Incidence of HIV-1 Drug Resistance Among Antiretroviral Treatment–Naive Individuals Starting Modern Therapy Combinations

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Background. Estimates of drug resistance incidence to modern first-line combination antiretroviral therapies against human immunodeficiency virus (HIV) type 1 are complicated by limited availability of genotypic drug resistance tests (GRTs) and uncertain timing of resistance emergence.

Methods. Five first-line combinations were studied (all paired with lamivudine or emtricitabine): efavirenz (EFV) plus zidovudine (AZT) (n = 524); EFV plus tenofovir (TDF) (n = 615); lopinavir (LPV) plus AZT (n = 573); LPV plus TDF (n = 301); and ritonavir-boosted atazanavir (ATZ/r) plus TDF (n = 250). Virological treatment outcomes were classified into 3 risk strata for emergence of resistance, based on whether undetectable HIV RNA levels were maintained during therapy and, if not, whether viral loads were >500 copies/mL during treatment. Probabilities for presence of resistance mutations were estimated from GRTs (n = 2876) according to risk stratum and therapy received at time of testing. On the basis of these data, events of resistance emergence were imputed for each individual and were assessed using survival analysis. Imputation was repeated 100 times, and results were summarized by median values (2.5th–97.5th percentile range).

Results. Six years after treatment initiation, EFV plus AZT showed the highest cumulative resistance incidence (16%) of all regimens (<11%). Confounder-adjusted Cox regression confirmed that first-line EFV plus AZT (reference) was associated with a higher median hazard for resistance emergence, compared with other treatments: EFV plus TDF (hazard ratio [HR], 0.57; range, 0.42–0.76), LPV plus AZT (HR, 0.63; range, 0.45–0.89), LPV plus TDF (HR, 0.55; range, 0.33–0.83), ATZ/r plus TDF (HR, 0.43; range, 0.17–0.83). Two-thirds of resistance events were associated with detectable HIV RNA level ≤500 copies/mL during treatment, and only one-third with virological failure (HIV RNA level, >500 copies/mL).

Conclusions. The inclusion of TDF instead of AZT and ATZ/r was correlated with lower rates of resistance emergence, most likely because of improved tolerability and pharmacokinetics resulting from a once-daily dosage.

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The combination of antiretroviral drugs against human immunodeficiency virus type 1 (HIV-1) infection from different classes has proven to be highly effective in suppressing viral replication and has dramatically reduced HIV-1 infection–related morbidity and mortality [1, 2]. Nevertheless, viral breakthrough caused by incomplete drug intake and/or emergence of drug resistance still poses a challenge for clinicians [3]. For example, in the Swiss HIV Cohort Study (SHCS), we observed rates of virological treatment failures with HIV RNA levels

>500 copies/mL of 2.4 and 2.7 cases per 100 person-years during therapy with nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based and ritonavir-boosted protease inhibitor (PI/r)–based regimens, respectively, which often were accompanied by the emergence of drug resistance mutations, especially among NNRTI users [4].

Nevertheless, the proportion of individuals with HIV RNA replication suppressed to <50 copies/mL has increased continuously over the past decade [5, 6]. Whether the emergence of HIV-1 drug resistance has been reduced by the same extent is uncertain and difficult to assess [7, 8]. Incidence studies based on genotypic drug resistance test (GRT) data from clinical practice often struggle with limitations, namely, that patients subjected to GRTs may not be representative for all potentially eligible individuals or the whole population of HIV-infected, treated individuals [3, 9]. Moreover, the actual event of resistance emergence is usually not observable, and therefore, the timing of the event is unclear.

Building on a previously published method to estimate the prevalence of drug resistance among treated individuals [7], we are presenting an imputation approach that can mitigate these limitations. The general concept is as follows: On the basis of HIV RNA level quantifications, we identify events during patients' treatments that are associated with risk for drug resistance emergence. Next, we try to quantify these risks by analyzing GRT data, which were obtained under circumstances similar to the risk event of interest in terms of ongoing or previous virological failures and treatment received at time of drug resistance testing. By using a simulation technique, we then explore the range of possible incidence estimates under varying parameter estimates for risks of resistance emergence.

METHODS

Patients and Data

Patients from the SHCS [10] were included who initiated combination antiretroviral therapy (cART) from 1 January 1999 to 31 December 2010 with 1 of the 5 most frequently used combinations [11]: efavirenz (EFV), zidovudine (AZT), and lamivudine (3TC) (EFV+AZT group); lopinavir (LPV), AZT, and 3TC (LPV+AZT group); EFV, tenofovir (TDF), and 3TC or emtricitabine (FTC; EFV+TDF group); LPV, TDF, and 3TC or FTC (LPV+TDF group); and ritonavir-boosted atazanavir (ATZ/r), tenofovir (TDF), and 3TC or FTC (ATZ/r+TDF group). The majority (>78%) of the 3 TDF-based regimens contained FTC instead of 3TC. A minimum follow-up time of 180 days after treatment start was required for inclusion.

