MAJOR ARTICLE

Predictors of HIV Drug-Resistance Mutations in a Large Antiretroviral-Naive Cohort Initiating Triple Antiretroviral Therapy

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Objective. The objective of this study was to systematically characterize the incidence and determinants of antiretroviral resistance in the HOMER (Highly Active Antiretroviral Therapy [HAART] Observational Medical Evaluation and Research) cohort of 1191 human immunodeficiency virus–infected, antiretroviral-naive adults initiating HAART in British Columbia, Canada.

Methods. All plasma samples with plasma virus loads (pVLs) >1000 copies/mL collected during the first 30 months of follow-up were genotyped for drug resistance. The primary outcome measure was time to the first detection of major drug-resistance mutation(s). Cox proportional hazard regression was used to identify factors significantly associated with the detection of drug-resistance mutations.

Results. Drug-resistance mutations were detected in 298 subjects (25%). Factors significantly associated with detection of drug-resistance mutations included high baseline pVL (multivariate hazard ratio [HR], 1.59; $P <$ .001) and adherence (estimated using prescription-refill data and/or untimed plasma drug-concentration measurements). When compared with subjects with low (0%–<20%) prescription-refill percentages, subjects at an elevated risk of harboring drug-resistance mutations were those with relatively high but imperfect prescription-refill percentages (80%–<90%; multivariate HR, 4.15; $P < .001$) and those with essentially perfect (≥95%) refill percentages but with 2 plasma drug concentrations below the steady-state trough concentration minus 1 standard deviation (multivariate HR, 4.57; $P < .001$). Initial use of nonnucleoside reverse-transcriptase inhibitor–based HAART was significantly associated with multiclass drug resistance (multivariate HR, 1.84; $P = .001$).

Conclusion. High baseline pVLs and substantial but imperfect levels of adherence were major predictors of antiretroviral resistance.

The introduction of highly active antiretroviral therapy (HAART) has resulted in dramatic improvements in the clinical status of many patients with HIV-1 infection [1, 2]. It is estimated, however, that virologic failure occurs in as many as 30%–50% of HIV-1–infected individuals within 2 years of initiation of HAART [3, 4]. Determinants of virologic failure have been extensively evaluated in population-based cohort studies [3–8], and it is now recognized that selection of drug-resistant HIV-1 compromises HAART efficacy [9].

Although it has been demonstrated that antiretroviral resistance is an independent risk factor for virologic failure in both antiretroviral-naive and -treated HIV-1–infected populations [7–13], factors associated with the development of resistance are less well defined. The development of resistance is influenced by a number of factors, most notably adherence [14–16] and pharmacokinetics of antiretrovirals [8, 17]. To date, cohort studies investigating determinants of drug resistance have been limited to (and complicated by) the evaluation of previously treated individuals [18, 19], and comparatively little data exist describing the incidence and determinants of drug resistance in antiretroviral-naive populations initiating HAART. To address the

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relatively high rate of virologic failure in the era of HAART, an understanding of the factors associated with development of drug resistance is of critical importance.

We therefore undertook a retrospective longitudinal analysis of antiretroviral resistance in the HAART Observational Medical Evaluation and Research (HOMER) cohort, consisting of all HIV-1–infected antiretroviral-naive adults initiating HAART in the province of British Columbia, Canada, between 1 August 1996 and 30 September 1999. Our primary objectives were to characterize the incidence and determinants of antiretroviral-resistance mutations emerging in routine clinical practice in the HAART era.

MATERIALS AND METHODS

Study Population

In the province of British Columbia, Canada, antiretrovirals are distributed free of charge to HIV-1–infected individuals through a centralized HIV/AIDS drug-treatment program (DTP). Antiretrovirals are prescribed according to specific guidelines set by the British Columbia Therapeutic Guidelines Committee; these guidelines are in accordance with international guidelines (for more detail, see [6]).

The study population is very similar to a cohort described elsewhere [6, 20]. In brief, all HIV-1–positive, antiretroviral-naive adults who initiated HAART (consisting of 2 nucleoside reverse-transcriptase inhibitors [NRTIs] and either a protease inhibitor [PI] or a nonnucleoside reverse-transcriptase inhibitor [NNRTI]) in British Columbia between 1 August 1996 and 30 September 1999 were eligible for inclusion in the study ($n = 1312$). Of these, 121 (9.2%) were excluded for not having pretherapy CD4 cell count and plasma virus load (pVL) data available; the study sample was therefore made up of the remaining 1191 subjects, who form an open treatment-based cohort known as the HOMER cohort. In the present study, patients were followed for up to 30 months after initiation of HAART. Ethical approval for this study was obtained from the institutional ethics board of Providence Health Care/University of British Columbia.

Drug-Resistance Genotyping

pVLs were measured at baseline, after 1 month, and approximately quarterly thereafter, by use of the Roche Amplicor Monitor assay (Roche Molecular Systems), as part of routine patient monitoring. In addition, plasma samples were frozen for future use.

