE:
Provera, Cycrin, Amen, Prometrium, Micronor, Nor-QD

COMBINATION ESTROGEN/PROGESTERONE:
Premphase, Prempro, Combipatch

OTHER HRT:
Tamoxifen, Raloxifene, Testosterone patch or cream, Estratest (combination Estrogen/Testosterone), Birth Control Pills

YES………………………………1
NO………………………………..2 **GO TO QE9**

WIHS ID#

A. INTERVIEWERS: BASED ON PARTICIPANT RESPONSE IN E8, CODE BELOW THE TYPE OF HRT THE PARTICIPANT REPORTED ABOVE:

ESTROGEN 1
PROGESTERONE 2
COMBINATION......................................3
OTHER HRT 4

SPECIFY: ____

B. What are the main reasons you are taking hormone replacement therapy? Is it for:

		YES	NO
a.	Menopause related symptoms (the change, hot flashes, vaginal dryness, sweating)...	1	2
b.	Depression, anxiety, or emotional distress	1	2
c.	Replacement after hysterectomy or removal of ovaries	1	2
d.	Osteoporosis, or to prevent or treat bone loss...............................	1	2
e.	Prevention of heart disease	1	2
f.	Irregular menstrual periods (spotting)	1	2
g.	Other reason (specify)	1	2 **(E9)**

Specify:____

E9. Have you ever been treated with radioactive iodine or any other medication for an overactive thyroid (hyperthyroidism)?

YES 1
NO ....2 **(E10)**

HAND PARTICIPANT RESPONSE CARD D4i: HYPERTHYROID MEDICATIONS

	Did these include any of the following:	i) Have you taken this in the past 6 months? YES	NO	ii) Have you taken this in the past 5 days? YES	NO
a.	Propylthiouracil (PTU)	1	2 **(b)**	1	2
b.	Beta blockers (propranolol, Inderal)	1	2 **(c)**	1	2
c.	Methimazole (Tapazole)	1	2 **(d)**	1	2
d.	Radioactive iodine (RAI)	1	2 **(E10)**	1	2

WIHS ID#

E10. Since your (MONTH) visit, have you taken any other **PRESCRIBED** medications NOT previously mentioned?

YES 1
NO .... 2 **GO TO F1**

START F22MEDS6

SPECIFY:

Name of Drug:	Name of Drug:
Name of Drug:	Name of Drug:
Name of Drug:	Name of Drug:
Name of Drug:	Name of Drug:
Name of Drug:	Name of Drug:
Name of Drug:	Name of Drug:

END F22MEDS6

SECTION F. ALTERNATIVE/COMPLEMENTARY MEDICATION USE

F1. **PROMPT: HAND PARTICIPANT RESPONSE CARD D5.**
In addition to standard medication therapies, we are interested in collecting information on complementary and alternative therapies.

A. Since your (MONTH) visit, have you used any complementary or alternative medications that you take by mouth either as a pill or liquid, that you apply to your skin, or that you insert in your rectum or vagina. Please include any enzyme therapies, flower remedies, herbs, homeopathic remedies and nutritional supplements such as vitamins or minerals you may have taken. Do not include commercial herbal tea preparations (i.e., tea bags), but please include tea remedies made from fresh bulk herbs.

YES... 1
NO... 2 **GO TO QG1**

START F22MEDS5

WIHS ID#

B. Please name those complementary and alternative medications that you have taken.
PROMPT: CHECK THE COMPLEMENTARY AND/OR ALTERNATIVE MEDICATION(S) NAMED. SPECIFY THOSE NOT ON THE LIST UNDER "OTHER" AND FILL IN THE CORRESPONDING THREE-DIGIT DRUG CODE FROM DRUG LIST 3.

	Treatments		Frequency of Use: Every or almost every day	Frequency of Use: Only as needed	Currently taking?	MAIN reason for taking?
621 __	**Enzyme Therapies (plant or pancreatic)**		1	2	Y N	
622 __	**Flower Remedies**		1	2	Y N	
	Herbs (Chinese/Asian, Native American, South American, Indian/Ayurvedic)					
	613 __ Cat claw		1	2	Y N	
	615 __ Chinese herbs in combination		1	2	Y N	
	620 __ Echinacea (with or without Goldenseal)		1	2	Y N	
	624 __ Garlic		1	2	Y N	
	632 __ Milk thistle		1	2	Y N	
	167 __ St. John's Wort (Hypericin)		1	2	Y N	
	539 __ Other herbs, unspecified		1	2	Y N	
629 __	**Homeopathic Remedies**		1	2	Y N	
	Nutritional Supplements (such as vitamins, minerals)					
	602 __ Acidophilus		1	2	Y N	
	601 __ A-Vitamins		1	2	Y N	
	610 __ Beta-carotene		1	2	Y N	
	607 __ B-Complex		1	2	Y N	
	608 __ B-Vitamins (B1 Thiamine, B2 Riboflavin, B5 Pantothenic Acid, B6 Pyridoxine, B12)		1	2	Y N	
	612 __ C-Vitamins (Rosehips)		1	2	Y N	
	196 __ Coenzyme Q-10		1	2	Y N	
	161 __ DHEA		1	2	Y N	
	619 __ E-Vitamins		1	2	Y N	
	623 __ Folic Acid		1	2	Y N	
	630 __ Multivitamin / Mineral		1	2	Y N	
	631 __ Megadose Vitamins		1	2	Y N	
	633 __ Omega-3 Type Oils		1	2	Y N	
	634 __ Protein Powder		1	2	Y N	
	640 __ Zinc		1	2	Y N	
	503 __ Other nutritional supplements, unspecified		1	2	Y N	
188 __	**NAC (N-acetyl-cysteine)**		1	2	Y N	
173 __	**Ozone**		1	2	Y N	
635 __	**SPV-30**		1	2	Y N	
637 __	**Thymus Glandular**		1	2	Y N	
__	**Other treatment(s) (from Drug List 3)**					
Specify:		→Drug code: |__|__|__|	1	2	Y N	
Specify:		→Drug code: |__|__|__|	1	2	Y N	
Specify:		→Drug code: |__|__|__|	1	2	Y N	

HAND PARTICIPANT RESPONSE CARD D6. ***Reasons for taking complementary/alternative medications:***

01=to treat or reduce side effects from "standard" medications
02=to boost immune system
03=to prevent opportunistic and general infections
04=to treat HIV infection
05=for general health
06=beneficial without causing side effects
07=standard HIV medications don't work
99=other

WIHS ID#

END F22MEDS5

F2. Who prescribes or guides your use of these alternative medications? **CIRCLE ONE ANSWER.**

Primary care provider (non-C/A practitioner) .... 1 **GO TO QG1**
Self-medicated 2
Complementary/Alternative practitioner (Homeopath, Herbalist, Naturopath).... 3
Health Store Staff... 4
Other 5

F3. Have you discussed your use of this medication with your primary care provider?

YES.. 1 **GO TO QG1**
NO 2
DON'T HAVE A PRIMARY CARE PROVIDER .... 3 **GO TO QG1**

F4. **PROMPT: HAND PARTICIPANT RESPONSE CARD D7.**

If no, what is the MAIN reason you have not told him/her? **CIRCLE ONE ANSWER.**

He/She didn't ask.. 1
I didn't think it was important 2
I don't think he/she would approve of its use 3
I think he/she would ask me to stop taking it............................... 4
He/She is not knowledgeable about alternative medications...................... 5
Other 6

WIHS ID#

SECTION G. SYMPTOMS

G1. **PROMPT: HAND PARTICIPANT RESPONSE CARD D8.**
Now I am going to ask you some questions about symptoms that may occur due to the stress of daily life or aging. These symptoms also occur in a small number of people as a result of taking certain medications.

