Pharmacology
Report for WIHS Advisory Board on Pharmacology Projects in WIHS over Past Funding Cycle

The major pharmacology projects in the WIHS over the past two funding cycles have focused on two major initiatives: the WIHS Intensive PK studies and the WIHS Hair Exposure Studies.

I. WIHS Intensive PK Studies


The genesis of this project arose from the observation that intensive PK studies of ARVs are typically performed for dose-finding purposes after short-term use in the context of phase I clinical trials. These PK studies are usually performed in small, homogeneous populations (sometimes HIV-noninfected) in regards to race/ethnicity, gender and comorbidities. The generalizability of PK evaluations performed in later stage clinical trial settings is also limited due to restrictive eligibility criteria. The typical PK component of clinical trials does not thoroughly investigate the range of individual characteristics (e.g. concurrent medical conditions, dietary patterns, weight differences, ethnicity and gender, use of concomitant medications or recreational drugs) common among patients who will eventually receive ARV prescriptions. The end result can be the revelation of unanticipated adverse effects and treatment failures after drug approval and dissemination.

To address the limitation in the literature and to identify relevant clinical factors that contribute to PK variability in real-world populations, we performed intensive PK studies over 12 to 24 hours for 120 women on each of six ARVs during WIHS IV, specifically nevirapine, efavirenz, atazanavir, lopinavir/ritonavir, tenofovir and raltegravir. Intensive PK data from this large heterogeneous population allowed us to identify a number of clinical factors in multivariate modeling that contribute to increases or decreases in ARV exposure as represented by areas-under-the-curve (AUC), such as hepatic transaminase levels, cocaine use, diet, body mass index, orange juice consumption and renal function. Moreover, we have recently assessed the contribution of genetic traits in addition to non-genetic factors to ARV exposure in the WIHS intensive PK studies by linking with the WIHS Genetics Working Group.

In addition to the rich dataset of intensive PK parameters for large numbers of women in the cohort, and extensive data on possible non-genetic and genetic contributors to exposure, we have been collecting sparse PK levels (single plasma concentrations of the target ARV) at each WIHS study visit. We have now initiated collaborated with an analytic group at UCSF with expertise in nonlinear mixed effects modeling (NONMEM analysis) to assist us in modeling the sparse and intensive PK data in WIHS. We do not plan to perform any additional intensive PK studies of ARVs during WIHS V, but will continue analyzing this unprecedented database of PK and pharmacogenetic data over the next five years, with additional funding to be sought for analytic efforts via linked RO1s.

I. WIHS Hair Exposure Studies


The WIHS UCSF site has pioneered the use of small hair samples to monitor ARV adherence and
exposure for patients on combination ARVs after another group presented proof-of-concept analyses that indinavir could be measured in hair samples and correlated with clinical outcomes. We have now developed methods to extract and analyze nucleoside reverse transcriptase inhibitors (NRTIs), prevalent-use protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs), from hair and demonstrated that hair concentrations of ARVs are the strongest independent predictor of virologic success in large prospective cohorts of HIV-infected patients in the WIHS. We have also examined factors that contribute to hair concentrations of ARVs in the cohort, including genetic contributors to exposure. This particular exposure project initiated in the WIHS study has now been translated to a number of research settings around the world.

Development of methods to analyze lopinavir (LPV), ritonavir (RTV), atazanavir (ATV), nevirapine (NVP), tenofovir (TFV), and emtricitabine ( FTC) in very small samples of human hair by our laboratory using sensitive methods have been developed and reported. We have also developed methods for measuring the HIV protease inhibitor, darunavir (DRV), and the HIV integrase inhibitor, raltegravir (RAL) in hair (manuscript is in preparation) with performance characteristics similar to those we have reported for other ARVs. Analysis of PI, NNRTI and integrase inhibitor levels requires 20-30 strands of human hair, whereas analysis of TFV and FTC requires 50-100 strands.

During WIHS V, we will implement new measures of tenofovir exposure, while maintaining our ongoing measurement of PIs, NNRTIs and integrase inhibitors in hair. For women on TFV-containing regimens, we propose to modify the WIHS protocol to collect 50-100 strands of hair instead of the usual 20 strands. ARV exposure will be assessed as a contributor to key HIV-related outcomes, such as virologic and immunologic responses to therapy, as well as HIV-related outcomes, such as neurocognitive status, bone mineral density, hepatic fibrosis, renal function (especially in relationship to TFV exposure), metabolic outcomes, and vascular injury. Since hair concentrations of ARVs represent an integrated measure of behavior (adherence) and biology (individual pharmacokinetics), we propose that analyses performed in WIHS from this point forward incorporate hair levels of ARVs as markers of adherence and/or exposure. We will also study determinants of ARV exposure using hair measures such as host genetic traits, and develop methods to assess exposure to new HCV medications in hair during WIHS V.

References:
A Single-Nucleotide Polymorphism in CYP2B6 Leads to >3-Fold Increases in Efavirenz Concentrations in Plasma and Hair Among HIV-Infected Women

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Background. Efavirenz exhibits marked interindividual variability in plasma levels and toxicities. Prior pharmacogenetic studies usually measure exposure via single plasma levels, examine limited numbers of polymorphisms, and rarely model multiple contributors. We analyzed numerous genetic and nongenetic factors impacting short-term and long-term exposure in a large heterogeneous population of human immunodeficiency virus (HIV)–infected women.

Methods. We performed 24-hour intensive pharmacokinetic studies in 111 women receiving efavirenz under actual-use conditions and calculated the area-under-the-concentration-time curve (AUC) to assess short-term exposure; the efavirenz concentration in hair was measured to estimate long-term exposure. A total of 182 single-nucleotide polymorphisms (SNPs) and 45 haplotypes in 9 genes were analyzed in relationship to exposure by use of multivariate models that included a number of nongenetic factors.

Results. Efavirenz AUCs increased 1.26-fold per doubling of the alanine aminotransferase level and 1.23-fold with orange and/or orange juice consumption. Individuals with the CYP2B6 516TT genotype displayed 3.5-fold increases in AUCs and 3.2-fold increases in hair concentrations, compared with individuals with the TG/GG genotype. Another SNP in CYP2B6 (983TT) and a p-glycoprotein haplotype affected AUCs without substantially altering long-term exposure.

Conclusions. This comprehensive pharmacogenomics study showed that individuals with the CYP2B6 516TT genotype displayed >3-fold increases in both short-term and long-term efavirenz exposure, signifying durable effects. Pharmacogenetic testing combined with monitoring of hair levels may improve efavirenz outcomes and reduce toxicities.

Achievement of the full benefits of antiretroviral therapy requires lifelong exposure to adequate but nontoxic levels of these medications. However, significant interindividual variability in drug concentrations occurs, owing to the influence of factors such as treatment adherence, concomitant conditions and medications, illicit substance use, diet, body mass, renal and/or hepatic function, and host genetics. Pharmacogenetic assessment is optimally performed via a comprehensive approach in which genetic traits are assessed in parallel with nongenetic determinants in representative populations. Moreover, since a single plasma drug level does not provide information on long-term exposure, pharmacogenetic studies are best performed using more comprehensive drug exposure measures.
We have previously described methods to measure antiretroviral levels in small samples of hair to assess long-term exposure to treatment [1, 2]. We have shown that protease inhibitor and non-nucleoside reverse transcriptase inhibitor concentrations in hair are consistently powerful independent predictors of virologic outcome [3, 4]. We have also performed intensive pharmacokinetic (PK) studies over 12–24 hours in a large heterogeneous population that we now use to model the contribution of genetic and nongenetic factors to antiretroviral exposure [5, 6]. The area-under-the-concentration-time curve (AUC) calculated from intensive PK sampling data and the concentration in hair samples can be used to measure short-term and long-term exposure, respectively.