Drug-resistance genotyping was attempted on all plasma samples with pVLs ⩾1000 copies/mL collected during the 30 months after HAART initiation ($n = 2805$). A further 732 baseline samples were also genotyped. Samples with pVLs <1000 copies/mL were not systematically genotyped (since genotyping does not yield consistent results for samples with low pVLs) and were assumed to have no drug-resistance mutations. However, available genotypes for 41 samples with pVLs <1000 copies/mL were included in the analysis. HIV-1 RNA was extracted from plasma by use of the QIAGEN viral RNA kit with a BioRobot 9600/9604 or was extracted manually by use of guanidinium-based buffer followed by isopropanol and ethanol washes. HIV-1 protease (PR) and reverse transcriptase (RT) genes were amplified using nested RT polymerase chain reaction (RT-PCR) and sequenced in both the 5′ and 3′ directions, as described elsewhere [21]. Results were reported as amino acid changes in the HIV-1 PR and RT with respect to a wild-type reference sequence (HIV-1 HXB2). Pretherapy genotype data were not available for all subjects; for simplicity, it was assumed that subjects did not harbor baseline drug resistance. The effect of this assumption was tested in the 732 individuals for whom pretherapy genotypes were available.

Interpretation of Major Drug-Resistance Mutations

HIV-1 isolates were assigned to 1 of 4 drug-resistance categories on the basis of a modification of the International AIDS Society–USA table [22]: (1) lamivudine (3TC) resistance (184I/V), (2) any other NRTI resistance (41L, 62V, 65R, 67N, 69D or insertion, 70R, 74V, 75I, 151M, 210W, 215F/Y, or 219E/Q), (3) any NNRTI resistance (100I, 103N, 106A/M, 108I, 181C/I, 188C/H/L, 190A/S, 225H, 230L, or 236L), and (4) any PI resistance (30N, 46I/L, 48V, 50L/V, 54V/L/M, 82A/F/S/T, 84V, or 90M). 3TC resistance was classified in a separate category, because of the common appearance of the M184V/I mutation and the lack of NRTI cross-resistance conferred by this mutation.

Estimates of Adherence

Prescription-refill data. The proportion of time spent taking HAART during the first year, calculated by dividing the number of months of prescriptions dispensed by the number of months of follow-up, was employed as an adherence estimate [16, 23]. Subjects were stratified into 7 categories based on the proportion of time in the first year of therapy covered by prescription refills: 0%–<20%, 20%–<40%, 40%–<60%, 60%–<80%, 80%–<90%, 90%–<95%, and ⩾95% of prescriptions refilled.

Untimed plasma drug concentrations. Plasma concentrations of prescribed PIs and NNRTIs were determined for the first 2 plasma samples collected for pVL testing within the first year of follow-up by use of a sensitive, validated, simultaneous assay using reverse-phase high-pressure liquid chromatography coupled with tandem mass spectrometry [24]. Plasma drug concentrations were classified as "untimed" because the time of dosing relative to sampling was unknown. Plasma drug concentrations were categorized as "abnormally low" if they were lower than the steady-state trough concentration minus 1 SD ($C_{\text{trough}} - 1$ SD) reported in the product monographs [24], a

concentration unlikely to be observed in most randomly sampled patients.

Statistical Analysis

The primary outcome measure was time to detection of drug resistance, defined as the time from the date of HAART initiation to the date of collection of the first plasma sample containing at least 1 drug-resistance mutation. Event-free subjects were censored on their last pVL date (table 1).

Cox proportional hazard regression was used to calculate univariate and multivariate risk ratios [25] associated with the following baseline variables: pVL (per $\log_{10}$ increment), CD4 cell count (per 100 cells/μL decrement), AIDS diagnosis (yes vs. no), age (per 10-year increment), sex (male vs. female), calendar year of initial HAART, adherence (prescription-refill percentage and untimed drug-concentration data), initial use of NNRTIs (yes vs. no), and history of injection drug use (self- and/or physician-reported, yes vs. no). All tests for significance were 2-sided, with $P < .05$ indicating statistical significance. Subjects with missing baseline values were censored on HAART initiation date.

RESULTS

Study population. The HOMER cohort of 1191 antiretroviral-naive individuals (1004 [84.3%] men and 187 [15.7%] women) initiating HAART has been described elsewhere [6, 20]. At the time of HAART initiation, the median age of participants was 37 years (interquartile range [IQR], 32–44 years), the median CD4 cell count was 280 cells/μL (IQR, 130–420 cells/μL), and the median pVL was 120,000 copies/mL (IQR, 42,000–310,000 copies/mL). Study subjects received a total of 26 different initial HAART combinations. More than one-half (885; 74.3%) of these participants initiated HAART with a PI. The PI used was predominantly indinavir (672; 75.9%), followed by nelfinavir (105; 11.9%), saquinavir (75; 8.5%), and ritonavir (33; 3.7%). The rest of the study participants (306; 25.7%) received a triple-drug regimen that included an NNRTI. Among these, the vast majority received nevirapine (288; 94.1%), whereas efavirenz (8; 2.6%) and delavirdine (10; 3.3%) were prescribed less frequently. Of the 1191 subjects, 842 (70.7%) initiated HAART after July 1997, when HAART became universally recommended for individuals initiating therapy in British Columbia.

