

Emergence of HIV-1 Drug Resistance in Previously Untreated Patients Initiating Combination Antiretroviral Treatment

A Comparison of Different Regimen Types

Viktor von Wyl, MSc; Sabine Yerly, MSc; Jürg Böni, DVM; Philippe Bürgisser, PhD; Thomas Klimkait, PhD; Manuel Battegay, MD; Hansjakob Furrer, MD; Amalio Telenti, MD; Bernard Hirschel, MD; Pietro L. Vernazza, MD; Enos Bernasconi, MD; Martin Rickenbach, MD; Luc Perrin, MD; Bruno Ledergerber, PhD; Huldrych F. Günthard, MD; for the Swiss HIV Cohort Study

Background: Standard first-line combination antiretroviral treatment (cART) against human immunodeficiency virus 1 (HIV-1) contains either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI/r). Differences between these regimen types in the extent of the emergence of drug resistance on virological failure and the implications for further treatment options have rarely been assessed.

Methods: We investigated virological outcomes in patients from the Swiss HIV Cohort Study initiating cART between January 1, 1999, and December 31, 2005, with an unboosted PI, a PI/r, or an NNRTI and compared genotypic drug resistance patterns among these groups at treatment failure.

Results: A total of 489 patients started cART with a PI, 518 with a PI/r, and 805 with an NNRTI. A total of 177 virological failures were observed (108 [22%] PI failures, 24 [5%] PI/r failures, and 45 [6%] NNRTI failures). The failure rate was highest in the PI group (10.3 per 100 person-years; 95% confidence interval [CI], 8.5-

12.4). No difference was seen between patients taking a PI/r (2.7; 95% CI, 1.8-4.0) and those taking an NNRTI (2.4; 95% CI, 1.8-3.3). Genotypic test results were available for 142 (80%) of the patients with a virological treatment failure. Resistance mutations were found in 84% (95% CI, 75%-92%) of patients taking a PI, 30% (95% CI, 12%-54%) of patients taking a PI/r, and 66% (95% CI, 49%-80%) of patients taking an NNRTI ($P < .001$). Multidrug resistance occurred almost exclusively as resistance against lamivudine-emtricitabine and the group-specific third drug and was observed in 17% (95% CI, 9%-26%) of patients taking a PI, 10% (95% CI, 0.1%-32%) of patients taking a PI/r, and 50% (95% CI, 33%-67%) of patients taking an NNRTI ($P < .001$).

Conclusions: Regimens that contained a PI/r or an NNRTI exhibited similar potency as first-line regimens. However, the use of a PI/r led to less resistance in case of virological failure, preserving more drug options for the future.

Arch Intern Med. 2007;167(16):1782-1790

CURRENT GUIDELINES recommend the initiation of combination antiretroviral treatment (cART) against human immunodeficiency virus 1 (HIV-1) with 2 nucleoside reverse transcriptase inhibitors (NRTIs) and a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI/r).¹ These different drug classes vary considerably in the number of mutations required to confer resistance and in the degree of cross-resistance to drugs of the same class. Selection of a single mutation leads to the emergence of high-level resistance against NNRTIs, and resistance usually emerges quickly if treatment failure occurs.^{2,3} In contrast, virological failure with boosted

PIs is associated with a reduced risk of selection of drug resistance mutations.⁴

Few data from observational studies exist on how these differences in genetic barriers, defined as the number of amino acid changes required to confer resistance, and the degree of cross-resistance may drive the emergence of drug resistance and hence affect the success of first-line therapy and beyond.⁵⁻⁷ Moreover, to our knowledge, only 1 head-to-head comparison of boosted PI (lopinavir) vs NNRTI-based (efavirenz) first-line combination therapy has been conducted to date. The AIDS Clinical Trial Group 5142 presented preliminary data on this randomized, open-label trial and observed no difference in rates of primary study end points, which were virological failure and/or treatment dis-

Author Affiliations are listed at the end of this article.

Group Information: The members of the Swiss HIV Cohort Study are listed at the end of this article.

continuation due to toxic effects.⁸ However, the authors noted trends for a shorter time to those end points in the lopinavir arm and for more resistance accumulation on virological failure in the efavirenz arm.

Our objectives were to study the frequency and patterns of the emergence of viral drug resistance in patients from a large cohort in whom first-line cART failed in a routine clinical setting and to assess the potential impact on future therapies. Therefore, we analyzed patients from the Swiss HIV Cohort Study (SHCS) who were grouped according to their third drug, defined as the additional drug besides NRTIs or lamivudine or emtricitabine in standard 2-class combination therapy (NNRTI, PI, or PI/r). Further analyses were performed to compare the 3 regimen types with regard to virological failure and treatment discontinuation.

METHODS

STUDY DESIGN AND PARTICIPANTS

Included in this analysis were the cART-naïve patients from the SHCS^{9,10} who had initiated antiretroviral treatment between January 1, 1999, when genotyping became available in clinical practice in Switzerland, and December 31, 2005, with either 2 NRTIs and 1 NNRTI or 2 NRTIs and 1 PI (PI/r or unboosted). In case patients experienced an early adverse reaction to the initial treatment, the second cART regimen was considered if the initial regimen was followed for less than 30 days and if no treatment interruption occurred between the first and second regimens. As another inclusion criterion, 1 additional on-treatment HIV RNA measurement after the attainment of undetectable viral load levels of less than 50 copies/mL had to be available, or in patients in whom viral suppression was not achieved, 1 viral load after 180 days or more of continuous therapy. This criterion was required to evaluate our study participants with regard to virological failure while receiving therapy, which was defined as follows: (1) viral rebound with 2 consecutive viral loads greater than 500 copies/mL after previous attainment of undetectable HIV RNA levels, (2) 1 value greater than 500 copies/mL followed by a stop or a modification of the current therapy in patients with previous suppression of viral replication to undetectable levels, or (3) 1 viral load greater than 500 copies/mL after 180 days of continuous treatment in patients without previous suppression of viral load to undetectable levels. The date of the first HIV RNA measurement that indicated a virological failure was considered the failure date.