Efavirenz is a mainstay of combination antiretroviral therapy, and its dosing in adults is frequently uniform (600 mg daily), despite marked interindividual variability in plasma levels [7, 8]. Although efavirenz is available in a fixed-dose combination administered once daily, rates of discontinuation of this medication in real-world settings, usually due to adverse effects, are high (up to 50%) [9–12]. Central nervous system (CNS) side effects are common and have been linked with high plasma efavirenz levels [13, 14].

Efavirenz is primarily metabolized by the cytochrome p450 (CYP) 2B6 enzyme, and several single nucleotide polymorphisms (SNPs) in the CYP2B6 gene are known to influence plasma levels, responses, and CNS side effects [15–17]. Polymorphisms in other cytochromes have also been linked with variations in efavirenz levels [18], as have SNPs in the multiple drug resistance (MDR1/ABCB1) gene [19]. After SNPs were identified that appeared to influence efavirenz pharmacokinetics, investigators conducted replication studies to examine their associations with efavirenz levels. However, a systematic evaluation of SNPs encompassing the majority of genetic variability in key genes implicated in efavirenz absorption, distribution, metabolism, and excretion (ADME) [20] has not been performed.

The goal of the current study was to examine the contributions of ADME gene polymorphisms to efavirenz exposure when modeled with nongenetic factors. To conduct this study, the association of literature-based SNPs and tagging SNPs selected to capture the majority of genetic variability in CYP2B6 and other implicated ADME genes with efavirenz exposure was examined in a diverse unselected population of HIV-infected women. The effects of these polymorphisms on short-term exposure (evaluated using plasma AUCs) and long-term exposure (evaluated using hair levels) to efavirenz are presented.

METHODS

Study Population and Protocol

The Women’s Interagency HIV Study (WIHS) is a large, multicenter cohort study of women with or without HIV infection [5] and is highly representative of HIV-infected women in the United States in terms of factors such as age, race/ethnicity, and socioeconomic characteristics. We previously described the “WIHS Intensive PK Study,” in which 121 participants receiving efavirenz-based therapy underwent 24-hour sampling after a witnessed dose under conditions of routine use at steady-state [6]. The only eligibility criterion for participation in this study was use of an efavirenz-containing antiretroviral regimen.

Laboratory Procedures

Measurement of Short-Term Exposure: Efavirenz AUC Using Intensive Plasma Sampling

Procedures for measuring efavirenz blood levels have been described previously [21]. Plasma was analyzed for efavirenz by standard techniques of liquid chromatography/tandem mass spectrometry (LC/MS/MS) [22]. 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% [21]. The plasma EFV assay was validated according to the current Food and Drug Administration guidelines for bioanalytical method validation, and all quality control samples were within 15% of their respective nominal value.

Measurement of Long-Term Exposure: Efavirenz Concentration in Hair

Methods to analyze efavirenz levels in hair have been described elsewhere [1]. A small thatch of hair (approximately 20 strands) is cut as close as possible to the scalp, and the distal portion is labeled. Extraction methods have been described and measurement of efavirenz is performed by LC/MS/MS [1]. This method has been validated for detection of a range of .05–20 nanograms of efavirenz per milligram of hair (ng/mg), with good linearity (R^2> .99) and reproducibility (coefficient of variation, <15%).

Blood Collection and Genotyping

Of the 121 participants recruited, genomic DNA could be isolated from cell pellets and successfully genotyped for 111. Genotyping was performed by individuals who were blinded to 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.

Gene Selection

A literature search identified genes implicated in efavirenz ADME. The custom array was designed to interrogate each candidate gene by using literature-driven SNPs, as well as tagging SNPs. Tagging SNPs were selected to capture
neighboring regions in high linkage disequilibrium (LD) across coding and noncoding regions of genes implicated in ADME. The 9 genes studied in each of the 111 participants included adenosine triphosphate–binding cassette protein (ABC) B1 (ABCB1), ABCC2, CYP2B6, CYP2C19, CYP2D6, CYP3A4, CYP3A5, solute carrier-like protein (SCL) A6 (SCL22A6), and uridyl diphosphate glucuronosyltransferase-1 family, polypeptide A1 (UGT1A1).

**SNP Selection**

Tagging SNPs were required to be common (defined as a minor allele frequency of ≥.05) in public databases (eg, HapMap). To ensure robust analyses, quality control filtering of SNPs was performed, and SNPs with call rates of <93.5% (n = 8) or Hardy-Weinberg P values of <.001 (n = 9) were excluded. SNPs with allele frequencies of <5% (n = 35) were excluded from analysis.

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; CYP3A5/ CYP3A4: 21 of 30 SNPs; SCL22A6: 5 of 8 SNPs; and UGT1A1: 22 of 26 SNPs) passed all quality control filters. Figure 1 shows the LD map of the literature-driven and tagging SNPs assessed in CYP2B6.

**Study Measurements**

**Outcome Variables**

We evaluated factors impacting drug exposure, using 2 methods. First, exposure was assessed by calculation of the AUC divided by dose, in which the plasma AUC was calculated using the trapezoidal rule from 24-hour intensive PK studies, and the dose was the efavirenz dose witnessed at the start of the PK sampling. Second, we determined exposure by

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**Figure 1.** Linkage disequilibrium (LD) map of single-nucleotide polymorphisms (SNPs) assessed in CYP2B6 in relationship to efavirenz exposure. An ideogram of CYP2B6 is presented at the top, which represents the physical distance along human chromosome 19 (46 187 595 to 46 230 064; genome build 36.3, contig NT_011109.15). Exons are represented as boxes, with coding regions rendered in gray and untranslated regions rendered in pink; gray lines connecting exons represent introns. Reference sequence (rs) identifiers for each SNP are plotted both in terms of their physical distance (ie, the white bar at the top of the figure) and also equidistantly to render the pairwise LD estimates, which were calculated and visualized with Haploview 4.2. The gene structure for CYP2B6 (ie, reference sequence NM_000767) was rendered with FancyGene 1.4. The correlation statistics ($r^2$ and D') are provided in the heat map. LD-based haplotype block definition was based on the D' confidence interval. The haploblock is indicated in a bolded triangle, and its component SNPs are also rendered in bold font, with dashed regions indicating intervening SNPs that are not part of the haploblock. Pairwise D' values (range, 0–1) were rendered in color, with darker red diamonds representing D' values approaching 1.0, and progressively lighter red to pink colored diamonds representing progressively smaller D' values. Grey diamonds represent pairwise D' values of 1.0 but with log of odds values of <2 (ie, below the significance threshold of 2.0). When the $r^2$ values (range, 0–100) are not equal to 0 or 100, they are provided in a given diamond.
measuring efavirenz concentrations in small samples of hair, which is indicative of drug uptake over at least 1 month.

**Predictor Variables**
The 2 exposure outcomes were analyzed in relation to a number of factors that may influence PK. Variables included race, age, hepatitis B/C virus infection status, ovulatory cycle phase or menopausal status, pregnancy status, smoking status, alcohol and substance use, percentage of fat in the typical diet [23], diarrhea, other concurrent symptoms or infections, concomitant medications with potential drug-drug interactions, calculated creatinine clearance rate, body mass index and fat free mass, and results of hepatic function tests [6]. The models examining the hair exposure measure included self-reported adherence to antiretroviral therapy, as estimated by the percentage of prescribed doses consumed over 6 months (using visual analog scales). Level of adherence was categorized as ≤74%, 75%–94%, or ≥95% of prescribed doses.