Factors associated with selection of drug-resistance mutations. Drug-resistance genotyping was performed on all follow-up plasma samples with pVLs $\geq$1000 copies/mL collected during the first 30 months of HAART (median, 2 genotypes/study subject; range, 0–13 genotypes/study subject). Pretherapy samples and those with pVLs <1000 copies/mL were not routinely genotyped and were assumed to have no drug-resistance mutations. A total of 360 individuals (32.2%) maintained pVLs <1000 copies/mL over the entire study follow-up period (table 1).

Four broad drug-resistance categories were used to classify samples with key resistance mutations: 3TC resistance, other NRTI resistance, NNRTI resistance, and PI resistance. During the study follow-up period, mutations linked to any drug-resistance category were observed in 298 (25%) subjects (table 1). 3TC resistance was most commonly observed ($n = 204$; 68.5%), followed by NNRTI ($n = 120$; 40.3%), NRTI ($n = 98$; 32.9%) and PI ($n = 68$; 22.8%) resistance. (Note that, as a result of multidrug resistance, these total to >100%.) Among subjects who developed at least 1 drug-resistance mutation, the median time to resistance was 8.2 months. The time course of detection of drug-resistance mutations is presented in figure 1*A*.

Cox proportional hazard regression was used to identify factors associated with the development of any drug-resistance mutations after initiation of HAART (table 2). In univariate analyses, significant determinants of detection of drug-resistance mutations were high baseline pVL, low CD4 cell count, history of injection drug use, and <95% prescription-refill percentage. Age, sex, baseline AIDS diagnosis, calendar year of initiation of HAART, and type of HAART at initiation (NNRTI vs. PI based) were not associated with detection of drug-resistance mutations. In multivariate analyses, baseline pVL (multivariate hazard ratio [HR], 1.59; $P < .001$), baseline CD4 cell count (multivariate HR, 1.08; $P = .013$), history of injection drug use (multivariate HR, 1.33; $P = .023$), and prescription-refill percentage remained significant predictors of detection of drug-resistance mutations (table 2).

Table 1. Drug-resistance status of antiretroviral-naive subjects initiating highly active antiretroviral therapy and followed for 30 months.

Characteristic	No. (%)
No follow-up HIV-1 pVL (censored at baseline)	36 (3.0)
All HIV-1 pVLs <1000 copies/mL during entire study follow-up; assumed nonresistant (censored at last pVL)	360 (30.2)
At least 1 HIV-1 pVL $\geq$1000 copies/mL, but resistance test failed (censored at baseline)	3 (0.3)
At least 1 HIV-1 pVL $\geq$1000 copies/mL; no resistance detectable at any time (censored at last pVL)	494 (41.5)
Any detectable resistance	298 (25.0)
Total	1191

NOTE. pVL, plasma virus load.