The SHCS is a multicenter, clinic-based cohort study that includes 45% of all individuals who receive a diagnosis of HIV infection in Switzerland, 70% of all patients receiving ART, and 75% of all patients with AIDS [10, 12]. The SHCS has been approved by ethical committees of all participating institutions, and written informed consent has been obtained from participants. The collection of GRT data is described elsewhere [4, 13].

Definition of Risk States

Patients were classified into 3 risk states for the emergence of drug resistance mutations, which were updated continuously with each new viral load measurement. Situations in which individuals were either not receiving therapy or always had optimally suppressed HIV viremia (ie, HIV RNA level <50 copies/mL) were considered to be at low risk for resistance emergence; detectable viremia (HIV RNA level ≤500 copies/mL) without subsequent virologic suppression to HIV RNA level <50 copies/mL during the same therapy (to exclude blips in HIV RNA level [14, 15]) were classified as intermediate risks; and virological failures with viral loads >500 copies/mL with the same regimen were considered as high-risk events. For a given line of treatment, patients could only move from a lower to a higher risk state but not backward.

Estimation of Risk- and Treatment-Adjusted Probabilities of Resistance

On the basis of a set of 2876 GRTs performed after ≥30 days of continuous ART exposure and detailed treatment histories, we calculated the probability for the detection of ≥1 International AIDS Society (IAS)–USA drug resistance mutation [16]. These analyses were stratified by the 3 aforementioned risk states and treatments received at time of genotypic testing according to 6 categories: cART with boosted PI, unboosted PI, or NNRTI; abacavir/3TC/AZT (Trizivir); ≤3 nucleoside reverse-transcriptase inhibitors (NRTIs) (excluding Trizivir); and any other treatment. Genotypic tests following virological failure events were stratified further by whether they had been performed after the first or later therapy failures. To limit the analysis to emerging resistance during treatment, only mutations were evaluated that corresponded to the treatment received at time of genotypic testing. Five groups of IAS-USA drug resistance mutations were analyzed: thymidine analogue mutations (TAMs), M184V and M184I, other NRTI mutations (eg, K65R and L74V), major PI mutations, and NNRTI mutations.

Simulation

Given the risk state definitions and the associated probabilities for detection of newly selected drug resistance mutations, we scored each patient's treatment history with respect to his or her specific risk for the emergence of certain resistance mutation groups. Using these individual risk assessments, we then generated new datasets, in which resistance emergence events were imputed randomly around the time of transitions to higher risk states (Figure 1). Next, incidence rates were calculated on the basis of imputed risk events, and the range of possible incidence estimates was explored by repeating the imputation procedure