The variables identified in our previous analyses that independently influenced exposure include age, alanine aminotransferase (ALT) level, albumin level, ideal body weight, orange or orange juice consumption in the preceding 5 days, amenorrhea for ≥12 months, tenofovir use, and race [6]. SNPs were first assessed in relationship to exposure in a bivariate fashion and then added to multivariate models with these nongenetic predictors. For the SNPs, allele and genotype frequencies were determined by gene counting, and Hardy-Weinberg equilibrium was assessed by the \( \chi^2 \) exact test. Measures of LD (ie, \( D' \) and \( r^2 \)) were computed from the participants’ genotypes with Haploview 4.1. LD-based haplotype block definition was based on the \( D' \) confidence interval (CI) [24].

For SNPs in the same haplotype, haplotype analyses were conducted to localize the association signal within each gene and to determine whether haplotypes improved the strength of the association. Haplotypes were constructed using the program PHASE, version 2.1 [25]. To improve the stability of haplotype inference, the haplotype construction procedure was repeated 5 times. Only haplotypes that were inferred with probability estimates of ≥0.85 across the 5 iterations and estimated to occur at a frequency of ≥20% were retained for downstream analyses.

Ancestry informative markers (AIMs) were used to estimate ancestry and as a tool to minimize bias due to population stratification [26]. Homogeneity in ancestry among participants was verified by cluster and principal component (PC) analysis [27]. The number of PCs were sought that distinguished the major racial/ethnic groups in the sample (ie, European, African, and Asian) by visual inspection of scatter plots of orthogonal PCs (ie, PC 1 vs PC2, and PC2 vs PC3). The first 3 PCs were selected to adjust for potential confounding due to population substructure (ie, race/ethnicity) by including them in all multiple regression models. AIMs and their PCs were available for 97 of 111 participant samples.

**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 sample characteristics. The AUC and hair outcomes were log transformed for linear regression (modified for hair to account for undetectable levels [28]), but we added 1.0 to the dose-normalized hair level (hair level × 600 mg/dose) before taking logs, to prevent very low levels in hair from becoming large negative values. Four genetic models were assessed for each SNP: unstructured, additive, dominant, and recessive. The genetic model that best fit the data by maximizing the significance of the \( P \) value was selected for each SNP. For race/ethnicity, both genetic (AIM-derived PCs) and nongenetic (self-reported) parameters were examined. Since AIMs were only available in a subset of study participants (97 of 111), models with either self-reported race (African American or not) or both self-reported race and AIM PCs were also examined in the same subset and compared with the models in the full sample (n = 111). Although the precision of the estimates was typically less, the models including AIM-derived PCs were essentially the same as those including self-reported race alone.

The first set of models (Supplementary Table 1) show the effect of each individual SNP on log-transformed AUC over dose when combined with nongenetic factors previously shown to influence exposure. For the multivariate models in Tables 1 and 2, genetic predictors were selected in a forward stepwise manner, with adjustment for nongenetic factors, until no remaining genetic candidates met our a priori significance threshold of \( P < .001 \). To obtain a more parsimonious final model, we then applied backward elimination to the nongenetic factors until all of the remaining predictors demonstrated \( P \) values of <.05. Because efavirenz levels in hair were available for fewer participants, and to allow for a comparison of the findings, we modeled log levels in hair with the same final set of predictors as for log AUC/dose. We then evaluated each remaining genetic factor as a single addition to the hair models.

**RESULTS**

**Effect of Individual SNPs on Short-Term Efavirenz Exposure**
We previously summarized the diverse characteristics of this intensively studied population, including a range of factors that could influence exposure [6]. 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 the participants was 43.1 years (range, 20.6–60.4 years).

The mean efavirenz AUC among these participants was 78 μg × hours/mL; the median AUCs were 54 μg × h/mL (range, 11–519 μg × h/mL). The majority of the participants in this study were taking 600 mg of efavirenz daily, and only 4 of 111 were taking alternative doses.

A total of 230 polymorphisms were selected to interrogate the 9 ADME candidate genes; 48 SNPs failed to meet at least 1 quality control criterion. A total of 182 SNPs and 45 haplotypes were analyzed in relationship to exposure, using linear regression. Supplementary Table 1 shows the relationship between each SNP or haplotype and log-transformed AUC/dose when a single genetic predictor is added to models containing nongenetic predictors. Of the polymorphisms studied in each gene, 1 of 7 in ABCB1, 2 of 23 in ABCC2, 16 of 27 in CYP2B6, and 9 of 32 in UGT1A1 were associated with exposure with a P value of <.01 when combined with nongenetic predictors.

### Table 1. Factors Associated With Short-Term Efavirenz Exposure Among 111 Subjects From the Women’s Interagency HIV Study

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fold-Effect on AUC (95% CI)</th>
<th>P</th>
<th>Distribution of Factor in Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oranges or orange juice in preceding 5 d</td>
<td>1.26 (1.05–1.50)</td>
<td>.012</td>
<td>Consumed by 76 (68.5%)</td>
</tr>
<tr>
<td>For every doubling of ALT level</td>
<td>1.23 (1.11–1.36)</td>
<td>.0001</td>
<td>Median ALT level, 23 IU/L (range, 8–117 IU/L)</td>
</tr>
<tr>
<td>CYP2B6 516 G&gt;T (rs3745274)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1 dose of minor allele (GG, GT)</td>
<td>1.00</td>
<td>1.4 × 10⁻¹⁸</td>
<td>53 (47.7%), 0 doses</td>
</tr>
<tr>
<td>2 doses of minor allele (TT)</td>
<td>3.5 (2.7–4.5)</td>
<td></td>
<td>44 (39.6%), 1 dose</td>
</tr>
<tr>
<td>CYP2B6 983 T&gt;C (rs28399499)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 doses of minor allele (TT)</td>
<td>1.00</td>
<td>2.2 × 10⁻¹⁰</td>
<td>95 (85.6%), 0 doses</td>
</tr>
<tr>
<td>1 or 2 doses of minor allele (TC, CC)</td>
<td>1.96 (1.54–2.5)</td>
<td></td>
<td>15 (13.5%), 1 dose</td>
</tr>
<tr>
<td>ABCB1 haplotype (2 SNPs: rs7779562 and rs4148745)</td>
<td></td>
<td></td>
<td>1 (0.9%), 2 doses</td>
</tr>
<tr>
<td>0 doses of the haplotype</td>
<td>1.00</td>
<td></td>
<td>48 (43.2%), 1 dose</td>
</tr>
<tr>
<td>1 or 2 doses of the haplotype</td>
<td>1.60 (1.24–2.1)</td>
<td>.0004</td>
<td>49 (44.1%), 2 doses</td>
</tr>
</tbody>
</table>

Short-term exposure was evaluated in terms of the AUC per dose.
Abbreviations: ALT, alanine amino transferase; AUC, area under the concentration-time curve; CI, confidence interval.