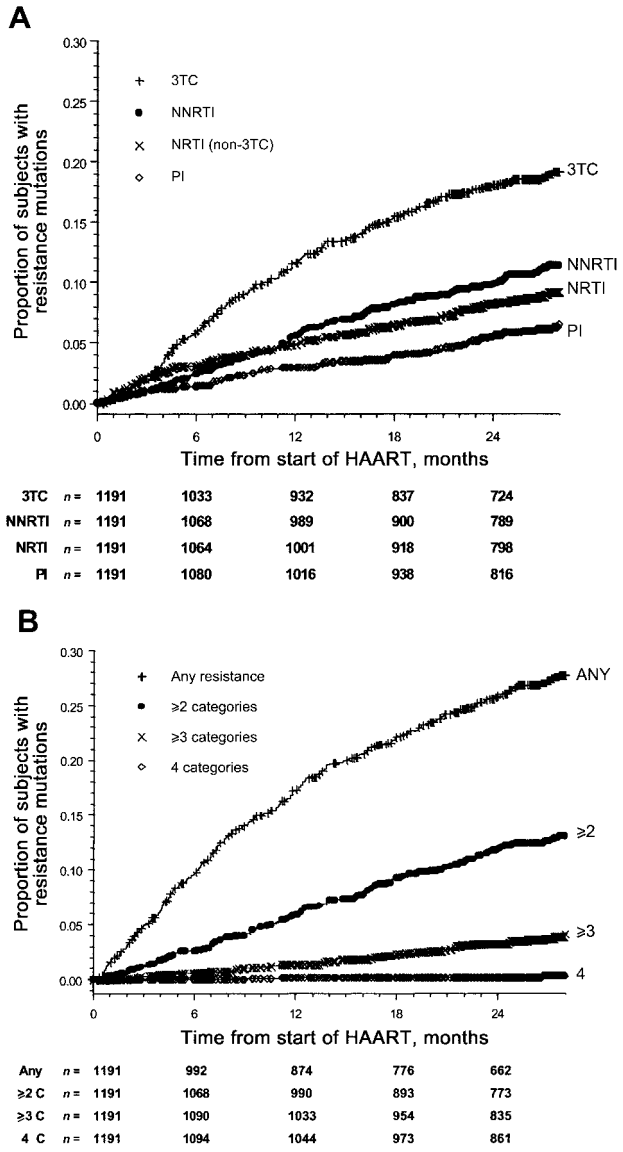

Figure 1. Detection of HIV-1 drug-resistance mutations during the 30 months after initiation of highly active antiretroviral therapy (HAART). The graphs show Kaplan-Meier analyses of drug-resistance events assessed in terms of resistance category *(A)* and combinations of resistance categories *(B)*. Drug-resistance categories were defined as lamivudine (3TC) resistance (184I/V), any other nucleoside reverse-transcriptase inhibitor (NRTI) resistance (41L, 62V, 65R, 67N, 69A/N/D or insertion, 70R, 74V, 75I, 151M, 210W, 215F/Y, or 219E/Q), nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance (100I, 103N, 106A, 108I, 181C/I, 188C/H/L, 190A/S, or 236L), and protease inhibitor (PI) resistance (30N, 46I/L, 48V, 50L/V, 54L/M, 82A/F/S/T, 84V, or 90M). *n* represents the no. of participants remaining at risk for a resistance event at 0, 6, 12, 18, or 24 months after initiation of HAART.

Factors associated with multicategory drug resistance. Mutations linked to multiple drug-resistance categories were detected in 135 subjects over the course of the study follow-up period (figure 1*B*). Multicategory resistance occurred most commonly as a combination of 3TC resistance plus resistance to another category (3TC + NRTI, $n = 16$ of 135 [11.9%]; 3TC + NNRTI, $n = 32$ [23.7%]; 3TC + PI, $n = 23$ [17.0%]; NRTI + NNRTI, $n = 16$ [11.9%]; NRTI + PI, $n = 3$ [2.2%]; NNRTI + PI, $n = 2$ [1.5%]; 3TC + NRTI + NNRTI, $n = 17$ [12.6%]; 3TC + NRTI + PI, $n = 19$ [14.1%]; 3TC + NNRTI + PI, $n = 3$ [2.2%]; NRTI + NNRTI + PI, $n = 1$ [0.7%]; and all 4 categories, $n = 3$ [2.2%]). Among individuals in whom multicategory drug resistance was detected, the median time to multicategory resistance was 12.3 months. In multivariate analyses, the main determinants of multicategory drug resistance were similar to those for detection of any resistance (table 2), with the following main exceptions: subjects initiating NNRTI-based HAART were at 1.84 times higher risk of developing multicategory drug resistance ($P = .001$), and injection drug use was not associated with multicategory resistance. Plasma samples containing HIV-1 with mutations in 3 or all 4 drug-resistance categories were detected in 40 and 3 subjects, respectively. Multivariate analyses were not attempted in these subgroups.

Relationship between adherence estimates and detection of drug-resistance mutations. Prescription-refill percentages were available for all 1191 subjects. A total of 671 (56.3%) subjects had ⩾95% prescription-refill percentages during the first year of HAART. Untimed plasma levels of PIs and NNRTIs were obtained for 822 (69.0%) subjects for whom the first 2 plasma samples collected within the first year of HAART were available. Of these, 400 (48.7%) subjects registered 2 drug concentrations above the $C_{trough} - 1$ SD level (figure 2).

Both prescription-refill and drug-concentration data were independently associated with the detection of resistance-associated mutations. When compared with subjects with 0%–<20% prescription-refill percentages, those with relatively high but imperfect prescription-refill percentages had a markedly increased risk of developing drug-resistance mutations, with an 80%–<90% prescription-refill percentage being associated with the highest risk of detecting mutations in any resistance category (multivariate HR, 4.15; $P < .001$) (figure 3*A*) or multiple resistance categories (multivariate HR, 6.99; $P = .010$) (figure 3*B*). The time course of detection of drug-resistance mutations, stratified by untimed drug concentrations, are presented in figure 2. Individuals with 1 ($n = 229$) or 2 ($n = 193$) abnormally low drug concentrations in their first 2 posttherapy plasma samples were significantly more likely to develop drug-resistance mutations, compared with those without abnormally low drug concentrations ($n = 400$) (multivariate HR, 1.45 and 2.55, respectively; $P < .05$). Untimed drug concentrations were not significantly associated with detection of multicategory resistance (data not shown).

Since a perfect prescription-refill percentage does not necessarily imply consistent adherence, we elected to refine our

Table 2. Determinants of time to development of drug-resistance mutations in single and multiple categories ($n = 1191$).