Drug resistance tests and clinical data collected up until February 1, 2007, were used for this analysis. Resistance tests were included in statistical analyses if the blood sampling was performed on or after the presumed date of virological failure or within 1 month after stopping the initial regimen if the patient continued not to receive treatment. Mean HIV RNA levels at the time of resistance testing were 3.55, 3.33, and 3.23 log copies/mL for the NNRTI group, the PI group, and the PI/r group, respectively (Kruskal-Wallis test, $P = .66$). Resistance data stem from routine clinical testing (60% of all tests) performed by the 4 laboratories in Switzerland authorized by the Federal Office of Public Health and tests specifically performed for this project from frozen repository samples (40%) if specimens were available. All laboratories performed population-based sequencing of the full protease gene and in minimum codons 28 to 225 of the reverse transcriptase gene using commercial assays (Virorseq vs 1, PE Biosystems, Rotkreuz, Switzerland; Virsoseq vs 2, Abbott AG, Baar, Switzerland; and vircoTYPE HIV-1 Assay,

Virco Laboratory, Mechelen, Belgium) and in-house methods.¹¹ Resistance data were collected and stored using SmartGene's Integrated Database Network System (version 3.3.0, SmartGene, Zug, Switzerland). The degree of drug resistance was evaluated using the Stanford algorithm¹² version 4.2.6 and a modified version of the Fall 2006 International AIDS Society–USA drug mutation list,¹³ in which the following major mutations were included: NRTIs: M41L, A62V, K65R, D67N, K70E/R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, K219Q/E, and insertions at codon 69; NNRTIs: L100I, K103N, V106M/A, V108I, Y181C/I, Y188C/L/H, G190S/A, P225H, and P236L; and PIs: D30N, V32I, L33F, M46I/L, I47V/A, G48V, I50V/L, L76V, V82A/F/T/S/L, I84V, N88S, and L90M. Mutations that confer resistance against entry inhibitors were not considered. The 5 resistance categories from the Stanford algorithm were regrouped for this analysis: viruses with a genotypic sensitivity score less than 15 were considered susceptible, those with a genotypic sensitivity score in the range of 15 to 59 were considered to have intermediate resistance, and those with a genotypic sensitivity score greater than 59 were considered to have high resistance. Throughout this study, lamivudine and emtricitabine were considered separate drug classes. In these drugs, the emergence of resistance on failure follows a pattern that is distinct from other NRTIs because only a single mutation at codon 184 is required to confer high resistance.¹⁴

The SHCS was approved by ethics committees of all participating institutions, and written informed consent was obtained from all participants.

STATISTICAL ANALYSES

All statistical analyses were stratified by regimen type (PI group [cART that contained 1 unboosted PI], PI/r group [1 PI/r], or NNRTI group [1 NNRTI]). Patterns of drug resistance were assessed using the Kruskal-Wallis test for continuous data and Fisher exact test for categorical data. Failure rates were analyzed using Kaplan-Meier life tables and univariate and multivariate Cox regression. We performed a competing risk analysis to estimate time receiving first-line therapy, which took into account that some patients were stopping cART because of reasons other than virological failure, such as ART-related toxic effects.^{15,16} The primary event of interest was stopping or modification of a regimen because of virological failure, and stopping or modification of a regimen owing to adverse events (adverse effects and laboratory abnormalities) was considered a competing risk event. For patients not reaching these end points, the analysis was censored at the stop date of the initial cART regimen or the last date of follow-up, whichever occurred first. For the estimation of Cox model parameters, the type of end point (virological failure or adverse event) and year of cART initiation were included as strata. Modeling assumptions were verified using the Schoenfeld residuals method.¹⁷ Reasons for stopping cART are systematically assessed in the SHCS using the Data Collection on Adverse Events of Anti-HIV Drugs reporting scheme.¹⁸

Models were adjusted for baseline demographics and the following potential confounders: HIV RNA viral load and CD4 cell count at therapy initiation as well as adherence.¹⁹ Baseline measurements for HIV RNA and CD4 cell counts were missing for approximately one-fifth of all patients (irrespective of treatment group). Hence, those variables were stratified (<1000, 1000–99 999, and $\geq 100\,000$ copies/mL for HIV RNA; <200, 200–349, and ≥ 350 cells/ μ L for CD4 cell count), and additional strata were included for missing values.

All statistical tests were 2-sided. $P < .05$ was considered statistically significant. No adjustments for multiple testing were made. Analyses were performed using Stata SE 9.2 (Stata-Corp, College Station, Texas).

Table 1. Characteristics of 1812 Patients Initiating cART Between January 1, 1999, and December 31, 2005

Characteristic	No. (%) of Patients ^a		
	PI (n=489)	PI/r (n=518)	NNRTI (n=805)
Age, median (range), y	36 (30-42)	38 (32-45)	38 (32-44)
Male	300 (61)	371 (72)	551 (68)
Transmission category			
Homosexual-bisexual contact	120 (24)	197 (38)	269 (33)
Heterosexual contact	246 (50)	222 (43)	400 (50)
Injection drug use	107 (22)	69 (13)	101 (12)
Other	16 (3)	30 (6)	35 (4)
CDC stage C	89 (18)	120 (23)	157 (20)
Year of cART initiation, median (IQR)	2000 (1999-2001)	2003 (2002-2004)	2002 (2000-2004)
CD4 cell count at cART initiation			
Median (IQR)	198 (108-334)	182 (76.5-304)	209 (124-297)
<200 cells/ μ L	203 (42)	233 (45)	321 (40)
Missing	84 (17)	78 (15)	126 (16)
HIV RNA at cART initiation			
Median log (IQR)	4.76 (4.11-5.20)	5.08 (4.58-5.59)	4.90 (4.38-5.34)
\geq 100 000 copies/mL	133 (27)	234 (45)	285 (35)
Missing	98 (20)	100 (19)	149 (18)
Adherence			
100	116 (74)	332 (81)	459 (77)
95-99	35 (22)	75 (18)	123 (21)
<95	5 (3)	4 (1)	14 (2)
Missing	333 (68)	107 (21)	209 (26)
NRTI			
Zidovudine-lamivudine	316 (65)	359 (69)	552 (69)
Stavudine-lamivudine	96 (20)	21 (4)	52 (6)
Didanosine-stavudine	50 (10)	12 (2)	28 (4)
Tenofovir disoproxil fumarate-emtricitabine	0	28 (5)	26 (3)
Tenofovir-lamivudine	4 (0.8)	60 (12)	102 (13)
Abacavir sulfate-containing combination	10 (2)	15 (3)	14 (2)
Other	13 (3)	23 (4)	31 (4)
Frequency of lamivudine- or emtricitabine-containing backbone combinations	430 (88)	493 (95)	762 (95)
Third drug (PI or NNRTI)			
Nelfinavir mesylate	430 (88)	0 (0)	0
Indinavir sulfate	24 (5)	56 (11)	0
Atazanavir sulfate	21 (4)	46 (9)	0
Lopinavir	0	391 (76)	0
Efavirenz	0	0	736 (91)
Other	14 (3)	25 (5)	69 (9)

Abbreviations: cART, combination antiretroviral treatment; CDC, Centers for Disease Control and Prevention; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated.