### Table 2. Factors Associated With Long-Term Efavirenz Exposure Among 87 Subjects From the Women’s Interagency HIV Study

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on Hair (95% CI)</th>
<th>P</th>
<th>Distribution of Factor in Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oranges or orange juice in preceding 5 d</td>
<td>1.19 (.88–1.60)</td>
<td>.27</td>
<td>Consumed by 57 (65.5%)</td>
</tr>
<tr>
<td>For every doubling of ALT level</td>
<td>1.10 (.93–1.30)</td>
<td>.25</td>
<td>Median ALT, 23 IU/L (range, 8–117 IU/L)</td>
</tr>
<tr>
<td>CYP2B6 516 G&gt;T (rs3745274)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1 dose of minor allele (GG, GT)</td>
<td>1.00 (1.0–1.0)</td>
<td>1.0 × 10⁻¹⁰</td>
<td>38 (43.7%), 0 doses</td>
</tr>
<tr>
<td>2 doses of minor allele (TT)</td>
<td>3.2 (2.2–4.7)</td>
<td></td>
<td>36 (41.4%), 1 dose</td>
</tr>
<tr>
<td>CYP2B6 983 T&gt;C (rs28399499)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 doses of minor allele (TT)</td>
<td>1.00 (1.0–1.0)</td>
<td>.021</td>
<td>77 (88.5%), 0 doses</td>
</tr>
<tr>
<td>1 or 2 doses of minor allele (TC, CC)</td>
<td>1.70 (1.09–2.7)</td>
<td></td>
<td>9 (10.3%), 1 dose</td>
</tr>
<tr>
<td>ABCB1 haplotype (2 SNPs: rs7779562 and rs4148745)</td>
<td></td>
<td></td>
<td>1 (1.1%), 2 doses</td>
</tr>
<tr>
<td>0 doses of the haplotype</td>
<td>1.00 (1.0–1.0)</td>
<td>.31</td>
<td>38 (43.7%), 1 dose</td>
</tr>
<tr>
<td>1 or 2 doses of the haplotype</td>
<td>1.24 (.82–1.88)</td>
<td></td>
<td>38 (43.7%), 2 doses</td>
</tr>
<tr>
<td>Self-reported adherence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤74%</td>
<td>1.00</td>
<td></td>
<td>4 (4.6%)</td>
</tr>
<tr>
<td>75–94%</td>
<td>.94 (.45–1.96)</td>
<td>.88</td>
<td>14 (16.1%)</td>
</tr>
<tr>
<td>≥95%</td>
<td>1.10 (.56–2.2)</td>
<td>.77</td>
<td>69 (79.3%)</td>
</tr>
</tbody>
</table>

Long-term exposure was evaluated in terms of the hair level per dose.
Abbreviations: ALT, alanine amino transferase; AUC, area under the concentration-time curve; CI, confidence interval.
Multivariate Models of Factors Associated With Short-Term Exposure

By using forward selection for the genetic predictors, we generated a final multivariate model of the factors associated with efavirenz AUCs (Table 1). Table 1 also shows the distribution of the SNPs in the sample independently associated with AUC with a P value of <.001 in the final model. Of note, when genetic predictors were included, 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 past 5 days. The model explained 53% of the interpatient variability. The effects of genetic factors on exposure were similar (all estimates were within 3%) in the larger models prior to backward elimination, so only the parsimonious models are presented.

Of the SNPs and haplotypes examined, 3 were associated with short-term exposure. Individuals homozygous for the CYP2B6 516T rare allele (“TT” genotype) displayed 3.5-fold ($P = 1.4 \times 10^{-18}$) increases in AUC as compared to carriers of the common “G” allele (“TG” and “GG” genotypes). Individuals homozygous or heterozygous for the CYP2B6 983C common allele (“CC” or “CT” genotype) displayed 1.96-fold ($P = 2.2 \times 10^{-10}$) 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 the transporter ABCB1 was associated with AUC increases of 1.60-fold ($P = .0004$). Fitting the model for the exposure outcome of $C_{\text{max}}$ per dose instead of AUC per dose produced similar results. The direction of the association between elevated ALT level and exposure could not be assessed in these cross-sectional analyses. However, repeating the final models without ALT levels did not substantially alter the fixed predictor effects (orange juice consumption and genetic predictors) on exposure.

Multivariate Models of Factors Associated With Long-Term Exposure

Of the 111 participants, long-term exposure measures, as estimated by hair levels, were available for 87. The majority of these 87 participants were taking 600 mg of efavirenz daily; only 3 were receiving alternative doses. The mean concentration of efavirenz in hair was 5.92 ng/mg, and the median concentration was 3.11 ng/mg (range, <.05–41.4). Unlike the intensive PK studies conducted after witnessed dosing, long-term exposure may be affected by adherence, so a self-reported adherence measure was included in these models. Table 2 shows the final multivariate model in relationship to dose-normalized hair levels, along with the distribution of the covariates in the 87 participants. This model explained 34% of the interpatient variability. ALT level and orange juice consumption were not statistically significantly associated with hair levels, although the associations were in the same direction and the estimate for orange juice consumption was reduced only modestly. Self-reported adherence was also not substantially associated with hair concentrations.

The major CYP2B6 SNP independently associated with short-term efavirenz exposure was also associated with long-term exposure to efavirenz. Individuals homozygous for the CYP2B6 516T rare allele (“TT” genotype) displayed 3.2-fold increases ($P = 1 \times 10^{-18}$) in efavirenz levels in hair as compared to carriers of the common “G” allele, similar to the 3.5-fold increase seen with AUC. The effects of the CYP2B6 983CC/CT polymorphisms and the ABCB1 haplotype on efavirenz hair levels were in the same direction as their effects on AUC but with smaller estimated effects and larger P values.

We then evaluated each genetic predictor as a single addition to the hair model. One of the genetic factors (an ABC2 SNP) not selected for in the AUC model met our threshold P value of <.001 when added to factors in Table 2; the unstructured model for this SNP (rs2002042) had an overall P value of $5.0 \times 10^{-5}$, with those with 1 copy of the rare allele averaging 0.55-fold lower levels in hair than those with no copies (95% CI, .40–.75; P = .0002) and 0.38-fold lower levels than those with 2 copies (95% CI, .24–.61; P = .0001). This pattern did not hold in the corresponding model for AUC.

DISCUSSION

To our knowledge, this is the first report on the association of polymorphisms in CYP2B6 516 with long-term concentrations of efavirenz using hair samples. Since lifelong daily therapy is required for the treatment of HIV infection, studies that identify SNPs that can influence antiretroviral exposure over prolonged periods may guide dose-optimization strategies. Given the extent of treatment discontinuation, toxicities [29], and adherence difficulties in HIV-infected individuals, use of pharmacogenetics to personalize care for these individuals should improve treatment selection, dosing, and outcomes [30]. Indeed, HIV medicine is one of the few fields that routinely uses pharmacogenetic screening to reduce rates of a medication-associated reaction already.

With the availability of new genotyping technology, the paradigm in pharmacogenetics has shifted from examining a small number of genes for a limited number of allelic variants to systematically assessing SNPs in a range of genes relevant for ADME [20]. In a large heterogeneous population of HIV-infected patients, we performed a comprehensive search of 182 SNPs and 45 haplotypes in 9 genes implicated in efavirenz ADME and modeled them with nongenetic traits previously found to influence efavirenz AUC. Host traits were examined in combination with other factors that influence drug metabolism, to identify both genetic and nongenetic contributors to exposure.

Orange juice consumption and 2 additional SNPs (CYP2B6 983C and an ABCB1 haplotype, both of which have been
found to influence plasma levels in the literature [15]) were associated with modest elevations in AUC, but less so with hair levels. Estimated effects on hair levels were generally smaller than those on AUCs, possibly reflecting imprecision in our self-reported adherence measure, as well as the need to add 1 before logarithmic transformation [28]. Inhibition of intestinal p-glycoprotein transport or downregulation of enteric CYP3A4 by citrus components in oranges or orange juice could lead to enhanced bioavailability and increased short-term exposure to efavirenz. We identified 1 additional genetic association (rs2002042 in the ABCG2 transporter) that influenced hair levels but not AUCs. The unusual pattern of heterozygotes having lower levels in hair than either type of homozygote must be regarded as preliminary. The most important finding of this study was the consistent effect of the CYP2B6 516T rare allele on tripling efavirenz exposure as measured via AUCs and hair levels.