	Any key mutation associated with drug resistance				Resistance to ⩾2 categories			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Variable[a]	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Baseline pVL per log increment	1.85 (1.51–2.26)	<.001	1.59 (1.29–1.96)	<.001	1.78 (1.32–2.41)	<.001	1.60 (1.17–2.18)	.003
Baseline CD4 cell count per 100 CD4 cell/μL decrement	1.11 (1.05–1.18)	<.001	1.08 (1.02–1.14)	.013	1.24 (1.12–1.36)	<.001	1.18 (1.07–1.31)	<.001
IDU	1.41 (1.11–1.79)	.005	1.33 (1.04–1.71)	.023	1.00 (0.69–1.45)	.995	…	…
NNRTI in first HAART	1.05 (0.81–1.36)	.700	…	…	1.51 (1.06–2.15)	.024	1.84 (1.28–2.65)	.001
Sex (male)	0.89 (0.66–1.20)	.432	…	…	0.92 (0.59–1.46)	.732	…	…
Baseline AIDS diagnosis	1.12 (0.81–1.55)	.500	…	…	1.06 (0.64–1.74)	.823	…	…
Calendar year	0.96 (0.85–1.09)	.523	…	…	1.12 (0.94–1.34)	.205	…	…

NOTE. CI, confidence interval; HAART, highly active antiretroviral therapy; IDU, injection drug use; NNRTI, nonnucleoside reverse-transcriptase inhibitor; pVL, plasma virus load.

[a] See figure 3 for multivariate hazard ratios for prescription-refill percentages.

estimate of adherence by combining prescription-refill and drug-concentration data for the subset of individuals with ⩾95% prescription-refill percentages ($n = 671$). Of these, 496 had drug-concentration data available. In this subset, individuals with 2 abnormally low drug concentrations were at the highest risk of developing any drug-resistance mutations (multivariate HR, 4.57; $P < .001$) and mutations in multiple resistance categories (multivariate HR, 5.54; $P = .025$), compared with subjects with 0%–<20% prescription-refill percentages (figure 3, *inset*). In contrast, those with 2 drug concentrations above $C_{trough} - 1$ SD were at as low a risk as those with 0%–<20% prescription-refill percentages. The risk for individuals with 1 of 2 drug concentrations above $C_{trough} - 1$ SD fell between these extremes. Combining prescription-refill and drug-concentration data was not attempted for prescription-refill-percentage categories <95%, because of the relatively small sample sizes in these groups. In multivariate models accounting for combined adherence measures, injection drug use was no longer associated with drug resistance (data not shown).

Secondary analyses. In primary analyses of drug resistance after initiation of HAART, samples with pVLs <1,000 copies/mL were not genotyped and were assumed to carry no major resistance mutations. To test the sensitivity of this assumption, we restricted the analyses to only those subjects for whom drug-resistance genotyping was successfully performed ($n = 792$). Consistent with the primary analysis, the baseline factors associated with the detection of any drug-resistance mutation(s) in this subset were a higher pVL (multivariate HR, 1.31; $P = .012$), a lower CD4 cell count (multivariate HR, 1.07; $P = .019$), and a relatively high but imperfect (80%–<90%) prescription-refill percentage (multivariate HR, 4.51; $P < .001$). The secondary analysis differed from the primary analysis in that NNRTI-based initial HAART was significantly associated with detection of drug-resistance mutations (multivariate HR, 1.58; $P = .002$), and history of injection drug use was not.

We also wished to test the sensitivity of our assumption that pretherapy plasma samples harbored no drug-resistance mutations. Pretherapy genotypes (within 6 months of HAART initiation) were available for 717 of 1191 subjects. Of these, 53 (7.4%) had mutations associated with ⩾1 resistance category. A variety of contributing factors, including the transmission of drug-resistant HIV-1, could explain these findings. In order to investigate the accumulation of additional drug-resistance mutations over time, we chose not to exclude these 53 individuals from the original analysis. Restricting the analysis to subjects with no baseline drug-resistance mutations ($n = 664$) yielded results consistent with those of the primary analyses: baseline factors associated with resistance in this subset included a higher baseline pVL (multivariate HR, 2.19; $P < .001$), a lower CD4 cell count (multivariate HR, 1.12; $P = .009$), a history of injection drug use (multivariate HR, 1.64; $P = .004$), and an 80%–<90% prescription-refill percentage (multivariate HR, 4.60; $P = .006$). In addition, excluding the 53 patients with baseline mutations from the primary analysis did not affect the results (data not shown).