RESULTS

Between January 1, 1999, and December 31, 2005, 2751 study participants in the SHCS initiated therapy with cART, of which 939 patients (34%) were excluded from our primary analyses because of short treatment duration with the same regimen or missing HIV RNA values. Patient characteristics associated with exclusion were following a PI-based therapy, use of injection drugs, female sex, or having higher CD4 cell counts, all characteristics known to be associated with premature therapy discontinuation.^{20,21} The second treatment was considered for 141 patients (44 in the PI group, 48 in the PI/r group, and 49 in the NNRTI group). Of the remaining 1812 patients who contributed 3789 person-years to the competing risk analysis, 489 (27%) initiated cART with unboosted PIs, 518

(29%) with boosted PIs, and 805 (44%) with NNRTIs as the group-specific third drug. A median of 5.7 viral load values per patient per year were obtained.

Baseline characteristics at the time of cART initiation for the 1812 patients are given in **Table 1**. Of note, patients who initiated cART with boosted PIs in general had a lower CD4 cell count and a higher HIV viral load at baseline compared with the PI group and the NNRTI group. Most patients received lamivudine as part of their treatment, ranging from 88% in the unboosted PI group to 95% in the 2 other groups. The most common third drug in the 3 groups was unboosted nelfinavir mesylate (88%), boosted lopinavir (76%), and efavirenz (91%).

In total, 177 patients experienced a virological failure (108 in the unboosted PI group, 24 in the boosted PI/r group, and 45 in the NNRTI group). For those pa-

Table 2. Class Resistance Patterns in 177 Patients in Whom First-Line cART Failed Virologically

Pattern	No. (%) of Patients ^a			P Value
	PI (n=108)	PI/r (n=24)	NNRTI (n=45)	
Resistance test at baseline ^b	64 (59)	14 (58)	34 (76)	
Resistance test at failure	84 (78)	20 (83)	38 (84)	
HIV-1 subtype available	95 (88)	21 (88)	44 (98)	
Subtype B	59 (62)	13 (62)	25 (57)	
Subtype other than B	36 (38)	8 (38)	19 (43)	
No. of IAS-USA mutations, median (IQR)	2 (1-2.5)	0 (0-1)	2 (0-3)	<.001 ^c
No. of drug classes affected (intermediate or high-level resistance against at least 1 drug according to Stanford algorithm), median (IQR)	2 (1-2)	0 (0-1)	2 (0-2)	<.001 ^c
Virus susceptible to all drugs in class according to Stanford algorithm				
NRTI	72 (86)	18 (90)	30 (79)	.46 ^d
Lamivudine or emtricitabine	23 (27)	15 (75)	16 (42)	<.001 ^d
Third drug	40 (48)	18 (90)	20 (53)	<.001 ^d
Drug classes affected (high-level resistance against at least 1 drug per class according to Stanford algorithm)				
By class type				
NRTI	2 (2)	1 (5)	3 (8)	.27 ^d
Lamivudine or emtricitabine	61 (73)	5 (25)	22 (58)	<.001 ^d
Third drug	17 (20)	1 (5)	21 (55)	<.001 ^d
By class combination				
No resistance	18 (21)	15 (75)	13 (34)	
Lamivudine or emtricitabine (1 class)	48 (57)	3 (15)	4 (10)	
NRTI (1 class)	1 (1)	0	0	
Third drug (1 class)	3 (4)	0	2 (5)	
Lamivudine or emtricitabine plus NRTI (2 classes)	0	1 (5)	0	
Lamivudine or emtricitabine plus third drug (2 classes)	13 (16)	1 (5)	16 (42)	
NRTI plus third drug (2 classes)	1 (1)	0	1 (3)	
Lamivudine or emtricitabine plus NRTI plus third drug (3 classes)	0	0	2 (5)	

Abbreviations: cART, combination antiretroviral treatment; HIV-1, human immunodeficiency virus 1; IAS-USA, International AIDS Society–USA; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated. Percentages may not total 100% because the numbers of patients who had at least 1 resistance mutation, the numbers of patients with NRTI, lamivudine, and third drug mutations, and the numbers of patients without high resistance against NRTI, lamivudine, or the third drug are not shown.

^bThe following baseline resistance mutations were observed: PI group: 1 patient with reverse transcriptase gene (*RT*) 219Q, 1 patient with *RT* 41L and 210W, 1 patient with *RT* 65R, and 1 patient with *PR* 33F; PI/r group: 1 patient with *RT* 103N; and NNRTI group: 1 patient with *RT* 103N.

^cKruskal-Wallis test.

^dFisher exact test.

tients, 112 baseline resistance test results (64 [59%], 14 [58%], and 34 [76%] of patients in the PI, PI/r, and NNRTI groups, respectively; **Table 2**) were available, and pre-existing resistance was detected in 6 samples (5%). Resistance test results at the time of virological failure could be obtained for 142 patients (84 [78%], 20 [83%], and 38 [84%] in the PI, PI/r, and NNRTI groups, respectively) **Table 3**. At least 1 relevant resistance mutation was detected in 71 of the 84 patients (84%; 95% confidence interval [CI], 75%-92%) in the unboosted PI group, 6 of 20 patients (30%; 95% CI, 12%-54%) in the boosted PI/r group, and 25 of 38 (66%; 95% CI, 49%-80%) in the NNRTI group (Fisher exact test, $P < .001$; **Figure 1**). Patients in the unboosted PI and NNRTI groups acquired a median of 2 mutations during treatment (interquartile range [IQR], 1-2.5 and 0-3, respectively), whereas the median number of mutations in the boosted PI group was 0 (IQR, 0-1) (Kruskal-Wallis test, $P < .001$; **Figure 1**).