Efavirenz is prescribed commonly for HIV infection, and a number of studies have examined the relationship between host traits and plasma efavirenz levels [15, 16, 18, 31–44]. Certain CYP2B6 SNPs have consistently been linked to higher plasma efavirenz levels and higher rates of early discontinuation [45, 46]. Individuals homozygous for the CYP2B6 516T rare allele (TT), referred to as “slow metabolizers,” consistently demonstrate higher plasma levels than those in individuals carrying the TG/GG genotypes. However, dose modification of efavirenz on the basis of the presence of SNPs that might transiently increase plasma levels is not routinely performed. Although authors have argued for the synthesis of pharmacogenetic testing with therapeutic drug monitoring to individualize dosing [47], the lack of a “gold standard” to monitor exposure to antiretroviral therapy and the uncertain significance of a SNP’s influence on a plasma antiretroviral level remain significant barriers to dose-optimization protocols.

Plasma levels as a measure of exposure have a number of limitations, including insensitivity to day-to-day variation [48], dependence on the accurate reporting of last dose taken, intrindividual variations in diet, medications, and illicit substance use. Plasma levels may not reflect typical patterns of medication use and are subject to “white-coat” effects, in which adherence improves prior to medical visits [49]. Previous studies examining the relationship between genetic traits and efavirenz exposure have used single plasma levels or nonlinear mixed effects modeling of plasma concentrations to calculate population pharmacokinetic parameters as estimates of exposure. Short-term exposure is more robustly estimated by AUCs from intensive PK sampling, which was the measure used in this analysis. Although AUC measurement after witnessed dosing more accurately estimates exposure than single plasma levels, intensive PK sampling is cumbersome and expensive for the routine clinical setting.

We measured long-term drug exposure by monitoring efavirenz concentrations in small samples of hair. Because the concentrations of medications in hair reflect drug uptake from the systemic circulation over weeks to months, they are not subject to bias from the “white-coat” effect or inaccurate recall of the time at which the last dose was taken. Drug levels in hair therefore provide an advantage over plasma concentrations in estimating an average level of exposure over time [50] and provide a more feasible exposure measure in the clinical setting than AUCs. Genetic traits that influence both AUCs and hair concentrations of medications are likely to be durable in their effects.

Given the high rates of efavirenz discontinuation in clinical settings, identifying traits that lead to durable effects on exposure is important. This comprehensive search for SNPs in genes associated with efavirenz ADME demonstrated that individuals homozygous for the CYP2B6 516T (“TT” genotype) had >3-fold increases in short-term and long-term efavirenz exposure. This SNP’s effect on exposure over the prolonged duration represented by hair levels is reported for the first time. Hair testing for exposure has feasibility and cost advantages over intensive PK sampling. Polymorphisms that affect exposure may be more important to assess in individuals whose capacity to clear a medication is already limited by nongenetic factors. Therefore, genetic testing coupled with hair measurement may be helpful in optimizing efavirenz dosing in the clinical setting, particularly when other risk factors for high exposure are present.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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References


Atazanavir Concentration in Hair Is the Strongest Predictor of Outcomes on Antiretroviral Therapy

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Background. Adequate exposure to antiretrovirals is important to maintain durable responses, but methods to assess exposure (eg, querying adherence and single plasma drug level measurements) are limited. Hair concentrations of antiretrovirals can integrate adherence and pharmacokinetics into a single assay.

Methods. Small hair samples were collected from participants in the Women’s Interagency HIV Study (WIHS), a large cohort of human immunodeficiency virus (HIV)-infected (and at-risk noninfected) women. From 2003 through 2008, we analyzed atazanavir hair concentrations longitudinally for women reporting receipt of atazanavir-based therapy. Multivariate random effects logistic regression models for repeated measures were used to estimate the association of hair drug levels with the primary outcome of virologic suppression (HIV RNA level, <80 copies/mL).

Results. 424 WIHS participants (51% African-American, 31% Hispanic) contributed 1443 person-visits to the analysis. After adjusting for age, race, treatment experience, pretreatment viral load, CD4 count and AIDS status, and self-reported adherence, hair levels were the strongest predictor of suppression. Categorized hair antiretroviral levels revealed a monotonic relationship to suppression; women with atazanavir levels in the highest quintile had odds ratios (ORs) of 59.8 (95% confidence ratio, 29.0–123.2) for virologic suppression. Hair atazanavir concentrations were even more strongly associated with resuppression of viral loads in subgroups in which there had been previous lapses in adherence (OR, 210.2 [95% CI, 46.0–961.1]), low hair levels (OR, 132.8 [95% CI, 26.5–666.0]), or detectable viremia (OR, 400.7 [95% CI, 52.3–3069.7]).

Conclusions. Antiretroviral hair levels surpassed any other predictor of virologic outcomes to HIV treatment in a large cohort. Low antiretroviral exposure in hair may trigger interventions prior to failure or herald virologic failure in settings where measurement of viral loads is unavailable. Monitoring hair antiretroviral concentrations may be useful for prolonging regimen durability.

The use of combination antiretroviral therapy (cART) is the prime determinant of longevity among human immunodeficiency virus (HIV)-infected individuals, and the focus of treatment has now shifted to maintaining durable responses on existing regimens. Toward this end, robust predictive models of treatment response are needed to identify factors that may threaten virologic response and herald resistance. By the time a regimen has failed virologically, important opportunities for adherence interventions and preventing resistance have been missed. In resource-limited settings, where HIV viral load monitoring may not be routinely available, resistance mutations can accumulate by the time a regimen fails clinically or immunologically [1, 2]. A low-cost method that provides an early predictor of virologic failure would be useful in both industrialized and nonindustrialized settings to monitor responses to long-term cART [3].

Adherence to treatment, commonly assessed by self-report, is a strong contributor to cART outcomes.
However, limitations of the accuracy of self-reporting and other commonly used adherence measures are well described [4]. Furthermore, interindividual variations in pharmacokinetics can lead to outcome disparities even when adherence is high, indicating the utility of an objective measure that could integrate both combined effects of adherence and individual pharmacokinetic parameters. We have developed methods for monitoring antiretroviral exposure by means of determination of antiretroviral concentrations in small-volume hair samples [5–9]. We previously reported that hair levels of protease inhibitors (PIs) were the strongest independent factor associated with short-term virologic response in individuals initiating new PI-based regimens [6]. Drug levels in hair were more closely associated with HIV RNA suppression six months after starting therapy than were self-reported adherence and other commonly applied factors, including pretreatment viral load and CD4 cell count, extent of antiretroviral or PI experience, age, or race. A better test of the ultimate value of drug levels in hair is whether these measures predict impending virologic failure. We report here on a longitudinal study that assessed how well atazanavir levels measured in hair predict treatment outcomes in a multisite observational cohort of HIV-infected women.

**METHODS**

**The Women's Interagency HIV Study Cohort and Study Sample**

The Women's Interagency HIV Study (WHIS) is the largest cohort of HIV-infected women and at-risk HIV-noninfected women studied in the United States [10]. This ongoing prospective multicenter study with sites in the San Francisco and Los Angeles, California; Chicago, Illinois; Bronx and Brooklyn, New York; and Washington, DC metropolitan areas observes participants via visits that occur at 6-month intervals. Interviewer-administered survey instruments, physical examination, and specimen collection are performed at each visit. A small sample of hair (10–20 strands, or 1–3 mg) is cut from the occipital region of the scalp at each study visit from every consenting HIV-seropositive woman reporting cART. All participants who reported taking an atazanavir-based reporting at any study visit during the period from April 2003 through April 2008 were included in this particular analysis. WHIS study protocols and consent materials were reviewed and approved by institutional review boards at all participating institutions.