Since your last visit, please tell me if you have experienced any of the following symptoms and, if you have, whether the symptom was not bad, bad, very bad or terrible.

PROMPT: IF PARTICIPANT IS NOT TAKING ANY PRESCRIBED OR ALTERNATIVE MEDICATIONS, CODE SUBQUESTIONS i AND ii AS "N/A."

Since your last visit, have you had…						Do you feel that this symptom was a side effect of your…	
	Not at all	Not bad	Bad	Very bad	Terrible	i. Prescribed medications	ii. Alternative therapies
a. Headaches	0 **(b)**	1	2	3	4	Y N N/A	Y N N/A
b. Fever	0 **(c)**	1	2	3	4	Y N N/A	Y N N/A
c. Chills	0 **(d)**	1	2	3	4	Y N N/A	Y N N/A
d. Rash	0 **(e)**	1	2	3	4	Y N N/A	Y N N/A
e. Lack of appetite	0 **(f)**	1	2	3	4	Y N N/A	Y N N/A
f. Drowsiness / tiredness	0 **(g)**	1	2	3	4	Y N N/A	Y N N/A
g. Nausea and/or vomiting	0 **(h)**	1	2	3	4	Y N N/A	Y N N/A
h. Pain / tingling in feet or hands	0 **(i)**	1	2	3	4	Y N N/A	Y N N/A
i. Dizziness or lack of concentration	0 **(j)**	1	2	3	4	Y N N/A	Y N N/A
j. Muscle aches or pains	0 **(k)**	1	2	3	4	Y N N/A	Y N N/A
k. Abdominal pains or cramps	0 **(l)**	1	2	3	4	Y N N/A	Y N N/A
l. Kidney stones	0 **(m)**	1	2	3	4	Y N N/A	Y N N/A
m. Dry mouth	0 **(n)**	1	2	3	4	Y N N/A	Y N N/A
n. Shifting of your body fat	0 **(o)**	1	2	3	4	Y N N/A	Y N N/A
o. Diarrhea	0 **(p)**	1	2	3	4	Y N N/A	Y N N/A
p. Constipation	0 **(q)**	1	2	3	4	Y N N/A	Y N N/A
q. Low red blood cell count (Anemia)	0 **(r)**	1	2	3	4	Y N N/A	Y N N/A
r. Low white blood cell count (leukopenia)	0 **(s)**	1	2	3	4	Y N N/A	Y N N/A
s. Other: ________________	0 **(t)**	1	2	3	4	Y N N/A	Y N N/A
t. Other: ________________	0 **(G2)**	1	2	3	4	Y N N/A	Y N N/A

WIHS ID#

G2. HAND PARTICIPANT RESPONSE CARD 12.
I am going to read to you some things people say about HIV. Please tell me if you strongly agree, agree, if you are uncertain or if you disagree or strongly disagree.

	STRONGLY AGREE	AGREE	UNCERTAIN	DISAGREE	STRONGLY DISAGREE
a. HIV is no longer the threat it used to be	1	2	3	4	5
b. A person with a higher viral load is more likely to pass HIV to sexual partners if she has unprotected sex	1	2	3	4	5
c. Because of combination drug treatments for HIV, I am less concerned about getting HIV or infecting someone else	1	2	3	4	5
d. Being on combination drug treatments decreases a person's chances of giving HIV to other people..	1	2	3	4	5
e. People who are sicker because of their HIV are more likely to pass the virus on to others............	1	2	3	4	5
f. HIV is now a controllable disease, like diabetes	1	2	3	4	5
g. People who always take their combination drug treatments as prescribed are less likely to pass HIV to sexual partners than those who do not take their drugs as prescribed..............................	1	2	3	4	5
h. Because of the new combination drug treatments, fewer people in the future will be infected with HIV	1	2	3	4	5
i. Because of the new combination drug treatments, fewer women will give HIV to their babies during pregnancy and childbirth	1	2	3	4	5

G3. TIME MODULE ENDED |__|__| : |__|__| AM............1
PM 2

STOP HERE

WOMEN'S INTERAGENCY HIV STUDY

FOLLOW-UP HEALTH HISTORY

FORM 22 HX

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE

|__|- |__|__| - |__|__|__|__| - |__|

A2. WIHS STUDY VISIT #: ___ ___

A2a. WIHS Core Visit 1
3 Month VRS Visit...............2

A3. FORM VERSION: 0 4 / 0 1 / 0 3 (M / D / Y)

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___ (M / D / Y)

A5. INTERVIEWER'S INITIALS: ___ ___ ___

A6. DATE OF LAST STUDY VISIT (FROM VISIT CONTROL SHEET) ___ ___ / ___ ___ / ___ ___ (M / D / Y)

A7. TIME MODULE BEGAN: |__|__| : |__|__|

AM 1
PM............... 2

INTRODUCTION TO PARTICIPANT:

Now, I am going to ask you some questions about your health history. I will be asking you a series of questions about diseases and symptoms you may have had since your study visit on __ __ / __ __ / __ __ (M D Y).
Also, if at any point in the interview you wish to stop, let me know.

Finally, I need to re-emphasize that all your answers are confidential, and the responses you provide will in no way affect your clinical care.

WIHS ID#

SECTION B. SYMPTOMS

Since your (MONTH) study visit, have you experienced any of the following:

		YES	NO
B1.	a fever for more than one month straight, with a temperature over 100 degrees	**1**	2
B3.	major problems with memory or concentration that interfered with your normal, everyday activities, and that lasted for more than two weeks	**1**	2
B4.	numbness, tingling, or burning sensations in your arms, legs, hands or feet that lasted for more than two weeks.	**1**	2
B5.	an unintentional weight loss, of 10 pounds or more, or have changed to a smaller clothing size, that lasted more than one month	**1**	2
B6.	confusion, getting lost in a familiar place or inability to perform routine mental tasks	**1**	2
B7.	drenching night sweats that soak night clothes or bedding	**1**	2

REFER FOR DIFFERENTIAL DIAGNOSIS TO PARTICIPANT'S MEDICAL PROVIDER

INTRODUCTION: The next series of questions asks about changes in the shape of your body that you may have noticed since your (MONTH) study visit. When thinking about these changes, please do not include any changes that have occurred due to being pregnant.

B8. Since your (MONTH) study visit, have you noticed any changes in the shape of your body or in the amount of your body fat (either loss or gain)?

YES 1
NO ....2 **(B9)**

To help me understand these changes, please tell me if you have noticed any of the following body changes since your (MONTH) study visit:

PROMPT: USE THE BODY DIAGRAM CARD TO POINT OUT THE LOCATION OF THE SUPRACLAVICULAR AND DORSOCERVICAL FAT PADS, AND AS NEEDED.

WIHS ID#

	Have you noticed…	YES	NO	Was this change in size an increase or a decrease? INCREASE	DECREASE	Was this change mild, moderate, or severe? MILD	MODERATE	SEVERE
a)	A change in the size of one or both of your breasts (unrelated to pregnancy)?	1	2 **(b)**	1	2	1	2	3
b)	A change in the size of your belly or abdominal fat?	1	2 **(c)**	1	2	1	2	3
c)	A change in the size of your waist?	1	2 **(d)**	1	2	1	2	3
d)	Any changes in the shape of your face?	1	2 **(e)**	1	2	1	2	3
e)	A change in the amount of fat in your cheeks, just next to your nose and mouth?	1	2 **(f)**	1	2	1	2	3
f)	A change in the amount of fat in your upper back?	1	2 **(g)**	1	2	1	2	3
g)	A change in the size of your neck?	1	2 **(h)**	1	2	1	2	3
h)	A change in the amount of fat in your arms?	1	2 **(i)**	1	2	1	2	3
i)	A change in the amount of fat in your legs?	1	2 **(j)**	1	2	1	2	3
j)	A change in the amount of fat in your buttocks?	1	2 **(B9)**	1	2	1	2	3

Mild – Only seen if looked for.
Moderate – Easily seen.
Severe – Obvious immediately.