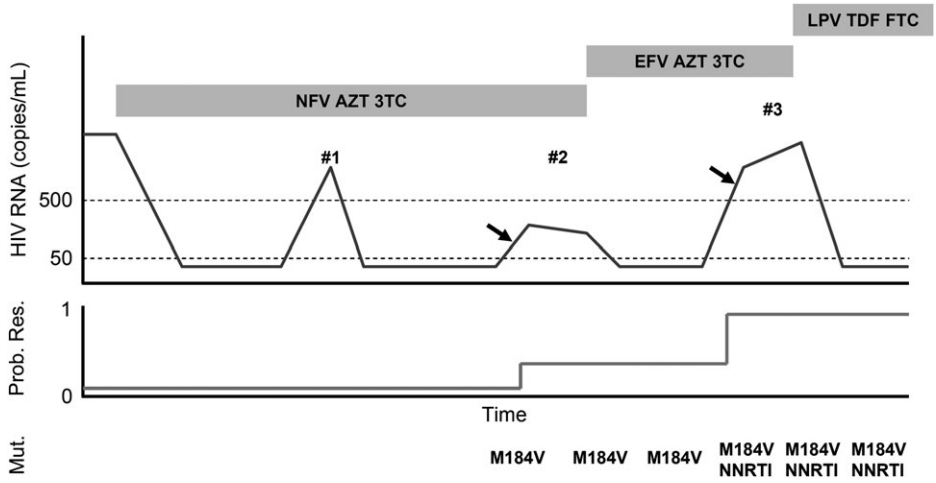

Figure 1. Risk state classification and simulation of drug resistance for a hypothetical patient (example). For classification into risk states (upper panel), longitudinal human immunodeficiency virus (HIV) RNA measurements and treatment histories were used to determine, at each HIV measurement, the risk state. Low risks were defined as either being off therapy or always having had HIV RNA <50 copies. Note that blips (indicated by #1) were ignored. Intermediate risks (#2) were defined as detectable viremia ≤500 copies without subsequent virologic suppression <50 copies on same therapy. High risks (#3) were defined as 2 consecutive HIV RNA >500 copies or 1 HIV RNA >500 copies and stop or modification of therapy (virological failure). The simulation of resistance was done as follows: at transitions to higher-risk states (indicated by #2 and #3, upper panel), a random number between 0 and 1 was drawn. If this number exceeded the risk for resistance as estimated from genotypic resistance tests (middle panel, data shown in Supplementary Table 1; online only), resistance mutations, corresponding to current antiretroviral therapy exposure, were assumed to have emerged (lower panel). Timing of emergence (indicated by arrows, upper panel) was randomly placed between the risk event (ie, the transition to a higher risk category, labels #2 and #3, upper panel) and preceding HIV RNA. For example, if this patient who previously had an intermediate risk event on an unboosted protease inhibitor therapy experienced the first virological failure on a combination treatment including nonnucleoside reverse-transcriptase inhibitors, this individual had a 70.2% chance to have at least 1 resistance mutation detected by genotypic testing (Supplementary Table 1; online only). To simulate resistance emergence in this individual, a decision was made whether the mutation has occurred with a 70.2% probability, and if so, when the event took place, which was sometime between the last undetectable viral load and the first viral load >500 copies/mL. These decisions were governed by draws of uniformly distributed random numbers ranging from 0 to 1; if this number was less or equal to the current risk for mutations (here 0.702), then mutations were assumed to be present. Similarly, a random number determined the fraction of the absolute time distance between the last undetectable and the first viral load >500 copies at which the event took place. These procedures were repeated 100 times to generate 100 datasets, in which resistance emergence events were assigned randomly, but taking into account patients' risk profiles.
Abbreviations: 3TC, lamivudine; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; Mut, type of mutations; NFV, nelfinavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; Prob. Res., probability for resistance emergence; TDF, tenofovir.

100 times, with newly sampled risks for resistance emergence from distributions generated via bootstrapping.

Statistical Analysis

The main study outcomes were the emergence of any mutations from the 5 groups and the emergence of triple-class drug resistance, defined as the presence of ≥1 mutation from the NNRTI and PI groups and ≥1 from any of the 3 groups of NRTI mutations. Drug resistance mutations observed in the lowest risk state (Supplementary Table 1; online only) were not considered as incident during therapy but as transmitted [17].

Each dataset of imputed resistance was analyzed by univariable and multivariable Cox regression models to estimate the relative hazard of drug resistance emergence across the 5 initial treatment groups. Exposure time started at cART initiation (baseline) and ended when the first simulated drug resistance mutation occurred. Analyses were censored after 6 years of follow-up, and comparisons were conducted on an intent-to-treat basis, which means that patients who underwent switches in their first-line regimens were also considered. Adjustments in the multivariable model consisted of age, sex, ethnicity, risk group, and HIV RNA level and CD4 cell count at treatment initiation. Unless stated otherwise, point estimates are presented as the median and the 2.5th and 97.5th percentiles over all simulation runs (ie, the range including 95% of all estimates). The simulation study was performed using Stata SE software, version 11.2 (StataCorp).

RESULTS

Study Population

A total of 1139 patients receiving NNRTI and 1124 patients receiving PI/r were included. Baseline characteristics, stratified by cART received, are shown in Table 1. Individuals receiving newer therapies based on EFV+TDF or ATZ/r tended to have higher CD4 cell counts. Moreover, patients with higher pretreatment viral loads tended to receive PI/r-based regimens

Table 1. Baseline Characteristics of Treatment Groups

Variable	EFV/AZT/3TC	EFV/TDF/FTC or 3TC	LPV/AZT/3TC	ATV/RTV/TDF/FTC or 3TC	LPV/TDF/FTC or 3TC	*P* value
No. of patients	524	615	573	250	301	
Mode of HIV acquisition						<.001
Heterosexual contacts	280 (53.4%)	258 (42.0%)	281 (49.0%)	86 (34.4%)	127 (42.2%)	
Intravenous drug use	64 (12.2%)	38 (6.2%)	65 (11.3%)	35 (14.0%)	32 (10.6%)	
Homosexual contacts	180 (34.4%)	319 (51.9%)	227 (39.6%)	129 (51.6%)	142 (47.2%)	
Female sex	166 (31.7%)	120 (19.5%)	197 (34.4%)	53 (21.2%)	77 (25.6%)	<.001
Median age at baseline (IQR)	38 (32–44)	40 (33–46)	36 (30–44)	40 (34–46)	40 (34–46)	<.001
Ethnicity						.326
White	393 (75.0%)	464 (75.4%)	421 (73.5%)	206 (82.4%)	224 (74.4%)	
Black	85 (16.2%)	91 (14.8%)	99 (17.3%)	26 (10.4%)	48 (15.9%)	
Unknown/Other	46 (8.8%)	60 (9.8%)	53 (9.2%)	18 (7.2%)	29 (9.6%)	
Median baseline CD4 cell count (IQR)	196 (110–288)	242 (154–317)	219 (110–357)	244 (160–323)	209 (92–303)	<.001
Median baseline log10 HIV RNA level (IQR)	5 (4.5–5.5)	4.7 (4.3–5.2)	5.1 (4.6–5.7)	4.9 (4.4–5.2)	5 (4.4–5.5)	<.001
Median year of ART initiation (IQR)	2002 (2001–2004)	2007 (2006–2008)	2005 (2003–2007)	2008 (2006–2009)	2007 (2006–2008)	<.001

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; ATV, atazanavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; IQR, interquartile range; LPV, lopinavir; RTV, ritonavir-boosted; TDF, tenofovir.

more frequently, as indicated by the higher 75th percentiles of baseline HIV RNA level in the 2 types of lopinavir-based treatments, compared with the other groups, reflecting recommendations by earlier guidelines [18, 19].