A similar pattern was observed when resistance against the 4 drug classes (NRTI, lamivudine or emtricitabine, NNRTI, and PI) was compared using the Stanford algorithm, whereby intermediate or high resistance against 1

or more drugs in a class signified class resistance. We observed that more classes were affected in unboosted PI-based (median, 2; IQR, 1-2) and NNRTI-based (median, 2; IQR, 0-2) regimens compared with boosted PI-based regimens (median, 0; IQR, 0-1; Kruskal-Wallis test, $P < .001$; **Figure 1**). This difference remained statistically significant when only high-level resistance was considered (data not shown). High-level resistance against lamivudine or emtricitabine was observed in 73% (95% CI, 62%-82%) of the PI group, 25% (95% CI, 9%-49%) of the PI/r group, and 58% (95% CI, 41%-74%) of the NNRTI group (Fisher exact test; $P < .001$). High-level resistance against the respective third drug was most prevalent in the NNRTI group at 55% (95% CI, 38%-71%), followed by the unboosted PI group at 20% (95% CI, 12%-30%) and the boosted PI group at 5% (95% CI, 0%-25%) (Fisher exact test, $P < .001$). Multidrug resistance predominantly occurred as resistance against the third drug and against lamivudine or emtricitabine and was also more frequent in the NNRTI group (50%; 95% CI, 33%-67%) than in the PI group (17%; 95% CI, 9%-26%) or PI/r group (10%; 95% CI, 0.1%-32%, respectively) (Fisher exact test, $P < .001$).

Table 3. International AIDS Society–USA Mutations in 177 Patients in Whom First-Line cART Failed

Mutation	No. (%) of Patients		
	PI (n=108)	PI/r (n=24)	NNRTI (n=45)
No. tested	84 (78)	20 (83)	38 (84)
NRTI or lamivudine or emtricitabine			
<i>RT</i> gene			
41L	4 (5)	1 (5)	1 (3)
62V	5 (6)	0	1 (3)
65R	0	0	3 (8)
67N	8 (10)	0	1 (3)
70R/E	5 (6)	1 (5)	1 (3)
75I	0	0	1 (3)
115F	0	0	1 (3)
184I/V	61 (73)	5 (25)	22 (58)
210W	0	1 (5)	1 (3)
215F/Y	3 (4)	1 (5)	4 (10)
219E/Q	2 (2)	0	1 (3)
Any <i>TAM1</i>	4 (5)	1 (5)	3 (8)
Any <i>TAM2</i>	12 (14)	1 (5)	2 (5)
Third drug (NNRTI or PI)			
<i>RT</i> gene			
103N	0	0	10 (26)
106A/M	0	0	1 (3)
108I	0	0	3 (8)
181C	0	0	5 (13)
188C	0	0	1 (3)
190A	0	1 (5)	2 (5)
225H	0	0	2 (5)
Protease gene			
30N	18 (21)	0	0
32I	1 (1)	0	0
33F	1 (1)	1 (5) ^a	0
46I/L	11 (13)	2 (10) ^a	1 (3)
82A/F/T/S/L	0	1 (5) ^a	0
84V	1 (1)	0	0
88S	12 (14)	0	0
90M	13 (15)	0	0
Any third drug	44 (52)	2 (10)	18 (47)

Abbreviations: cART, combination antiretroviral treatment; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor; *RT*, reverse transcriptase; *TAM1*, any *RT* gene mutation of the following: 41L, 210W, 215Y; *TAM2*, any *RT* gene mutation of the following: 67N, 70R, 215F, 219Q.

^aThe 46L and the 82A mutations were newly selected during therapy with boosted indinavir sulfate. The baseline sequence was available to exclude the presence of transmitted resistance.

We further assessed rates of virological failure and adverse events in the different treatment groups (**Figure 2**). Analyzing the 1812 included patients with respect to virological failure events, we found that crude rates (per 100 person-years) were highest in the unboosted PI group (10.3; 95% CI, 8.5-12.4) and comparable between the boosted PI group (2.7; 95% CI, 1.8-4.0) and the NNRTI group (2.4; 95% CI, 1.8-3.3; log-rank $P < .001$; **Table 4**). A multivariate Cox regression adjusted for potential confounders confirmed this finding. Using the NNRTI group as a reference, hazard ratios were 1.19 (95% CI, 0.71-2.0) for the boosted PI group and 3.83 (95% CI, 2.62-5.59) for the unboosted PI group.

Crude rates for toxic effects related to cART were higher in the PI-based regimens (unboosted PIs: 8.9; 95% CI,

7.3-10.9; boosted PIs: 10.9; 95% CI, 9.0-13.3) compared with 7.8 (95% CI, 6.6-9.2) in the group taking NNRTIs (log-rank test; $P = .03$). When considering both types of events (virological failure and toxic effects) simultaneously, patients taking unboosted PIs had the highest rate at 19.2 (95% CI, 16.7-22.1) followed by boosted PIs (13.6; 95% CI, 11.4-16.3) and NNRTIs (10.3; 95% CI, 8.9-11.8; log-rank test; $P < .001$), which was consistent with results from the multivariate competing risk Cox model. Compared with the NNRTI group, relative hazards for experiencing either a virological failure or a cART-related toxic effect were 1.36 (95% CI, 1.07-1.73) and 1.75 (95% CI, 1.41-2.18) for patients taking boosted PIs and unboosted PIs, respectively. When repeating the competing risk analysis including all patients who had started cART (intent to treat, $n = 2751$), we obtained a lower hazard ratio for the unboosted PI group (1.24; 95% CI, 1.05-1.46) but similar estimates for the boosted PI group (1.28; 95% CI, 1.07-1.52; **Table 4**). Reasons for premature therapy discontinuation of the 939 patients additionally included in the intent-to-treat analysis are shown in **Table 5**.

We also looked at subsequent virological failures in 1001 of 1812 patients (55%; including 141 of 177 patients in whom first-line therapy failed) who initiated a new treatment after stopping first-line therapy and who were not lost to follow-up (107; 6%). We observed that patients in whom first-line therapy had already failed virologically were more likely to experience second-line therapy failure (18 cases; incidence rate, 10.4 per 100 person-years; 95% CI, 6.6-16.5) compared with patients without previous failure (27 patients; incidence rate, 2.4 per 100 person-years; 95% CI, 1.7-3.6; log-rank test; $P < .001$). Because of the small number of cases, this analysis could not be stratified further.