WIHS ID#

B9. Now I am going to ask you about actions you may have intentionally taken to change or maintain the shape of your body. Since your (MONTH) study visit, have you taken any of the following actions to influence your body shape or fat distribution:

Have you…	YES	NO	
a) changed your diet?	1	2	
b) changed your HIV medications?	1	2	
c) changed your exercise habits?	1	2	
d) taken nutritional supplements?	1	2	
e) taken growth hormone or steroids? (i.e., anabolic steroids, androgens, growth factors, andros, Anadrol, roids, Android, juice, DHEA & (DHEA-S) danabol, nandrolone, Deca-Durabolin, Oxandrin)	1	2	
f) had cosmetic surgery such as liposuction, breast reduction or breast enlargement?	1	2	
g) Done anything else to influence your body shape?	1	2 **(B10)**	Specify: ________________________ ________________________

B10. What is your current bra size? I need both the chest and the cup size (for example, 36C.) **NOTE: If participant does not wear a bra or reports wearing a sports bra, code "CHEST SIZE" as 99 and enter –1 in "CUP SIZE."**

a. CHEST SIZE

|__|__|
(e.g., 36)

b. CUP SIZE

|__|__|__|
(e.g., C, DD ,etc…)

WIHS ID#

SECTION C: MEDICAL CONDITIONS AND CONCOMITANT ILLNESSES/SYMPTOMS

For the following questions, I am going to use the words "health care provider" to mean any doctor, nurse, physician's assistant or nurse practitioner you go to for medical care.

C1. a. Since your (MONTH) study visit, have you been told by a health care provider that you had cervical cancer?

YES……………………………………………………1
NO…………………………………………………...2 **(C2)**

b. Have you had surgery (been admitted to the hospital and had surgery in an operating room) to treat the cervical cancer?

YES 1
NO 2

c. Have you had a CAT or MRI scan of your abdomen (a big donut-shaped machine that takes special pictures)?

YES 1
NO 2

d. Have you been told that you need to have either surgery or radiation therapy?

YES 1
NO 2

C2. Since your (MONTH) study visit, have you been told by a health care provider that you had any other type of cancer, including skin cancer, lymphoma, Kaposi's sarcoma, Hodgkin's disease, breast cancer or cancer of the female organs – the ovaries or uterus?

YES 1
NO ....2 **(C12)**

WIHS ID#

What kind of cancer? Was it: **[READ C3 - C11]**

	YES	NO/NEVER HEARD OF IT
C3. Breast cancer	1	2 **(C4)**

a. Have you had a lump removed by a surgeon (not a needle biopsy, but an incision resulting in stitches)?

YES…………………1
NO…………………2

b. Have you had a mastectomy (removal of entire breast)?

YES…………………1
NO…………………2

	YES	NO/NEVER HEARD OF IT
C4. Cancer of the ovary	1	2
C5. Cancer of the uterus	1	2
C6. Kaposi's Sarcoma (KS)	1	2
C7. Lymphoma	1	2
C8. Lymphoma in the brain	1	2
C9. Hodgkin's disease	1	2
C10. Skin cancer (not KS)	1	2
C11. Other	1	2 **(C12)**

____
(SPECIFY)

C12. PLEASE RECORD THE TOTAL NUMBER OF CANCERS REPORTED AT THIS VISIT. DO NOT FORGET TO INCLUDE CERVICAL CANCER IF REPORTED IN QUESTION C1a, IN ADDITION TO ALL CANCERS REPORTED IN QUESTIONS C3 – C11.

|___|___|
CANCERS

PROMPT: IF QUESTION C12 = 00, SKIP TO QUESTION C27.

WIHS ID#

START F22HXS8

PROMPT: FOR EACH CANCER INDICATED IN QUESTION C12, COMPLETE QUESTIONS C13–C14. THE NUMBER OF BOXES COMPLETED MUST EQUAL THE VALUE RECORDED AT C12. INDICATE THE LOCATION OF EACH REPORTED CANCER IN a, THEN COMPLETE b–f AS INDICATED FOR EACH. IF THE TOTAL NUMBER OF REPORTED CANCERS IS GREATER THAN TWO, PLEASE XEROX THIS PAGE AND INSERT THE COPY AFTER PAGE 7.

C13. a. LOCATION OF REPORTED CANCER: ____________________

PROMPT: REPLACE (LOCATION) WITH THE LOCATION WRITTEN IN C13a.

Now I'm going to ask you a few more questions about your (LOCATION) cancer diagnosis.	YES	NO
b. Is this your first diagnosis of cancer?	1 **(c)**	2 **(e)**
c. When your (LOCATION) cancer diagnosis was made, were you told that it had also metastasized or spread to another part of your body?	1 **(d)**	2 **(C14)**
d. Spread to where? ____________________ **(C14)**		
e. Were you told that the cancer you are <u>now</u> reporting had metastasized or spread from the original cancer?	1 **(f)**	2 **(f)**
f. Where was the original cancer? ____________________ **(C14)**		

C14. a. LOCATION OF REPORTED CANCER: ____________________

PROMPT: REPLACE (LOCATION) WITH THE LOCATION WRITTEN IN C14a.

Now I'm going to ask you a few more questions about your (LOCATION) cancer diagnosis.	YES	NO
b. Is this your first diagnosis of cancer?	1 **(c)**	2 **(e)**
c. When your (LOCATION) cancer diagnosis was made, were you told that it had also metastasized or spread to another part of your body?	1 **(d)**	2 **(C15)**
d. Spread to where? ____________________ **(C15)**		
e. Were you told that the cancer you are <u>now</u> reporting had metastasized or spread from the original cancer?	1 **(f)**	2 **(f)**
f. Where was the original cancer? ____________________ **(C15)**		

END F22HXS8

PROMPT: IF ANY OF C1–C11 = YES, THEN COMPLETE ASCERTAINMENT TRACKING CHECKLIST (ATC) FOR EACH ILLNESS AND OBTAIN MEDICAL RECORD RELEASE. ALSO, IF EITHER C13c/C14c OR C13e/C14e = YES, THEN COMPLETE ATC FOR METASTATIC CANCER.

		YES	NO
C15.	Since your (MONTH) study visit have you received cancer chemotherapies?	1	2
C16.	Since your (MONTH) study visit have you received radiation treatments?	1	2

C27. Since your (MONTH) study visit, have you had a new diagnosis of asthma, or a worsening of your asthma?

YES 1
NO 2

C28. The next few questions are about tuberculosis. I will refer to tuberculosis as TB for short. Since your (MONTH) study visit, as far as you know, has anyone in your family or anyone you lived with, had TB?

YES 1
NO 2

C29. Since your (MONTH) study visit, have you had TB?

YES 1
NO .... 2 **(C30)**

Was it in your:	YES	NO
a. Lungs?	1	2
b. Other Location?	1	2
________________________ **(SPECIFY)**		
c. Did you have a chest X-ray?	1	2
d. Did you take medications for 3 months or more?	1	2

PROMPT: IF ANY OF C29a–d = YES, THEN COMPLETE ASCERTAINMENT TRACKING CHECKLIST FOR EACH ILLNESS AND OBTAIN MEDICAL RECORD RELEASE.

C30. Not including the test you had for this study, since your (MONTH) study visit, have you had a skin test for TB?

YES 1
NO ....2 **(C31)**
DON'T KNOW .... <-8> **(C31)**
DECLINED...................................... <-7> **(C31)**

a. When was the last time (most recent) you had a skin test for TB? I need the month and the year.

____ ____ / ____ ____ ____ ____
M Y

b. Were you told that the test was positive or showed that you had been exposed to TB?