Characteristics of the study population at the last follow-up visit or at year 6 after therapy start, whichever occurred first, are shown in Table 2. Over a mean follow-up time of 4.5 years, 325 individuals (14.4%) experienced intermediate risk events for drug resistance emergence (ie, low-level viremia while receiving cART), and 109 (4.8%) had virologically failed at least 1 therapy. Table 2 indicates that being female, being of black ethnicity, having acquired HIV infection through heterosexual contact, and having a baseline CD4 cell count ≤200 cells/μL were associated with virological failure. Among all individuals who experienced an intermediate risk event or a virological failure, 21% and 64%, respectively, also had GRTs performed (Supplementary Table 2; online only). No major testing biases were detected apart from the finding that more recent risk events were less likely to have corresponding genotypic data available. This is attributable to 2 factors: first, there is a certain delay in transferring GRT data to the SHCS, and second, treatment switches owing to suspected treatment failure have often occurred at plasma viral loads ≤500 copies/mL in more recent years, which affects chances for successful viral genotyping [20].

Rates of Resistance Emergence

Intent-to-treat rates of mutation emergence from the simulation are shown in Table 3. The EFV+AZT group exhibited the highest rates, with 2.6 events per 100 person-years of treatment exposure, whereas the 4 other groups showed rates between 1.45 (ATZ/r+TDF) and 1.92 (EFV+TDF) events per 100 person-years. This higher rate of mutation emergence among EFV+AZT recipients was partially driven by the frequent detection of the 3TC mutation M184V, which occurred more than twice as often as in any other treatment group. This observation also explained the marked difference in overall mutation rates between EFV+AZT and the second NNRTI group (EFV+TDF), whereas rates of NNRTI mutations were comparable between the 2 NNRTI groups.

Analyses of the origins of mutations in terms of risk stratum showed that, across all treatment groups, almost two-thirds of mutations (median over 100 simulations, 65%) emerged while individuals had experienced detectable viral loads ≤500 copies /mL during therapy. This proportion was lowest in the EFV+AZT group (52%) and highest in the LPV+TDF group (83%; also LPV+AZT: 73%; EFV+TDF: 69%; ATZ/r+TDF: 75%).

Time-to-Event Analyses of Resistance Emergence

When analyzing resistance emergence with the Kaplan-Meier method (Figure 2), the median intent-to-treat cumulative incidence of drug resistance mutations after 6 years of follow-up was highest in the EFV+AZT group (16% [2.5th–97.5th percentile, 14%–18%]), compared with other types of initial treatments (EFV+TDF: 9% [7%–11%]; LPV+AZT: 10% [8%–12%]; LPV+TDF: 7% [5%–11%]). Because of limited

Table 2. Characteristics of Study Population 6 Years After Therapy Initiation or Time of Last Follow-up (Whichever Occurred First)