**Hair Collection, Processing, and Analysis**

A hair specimen is collected in the WHIS if the participant reports taking antiretrovirals for at least 1 month. Field staff at all WHIS sites have been uniformly trained on the method of collecting hair. Briefly, a small thatch of hair is cut as close as possible to the scalp from the occiput and the distal portion labeled to denote directionality (Figure 1). Methods for extraction and analyses of most of the PIs, nonnucleoside reverse transcriptase inhibitors, and tenofovir have been developed and optimized in our laboratory and reported elsewhere [6–11].

Atazanavir and ritonavir levels were measured in hair samples collected at visits during which the participant reported current use. Using 2 mg of human hair, atazanavir is detected at levels as low as 0.05 ng/mg hair and ritonavir is detected at levels of as low as 0.01 ng/mg hair. The method has been validated in the range of 0.05–20 ng/mg hair for atazanavir and 0.01–4.0 ng/mg hair for ritonavir with good linearity and reproducibility. Of note, we have tested antiretrovirals in hair in this diverse WHIS cohort and found that median levels and interquartile ranges among participants with undetectable viral loads do not vary significantly by race or ethnicity.

**Statistical Analysis**

The primary outcome was virologic success (or “suppression”) at each study visit, defined as an HIV viral load of <80 copies/mL. Multivariate random effects logistic regression models for repeated measures were used to estimate the association of hair drug levels with the dichotomous outcome of virologic suppression. We used the hair level at each visit to predict virologic success,
RESULTS

Participant Demographic Characteristics

Table 1 summarizes demographic and other characteristics for the 424 WIHS participants receiving atazanavir-based CART at any time from April 2003 through April 2008. The racial and ethnic distribution of the study sample was 215 African-Americans (51%), 131 Hispanics (31%), 66 whites (15%), and 12 others (3% [Native Americans or Asian-Americans]). Each woman contributed 1–9 WIHS biannual study visits to the analysis (median, 3), for a total of 1443 person-visits. Of these 1443 person-visits, participants reported ritonavir coadministration with atazanavir, hair levels of each of these agents are substantially collinear, so separate models were run for atazanavir and ritonavir in the women receiving ritonavir-boosted atazanavir. All analyses were performed using SAS software, version 9.2 (SAS Institute).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-visits contributing to analysis</td>
<td>1443</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>43 (21–71)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>White (non-Hispanic)</td>
<td>66 (15)</td>
</tr>
<tr>
<td>African-American (non-Hispanic)</td>
<td>215 (51)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>131 (31)</td>
</tr>
<tr>
<td>Other</td>
<td>12 (3)</td>
</tr>
<tr>
<td>Past PI treatment</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>101 (24)</td>
</tr>
<tr>
<td>1 PI</td>
<td>156 (37)</td>
</tr>
<tr>
<td>&gt;2 PIs</td>
<td>167 (39)</td>
</tr>
<tr>
<td>Pretreatment viral load, copies/mL</td>
<td></td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>52 (12)</td>
</tr>
<tr>
<td>&lt;100,000</td>
<td>372 (88)</td>
</tr>
<tr>
<td>HIV viral load, median copies/mL (range)</td>
<td>5950 (80–2,500,000)</td>
</tr>
<tr>
<td>Pretreatment CD4 cell count, cells/mm³</td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>133 (31)</td>
</tr>
<tr>
<td>&gt;200</td>
<td>291 (69)</td>
</tr>
<tr>
<td>CD4 cell count, median cells/mm³ (range)</td>
<td>281 (5–2046)</td>
</tr>
<tr>
<td>Person-visits at which atazanavir is boosted with ritonavir</td>
<td>1136 (79)</td>
</tr>
<tr>
<td>Person-visits at which viral load is undetectable</td>
<td>918 (64)</td>
</tr>
<tr>
<td>Adherence during past 6 months (self-reported at person-visit)</td>
<td></td>
</tr>
<tr>
<td>0%–74%</td>
<td>83 (6)</td>
</tr>
<tr>
<td>75%–94%</td>
<td>244 (17)</td>
</tr>
<tr>
<td>&gt;95%</td>
<td>1116 (77)</td>
</tr>
<tr>
<td>Person-visits in each atazanavir hair level quintile (ng/mg), no. (%))</td>
<td></td>
</tr>
<tr>
<td>Quintile 1 (0.05 to &lt;0.658 ng/mg)</td>
<td>289 (20)</td>
</tr>
<tr>
<td>Quintile 2 (&gt;0.658 to &lt;1.78 ng/mg)</td>
<td>282 (19)</td>
</tr>
<tr>
<td>Quintile 3 (&gt;1.78 to &lt;3.13 ng/mg)</td>
<td>297 (21)</td>
</tr>
<tr>
<td>Quintile 4 (&gt;3.13 to &lt;5.19 ng/mg)</td>
<td>289 (20)</td>
</tr>
<tr>
<td>Quintile 5 (&gt;5.19 ng/mg)</td>
<td>286 (19)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of study participants unless otherwise indicated. PI, protease inhibitor.

a Median number of visits per patient was 3 (range, 1–9); 105 women had 1 visit; 70 had 2 visits; 53 had 3 visits; 81 had 4 visits; 86 had 5 visits; 33 had 6 visits; 23 had 7 visits; 11 had 8 visits; 2 had 9 visits.

b Threshold of detection, 80 copies/mL.

adherence (dichotomized into <95% vs ≥95%) was observed (F statistic P < .001).

Association of Atazanavir Hair Levels with Virologic Suppression

Figure 2 shows the percentage of person-visits during which viral loads were <80 copies/mL according to hair quintile. HIV RNA levels were undetectable in only 25% of visits in which hair concentrations of atazanavir were in the lowest quintile. In

and levels were analyzed both as continuous measures and as categorical variables (tertiles, quartiles, or quintiles). Also included in models were variables that could affect response, including age, race, viral load at the time of regimen initiation (continuous or dichotomized into <100,000 vs ≥100,000 copies/mL), prior antiretroviral treatment experience (dichotomized into yes vs no), and degree of PI experience (categorized into naïve to PIs, past experience with 1 PI, or treatment with ≥2 PIs), nadir and pretreatment CD4 cell counts, study year, nucleoside reverse transcriptase inhibitor (NRTI) backbone components, history of clinical AIDS, and self-reported adherence. Adherence to atazanavir was reported as the percentage of prescribed doses consumed during periods of 6 months, 30 days, and 3 days; visual analog scales aided in estimating percentages [11]. Level of adherence was analyzed either as a continuous measure or categorized into ≤74%, 75%–94%, or ≥95% over the time interval assessed. Because atazanavir is often coadministered with ritonavir, hair levels of each of these agents are substantially collinear, so separate models were run for atazanavir and ritonavir in the women receiving ritonavir-boosted atazanavir. All analyses were performed using SAS software, version 9.2 (SAS Institute).
In contrast, for person-visits in which atazanavir concentrations in hair were in the highest quintile (>5.19 ng/mg), the likelihood of maximal virologic suppression was 87%. Figure 2 also depicts the univariate relationship between hair atazanavir levels and the likelihood of virologic suppression in repeated measures analyses. The odds of viral load undetectability increased with each quintile; the odds ratio (OR) for viral suppression if the atazanavir level in hair is in the second quintile (0.658 to <1.78 ng/mg) is 4.3 (95% confidence interval [CI], 2.5–7.4; \( P < .001 \)), for example, and the OR for virologic response if atazanavir levels are in the top quintile is 63.3 (95% CI, 30.8–130.0; \( P < .001 \)).