COMMENT

In this study, we observed clinically relevant differences in the emergence of drug resistance for the 3 types of initial regimens. Although cART-containing boosted PIs and NNRTIs appeared to be equivalent with regard to the virological failure rate (ie, 2.7 and 2.4 per 100 person-years, respectively), significantly more resistance emerged in the NNRTI group at virological failure. Virus strains resistant against lamivudine or emtricitabine were present in 58% of patients with virological failure in the NNRTI group, and 55% of all viral samples in this group exhibited resistance against NNRTI. Even more worrisome, multidrug resistance against NNRTIs and at least 1 component of NRTIs and lamivudine or emtricitabine was observed in half of all patients with virological failure in this group (**Table 2**). Nonetheless, NNRTI-containing combinations showed better tolerability than PI-based regimens. Thus, our analysis demonstrates that despite similar potency, boosted PIs are superior to more tolerable NNRTI regimens when comparing the emergence of drug resistance in patients in whom therapy is failing.

The low genetic barrier of NNRTIs and the high potential for cross-resistance in this drug class have long been known from in vitro experiments and clinical trials,^{2,14,22}

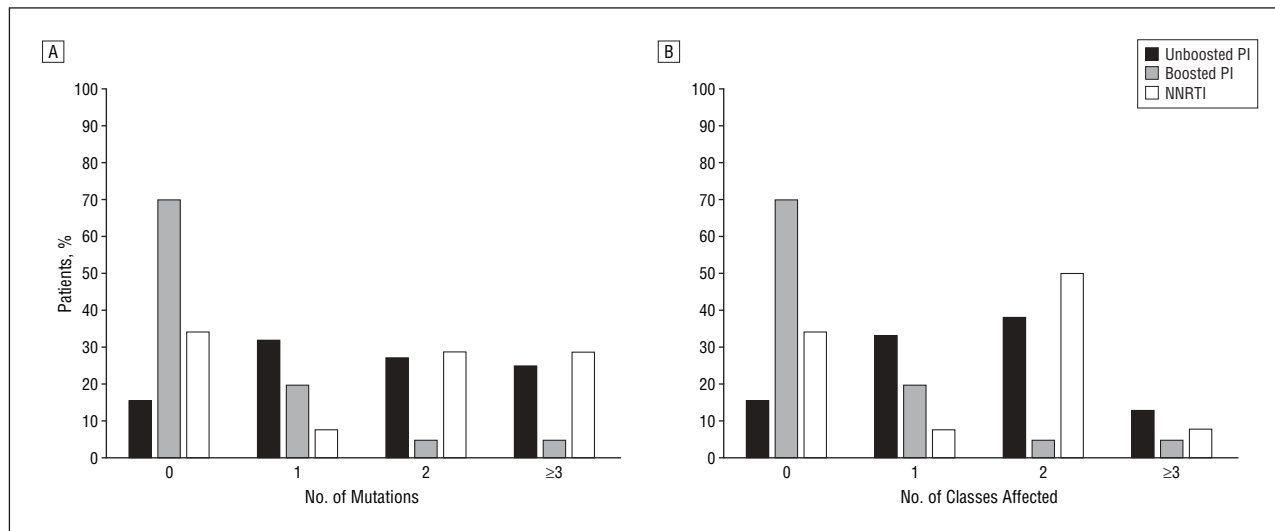


Figure 1. Drug resistance in patients experiencing virological failure of first-line combination antiretroviral treatment for whom genotypic resistance test results could be retrieved (142/177 [80%]). A, Number of International AIDS Society–USA mutations detected (excluding minor protease mutations). B, Frequency of class resistance, defined as at least 1 drug with a genotypic sensitivity score greater than or equal to 15 according to the Stanford algorithm. NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

but the impact of those aspects on the emergence of drug resistance in routine clinical practice and the consequences for future therapies have rarely been investigated. In a retrospective cohort analysis, Phillips et al⁶ reported a significantly higher risk of acquiring third drug-specific resistance and multidrug resistance for patients initiating cART with NNRTI compared with boosted PI. Preliminary results from AIDS Clinical Trial Group 5142 also indicated more drug resistance accumulation in NNRTI-based regimens compared with boosted PI regimens.⁸ However, no differences in virological failure and/or stopping because of toxic effects between NNRTI-based and boosted PI-based regimen types were observed.

Our results confirm and extend these previous observations. We found that virological failure of NNRTI-containing regimens led to the emergence of multidrug resistance in half of all cases, 5 times more often than in the boosted PI group. In addition, we observed that almost all cases of multiclass resistance (with 1 exception in the PI group and 1 in the NNRTI group; Table 2) involved the reverse transcriptase mutation *M184V/I*, whereas high-level resistance against NRTIs other than lamivudine or emtricitabine remained rare. Hence, our data demonstrate that NNRTI-based regimens carry a markedly higher risk of losing multiple drug classes in case of failure.

The inclusion of competing risks in our time-to-event analysis has allowed us to gain a more accurate picture of the success of initial cART. The differences in hazard ratios between the competing risk approach and the conventional approach of estimating Cox models imply that the benefits of higher potency of newer regimens compared with unboosted PIs are partially outweighed by the higher frequency of adverse events, especially in boosted PI-containing regimens. This loss of benefit was even more pronounced in the intent-to-treat analysis, in which event rates and relative hazards were no longer different between boosted and unboosted PIs. Further analyses showed that patients following boosted PI regimens had more adverse events related to cART in the first 180 days