Characteristic	Always optimally treated (n = 1829)	Ever low-level viremia while on ART (n = 325)	Ever virological treatment failure (n = 109)
Type of initial combination therapy			
NNRTI			
Efavirenz/zidovudine/lamivudine or emtricitabine	403 (22.0%)	74 (22.8%)	47 (43.1%)
Efavirenz/tenofovir/lamivudine or emtricitabine	539 (29.5%)	58 (17.8%)	18 (16.5%)
Boosted PI			
Lopinavir/zidovudine/lamivudine or emtricitabine	446 (24.4%)	100 (30.8%)	27 (24.8%)
Boosted atazanavir/tenofovir/lamivudine or emtricitabine	199 (10.9%)	42 (12.9%)	9 (8.3%)
Lopinavir/tenofovir/lamivudine or emtricitabine	242 (13.2%)	51 (15.7%)	8 (7.3%)
Characteristics by risk states			
Female sex	482 (26.4%)	91 (28.0%)	40 (36.7%)
Median age (IQR)	39 (32–45)	38 (31–46)	35 (30–42)
Ethnicity			
White	1398 (76.4%)	241 (74.2%)	69 (63.3%)
Black	258 (14.1%)	60 (18.5%)	31 (28.4%)
Unknown/Other	173 (9.5%)	24 (7.4%)	9 (8.3%)
Mode of HIV acquisition			
Heterosexual contacts	806 (44.1%)	161 (49.5%)	65 (59.6%)
Intravenous drug use	179 (9.8%)	40 (12.3%)	15 (13.8%)
Homosexual contacts	844 (46.1%)	124 (38.2%)	29 (26.6%)
Median baseline CD4 cells/μL (IQR)	228 (138–321)	204 (105–303)	166 (48–263)
Baseline CD4 ≤200 cells/μL	761 (41.6%)	158 (48.6%)	64 (58.7%)
Median baseline $\log_{10}$ HIV RNA (IQR)	4.9 (4.4–5.3)	5.2 (4.8–5.6)	5.0 (4.6–5.7)
Baseline HIV RNA ≥5 $\log_{10}$ copies/mL	773 (42.3%)	200 (61.5%)	56 (51.4%)
Median year of ART initiation (IQR)	2006 (2004–2008)	2006 (2003–2008)	2004 (2002–2006)
Ever had a GRT in current state	140 (7.7%)	67 (20.6%)	69 (63.3%)
Of those with GRT, ever resistance detected	11 (7.9%)	28 (41.8%)	46 (66.7%)
Treatment at last follow-up before or at year 6			
2 or 3 NRTIs + 1 boosted PI	747 (40.8%)	174 (53.5%)	55 (50.5%)
2 or 3 NRTIs + 1 NNRTI	922 (50.4%)	95 (29.2%)	25 (22.9%)
2 or 3 NRTIs + 1 unboosted PI	26 (1.4%)	8 (2.5%)	4 (3.7%)
Trizivir (zidovudine/lamivudine/abacavir)	85 (4.6%)	11 (3.4%)	2 (1.8%)
≤3 NRTIs (excl. Trizivir)	24 (1.3%)	21 (6.5%)	7 (6.4%)
Other combinations (eg, NNRTI+PI, mono PI)	25 (1.4%)	16 (4.9%)	16 (14.7%)
Median follow-up time in years (IQR)	3.9 (2.0–5.3)	3.8 (1.8–5.2)	4.9 (3.5–5.5)
Died before year 6	36 (2.0%)	16 (4.9%)	4 (3.7%)
Lost to follow-up before year 6	176 (9.6%)	50 (15.4%)	31 (28.4%)

Abbreviations: ART, antiretroviral therapy; GRT, genotypic drug resistance test; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

follow-up, only a 4-year cumulative incidence could be estimated with adequate precision for the ATZ/r+TDF group (5% [2%–8%]), which was lower than the smallest 4-year risk among the other 4 groups (LPV+TDF: 7% [5%–10%]). Of note, all these estimates were higher than estimates based on measured drug resistance or virological failure events (Figure 2). For instance, in the EFV+AZT group, measured drug resistance (7%) or virological failure (10%) 6 years after therapy initiation was considerably less frequent than simulated resistance (16%).

Next, simulated resistance emergence was analyzed with regression models. The median hazard ratios (2.5th–97.5th percentile) from unadjusted Cox regressions using EFV+AZT as reference were as follows: EFV+TDF: 0.53 (0.41–0.68); LPV+AZT: 0.62 (0.49–0.80); LPV+TDF: 0.50 (0.31–0.73); and ATV+TDF: 0.40 (0.18–0.67). These point estimates varied little when adjusted Cox regression analyses were performed (Table 4) and provided strong evidence for the inferiority of EFV/AZT/3TC with regard to drug resistance emergence relative to all other groups. Furthermore, having a high HIV RNA load

Table 3. Simulated Numbers and Rates of Occurrence of Specific Mutations Over 100 Simulation Runs[a]

	EFV/AZT/3TC (n = 524)	EFV/TDF/FTC or 3TC (n = 615)	LPV/AZT/3TC (n = 573)	LPV/TDF/FTC or 3TC (n = 301)	ATV/RTV/TDF/FTC or 3TC (n = 250)
Person-years of follow-up	3274	1722	2592	883	622
Any IAS-USA mutations					
Number of events	85 (81–88)	33 (30–36)	49 (46–52)	17 (15–18)	9 (8–11)
Rate per 100 person-years	2.60 (2.23–2.96)	1.92 (1.48–2.41)	1.90 (1.49–2.38)	1.91 (1.22–2.55)	1.45 (0.79–2.30)
M184V/I					
Number of events	66 (62–69)	13 (11–15)	32 (28–35)	9 (7–10)	3 (2–4)
Rate per 100 person-years	1.97 (1.63–2.34)	0.74 (0.51–1.03)	1.21 (0.86–1.61)	0.99 (0.44–1.35)	0.47 (0–0.96)
Thymidine analogue mutations					
Number of events	25 (23–28)	3 (2–4)	6 (5–8)	3 (2–4)	0 (0–0)
Rate per 100 person-years	0.71 (0.50–0.96)	0.17 (0.02–0.28)	0.22 (0.11–0.37)	0.32 (0–0.55)	0 (0–0)
Other NRTI mutations					
Number of events	9 (8–11)	12 (9–15)	11 (9–13)	3 (2–4)	2 (1–3)
Rate per 100 person-years	0.25 (0.11–0.45)	0.68 (0.28–1.13)	0.40 (0.18–0.63)	0.33 (0–0.77)	0.31 (0–0.80)
NNRTI mutations					
Number of events	58 (55–62)	26 (24–28)	28 (25–30)	6 (5–8)	3 (3–4)
Rate per 100 person-years	1.71 (1.40–2.03)	1.49 (1.08–1.86)	1.05 (0.76–1.34)	0.66 (0.33–1)	0.48 (0.16–0.96)
PI mutations					
Number of events	21 (19–24)	6 (4–7)	17 (14–19)	8 (6–9)	5 (4–6)
Rate per 100 person-years	0.59 (0.39–0.82)	0.34 (0.11–0.56)	0.63 (0.34–0.94)	0.88 (0.43–1.56)	0.79 (0.16–1.46)
Mutations against 3 classes					
Number of events	11 (10–13)	3 (2–4)	6 (4–7)	1 (1–2)	0 (0–1)
Rate per 100 person-years	0.31 (0.17–0.48)	0.17 (0–0.34)	0.22 (0.07–0.37)	0.11 (0–0.33)	0 (0–0.32)