DISCUSSION

Results from this population-based study of antiretroviral-naive individuals initiating HAART indicate that baseline parameters (CD4 cell count and particularly pVL) and imperfect levels of adherence (as estimated in terms of prescription-refill percentages and untimed drug-concentration measurements) are independent determinants of the development of resistance to commonly prescribed triple-drug combinations. Drug-resistance mutations were detected in ~25% of the study population during the follow-up period. These data have clinically relevant implications. Biologically plausible explanations for the strong association between baseline pVL and the development of drug resistance include incomplete viral suppression in individuals

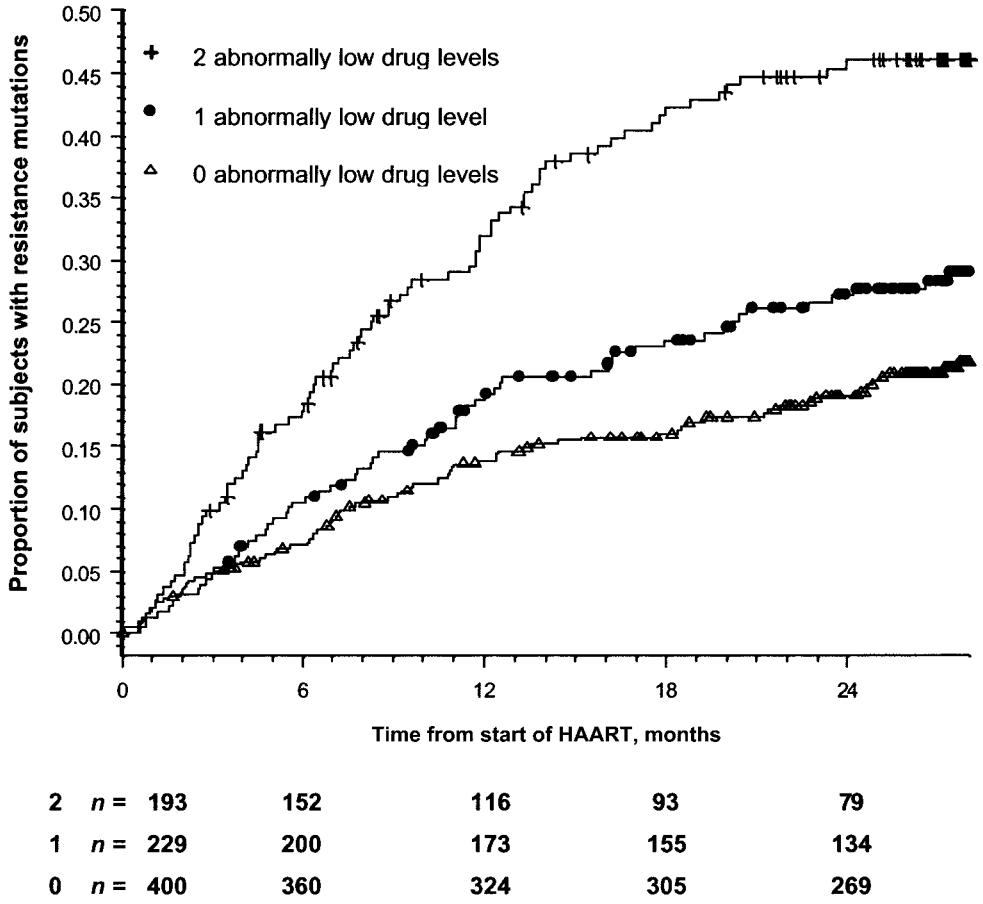

Figure 2. Association between untimed plasma drug concentrations and detection of HIV-1 drug-resistance mutations during the 30 months after initiation of highly active antiretroviral therapy (HAART). The graphs show Kaplan-Meier analyses of drug-resistance events stratified according to results from 2 untimed plasma drug-concentration measurements during the first year of HAART. Drug-resistance categories are defined as in figure 1. *n* represents the no. of participants remaining at risk for a resistance event at 0, 6, 12, 18, or 24 months after initiation of HAART.

with higher pVLs and/or the increased presence of drug-resistant minority HIV-1 variants in individuals with high pVL during untreated infection. Baseline CD4 cell count, the most significant predictor of survival after initiation of first HAART [6, 26], appears to be less strongly linked to the development of drug resistance. The association between CD4 cell count and drug resistance may have a biological basis; however, in this observational, nonrandomized setting, analyses may be at least partially confounded by the characteristics of patients who initiate HAART with very low CD4 cell counts.

The prevalence of drug resistance observed here after 2.5 years of triple therapy is lower than that reported in the largest survey of HIV-1 drug resistance to date, the US HIV Cost and Service Utilization Study (HSCUS) [27], which estimated that ~50% of the US population being treated for HIV-1 infection had virus with decreased susceptibility to antiretrovirals (predominantly to NRTIs and PIs). This proportion increased to 78% in individuals with pVLs >500 copies/mL. The lower prevalence of drug resistance observed in our study likely derives from the fact that the HSCUS group included individuals who initiated HAART much earlier and/or who started with mono- or dual-nucleoside therapy [27].