Table 2 presents the multivariate analysis for virologic response in which levels of atazanavir in hair are adjusted for age, race, extent of PI experience, pretreatment viral load, and self-reported adherence. A pretreatment HIV RNA level of <100,000 copies/mL was associated with a higher odds of achieving virologic suppression over time, compared with individuals who started atazanavir-based treatment with viral loads ≥100,000 copies/mL (OR, 3.2 [95% CI, 1.5–6.9]; \( P = .002 \)). Participants who were not African-American showed a trend toward a higher likelihood of virologic suppression while receiving cART than did African-Americans. Those who had been treated with ≥2 PIs prior to atazanavir showed a trend toward a lower likelihood of virologic suppression while receiving atazanavir. Self-reported adherence was associated with a higher odds of virologic suppression, with an OR of 4.0 (95% CI, 1.9–8.6; \( P < .001 \)) for visits when participants reported ≥95% adherence, compared with those when participants reported <75% adherence. Adding pretreatment CD4 cell count, history of clinical AIDS, study year, or the NRTI backbone agents to the multivariate models did not significantly alter the results, so these variables were removed from the final models.

In adjusted analyses, concentrations of atazanavir in hair were the best independent predictor of virologic suppression. An atazanavir level in the second quintile, compared with the first, yielded an OR for virologic suppression that is comparable to a self-reported adherence of ≥95%. Hair atazanavir levels in the higher quintiles were associated with progressively increasing odds of virologic suppression; women whose atazanavir levels were in the highest quintile had an OR of 59.8 (95% CI, 29.0–123.2; \( P < .001 \)) for virologic suppression.

When models were repeated focusing on the 1136 person-visits during which ritonavir was coadministered with atazanavir, we found that ritonavir levels in hair were similarly the strongest predictor of subsequent virologic response. Finally, when we looked at hair level at one visit as a predictor of virologic suppression at the subsequent WIHS visit, we also saw a strong and monotonic relationship between hair level and subsequent response.

**Virologic Suppression Rates in Different At-Risk Scenarios**

We then investigated the key question of whether higher atazanavir exposure as indicated by hair levels could reestablish virologic suppression after a preceding lapse in adherence, exposure, or virologic suppression. We examined three separate “at risk” subgroups of the 1443 person-visits in our study: (1) those preceded by a reported adherence level of ≤95% at any previous visit (405 person-visits from 152 participants); (2) those preceded by a hair atazanavir level in the lowest quintile (0.05–0.658 ng/mg) at any previous visit (377 person-visits from 125 participants); and (3) those with an HIV viral load >1000 copies/mL at any previous visit (356 person-visits from 139 participants). Each subgroup showed lower overall rates of virologic suppression than did the entire group over time;

![Figure 2. Percent of person-visits in each hair quintile where viral load suppressed (entire study sample) and odds ratio of virologic response per hair quintile (univariate relationship): ATV, atazanavir.](image-url)
subgroups 2 and 3 showed differences in suppression rates that were statistically significant (Table 3).

"Redemption" Through Improved Atazanavir Exposure

Although each at-risk subgroup had lower overall rates of virologic suppression than did the entire cohort (Table 3), each subgroup subsequently revealed a pattern of increasing rates of virologic suppression by increasing quintile of hair atazanavir level (Figures 3A, 3B, 3C). Indeed, increasing atazanavir levels in hair became even more determinative of virologic suppression (or resuppression for subgroup 3) in multivariate models for each subgroup than for the overall cohort (Table 4). The ORs for reaching a viral load of $<80$ copies for person-visits in which hair levels were in the highest quintile were 210.2 (95% CI, 46.0–961.1), 132.8 (95% CI, 26.5–666.0), and 400.7 (95% CI, 52.3–3069.7), respectively, for subgroups 1, 2, and 3.

DISCUSSION

In this multivariate analysis of a cohort of HIV-infected women over time, we reveal that antiretroviral concentrations in hair are the strongest independent predictor of virologic suppression. Levels of antiretroviral drugs in hair showed a monotonic relationship to the likelihood of viral suppression (OR for success, 4.3, 12.7, 22.9, and 59.8 for each increasing quintile of hair atazanavir concentration; each \( P \), .001) in multivariate models. Because low hair antiretroviral concentrations can predict virologic failure prior to its development, this measurement may be useful in designing interventions aimed at prolonging the durability of cART.

Concentrations of antiretroviral drugs in hair samples may provide an integrated measure of behavior and biology. Levels of medications in hair reflect drug uptake from the systemic circulation over periods of weeks to months [12] and capture average, as well as individual, pharmacokinetic information. Single adherence measures or plasma antiretroviral concentrations provide "snapshots" of exposure, but a level measured in hair synthesize adherence and pharmacokinetic variability over time to provide a robust exposure measure in a single assay [13]. The value of single plasma antiretroviral levels is further limited by the so-called "white coat effect," in which adherence transiently improves prior to clinic appointments [14], and by an inability to define meaningful therapeutic antiretroviral

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR of virologic response (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per decade</td>
<td>1.05 (0.78–1.42)</td>
<td>.75</td>
</tr>
<tr>
<td>Race</td>
<td>Not African-American 1.6 (0.99–2.7), African-American Reference</td>
<td>.06</td>
</tr>
<tr>
<td>Pretreatment HIV RNA level, copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100,000</td>
<td>3.2 (1.5–6.9), Reference</td>
<td>...</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>Reference</td>
<td>.002</td>
</tr>
<tr>
<td>Past PI treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Reference</td>
<td>...</td>
</tr>
<tr>
<td>1 PI</td>
<td>0.94 (0.49–1.8), Reference</td>
<td>.84</td>
</tr>
<tr>
<td>&gt;2 PIs</td>
<td>0.56 (0.29–1.08), Reference</td>
<td>.09</td>
</tr>
<tr>
<td>Adherence level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%–74%</td>
<td>Reference</td>
<td>...</td>
</tr>
<tr>
<td>75%–94%</td>
<td>2.5 (1.1–5.5), Reference</td>
<td>.03</td>
</tr>
<tr>
<td>&gt;95%</td>
<td>4.0 (1.9–8.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hair concentration, quintile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Reference</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>4.3 (2.5–7.4), Reference</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3</td>
<td>12.7 (7.1–22.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>22.9 (12.2–43.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5</td>
<td>59.8 (29.0–123.2)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Adjusting for pretreatment CD4 cell count, a history of clinical AIDS, study year, and/or nucleoside reverse transcriptase inhibitor backbone components used with atazanavir did not significantly alter the results of the analysis, and these variables were therefore removed from the final model. AA, African-American; CI, confidence interval; OR, odds ratio; PI, protease inhibitor.
Figure 3. Percent of person-visits in each hair quintile in which viral load was suppressed for 3 subgroups in which “failure” was previously revealed (Figure 3A: subgroup 1; Figure 3B: subgroup 2; Figure 3C: subgroup 3). ATV, atazanavir.
ranges because of substantial interindividual pharmacokinetic variability [15, 16]. Therefore, despite the importance of ensuring adequate exposure to the components of HIV regimens, no gold standard exists in current practice to assess antiretroviral exposure.