YES 1
NO 2
DON'T KNOW .... <-8>
DECLINED .... <-7>

PROMPT: IF C30b = YES, THEN COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

C31. Now I'm going to ask you about some other medical conditions that may require medical care. Have you had any of the following conditions, since your (MONTH) study visit?

		YES	NO	DON'T KNOW
a.	Sinusitis, a sinus infection that required antibiotics..............	1	2	<-8>
b.	UTI, a urinary tract infection or an infection in your bladder or kidneys that required antibiotics..........................	1	2	<-8>
c.	High blood pressure or hypertension	1	2	<-8>
d.	High blood sugar, diabetes, or sugar diabetes	1	2	<-8>
e.	High blood cholesterol, triglyceride or blood lipid level.......	1	2	<-8>
f.	Lupus or rheumatoid arthritis or any rheumatologic disease..	1	2	<-8>
g.	Depression	1	2	<-8>

C32. Since your (MONTH) study visit, has a health care provider told you that you had a thyroid problem?

YES…………………………………1
NO…………………………………...2 **(C33)**
DON'T KNOW……………………<-8> **(C33)**
DECLINED………………………..<-7> **(C33)**

b. Was it hyperthyroidism or an "overactive" thyroid?

YES…………………………………1
NO…………………………………...2 **(C32c)**
DON'T KNOW……………………<-8> **(C32c)**
DECLINED………………………..<-7> **(C32c)**

i. Did your overactive thyroid get better without any treatment?

YES .... 1 **(C33)**
NO .... 2 **(C33)**

WIHS ID#

c. Was it hypothyroidism, or an "underactive" thyroid, that may have required taking thyroid hormone replacement medicine?

YES……………………………….1 **(C33)**
NO………………………………...2
DON'T KNOW……………………<-8>
DECLINED……………………..<-7>

d. Was it another type of thyroid disease, for example cancer or goiter?

YES……………………………….1
NO………………………………...2 **(C33)**
DON'T KNOW……………………<-8> **(C33)**
DECLINED……………………..<-7> **(C33)**

Specify: ________________________

C33. Since your (MONTH) study visit, has a health care provider told you that you had broken or fractured your…

		YES	NO
a.	hip?	1	2 **(b)**
	2. Did that fracture occur….		
	i. As a result of a fall from standing height or less	1 **(b)**	2
	ii. Because of a harder fall	1 **(b)**	2
	iii. From a car accident or other severe trauma	1 **(b)**	2
	iv. Don't know	1	2
b.	wrist (not including forearm or hand)?	1	2 **(c)**
	2. Did that fracture occur….		
	i. As a result of a fall from standing height or less	1 **(c)**	2
	ii. Because of a harder fall	1 **(c)**	2
	iii. From a car accident or other severe trauma	1 **(c)**	2
	iv. Don't know	1	2
c.	spine?	1	2 **(C34)**
	2. Did that fracture occur….		
	i. As a result of a fall from standing height or less	1 **(C34)**	2
	ii. Because of a harder fall	1 **(C34)**	2
	iii. From a car accident or other severe trauma	1 **(C34)**	2
	iv. Don't know	1	2

WIHS ID#

C34. Now I'm going to ask you about some liver conditions that may require medical care. Have you had any of the following conditions, since your (MONTH) study visit?

		YES	NO	DON'T KNOW
b.	Liver disease such as liver inflammation, cirrhosis or yellow jaundice or other liver problem	1	2	<-8>
c.	Abnormal fluid in the belly (ascites)	1	2	<-8>
d.	Bleeding from enlarged veins in your esophagus or stomach	1	2	<-8>

C35. Since your (MONTH) study visit, have you had a new diagnosis of hepatitis C?

YES .... 1 **(C35b)**
NO 2
DON'T KNOW .... <-8>

a. Have you **ever** been told by a health care provider that you have hepatitis C?

YES 1
NO .... 2 **(C38)**
DON'T KNOW .... <-8> **(C38)**

b. Who first told you?

WIHS staff 1
Your primary care provider 2
Another health care provider 3
Other 4

C36. Are you aware that there are treatment options for hepatitis C?

YES 1
NO .... 2 **(C37)**

a. How did you first learn about treatment options for hepatitis C?

Your primary care provider 1
Another healthcare provider 2
A friend or relative 3
Pamphlets or other educational material 4
Other 5

b. Which of the following best describes your impression of how well hepatitis C treatment works?

It gets rid of the infection in most people who take it 1
It works in less than half of the people who take it 2
It doesn't work at all 3
I have no opinion 4

c. Has anyone ever offered you treatment for hepatitis C?

YES 1
NO...2 **(C37)**

d. Were you offered this treatment **since** your (MONTH) study visit or **prior to** your (MONTH) study visit?

Since my (MONTH) study visit..........................1 **(C37)**
Prior to my (MONTH) study visit.......................2

e. Did you agree to be treated for hepatitis C?

YES 1
NO...2 **(C37)**

f. Are you still in treatment for hepatitis C?

YES ....1**(C37)**
NO...2

g. Did you successfully complete the treatment?

YES ....1 **(C37)**
NO...2

h. What was the **main** reason or reasons you stopped treatment?

	YES	NO
i. Therapy was unsuccessful	1	2
ii. I had a low white blood cell count (leukopenia)	1	2
iii. I had a low red blood cell count (anemia)	1	2
iv. I had other blood test abnormalities	1	2
v. I had psychological side effects	1	2
vi. I became pregnant	1	2
vii. My health care provider stopped it, but I don't know why	1	2
viii. It required too many visits	1	2
ix. It was too expensive/my insurance didn't cover treatment	1	2
x. I was not able to keep all the appointments	1	2
xi. Other reason	1	2 **(C37)**

Specify reason: ___________________________

C37. Have you ever been referred to a special provider or clinic for patients with hepatitis C?

YES 1
NO...2 **(C38)**

a. Have you ever gone to a special provider or clinic for patients with hepatitis C?

YES 1
NO...2

C38. Since your (MONTH) study visit, has a health care provider recommended that you receive a liver biopsy?

YES .... 1 **(C38b)**
NO 2
DON'T KNOW .... <-8>

a. Has a health care provider **ever** recommended you have a liver biopsy?

YES 1
NO .... 2 **(C39)**
DON'T KNOW .... <-8> **(C39)**

b. Why was the liver biopsy recommended?

To see how much hepatitis C has affected your liver 1
For another reason 2

c. Did you have the liver biopsy?

YES .... 1 **(C39)**
NO 2

d. Why did you choose not to have the biopsy (CIRCLE ALL THAT APPLY):

		YES	**NO**
i.	I was scared	1	2
ii.	I'm not sure a biopsy would help me	1	2
iii.	I don't think I would accept treatment for hepatitis C even if I had the biopsy	1	2
iv.	The biopsy procedure was not explained well enough	1	2
v.	It was too expensive/ my insurance didn't cover it	1	2
vi.	Other	1	2

Specify reason: ____________________________

C39. Have you ever been told by a health care provider that you needed a liver transplant?

YES 1
NO .... 2 **(C42)**

C40. Have you ever had a liver transplant?

YES 1
NO .... 2 **(C41)**

a. In what year? |___|___|___|___|

C41. Are you currently on a waiting list for a liver transplant?

YES 1
NO 2

PROMPT: IF ANY OF C34b–C34d OR C35 OR C38 OR C40 = YES, THEN COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

WIHS ID#

C42. Have you had any of the following heart problems, since your (MONTH) study visit?

	YES	NO	DON'T KNOW
a. A new diagnosis of angina or chest pain related to heart disease	1	2	<-8>
b. A new diagnosis of congestive heart failure or CHF	1	2	<-8>
c. A heart attack or myocardial infarction or MI	1	2	<-8>
d. A stroke or CVA	1	2	<-8>

C43. Do you take aspirin three days or more of every week?