Drug-resistance mutations observed in clinical practice reflect both selective pressure and the prevalence of antiretroviral use—for example, PI use was very common, but mutations associated with PI resistance were relatively rare, reflecting the limited selection of PI mutations during early treatment [28, 29]. Initial NNRTI use was strongly associated with development of multicategory drug resistance, an observation that likely derives from the low barrier to resistance to both 3TC and the NNRTIs.

We have previously demonstrated how prescription-refill data strongly predict CD4 cell decline, virologic response, and mortality after initiation of HAART and how these data can adjust for the potentially confounding effects of treatment interruption [16, 23, 30]. Results from the present study confirm the suspected association between adherence (estimated by prescription-refill percentages) and the development of drug re-

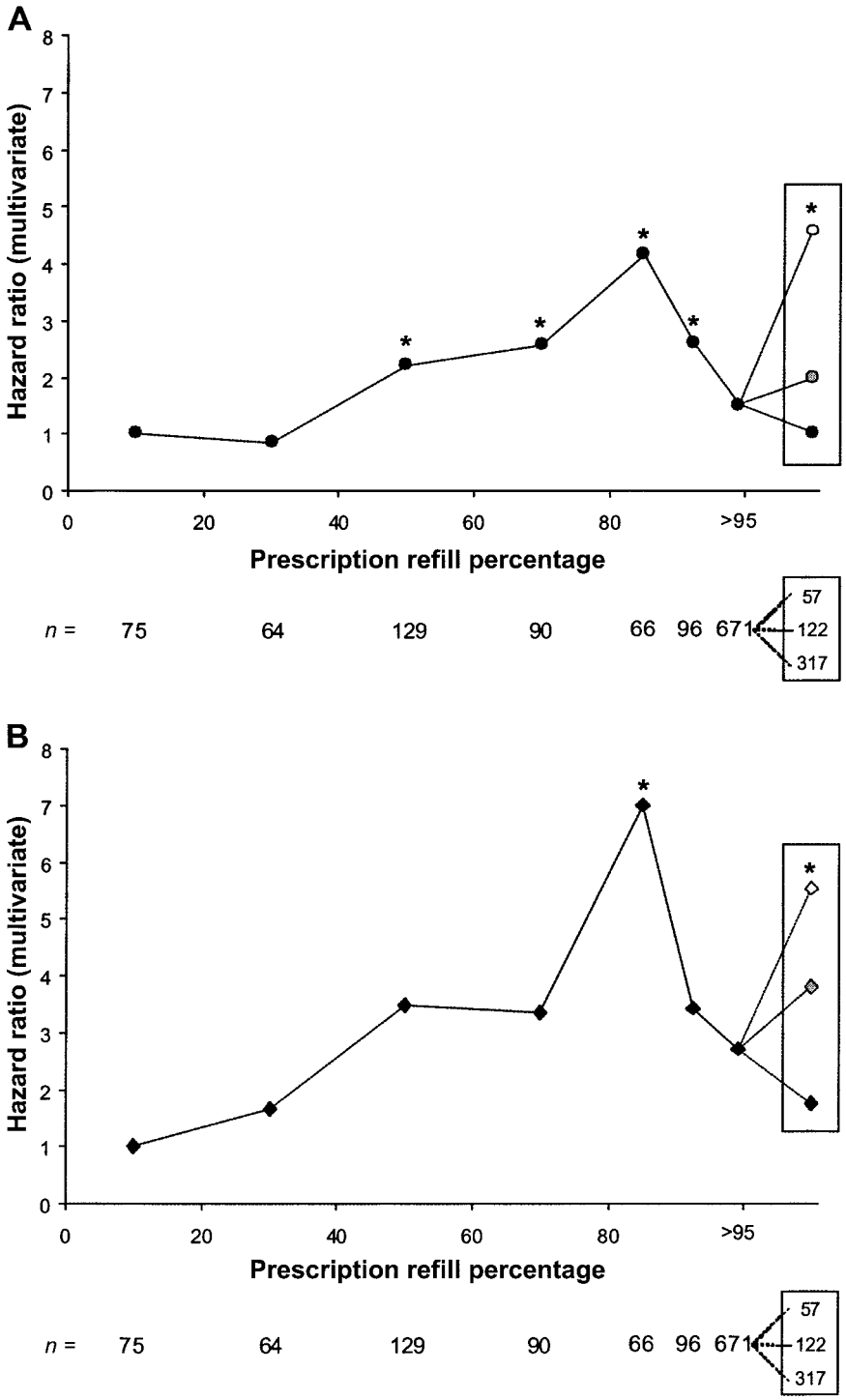

Figure 3. Association between adherence estimates and detection of HIV-1 drug-resistance mutations in antiretroviral-naive subjects initiating highly active antiretroviral therapy (HAART). The graphs show the association between adherence, estimated using prescription-refill percentages, and the risk of developing any drug resistance *(A, circles)* or resistance to multiple categories *(B, diamonds)* among 1191 patients initiating HAART and followed for 30 months. Drug-resistance categories are defined as in figure 1. *n* represents the no. of participants with prescription-refill percentages of 0%–<20%, 20%–<40%, 40%–<60%, 60%–<80%, 80%–<90%, 90%–<95%, and ⩾95%. A secondary analysis divided the subjects in the ⩾95% prescription-refill-percentage group into 3 subgroups on the basis of results from the first 2 available untimed nonnucleoside reverse-transcriptase inhibitor and protease inhibitor drug-concentration tests within the first year of follow-up *(inset).* Drug-concentration data were available for 496 of 671 subjects with ⩾95% prescription-refill percentages: 2 abnormally low drug concentrations *(white symbols),* 1 abnormally low drug concentration *(grey symbols),* and no abnormally low drug concentrations *(black symbols).* $^*P < .05$.

sistance and provide insight into the way in which adherence influences therapy outcome. Results demonstrate a skewed "bell-shaped" relationship between adherence and the detection of drug-resistance mutations, in which substantial (but imperfect) adherence is associated with the greatest risk of resistance [19, 31]. Even relatively small deviations of <15% from perfect prescription-refill percentages were associated with a markedly increased probability of detecting drug-resistance mutations. These observations are consistent with results from a recent study linking a 70%–89% adherence rate to viral rebound, including clinically significant drug resistance [19]. However, it should be noted that the study [19] used a different adherence assessment, which may not be directly comparable with our measures, and concentrated on individuals receiving NNRTIs. In addition, the observation that patients filling <40% of their prescriptions were at a low risk for the development of drug resistance is consistent with results from a recent study [17] that reported that lower adherence was associated with detection of fewer resistance mutations.

The use of untimed plasma drug-concentration measurements in the present study provided an independent and objective additional method of estimating adherence. Although interindividual differences in pharmacokinetics may influence drug concentrations, plasma drug concentrations have previously been shown to correlate with pill counts [32], future prescription-refill data [24], and treatment outcomes [24, 31], indicating that drug concentrations are useful estimators of adherence behavior. Unfortunately, since the time of dosing relative to the time of sampling was unknown, plasma drug concentrations could not be examined as a continuous variable. However, data from the present study indicate that plasma drug concentrations measured during the first year of HAART are independent predictors of development of drug resistance, with 2 abnormally low drug concentrations being associated with the highest risk of resistance.