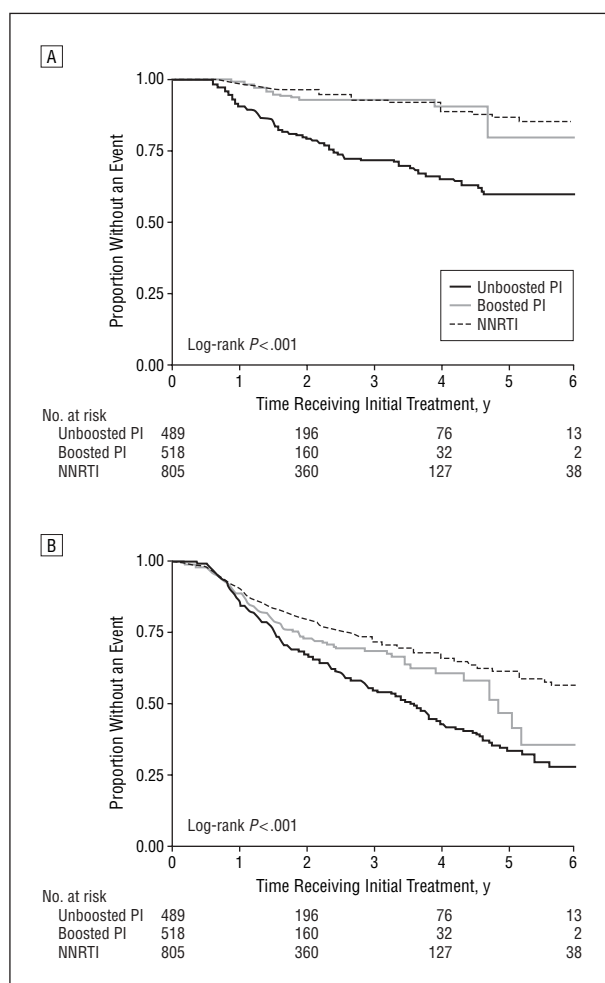


Figure 2. Kaplan-Meier curves for time to first-line therapy discontinuation. A, Therapy discontinuation because of virological failure. B, Therapy discontinuation because of virological failure and combination antiretroviral treatment-related adverse events. NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor. For more detailed group comparisons, see Table 4.

Table 4. Summary of Time-to-Event Analyses

Variable	No. (%) of Patients ^a			P Value
	PI	PI/r	NNRTI	
As-treated analysis (n = 1812)	489 (27)	518 (29)	805 (44)	ND
Median time undergoing first-line regimen, d (IQR)	489 (346-1092)	514.5 (327-835)	655 (364-1154)	<.001 ^b
Crude incidence rate of failures per 100 person-years (95% CI)	10.3 (8.5-12.4)	2.7 (1.8-4.0)	2.4 (1.8-3.3)	<.001 ^c
Treatment failures	108 (22)	24 (5)	45 (6)	ND
Crude incidence rate of adverse events per 100 person-years (95% CI)	8.9 (7.3-10.9)	10.9 (9.0-13.3)	7.8 (6.6-9.2)	.03 ^c
Treatment stopped because of cART-related adverse events (all)	94 (19)	98 (19)	144 (18)	ND
Abnormal fat distribution	27 (29)	9 (9)	18 (12)	
Concern of CVD	13 (14)	20 (20)	8 (6)	
Hypersensitivity reaction	1 (1)	1 (1)	2 (1)	
Toxic effects to abdomen or gastrointestinal tract	31 (33)	31 (32)	19 (13)	
Toxic effects to nervous system	9 (10)	3 (3)	64 (44)	
Toxic effects to kidneys	0 (0)	16 (16)	2 (1)	
Hematological toxic effects	1 (1)	9 (9)	11 (8)	
Lactate elevation	0	0	1 (0.7)	
Other toxic effects	12 (13)	9 (9)	19 (13)	
Crude incidence rate of stopping because of virological failure or adverse events per 100 person-years (95% CI)	19.2 (16.7-22.1)	13.6 (11.4-16.3)	10.3 (8.9-11.8)	<.001 ^c
Any event (failures or adverse events)	202 (41)	122 (24)	189 (23)	ND
Relative hazard of stopping cART because of adverse events or virological failure (competing risk analysis) (95% CI) ^d	1.75 (1.41-2.18)	1.36 (1.07-1.73)	1 [Reference]	NA
Intent-to-treat analysis (n=2751)	822 (30)	792 (29)	1137 (41)	NA
Crude incidence rate of stopping because of virological failure or adverse events per 100 person-years (95% CI)	25.0 (22.3-28.0)	25.9 (22.9-29.4)	18.4 (16.5-20.4)	.001 ^c
Any event (failures or adverse events)	294 (36)	249 (31)	358 (32)	NA
Relative hazard of stopping cART because of adverse events or virological failure (competing risk analysis) (95% CI) ^d	1.24 (1.05-1.46)	1.28 (1.07-1.52)	1 [Reference]	NA

Abbreviations: cART, combination antiretroviral treatment; CI, confidence interval; CVD, cardiovascular disease; IQR, interquartile range; NA, not applicable; ND, not done; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated. The on-treatment analysis includes patients with at least 1 additional HIV RNA measurement after the attainment of undetectable HIV viral loads or, if not the case, patients with at least 1 HIV RNA measurement after a minimum of 180 days of continuous treatment with first-line cART. The intent-to-treat analysis includes all patients who initiated cART between January 1, 1999, and December 31, 2005, with combination antiretroviral therapy.

^bKruskal-Wallis test.

^cLog-rank test.

^dCox model adjusted for age, sex, ethnicity, transmission category, baseline CD4 cell counts, and human immunodeficiency virus (HIV) RNA at time of cART initiation.

of therapy (39.4 per 100 person-years; 95% CI, 33.1-46.9) than any of the other groups, with 33.3 (95% CI, 28.7-38.7) and 25.0 (95% CI, 20.3-30.7) in patients taking NNRTIs and PIs, respectively. Overall, NNRTIs were generally better tolerated, and patients following those regimens continued with their initial therapy for a longer period in both the on-treatment analysis and the intent-to-treat analysis.

This study has some limitations. Potentially because of the somewhat shorter follow up-time, relatively few virological failures occurred in the group starting cART with newer regimen types and especially boosted PIs, but our results are largely consistent with other reports on resistance development.⁴ Like all observational studies, this analysis is prone to confounding. The time point of resistance testing might have affected our results because it has been shown that the longer maintenance of failing regimens leads to more resistance.²³ However, sensitivity analyses neither revealed a systematic confounding of our results by the time (per month) between presumed date of virological failure and the sampling date for resistance testing (odds ratio, 1.01; 95% CI, 0.97-

1.06) nor indicated any influence of viral load levels (per log increase) at the time of testing (OR, 0.94; 95% CI, 0.69-1.29).

Furthermore, studies²⁴ have demonstrated detrimental effects of transmitted resistance mutations on treatment success. In the present analysis, pre-cART resistance tests were available for 63% of all patients with a virological failure, and we therefore can not fully exclude potential confounding of our results by baseline resistance.