Our models show that antiretroviral exposure as measured in hair far surpasses commonly used covariates to predict HIV treatment outcomes [17]. Failed antiretroviral regimens result in substantial long-term adverse effects, including increased drug and diagnostic testing costs, as well as avoidable clinical and transmission events. Because patients who experience virologic failure on a regimen demonstrate attenuated rates of immune reconstitution on future regimens [18], substantial efforts should be made to optimize first-line cART. Preserving responses to first-line regimens are of particular import in resource-limited settings, where the average annual cost of second-line cART regimens can be up to 8 times that of first-line regimens [19]. The risk of viremia on therapy is highest in the first year after initiating cART and can be linked to increased HIV transmission rates [20]. Therefore, when initiating HIV treatment, the incorporation of an effective antiretroviral exposure measure, such as hair concentrations, during initial monitoring may avert early virologic failure, blunted responses to subsequent regimens, and the need for expensive or inaccessible second-line regimens.

Previous models of outcomes with atazanavir-based regimens have failed to define precise parameters of atazanavir exposure that increase the likelihood of virologic suppression [15, 21]. This failure could be a result of using single plasma atazanavir concentrations in these models to define exposure instead of a longer term measure. A recent report revealed that average adherence to dosage with boosted PI regimens was a better predictor of virologic suppression than was duration or frequency of missed doses [22]. Hair antiretroviral concentrations average daily exposure variability in a manner analogous to that of glycosylated hemoglobin A1C providing information on mean daily glucose levels in diabetic patients. A previous analysis by our group demonstrated that hair levels of antiretrovirals are more closely correlated with areas under the curve from intensive pharmacokinetic studies than are single plasma levels [23]. Therefore, it is not surprising that hair antiretroviral measurements predict treatment outcomes with greater accuracy than do single plasma levels. Another analysis by our group, directly comparing levels of antiretrovirals in hair with plasma levels to predict treatment responses, demonstrated the superiority of hair levels [23].

In addition to showing that atazanavir concentrations in hair predict virologic responses more strongly than self-reported adherence or other factors, we demonstrate that lapses in adherence, antiretroviral exposure, or virologic suppression are all associated with an increased likelihood of subsequent failure. Since adherence difficulties or the presence of detectable virus during therapy are well-known contributors to virologic failure, either state is likely to trigger corrective measures in the clinical setting. However, the inaccuracy of self-reported adherence, the lack of routine virologic monitoring in many resource-limited settings, and the fact that detectable viral loads when available may already indicate mutations [24] all increase the appeal of finding another tool to prospectively predict failure. Our models show that low antiretroviral hair levels portend a high risk of virologic failure; hair measures in the clinical setting could therefore trigger interventions to correct either adherence or low pharmacokinetic levels (eg, through assessing drug-drug interactions or diet) to extend regimen durability (Figure 4). Of note, only participants with hair atazanavir measurements in the higher quintiles during WIHS visits following a failure scenario had rates of virologic suppression similar to those in clinical trials. This supports the concept that the reasons for low drug levels in hair must be investigated and addressed by adherence intervention, change in regimen, or possibly dose increase (the latter requires additional study). Our group is currently planning a clinical trial assessing adherence interventions, regimen change, or dose modification of antiretrovirals based on hair measurements of anchor regimen components in a clinical setting.

### Table 4. Multivariate Models of Hair Atazanavir Concentrations and Virologic Success (3 Subgroups)

<table>
<thead>
<tr>
<th>Hair quintile</th>
<th>Subgroup 1</th>
<th>Subgroup 2</th>
<th>Subgroup 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person-visits</td>
<td>Person-visits</td>
<td>Person-visits</td>
</tr>
<tr>
<td>1</td>
<td>83</td>
<td>116</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>91</td>
<td>9.6 (3.4–27.2)</td>
<td>9.6 (3.4–27.2)</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
<td>30.0 (8.8–102.5)</td>
<td>30.0 (8.8–102.5)</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>24.1 (7.0–82.4)</td>
<td>24.1 (7.0–82.4)</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>132.8 (26.6–666.0)</td>
<td>132.8 (26.6–666.0)</td>
</tr>
</tbody>
</table>

**NOTE.** All P values are <.001. All models adjusted for age, race, previous treatment with protease inhibitors, pre-atazanavir HIV viral load, and self-reported adherence. Subgroup 1: self-reported adherence <95% at a previous visit (n = 405); subgroup 2: hair value in lowest quintile at a previous visit (n = 377); subgroup 3: viral load >1000 copies/mL at a previous visit (n = 356). CI, confidence interval; OR, odds ratio.
As we proposed previously [7], one possible algorithm for testing would involve measuring antiretroviral levels in hair soon after starting a new antiretroviral regimen and performing HIV viral load testing only if the hair levels fall into the lower quintiles as defined above (Figure 4). Data showing that the risk of viremia on therapy is highest during the first year after initiating cART [20] supports the use of these measures soon after regimen initiation. Data from the resource-limited setting showing that routine viral load monitoring decreases rates of virologic resistance over routine monitoring alone may argue for the use of hair measures as a surrogate for the former [25]. If the atazanavir level in hair is $<1.78$ ng/mg (first or second quintile), the rates of virologic failure approach 50% and intensive adherence interventions (vs a pharmacokinetic evaluation for low exposure if adherence is deemed adequate) should be triggered. After a patient is receiving stable HIV therapy, antiretroviral measurements using hair need not be performed routinely but only when clinical disease progression is observed (for settings where routine CD4 cell count or viral load monitoring are not available) or when an alteration in drug exposure is predicted, such as a new drug-drug interaction, pregnancy, change in dietary patterns, change in liver or renal function, and so on.

Unlike phlebotomy, hair collection is noninvasive and does not require specific skills, sterile equipment, or specialized storage conditions. The collection of hair samples for analysis merely requires a pair of scissors, and storage is at room temperature. Hair can be stored for long periods of time prior to analysis, shipped without precautions for biohazardous materials, and analyzed economically in a high-throughput hair analysis laboratory. These features may make this monitoring tool particularly advantageous in the resource-poor setting, especially when routine viral load monitoring is prohibitively expensive. We recently applied these hair measures in a nested case-control study in 2 South African public health clinics and found that low concentrations of lopinavir in hair had a high predictive value for virologic failure in that setting [25]. We are currently working on developing a lower cost, point-of-care method of analyzing antiretroviral levels in hair for resource-constrained settings to increase the feasibility of this tool. The results of the analyses presented here argue for the possibility of hair antiretroviral concentrations serving as a method of HIV therapeutic drug monitoring that may increase the durability of current antiretroviral regimens in a variety of settings.

Acknowledgments

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We thank the Women’s Interagency HIV Study (WIHS) participants who contributed data to this study. Data were collected by the WIHS Collaborative Study Group with centers (Principal Investigators) at New York City/Bronx Consortium (Kathryn Anastos, MD); Brooklyn, NY (Howard Minkoff, MD); Washington DC, Metropolitan Consortium (Mary Young, MD); The Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt, MD); Los Angeles County/Southern California Consortium (Alexandra Levine, MD); Chicago Consortium (Mardge Cohen, MD); and Data Coordinating Center (Stephen Gange, PhD).

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Authors’ contributions: M.G. developed the study protocol, provided study oversight, designed the analysis plan, interpreted the data, and wrote

Figure 4. Possible algorithm for use of atazanavir hair levels in the clinical setting. ATV, atazanavir.
the paper. R.M.G. contributed to the study concept and the analysis plan and interpretation. N.A. and P.B. provided data management, contributed to the analysis plan, and performed most of the statistical analyses. R.M.G., N.A., P.B., K.A., S.I.G., H.M., M.Y., J.M., M.H.C., and G.B.S. collected WIHS participant data, helped design protocols, and critically revised the manuscript. Y.H. developed the laboratory methods for analysis of antiretroviral levels in hair.

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References