Abbreviations: 3TC, lamivudine; ATV, atazanavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; IAS-USA, International AIDS Society–USA; LPV, lopinavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; RTV, ritonavir-boosted; TDF, tenofovir.

[a] Analyses were performed on an intent-to-treat basis, meaning that resistance emergence events occurring on later lines of regimens were still attributed to the respective group of first-line therapy. Data are presented as median (IQR) for number of events or median (2.5th-97.5th percentiles) for rates.

≥100 000 copies/mL at time of therapy start and black ethnicity showed strong associations with more frequent drug resistance emergence. Results changed little in predefined subanalyses, in which the Cox model estimation was restricted to individuals with baseline viral loads ≥100 000 copies/mL (n = 1029) or to modern TDF-containing treatments (n = 1166). In this latter analysis, none of the 3 TDF-based treatments showed significantly different rates of resistance emergence, although treatments with ATZ/r maintained the lowest hazard ratio (0.73 [0.37–1.31]), compared with LPV (0.91 [0.58–1.34]) or EFV (reference).

Comparison of Simulation Results With Standard Data Analyses

Finally, we aimed to compare the simulation results with those obtained from standard time to event analyses, with virological failure or detection of resistance as end points (Table 4). In general, the point estimates from the simulation study seemed to be compatible with the findings from standard analyses. Although in some cases not statistically significant, all 4 drug combinations showed hazard ratios of about the same magnitude as in the simulation study. In addition, black ethnicity emerged as a risk factor in all 3 analyses. Discrepancies were observed with respect to low CD4 cell counts ≤200 cells/μL at baseline, which was significantly associated with the detection of resistance mutations in the virologic failure study but not in the other analyses (possibly reflecting a testing bias; Supplementary Table 2; online only). In addition, although hazard ratios of baseline HIV RNA level ≥100 000 copies/mL indicated associations with higher outcome rates in all 3 analyses, statistical significance was only reached in the simulation study.

DISCUSSION

By applying a novel imputation method for estimation of drug resistance emergence, we observed surprisingly robust patterns across the 5 groups of cART under consideration. Over a mean observation period of 4.5 years, rates of emergence of any resistance ranged from 1.5 to 2.6 events per 100 person-years during treatment. The majority of events were related to emergence of 3TC or FTC resistance (ie, M184V; 33%–78% of mutations; Table 3) and, in individuals starting NNRTI-based