Another limitation of this study compared with clinical trials is the high variation of drug combinations within the 3 treatment groups. To verify our findings, we limited the time-to-event analyses to the most frequent drugs in the boosted PI group and the NNRTI group (lopinavir [76%] and efavirenz [91%]), which yielded similar results for the comparison of virological failure rates. For the competing risk analysis, however, the previously observed difference in the hazard ratios between boosted PIs and NNRTIs disappeared. A secondary analysis showed that this difference was mainly because of the exclusion of boosted indinavir sulfate, which exhibited

Table 5. Reasons for Premature Therapy Discontinuation in 939 Patients Treated for Less Than 6 Months or With Missing Human Immunodeficiency Virus Viral Load Values and Excluded From the Primary Risk Analyses

Reason	No. (%) of Patients		
	PI (n=333)	PI/r (n=274)	NNRTI (n=332)
Treatment stop because of cART-related adverse events (all)	92 (28)	127 (46)	169 (51)
Abnormal fat distribution	1 (1)	2 (2)	1 (0)
Concern of CVD	0	0	2 (1)
Hypersensitivity reaction	5 (5)	9 (7)	26 (15)
Toxicity from abdomen or gastrointestinal tract	27 (29)	55 (43)	28 (17)
Toxic effects to nervous system	4 (4)	3 (2)	44 (26)
Toxic effects to kidneys	2 (2)	12 (9)	3 (2)
Toxic effects to endocrine system	0	0	1 (0.6)
Hematological toxic effects	6 (6)	12 (9)	20 (12)
Other toxic effects (not further specified)	47 (51)	34 (27)	44 (26)
Treatment failure (virological, immunological, or clinical)	33 (10)	6 (2)	11 (3)
Patient's wish or physician's decision (not further specified)	92 (28)	74 (27)	81 (24)
Patient deceased	3 (0.9)	4 (2)	6 (2)
Unknown reason	113 (34)	63 (23)	65 (20)

Abbreviations: cART, combination antiretroviral treatment; CVD, cardiovascular disease; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

a far higher rate of treatment discontinuation caused by toxic effects (29.5 per 100 person-years; 95% CI, 20.4-42.7) compared with the remaining third drugs in the boosted PI group (8.7; 95% CI, 6.9-11.1). This finding implies that the apparent tolerability disadvantage of boosted PI compared with the NNRTI regimen might not be as pronounced or even absent with newer, more tolerable combinations that contain boosted atazanavir sulfate or lopinavir.

Previous studies²⁵ have demonstrated associations of adherence and pharmacokinetic drug levels with virological failure and the emergence of drug resistance. Systematic collection of adherence information in the SHCS started in the middle of 2003.¹⁹ Since we do not have complete assessments of adherence for all patients, we performed a sensitivity analysis by confining our time-to-event analyses to patients with at least 1 adherence measure while receiving first-line cART (n=927, data not shown). Of note, our findings were not altered by this, and we are therefore confident that the observed differences in virological failure rates truly reflect drug potency. Therapeutic drug monitoring has been introduced only recently in the SHCS, so systematic analyses of therapeutic drug monitoring are not possible at this time.

In summary, our findings have potentially important clinical implications. Given the risk of severely compromising future therapy options in case of virological failure while taking NNRTIs and the equivalent potency of boosted PI regimens, physicians should critically assess a patient's ability to adhere to NNRTI-based regimens and

to cope with the potential toxic effects, such as adverse effects on the central nervous system. If problems are expected, starting or changing to PI/r therapy most likely represents a safer choice, since a PI/r tends to accumulate less resistance in case of virological failure. Should our findings be confirmed, they should be implemented in treatment guidelines and also be considered for future treatment strategies in developing countries.

Accepted for Publication: April 17, 2007; final revision received April 24, 2007; accepted May 7, 2007.

Author Affiliations: Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich (Mr von Wyl and Drs Ledergerber and Günthard); Laboratory of Virology and AIDS Center (Ms Yerly and Dr Perrin) and Division of Infectious Diseases (Dr Hirschel), Geneva University Hospital, Geneva; National Center for Retroviruses, University of Zurich, Zurich (Dr Böni); Division of Immunology (Dr Bürgisser), and Institute of Microbiology (Dr Telenti), Lausanne University Hospital, University of Lausanne, Lausanne; Institute for Medical Microbiology (Dr Klimkait) and Division of Infectious Diseases and Hospital Epidemiology (Dr Battegay), University Hospital Basel, University of Basel, Basel; Division of Infectious Diseases, University Hospital Berne, Berne (Dr Furrer); Division of Infectious Diseases, Cantonal Hospital St Gallen, St Gallen (Dr Vernazza); Division of Infectious Diseases, Regional Hospital Lugano, Lugano (Dr Bernasconi); and Swiss HIV Cohort Study Data Center, Lausanne (Dr Rickenbach), Switzerland.

Correspondence: Huldrych F. Günthard, MD, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Rämistr 100, CH-8091 Zurich, Switzerland (huldrych.guenthard@usz.ch).

Author Contributions: Viktor von Wyl and Huldrych Günthard had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Günthard. *Acquisition of the data:* Böni, Bürgisser, Klimkait, Yerly, Battegay, Furrer, Telenti, Hirschel, Vernazza, Bernasconi, Rickenbach, and Perrin. *Analysis and interpretation of the data:* von Wyl, Ledergerber, and Günthard. *Drafting of the manuscript:* von Wyl, Ledergerber, and Günthard. *Critical revision of the manuscript for important intellectual content:* Böni, Bürgisser, Klimkait, Yerly, Battegay, Furrer, Telenti, Hirschel, Vernazza, Bernasconi, Rickenbach, and Perrin. *Statistical analysis:* von Wyl and Ledergerber. *Obtained funding:* Günthard, Yerly, and Ledergerber. *Study supervision:* Günthard.

Swiss HIV Cohort Study Members: Heiner Bucher, MD; Sandro Cattacin, PhD; Matthias Cavassini, MD; Rolf Dubs, PhD; Matthias Egger, MD; Lucia Elzi, MD; Peter Erb, PhD; Marek Fischer, PhD; Markus Flepp, MD; Adriano Fontana, MD; Patrick Francioli, MD (president of the SHCS, Centre Hospitalier Universitaire Vaudois, Lausanne); Meri Gorgievski, PhD; Hans Hirsch, MD; Irene Hösli, MD; Christian Kahlert, MD; Laurent Kaiser, MD; Urs Karrer, MD, PhD; Olivia Keiser, MSc; Christian Kind, MD; Gina Martinetti, MD; Begogna Martinez, MD; Nicolas Müller, MD;

David Nadal, MD; Milos Opravil, MD; Fred Paccaud, MD; Giuseppe Pantaleo, MD; Christoph Rudin, MD (chairman of the Mother & Child Substudy); Patrick Schmid, MD; Detlev Schultze, PhD; Jörg Schüpbach, MD; Roberto Speck, MD; Patrick Taffé, PhD; Philippe Tarr, MD; Alexandra Trkola, PhD; and Rainer Weber, MD.