YES 1
NO 2

PROMPT: IF ANY OF C42b–C42d = YES, THEN COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

SECTION D: SKIN AND ORAL CONDITIONS

ASK QUESTIONS D1 AND D3 FOR EACH CONDITION BELOW. EACH TIME A PARTICIPANT RESPONDS THAT SHE HAS HAD THE CONDITION, ASK SUBQUESTION "a" BEFORE PROCEEDING TO THE NEXT CONDITION.

D1-D3
Since your (MONTH) study visit, has a health care provider, either a doctor, dentist, nurse practitioner, nurse, or physician's assistant, told you that you had **(CONDITION)?**

D1a –D3a
How many different times in the past 6 months did you have this?

D1. Shingles (Herpes Zoster)?

YES 1
NO ....2 **(D3)**
DON'T KNOW ....<-8> **(D3)**
DECLINED ....<-7> **(D3)**

a. |__|__| # TIMES

b. Have you had 2 or more separate areas with shingles at the same time?

YES 1
NO 2

D3. Candida or thrush, yeast inside your mouth?

YES 1
NO ....2 **(E1)**
DON'T KNOW ....<-8> **(E1)**
DECLINED ....<-7> **(E1)**

a. |__|__| # TIMES

WIHS ID#

SECTION E: AIDS DEFINING ILLNESSES

We are now interested in finding out about diseases that some women experience. These diseases are rare and may occur in women who are HIV negative; however, they tend to occur more often in HIV positive women. As I read this list of diseases, please let me know whether or not you have had any of them. Many of the terms in this section are very technical and you may not have heard of them. If you've never heard of a term just say so.

E1. Since your (MONTH) study visit, has a health care provider told you that you had a CD4 count (T-cell count) less than 200 or less than 14%?

YES 1
NO/NEVER HEARD OF IT 2

E2. Since your (MONTH) study visit, has a health care provider told you that you had herpes simplex with ulcers or sores lasting longer than one month?

YES 1
NO/NEVER HEARD OF IT 2

E3. Since your (MONTH) study visit, have you had diarrhea (3 or more soft or liquid stools per day) that lasted for more than one month?

YES 1
NO ....2 **(E5)**

E4. Since your (MONTH) study visit, has a health care provider told you that any diarrhea you may have had was caused by:

	YES	NO/NEVER HEARD OF IT
i. Cryptosporidia?	1	2
ii. Microsporidia?	1	2
iii. Isospora?	1	2
iv. C-M-V?	1	2
v. M-A-I?	1	2

E5. Since your (MONTH) study visit, has a health care provider told you that you had herpes simplex infection of the lungs or esophagus, (the tube between your mouth and your stomach)?

YES 1
NO/NEVER HEARD OF IT 2

PROMPT: IF THE PARTICIPANT RESPONDED "YES" TO ANY OF QUESTIONS E4–E20, COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

E6. Since your (MONTH) study visit, has a health care provider told you that you had PCP, pneumocystis carinii pneumonia?

YES 1
NO/NEVER HEARD OF IT 2

E7. Since your (MONTH) study visit, has a health care provider told you that you had another type of pneumonia, lung infection? Do not answer yes if you were diagnosed only with bronchitis.

YES 1
NO/NEVER HEARD OF IT ....2 **(E8)**

a. In the past 12 months, how many times has a health care provider told you that you had pneumonia that required antibiotics, not counting PCP?

|___|___|
TIMES

b. Since your (MONTH) study visit, how many times have you had pneumonia that required antibiotics, not counting PCP?

|___|___|
TIMES

c. When was the last time you had pneumonia, not counting PCP? I need the month and the year?

____ ____ / ____ ____ ____ ____
M Y

E8. (Since your (MONTH) study visit, has a health care provider told you that you had) Candida or thrush, a yeast infection of the esophagus (the tube between your mouth and stomach) not just in your mouth?

YES 1
NO/NEVER HEARD OF IT 2

E9. (Since your (MONTH) study visit, has a health care provider told you that you had) Candida or thrush, a yeast infection of the lungs or airways (trachea or bronchi)?

YES 1
NO/NEVER HEARD OF IT 2

E10. (Since your (MONTH) study visit, has a health care provider told you that you had) an M-A-I infection, which is sometimes called M-A-C or MAC?

YES 1
NO/NEVER HEARD OF IT 2

PROMPT: IF THE PARTICIPANT RESPONDED "YES" TO ANY OF QUESTIONS E4–E20, COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

E11. (Since your (MONTH) study visit, has a health care provider told you that you had) Toxo infection, or toxoplasmosis of the brain?

YES 1
NO/NEVER HEARD OF IT 2

E12. (Since your (MONTH) study visit, has a health care provider told you that you had) C-M-V, cytomegalovirus:

	YES	NO	
a. in either eye (retinitis)?	1	2	
b. in your blood?	1	2	
c. in your intestine?..	1	2	
d. in your liver?...	1	2	
e. elsewhere in your body?	1	2	**(E13)**

____
(SPECIFY)

E13. Since your (MONTH) study visit, has a health care provider told you that you had meningitis related to HIV?

YES 1
NO/NEVER HEARD OF IT ....2 **(E14)**

a. Were you told that this was Crypto, Cryptococcal meningitis?

YES 1
NO/NEVER HEARD OF IT 2

E14. (Since your (MONTH) study visit, has a health care provider told you that you had) Cryptococcal infection:

	YES	NO	
a. in your blood?...	1	2	
b. elsewhere in your body?	1	2	**(E15)**

____
(SPECIFY)

E15. (Since your (MONTH) study visit, has a health care provider told you that you had) Histoplasmosis infection or Histo?

YES 1
NO/NEVER HEARD OF IT ....2 **(E16)**

a. Where in your body? ______________________________
(SPECIFY)

PROMPT: IF THE PARTICIPANT RESPONDED "YES" TO ANY OF QUESTIONS E4–E20, COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

WIHS ID#

E16. (Since your (MONTH) study visit, has a health care provider told you that you had) Cocci, coccidioidomycosis infection or Valley Fever?

YES 1
NO/NEVER HEARD OF IT 2

E17. (Since your (MONTH) study visit, has a health care provider told you that you had) wasting syndrome, in other words, severe weight loss?

YES 1
NO/NEVER HEARD OF IT ....2 **(E18)**

Have you had (CONDITION) that lasted for at least one month, during the same time that you experienced severe weight loss?

	YES	NO
a. chronic diarrhea (at least 3 loose stools per day for greater than or equal to 30 days?)	1	2
b. chronic weakness and documented fever (for greater than or equal to 30 days?)	1	2
c. were you told that [this symptom/these symptoms] [was/were] due to HIV/AIDS?	1	2

E18. (Since your (MONTH) study visit, has a health care provider told you that you had) dementia or encephalopathy, or that you had a memory problem or confusion caused by HIV?

YES 1
NO/NEVER HEARD OF IT 2

E19. (Since your (MONTH) study visit, has a health care provider told you that you had) an infection in the blood with a bacteria called salmonella?

YES 1
NO/NEVER HEARD OF IT ....2 **(E20)**

a. Have you had this more than once, since your (MONTH) study visit?

YES 1
NO 2

E20. (Since your (MONTH) study visit, has a health care provider told you that you had) PML, progressive multifocal leukoencephalopathy, a disease of the brain?

YES 1
NO/NEVER HEARD OF IT 2

PROMPT: IF THE PARTICIPANT RESPONDED "YES" TO ANY OF QUESTIONS E4–E20, COMPLETE ASCERTAINMENT TRACKING CHECKLIST AND OBTAIN MEDICAL RECORD RELEASE.

WIHS ID#

E21. (Since your (MONTH) study visit, has a health care provider told you that you had) AIDS?