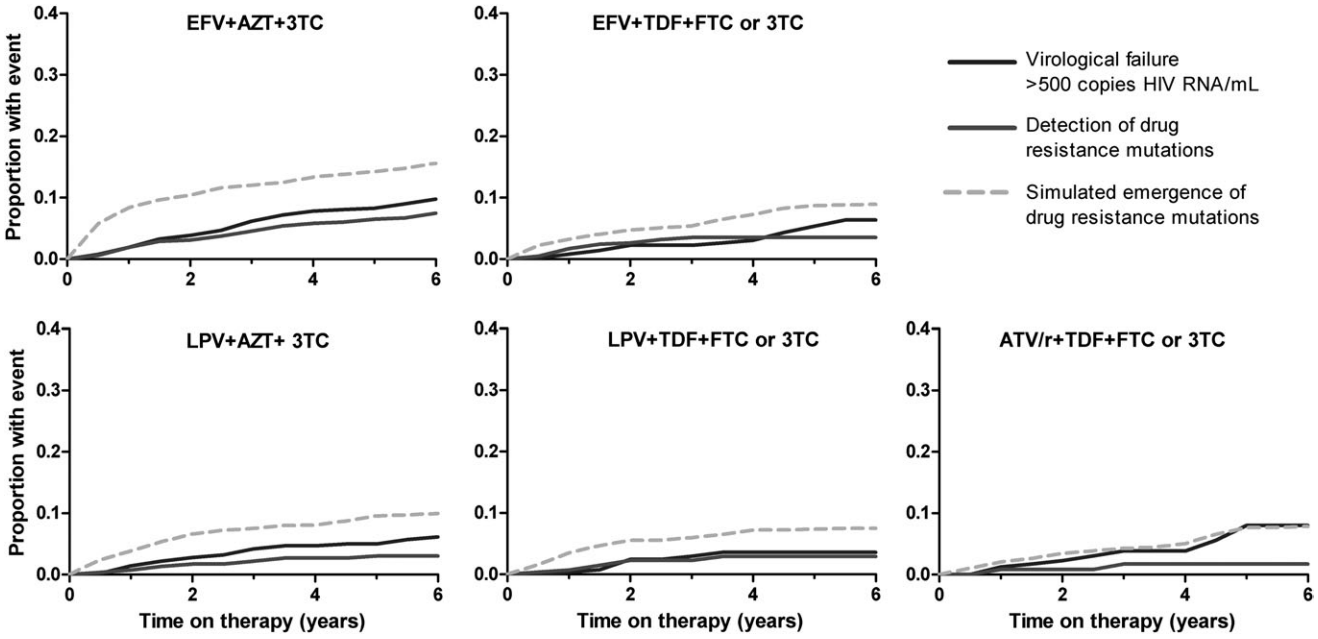

Figure 2. Kaplan-Meier curves for virological failure events (dark grey solid line), detection of drug resistance mutations (light grey solid line) as observed in data, or simulated emergence of drug resistance on the basis of patient-specific risk profiles (dashed line). Abbreviations: 3TC, lamivudine; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; TDF, tenofovir.

regimens, to NNRTI resistance mutations (68% and 79% of mutations in the EFV+AZT and EFV+TDF groups, respectively). In contrast, resistance to PIs among first-line PI users contributed less to overall resistance (35%–56%), which has been shown previously by observational data [4] and randomized clinical trials [21, 22]. In line with previous findings of improved response rates to ART over time [5, 6], resistance emergence seemed to have become rarer with novel treatment combinations. When compared with the simulation results from an outdated unboosted PI combination (NFV/AZT/3TC, with 5.5 [4.6–6.4] resistance emergence events per 100 person-years; data not shown), all 5 groups had markedly lower resistance rates. Moreover, newer TDF-based NNRTI treatments showed smaller rates of resistance (1.9 events per 100 person-years of treatment; Table 3) than did older AZT-based NNRTI treatments (2.6 events per 100 person-years). Similarly, newer treatments based on ATZ/r and TDF showed trends for lower rates of resistance emergence (1.5 events per 100 person-years) than did LPV+AZT (1.9 events per 100 person-years) or LPV+TDF (1.9 events per 100 person-years). After adjustment for potential confounders, the EFV+AZT group was the combination with the highest resistance rate, whereas the other 4 combinations seemed to be equivalent. These observations are in line with a recent survey on the durability of different first-line regimens in Switzerland that identified AZT/3TC-based NRTI backbones as a risk factor for toxicity-related treatment modifications [11]. The generally more favorable risk profile of TDF-based treatments for the emergence of drug resistance may therefore be mediated by better tolerability, less toxicity, and more favorable pharmacokinetics [23]. Overall, ATZ/r regimens showed the lowest rates for emergence of resistance. Although an influence of the somewhat shorter follow-up time in this group cannot be fully excluded—which would be relevant if resistance rates were increasing with prolonged treatment duration—ATZ/r treatments have also proven, in routine clinical care [11] and in randomized clinical trials [24], to be durable and well tolerated, thereby promoting adherence and, possibly, reducing the risk for the emergence of resistance. Another remarkable finding was the high proportion of resistance emerging from intermediate risk events (ie, HIV RNA level $\leq$500 copies/mL), which argues for close monitoring of antiretroviral therapy and immediate treatment switches even at relatively low HIV RNA levels if virological failure is suspected. It is likely that many of these intermediate-risk situations would develop into full virological failure, and early intervention, as is standard in Switzerland, can be a strategy to limit resistance and to preserve future treatment options.

Because of methodological challenges, few studies have attempted to assess the incidence of drug resistance with NNRTI or boosted PI therapy, and results are difficult to compare [9, 22, 25, 26]. The most similar study to ours was performed by the UK Collaborative Group on HIV Drug Resistance [9] and involved an assessment of 7891 individuals starting either NNRTI-based (83%) or PI/r-based (17%) combination treatments. Of those,

Table 4. Comparison of Results From the Simulation Analysis and Conventional Data Analyses Using Virological Failures >500 Copies/mL HIV RNA or Detection of any Drug Resistance by Genotypic Resistance Testing as Endpoints[a]