Financial Disclosures: Dr Klimkait has served on the advisory boards of Abbott, Bayer, Bristol-Myers Squibb, and Roche. Dr Battegay is a consultant for Roche Pharma Switzerland and Boehringer Ingelheim Switzerland. Drs Furrer and Vernazza have served on the advisory boards of Abbott, GlaxoSmithKline, Bristol-Myers Squibb, Roche, Gilead, Merck Sharp & Dohme, Boehringer-Ingelheim, and Tibotec (Dr Vernazza). The institution of Dr Furrer has received unrestricted educational grants from Abbott, GlaxoSmithKline, Bristol-Myers Squibb, Roche, Gilead, Merck Sharp & Dohme, and Boehringer-Ingelheim. Dr Bernasconi has received travel grants and honoraria from Gilead, Roche, GlaxoSmithKline, Pfizer, Boehringer Ingelheim, and Tibotec. Dr Ledergerber has received travel grants, grants, or honoraria from Abbott, Aventis, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck Sharp & Dohme, Roche, and Tibotec. Dr Günthard has been an adviser and/or consultant for GlaxoSmithKline, Abbott, Novartis, Boehringer Ingelheim, Roche, and Bristol-Myers Squibb and has received unrestricted research and educational grants from Roche, Abbott, Bristol-Myers Squibb, GlaxoSmithKline, and Merck Sharp & Dohme.

Funding/Support: This study has been financed in the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (SNF) (grant 3345-062041). Further support was provided by SNF grant 3247B0-112594/1 (Drs Günthard and Ledergerber and Ms Yerly) and SHCS project 470.

Role of the Sponsors: The funding sources had no role in study design; in collection, analysis, or interpretation of data; or in the writing of the report.

Previous Presentation: Results of this study were presented in part at the 15th International Drug Resistance Workshop; June 15, 2006; Sitges, Spain (abstract 72); and the 14th Conference on Retroviruses and Opportunistic Infections; February 26, 2007; Los Angeles, California (abstract M-102).

Additional Information: All sequences have been submitted to GenBank (accession Nos. EF449774 to EF450027).

Additional Contributions: Chantal Gaille (Laboratory of Virology and AIDS Center, Geneva University Hospital) provided the retrospective sequencing, SmartGene provided technical support, and Joseph Wong, MD, performed critical review of the manuscript. We thank the patients for participation in the SHCS, the physicians and study nurses for excellent patient care, and the laboratory technicians of the Swiss resistance laboratories for the quality of the data.

REFERENCES

1. Hammer SM, Saag MS, Schechter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *JAMA*. 2006; 296(7):827-843.
2. Delaugerre C, Rohban R, Simon A, et al. Resistance profile and cross-resistance of HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor-containing regimen. *J Med Virol*. 2001;65(3):445-448.
3. Whitcomb JM, Parkin NT, Chappay C, Hellmann NS, Petropoulos CJ. Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates. *J Infect Dis*. 2003;188(7):992-1000.
4. Kempf DJ, King MS, Bernstein B, et al. Incidence of resistance in a double-blind study comparing lopinavir/ritonavir plus stavudine and lamivudine to nelfinavir plus stavudine and lamivudine. *J Infect Dis*. 2004;189(1):51-60.
5. Gange SJ, Schneider MF, Grant RM, et al. Genotypic resistance and immunologic outcomes among HIV-1-infected women with viral failure. *J Acquir Immune Defic Syndr*. 2006;41(1):68-74.
6. Phillips AN, Dunn D, Sabin C, et al. Long term probability of detection of HIV-1 drug resistance after starting antiretroviral therapy in routine clinical practice. *AIDS*. 2005;19(5):487-494.
7. Zolopa AR, Shafer RW, Warford A, et al. HIV-1 genotypic resistance patterns predict response to saquinavir-ritonavir therapy in patients in whom previous protease inhibitor therapy had failed. *Ann Intern Med*. 1999;131(11):813-821.
8. Riddler SA, Haubrich R, DiRienzo G, et al. A prospective, randomized, phase III trial of NRTI-, PI-, and NNRTI-sparing regimens for initial treatment of HIV-1 infection—ACTG 5142. In: Program and abstract of the 16th International AIDS Conference; August 13-18, 2006; Toronto, Ontario.
9. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. *Lancet*. 1999;353(9156):863-868.
10. Ledergerber B, von Overbeck J, Egger M, Luthy R. The Swiss HIV Cohort Study: rationale, organization and selected baseline characteristics. *Soz Präventivmed*. 1994;39(6):387-394.
11. Yerly S, Vora S, Rizzardi P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. *AIDS*. 2001;15(17):2287-2292.
12. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res*. 2003;31(1):298-303.
13. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: fall 2006. *Top HIV Med*. 2006;14(3):125-130.
14. Boucher CA, Cammack N, Schipper P, et al. High-level resistance to (-) enantiomeric 2'-deoxy-3'-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob Agents Chemother*. 1993;37(10):2231-2234.
15. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995; 51(2):524-532.
16. Tai BC, Machin D, White I, GebSKI V. Competing risks analysis of patients with osteosarcoma: a comparison of four different approaches. *Stat Med*. 2001; 20(5):661-684.
17. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982;69(1):239-241.
18. Friis-Møller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med*. 2003;349(21):1993-2003.
19. Glass TR, De Geest S, Weber R, et al. Correlates of self-reported nonadherence to antiretroviral therapy in HIV-infected patients: the Swiss HIV Cohort Study. *J Acquir Immune Defic Syndr*. 2006;41(3):385-392.
20. d'Arminio Monforte A, Lepri AC, Rezza G, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naive patients. *AIDS*. 2000;14(5):499-507.
21. Mocroft A, Youle M, Moore A, et al. Reasons for modification and discontinuation of antiretrovirals: results from a single treatment centre. *AIDS*. 2001;15(2):185-194.
22. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol*. 1994; 68(3):1660-1666.
23. Kantor R, Shafer RW, Follansbee S, et al. Evolution of resistance to drugs in HIV-1-infected patients failing antiretroviral therapy. *AIDS*. 2004;18(11):1503-1511.
24. DeGruttola V, Dix L, D'Aquila R, et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. *Antivir Ther*. 2000; 5(1):41-48.
25. Harrigan PR, Hogg RS, Dong WW, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. *J Infect Dis*. 2005;191(3):339-347.