To refine our estimate of adherence, prescription-refill and drug-concentration data were combined in the subset of individuals with perfect prescription-refill percentages. Taken together, only 30% of our study population had perfect prescription-refill percentages (⩾95%) and plasma drug concentrations that were consistently within the expected range. Overall, subjects with an elevated risk for development of drug resistance were those who were ⩾95% adherent, as estimated by prescription-refill percentages, but for whom drug-concentration testing consistently revealed abnormally low drug concentrations. In contrast, the risk of development of drug resistance in individuals with ⩾95% prescription-refill percentages and consistently normal drug concentrations was relatively low and was comparable to the risk in individuals with little (<40%) exposure to antiretrovirals during the first year of HAART. These results differ from those of a recent study [33] that reported that high levels of adherence (as high as 92%–100%) do not prevent accumulation of drug-resistance mutations; the difference is likely a result of that study [33] including individuals with considerable prior antiretroviral experience, whereas the patient population in the present study was initially antiretroviral naive.

Although it is tempting to conclude that high levels of adherence protect against development of drug resistance, we must be cautious in our interpretation of the results. Resistance mutations sufficient to compromise effectiveness of HAART were assumed not to be present in individuals with pVLs <1000 copies/mL, but the presence of mutations in these individuals cannot be ruled out. In addition, subjects with high levels of adherence are most likely to achieve pVL suppression; therefore, a potential bias is created by this assumption, which may result in artificially low estimates of drug resistance in adherent subjects. We therefore conducted a secondary analysis restricted to subjects for whom drug-resistance genotypes were determined; the results were consistent with those of the primary analysis. Other limitations of this study include its retrospective nature and the fact that plasma samples were available at 3-month intervals only. In addition, the fact that some individuals were lost to follow-up may have resulted in slight underestimates of drug resistance in this population, especially if loss to follow-up is associated with adherence. However, it is worth noting that virtually all medication in the province of British Columbia is dispensed and monitored through a centralized HIV-1–treatment program, so the vast majority of subjects lost to follow-up are likely to have discontinued HAART entirely. The magnitude of any underestimation of drug resistance due to subjects being lost to follow-up is therefore likely to be small. Finally, it should be noted that no phenotypic drug-resistance data were available and that genotypic resistance tests typically detect only predominant circulating viral strains and may miss minority species.

This study represents one of the first population-based evaluations of predictors of drug resistance in individuals initiating HAART. High baseline pVLs, low CD4 cell counts, and substantial but imperfect levels of adherence were the strongest predictors of detection of antiretroviral resistance. These results underscore the need for measures to help HIV-1–infected individuals successfully integrate complex antiretroviral regimens into their daily routine.

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