	Simulated IAS-USA mutations	Detected IAS-USA mutations	Virological Failures
Number of events	253 (median)	85	109
Female sex	1.06 (0.81–1.36)	0.86 (0.51–1.44)	0.90 (0.57–1.40)
Age (per additional year)	0.94 (0.85–1.07)	0.98 (0.96–1.00)	0.97 (0.95–1.00)
Ethnicity			
White	Reference	Reference	Reference
Black	**1.58 (1.11–2.09)**	**2.78 (1.55–4.98)**	**1.88 (1.12–3.17)**
Unknown/Other	0.89 (0.52–1.33)	0.79 (0.33–1.89)	0.92 (0.45–1.91)
Mode of HIV acquisition			
Heterosexual contact	Reference	Reference	Reference
Intravenous drug use	1.16 (0.81–1.65)	1.11 (0.51–2.42)	1.27 (0.70–2.30)
Homosexual contact	0.75 (0.56–1.01)	1.32 (0.76–2.32)	0.66 (0.39–1.11)
Baseline CD4 ≤200 cells/μL	1.14 (0.93–1.44)	1.39 (0.94–2.04)	**1.70 (1.12–2.59)**
Baseline HIV RNA ≥5 log10 copies/mL	**1.49 (1.19–1.81)**	1.12 (0.76–1.64)	1.45 (0.95–2.19)
Type of initial combination therapy			
EFV/AZT/3TC	Reference	Reference	Reference
EFV/TDF/FTC or 3TC	**0.57 (0.42–0.76)**	0.69 (0.40–1.18)	**0.54 (0.31–0.94)**
ATV/TDF/FTC or 3TC	**0.43 (0.17–0.83)**	0.47 (0.18–1.20)	0.81 (0.39–1.68)
LPV/AZT/3TC	**0.63 (0.45–0.89)**	**0.51 (0.30–0.87)**	**0.57 (0.36–0.91)**
LPV/TDF/FTC or 3TC	**0.55 (0.33–0.83)**	0.55 (0.26–1.14)	**0.46 (0.21–0.97)**

Numbers printed in bold are statistically significant, because their simulation intervals or the confidence intervals do not contain the value 1.

Abbreviations: 3TC, lamivudine; ATV, atazanavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; IAS-USA, International AIDS Society–USA; LPV, lopinavir; RTV, ritonavir-boosted; TDF, tenofovir.

[a] Data are presented as median hazard ratios (2.5th and 97.5th percentiles) over 100 simulation runs (column 1) or point estimates and likelihood-ratio confidence intervals from standard Cox proportional hazard regressions (columns 2 and 3).

1359 patients (17%) experienced virological failure; however, as a limitation, only 48% had been genotyped for drug resistance mutations. The authors reported cumulative incidences of 14.9% (NNRTI) and 14.7% (PI/r) for detection of any drug resistance 6 years after treatment initiation. In contrast, the 6-year cumulative incidence estimates from this study were 12.9% for the pooled NNRTI group and 9.0% for the pooled PI/r group. It should be noted, however, that the majority of resistance in our simulation emerged from intermediate-risk events, which were not considered in the UK study. Moreover, the failure rates observed in our study differed markedly from the one reported by the UK group (5% vs 25%), possibly because of the higher proportion of individuals starting therapy with more modern drugs, such as TDF or ATZ/r.

A second study analyzed drug resistance emergence among individuals newly starting NNRTI- or PI/r-based combination therapy in Denmark [25]. The authors recorded 247 virological failures among 1829 ART initiators, of whom 23% had genotypic data available (compared with ∼65% in our study). With 0.6 events per 100 person-years, their incidence estimate was much lower than our rates (range, 1.5–2.6 events per 100 person-years) (Table 3). Because the focus of the Danish study was on time trends, they chose a very conservative study design, thereby accepting an underestimation of actual event rates.

The validity of our incidence estimates relies on assumptions. The accuracy of the simulation is dependent on the quality of the dataset, such as the frequency and completeness of HIV RNA measurements and treatment information. In addition, it is important that the risk state–specific estimates for the presence of resistance mutations are not affected unduly by testing biases. This latter assumption can safely be considered to be fulfilled for the high and intermediate risk states (Supplementary Table 2; online only). However, this is not the case for estimates derived from genotypic tests performed while an individual was still at low risk for the emergence of resistance. The estimate for any resistance mutations in this stratum was around approximately 16% and was mostly driven by NNRTI resistance (Supplementary Table 1; online only), thus considerably above levels of transmitted resistance in Switzerland [17]. An inspection of treatments and longitudinal CD4 cell count and viral load measurements revealed that these genotypic tests were often performed in situations in which a patient's treatment history was uncertain or after single or repeated low-level viremia with subsequent suppression to undetectable HIV RNA levels during the same treatment (this latter

criterion was responsible for the classification into the low-risk stratum). Although these observations are suggestive for the emergence of resistance mutations in the presence of low-level viremia, we chose to ignore these risk estimates from the lowest risk stratum for the simulation, because it was not possible to identify such elevated risks specifically. Moreover, a simulation of resistance in the low-risk strata on the basis of the observed resistance probabilities (Supplementary Table 1; online only) would lead to highly exaggerated incidence rates.

To conclude, this newly developed simulation approach complements recent methodological advances in estimating drug resistance prevalence among ART-exposed individuals. The concept can readily be implemented to any observational setting with treatment and laboratory data collections and corresponding genotypic data available. Despite the large variability of parameter estimates, the incidence estimates were surprisingly robust, and they document the continuous progress in HIV treatment, marked by reductions in the emergence of resistance and increased proportions of treated individuals with continuously undetectable HIV RNA levels.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). 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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