YES 1
NO 2

E22. Since your (MONTH) study visit, have you had a biopsy? A biopsy is when tissue, sometimes a lump or a mass, is removed with a needle or by making an incision. (DO NOT include biopsies that have been taken at WIHS gynecologic exams, including WIHS colposcopic examinations.)

YES 1
NO ....2 **(E23)**

Where in your body? Was it a:	YES	NO
a. Lung biopsy?	1	2
b. Skin biopsy?	1	2
c. Bone marrow biopsy?	1	2
d. Cervical biopsy?	1	2
e. Liver biopsy?	1	2
f. Breast biopsy?	1	2
g. Other biopsy?	1	2

____
(SPECIFY)

PROMPT: IF THE PARTICIPANT RESPONDED "YES" TO ANY OF QUESTIONS E22a–g, COMPLETE AN AIDS AND CANCER SPECIMEN RESOURCE ASCERTAINMENT TRACKING CHECKLIST (ACSR ATC) FOR EACH REPORTED BIOPSY AND OBTAIN MEDICAL RECORD RELEASE.

E23. Since your (MONTH) study visit, have you been admitted to the hospital for any reason? This would include staying overnight or being admitted for a procedure that was done in one day. Please include all medical and psychiatric hospitalizations. This doesn't include being treated in the emergency room and later released.

YES 1
NO ....2 **(E24)**
DON'T KNOW ....<-8> **(E24)**

a. How many times since your (MONTH) study visit?

|___|___|
TIMES

E24. TIME MODULE ENDED |__|__| : |__|__| AM............1
PM 2

PROCEED TO F22 MED

WOMEN'S INTERAGENCY HIV STUDY
PSYCHOSOCIAL MEASURES
FORM 26

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: ENTER NUMBER HERE ONLY IF ID LABEL IS NOT AVAILABLE

|__|- |__|__| - |__|__|__|__| - |__|

A2. WIHS STUDY VISIT #: ___ ___

A3. FORM VERSION: 10 / 01 / 98

A4. DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___
M D Y

A5. INTERVIEWER'S INITIALS: ___ ___ ___

A6. DATE OF LAST STUDY VISIT AT WHICH F26 WAS ADMINISTERED ___ ___ / ___ ___ / ___ ___
M D Y

A7. TIME MODULE BEGAN: |__|__| : |__|__|
AM 1
PM 2

INTRODUCTION TO PARTICIPANT:

At this time, I am going to ask you about your thoughts and feelings since your study visit on
__ __ / __ __ / __ __.
M D Y

WIHS ID#

SECTION B: QUALITY OF LIFE SCALE

B1. In general, would you say your health is:

Excellent 1
Very Good 2
Good 3
Fair 4
Poor 5

B2. During the past 4 weeks, has your health kept you from working at a job, doing work around the house, going to school or taking care of children:

All of the time 1
Some of the time 2
None of the time 3

B3. How much bodily pain have you generally had during the past 4 weeks:

None 1
Very Mild 2
Mild 3
Moderate 4
Severe 5
Very Severe 6

B4. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups:

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

B5. During the past 4 weeks, have you been unable to do certain kinds or amounts of work, housework, school work or caring for children because of your health:

All of the time 1
Some of the time 2
None of the time 3

B6. During the past 4 weeks, how much did bodily pain interfere with normal work (including work outside the house and housework):

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

B7. **HAND PARTICIPANT RESPONSE CARD 13A.**
How much, if at all, does your health limit you in each of the following activities? Please tell me if you are limited a lot, limited a little, or not at all limited.

How much does your health limit:	LIMITED A LOT	LIMITED A LITTLE	NOT AT ALL LIMITED
a. The kinds or amounts of vigorous activities you can do, like lifting heavy objects, running, or participating in strenuous sports?	1	2	3
b. The kinds or amounts of moderate activities you can do, like moving a table, or carrying groceries?	1	2	3
c. Walking uphill or climbing a few flights of stairs?	1	2	3
d. Eating, dressing, bathing, or using the toilet?.	1	2	3

B8. **HAND PARTICIPANT RESPONSE CARD 13.**
For each of the following questions, please tell me the answer that comes closest to the way you have been feeling during the past 4 weeks. Please tell me if you have been feeling that way all of the time, most of the time, a good bit of the time, some of the time, a little of the time, or none of the time.

How much of the time during the past 4 weeks:	ALL OF THE TIME	MOST OF THE TIME	A GOOD BIT OF THE TIME	SOME OF THE TIME	A LITTLE OF THE TIME	NONE OF THE TIME
a. Has your physical health or emotional problems limited your social activities (like visiting with friends or close relatives)?	1	2	3	4	5	6
b. Did you have trouble keeping your attention on an activity for long?	1	2	3	4	5	6

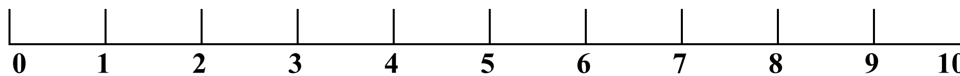
WIHS ID#

How much of the time during the past 4 weeks:	ALL OF THE TIME	MOST OF THE TIME	A GOOD BIT OF THE TIME	SOME OF THE TIME	A LITTLE OF THE TIME	NONE OF THE TIME
c. Did you have difficulty reasoning and solving problems?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Have you been downhearted and blue?	1	2	3	4	5	6
f. Did you feel tired?	1	2	3	4	5	6
g. Did you have enough energy to do the things you want to do?	1	2	3	4	5	6
h. Have you been happy?	1	2	3	4	5	6

B9. **HAND PARTICIPANT RESPONSE CARD 14.**
Please indicate the extent to which the following statements are true or false for you. Are they definitely true, mostly true, are you not sure, are they mostly false or definitely false?

	DEFINITELY TRUE	MOSTLY TRUE	NOT SURE	MOSTLY FALSE	DEFINITELY FALSE
a. My health is excellent	1	2	3	4	5
b. I have been feeling bad lately	1	2	3	4	5

B10. **HAND PARTICIPANT RESPONSE CARD 15.**
Overall, how would you rate your quality of life. Please tell me which number is closest with "0" being the worst possible quality of life and "10" being the best possible quality of life.



WORST POSSIBLE QUALITY OF LIFE (AS BAD OR WORSE THAN BEING DEAD)

HALF-WAY BETWEEN WORST AND BEST

BEST POSSIBLE QUALITY OF LIFE

WIHS ID#

SECTION C: CES-D DEPRESSION SCALE

HAND PARTICIPANT RESPONSE CARD 16.
First, I am going to read a list of the ways you might have felt or behaved in the past week. Please tell me how often you have felt this way during the past week.

NOTE THAT RESPONSE CARD CATEGORIES ARE AS FOLLOWS:
1 = Rarely or none of the time (less than 1 day)
2 = Some or a little of the time (1–2 days)
3 = Occasionally or moderate amount of time (3–4 days)
4 = Most or all of the time (5–7 days)

During the past week...		RARELY (Less than one day)	SOME (1–2 days)	OCCASIONALLY (3–4 days)	MOST (5–7 days)
C1.	I was bothered by things that usually don't bother me.	1	2	3	4
C2.	I did not feel like eating; my appetite was poor	1	2	3	4
C3.	I felt that I could not shake off the blues even with help from my family or friends	1	2	3	4
C4.	I felt that I was just as good as other people	1	2	3	4
During the past week...					
C5.	I had trouble keeping my mind on what I was doing	1	2	3	4
C6.	I felt depressed	1	2	3	4
C7.	I felt that everything I did was an effort	1	2	3	4
C8.	I felt hopeful about the future	1	2	3	4
During the past week...					
C9.	I thought my life had been a failure.	1	2	3	4
C10.	I felt fearful.	1	2	3	4
C11.	my sleep was restless.	1	2	3	4

WIHS ID#

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4 = Most or all of the time (5–7 days)

	RARELY (Less than one day)	SOME (1–2 days)	OCCASIONALLY (3–4 days)	MOST (5–7 days)
C12. I was happy... .	1	2	3	4
C13. I talked less than usual............................. .	1	2	3	4
During the past week...				
C14. I felt lonely	1	2	3	4
C15. People were unfriendly.............................	1	2	3	4
C16. I enjoyed life..	1	2	3	4
C17. I had crying spells....................................	1	2	3	4
During the past week...				
C18. I felt sad	1	2	3	4
C19. I felt that people dislike me	1	2	3	4
C20. I could not get “going”	1	2	3	4

SECTION D: SOCIAL SUPPORT

I am now going to ask you some questions about any type of help you may have received from family, friends, or your partner.

D1. At times people may need help with caring for children, getting a ride somewhere or we may need to borrow something. Within the past month did you get this kind of help from family, friends and/or your partner?

YES 1
NO 2
DECLINED .... <-7>
DON’T KNOW .... <-8>

WIHS ID#

D2. Within the past month, have family, friends, and/or your partner given you comfort and encouragement?

YES 1
NO 2
DECLINED .... <-7>
DON'T KNOW .... <-8>

D3. During the past month, did family, friends, and/or your partner listen and/or try to understand your concerns (worries/troubles)?

YES 1
NO 2
DECLINED .... <-7>
DON'T KNOW .... <-8>

SECTION E: CHILDREN

E1. These next few questions are about your children. By children we mean children you have given birth to, adopted, step or foster children **that are under 18 years of age**. Please bear with me as I ask you something you may have previously told me. Do you have any living children under 18 years of age?

YES 1
NO 2

E2. This question is about changes in the status of your children that are under 18 years of age. Since your (MONTH) study visit, have any children been born or died; been adopted by you or become your foster children; been placed in foster care; or come into or left your care for some other reason?

YES	1	**(E3)**
NO	2	**(PROMPT BELOW)**
DON'T KNOW	<-8>	**(PROMPT BELOW)**
DECLINED	<-7>	**(PROMPT BELOW)**

PROMPT: **IF E1 = YES, SKIP TO QUESTION E10.**
IF E1 = NO, DO NOT ADMINISTER ANY ADDITIONAL SECTION E QUESTIONS. READ PROMPT ON PAGE 10 AND THEN PROCEED TO F1 OR H4 AS DIRECTED.

E3. Have you given birth to any children since your (MONTH) study visit?

YES.. 1
NO.. 2 **(E4)**
DON'T KNOW....................................<-8> **(E4)**
DECLINED..<-7> **(E4)**

a. Is this child/these children living with you?

YES.. 1
NO 2
NO/NO LONGER LIVING.............................. 3
DON'T KNOW ....<-8>
DECLINED ....<-7>

E4. Did you have any foster, step, adopted or other children come into your care since your (MONTH) study visit?

YES.. 1
NO 2
DON'T KNOW ....<-8>
DECLINED ....<-7>

E5. Did any children under 18 years of age leave your care since your (MONTH) study visit?

YES.. 1
NO 2
DON'T KNOW ....<-8>
DECLINED ....<-7>

E6. These next few questions are about any of your children who may have died. Since your (MONTH) study visit, have any of your biological children, adopted children, step or foster children died?

YES.. 1 **(E6a)**
NO.. 2 **(PROMPT BELOW)**
DON'T KNOW....................................<-8> **(PROMPT BELOW)**
DECLINED..<-7> **(PROMPT BELOW)**

PROMPT: IF E1 = YES, SKIP TO QUESTION E10.
IF E1 = NO, DO NOT ADMINISTER ANY ADDITIONAL SECTION E QUESTIONS. READ PROMPT ON PAGE 10 AND THEN PROCEED TO F1 OR H4 AS DIRECTED.

a. Since that time, how many of your children have died? |___|___|
CHILDREN

START F26S1

WIHS ID#

PROMPT:	COMPLETE THIS SERIES OF QUESTIONS FOR EACH CHILD REPORTED AT E6a. YOU MAY GET A NAME OR INITIAL FOR EACH CHILD TO FACILITATE ADMINISTRATION. HOWEVER, DO NOT RECORD THE CHILD'S NAME ON THE FORM.

INTRODUCTION: These next few questions ask about [this child/these children]. We would like to find out something about the child's age and health before he or she died. If you need some time, just let me know and we will take a break.

[PAUSE OR STOP UNTIL THE PARTICIPANT SEEMS READY TO BEGIN.]
Are you ready to begin?

[IF PARTICIPANT REPORTED MORE THAN ONE CHILD HAVING DIED, PROMPT AS FOLLOWS: Let's begin with the first child... Now let's go on to the next child...**]**

	a. Was (__) a biological, adopted, or step/foster child?	b. What was (___)'s age at the time of her/his death?	c. Was she/he living with you?	d. Was she/he HIV positive?	e. What was the cause of her/his death?
E7.	BIOLOGICAL .... 1 ADOPTED .... 2 STEP/FOSTER 3	|__|__| MONTH.. 1 YEAR...... 2	YES.............. 1 NO................ 2	YES .... 1 NO......................... 2 DON'T KNOW ... <-8> DECLINED......... <-7>	____________________ ____________________ ____________________ (SPECIFY)
E8.	BIOLOGICAL .... 1 ADOPTED .... 2 STEP/FOSTER 3	|__|__| MONTH.. 1 YEAR...... 2	YES.............. 1 NO................ 2	YES .... 1 NO......................... 2 DON'T KNOW ... <-8> DECLINED......... <-7>	____________________ ____________________ ____________________ (SPECIFY)
E9.	BIOLOGICAL .... 1 ADOPTED .... 2 STEP/FOSTER 3	|__|__| MONTH.. 1 YEAR...... 2	YES.............. 1 NO................ 2	YES .... 1 NO......................... 2 DON'T KNOW ... <-8> DECLINED......... <-7>	____________________ ____________________ ____________________ (SPECIFY)

PROMPT:	IF E1 = NO, SKIP TO PROMPT, PAGE 10. [DO NOT ASK E10-E13 IF PARTICIPANT REPORTS NO LIVING CHILDREN UNDER 18 YEARS OF AGE.]

END F26S1

E10. Do you know the health status of any of your children?

YES.............................. 1 **(E11)**
NO................................ 2 **(PROMPT, PAGE 10)**

WIHS ID#

E11. **HAND PARTICIPANT RESPONSE CARD 17.**
During the past 6 months, how worried or concerned have you been about:

	Not at all	A little bit	Some/ Moderately	Quite a bit	A lot/ Extremely
a. Your child(ren)'s health?.........	1	2	3	4	5
b. Not being able to take care of your child(ren)?	1	2	3	4	5
c. Having your child(ren) taken away?	1	2	3	4	5
d. Your child(ren) possibly growing older without you?.....	1	2	3	4	5
e. Whether your child(ren) have HIV or AIDS?.........................	1	2	3	4	5
f. Giving HIV to your child(ren) while you are caring for them?	1	2	3	4	5

E12. During the past 6 months:

	Not at all	A little bit	Some/ Moderately	Quite a bit	A lot/ Extremely
a. How sick [has/have] your child(ren) been?	1	2	3	4	5
b. How difficult has it been to care for your child(ren)?................	1	2	3	4	5

E13. Since your (MONTH) study visit, how many times have any of your children been hospitalized?

|___|___| TIMES **(CODE "00" IF NONE)**

PROMPT:	**FOR CALIFORNIA SITES AND ALL PARTICIPANTS UNDER 18 YEARS OF AGE READ: "**Thank you very much for your responses; we have completed the interview"**AND SKIP TO H4.**

WIHS ID#

SECTION F: SEXUAL ABUSE

INTRODUCTION: At times we may be in difficult situations or things may happen to us that we cannot control, like sexual abuse or physical harm. We realize recalling such experiences can be difficult, so if you need to have some time during these next few sections, just let me know and we will take a break for a few minutes.

F1. Since your (MONTH) study visit, has anyone pressured or forced you to have sexual contact? By sexual contact I mean them touching your sexual parts, you touching their sexual parts, or sexual intercourse.

YES.. 1
NO.. 2 **(G1)**
DON'T KNOW .... <-8> **(G1)**
DECLINED .... <-7> **(G1)**

F2. I need to ask you who the person or persons were who pressured or forced you to have sexual contact. (I don't need their names, I just need their relationship to you.)
(**PAUSE OR STOP UNTIL THE PARTICIPANT SEEMS READY TO BEGIN**)
Okay. Are you ready to begin?

YES, PARTICIPANT WILL PROCEED............ 1
NO, PARTICIPANT DECLINED....................... 2 **(F4)**

F3. Please tell me who this person or these persons were (or are). (I don't need their names, I just need to know their relationship to you.)
[CIRCLE "1" FOR ALL PERSON(S) MENTIONED AND ASK "i". CIRCLE "2" (NO) FOR THOSE NOT MENTIONED]
(**PROBE**: Anyone else?)

RELATIONSHIP	MENTIONED		i. Has it stopped?	
	YES	NO	YES	NO
a. MOTHER/STEPMOTHER/FOSTER	1	2 **(b)**	1	**2**
b. FATHER	1	2 **(c)**	1	**2**
c. STEP/FOSTER FATHER	1	2 **(d)**	1	**2**
d. SIBLING/STEP/FOSTER	1	2 **(e)**	1	**2**
e. MOTHER'S BOYFRIEND/PARTNER	1	2 **(f)**	1	**2**
f. OTHER RELATIVE ________ **(SPECIFY)**	1	2 **(g)**	1	**2**
g. INTIMATE PARTNER/ SPOUSE/BOYFRIEND/ GIRLFRIEND	1	2 **(h)**	1	**2**
h. FRIEND	1	2 **(i)**	1	**2**
i. ACQUAINTANCE	1	2 **(j)**	1	**2**
j. STRANGER	1	2 **(k)**	1	**2**
k. OTHER ________ **(SPECIFY)**	1	2 **(F4)**	1	**2**
l. OTHER ________ **(SPECIFY)**	1	2 **(F4)**	1	**2**
m. OTHER ________ **(SPECIFY)**	1	2 **(F4)**	1	**2**

F4. Since your (MONTH) study visit, have you been forced to have sex with someone who you now know was HIV positive or had AIDS?

YES	**1**
NO	2
DON'T KNOW	<-8>
DECLINED	<-7>

REFER PARTICIPANT TO COUNSELOR

WIHS ID#

SECTION G: DOMESTIC VIOLENCE

Since your (MONTH) study visit, has a current or previous partner: **[ASK G1–G7] FOR EACH "YES" ASK "a"**	YES	NO	a. **HAND PARTICIPANT RESPONSE CARD 18.** When was the most recent time your partner (G1–G7)? Was it: 1 = Within the past week 2 = More than a week ago, but within the past month 3 = More than 1 month ago, but within the past 6 months 4 = More than 6 months ago			
G1. threatened to hurt you or kill you?	1	2 **(G2)**	1	2	3	4
G2. prevented you from leaving or entering your house?	1	2 **(G3)**	1	2	3	4
G3. prevented you from seeing friends?	1	2 **(G4)**	1	2	3	4
G4. prevented you from making phone calls?	1	2 **(G5)**	1	2	3	4
G5. prevented you from getting or keeping a job?	1	2 **(G6)**	1	2	3	4
G6. prevented you from continuing your education?	1	2 **(G7)**	1	2	3	4
G7. prevented you from seeking medical attention?	1	2 **(G8)**	1	2	3	4

REFER PARTICIPANT TO COUNSELOR

G8. Since your (MONTH) study visit, have you talked with your current or previous partner about using a condom or other barrier method (such as dental dams)?

YES.. 1
NO .... 2 **(G10)**

G9. Since your (MONTH) study visit, has your current or previous partner threatened you when you talked about using a condom or other barrier method (such as dental dams)?

YES.. 1
NO 2

G10. Since your (MONTH) study visit, have you been afraid that your current or previous partner would threaten you or hurt you if you asked him/her to use a condom or other barrier method (such as dental dams)?

YES.. 1
NO 2

WIHS ID#

G11. The next few questions are about a relationship that you may currently have with a partner. Before I ask you these questions, please remind me if you are currently in a relationship with someone that you think of as your partner? **(PROBE:** This could be your lover, boyfriend, girlfriend, husband, etc.**)**

YES 1
NO .... 2 **(G14)**

G12. Do you feel afraid of your partner?

YES **1**
NO 2

G13. Do you ever feel that your partner might try to kill you?

YES **1**
NO 2
DECLINED .... **<-7>**

G14. Are you afraid to go home?

YES **1**
NO 2
DON'T KNOW ....<-8>
DECLINED .... **<-7>**

REFER PARTICIPANT TO COUNSELOR

WIHS ID#

SECTION H: PHYSICAL VIOLENCE

H1. Since your (MONTH) study visit, have you experienced serious physical violence (physical harm by another person)? By that I mean were you ever hurt by a person using an object or were you ever slapped, hit, punched, kicked?

YES.. 1
NO... 2 **(H4)**
DON'T KNOW .... <-8> **(H4)**
DECLINED .... <-7> **(H4)**

H2. I need to ask you who the person or persons were who injured you. (I don't need their names, I just need to know their relationship to you.)
(**PAUSE OR STOP UNTIL THE PARTICIPANT SEEMS READY TO BEGIN**)
Okay. Are you ready to begin?

YES, PARTICIPANT WILL PROCEED............ 1
NO, PARTICIPANT DECLINED....................... 2 **(H4)**

WIHS ID#

H3. Please tell me who this person or these persons were (or are). (I don't need their names, I just need to know their relationship to you.)
[CIRCLE "1" FOR ALL PERSON(S) MENTIONED AND ASK "i". CIRCLE "2" (NO) FOR THOSE NOT MENTIONED]
(**PROBE**: Anyone else?)

RELATIONSHIP	MENTIONED		i. Has it stopped?	
	YES	NO	YES	**NO**
a. MOTHER/STEPMOTHER/FOSTER	1	2 **(b)**	1	**2**
b. FATHER	1	2 **(c)**	1	**2**
c. STEP/FOSTER FATHER	1	2 **(d)**	1	**2**
d. SIBLING/STEP/FOSTER	1	2 **(e)**	1	**2**
e. MOTHER'S BOYFRIEND/PARTNER	1	2 **(f)**	1	**2**
f. OTHER RELATIVE ____________ **(SPECIFY)**	1	2 **(g)**	1	**2**
g. INTIMATE PARTNER/ SPOUSE/BOYFRIEND/ GIRLFRIEND	1	2 **(h)**	1	**2**
h. FRIEND	1	2 **(i)**	1	**2**
i. ACQUAINTANCE	1	2 **(j)**	1	**2**
j. STRANGER	1	2 **(k)**	1	**2**
k. OTHER ____________ **(SPECIFY)**	1	2 **(H4)**	1	**2**
l. OTHER ____________ **(SPECIFY)**	1	2 **(H4)**	1	**2**
m. OTHER ____________ **(SPECIFY)**	1	2 **(H4)**	1	**2**

REFER PARTICIPANT TO COUNSELOR

H4. Thank you very much for your responses; we have completed the interview.

TIME MODULE ENDED: |__|__| : |__|__| AM............ 1
PM............ 2

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Date