REVIEW

Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-Based Antiretroviral Treatment Failure

A Systematic Review and Pooled Analysis

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Context Presence of low-frequency, or minority, human immunodeficiency virus type 1 (HIV-1) drug resistance mutations may adversely affect response to antiretroviral treatment (ART), but evidence regarding the effects of such mutations on the effectiveness of first-line ART is conflicting.

Objective To evaluate the association of preexisting drug-resistant HIV-1 minority variants with risk of first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)–based antiretroviral virologic failure.

Data Sources Systematic review of published and unpublished studies in PubMed (1966 through December 2010), EMBASE (1974 through December 2010), conference abstracts, and article references. Authors of all studies were contacted for detailed laboratory, ART, and adherence data.

Study Selection and Data Abstraction Studies involving ART-naive participants initiating NNRTI-based regimens were included. Participants were included if all drugs in their ART regimen were fully active by standard HIV drug resistance testing. Cox proportional hazard models using pooled patient-level data were used to estimate the risk of virologic failure based on a Prentice weighted case-cohort analysis stratified by study.

Data Synthesis Individual data from 10 studies and 985 participants were available for the primary analysis. Low-frequency drug resistance mutations were detected in 187 participants, including 117 of 808 patients in the cohort studies. Low-frequency HIV-1 drug resistance mutations were associated with an increased risk of virologic failure (hazard ratio (HR], 2.3 [95% confidence interval {CI}, 1.7-3.3]; $P < .001$) after controlling for medication adherence, race/ethnicity, baseline CD4 cell count, and plasma HIV-1 RNA levels. Increased risk of virologic failure was most strongly associated with minority variants resistant to NNRTIs (HR, 2.6 [95% CI, 1.9-3.5]; $P < .001$). Among participants from the cohort studies, 35% of those with detectable minority variants experienced virologic failure compared with 15% of those without minority variants. The presence of minority variants was associated with 2.5 to 3 times the risk of virologic failure at either 95% or greater or less than 95% overall medication adherence. A dose-dependent increased risk of virologic failure was found in participants with a higher proportion or quantity of drug-resistant variants.

Conclusion In a pooled analysis, low-frequency HIV-1 drug resistance mutations, particularly involving NNRTI resistance, were significantly associated with a dose-dependent increased risk of virologic failure with first-line ART.

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GENOTYPIC TESTS FOR HUMAN immunodeficiency virus type 1 (HIV-1) drug resistance use polymerase chain reaction (PCR) amplification and population sequencing techniques that detect resistance-associated mutations present in at least 15% to 25% of the viral population.[1,2] Using these traditional assays, the prevalence of transmitted drug resistance mutations is estimated to be between 8% and 16% among HIV-1 infected persons in North America and Europe.[3,4] These assays fail to detect the presence of low-frequency, or minority, drug resistance mutations within the population of HIV-1 quasispecies in an infected individual. Compared with standard population sequencing, a number of ultrasensitive assays, including allele-specific PCR and deep sequencing, can detect mutations

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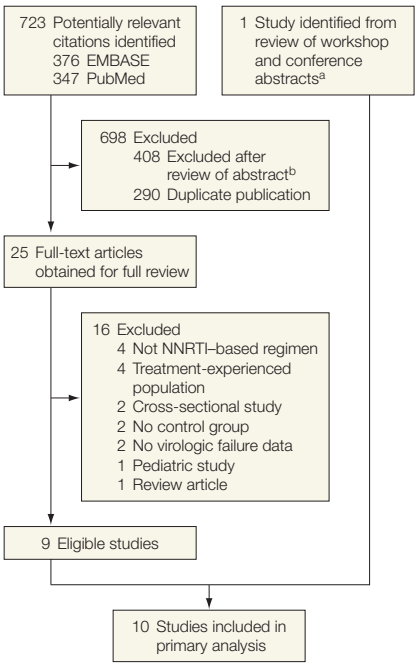
Figure 1. Study Selection



NNRTI indicates nonnucleoside reverse transcriptase inhibitor.
[a] International HIV Drug Resistance Workshop and Conference on Retroviruses and Opportunistic Infections (2007-2010).
[b] Common reasons for exclusion include: does not involve low-frequency resistance variants, review article, epidemiologic study, and treatment-experienced population only.

present at a far lower frequency.[5-7] Presence of these minority variants may adversely affect the response to antiretroviral treatment (ART), but their clinical significance continues to be the subject of considerable debate and uncertainty.

Nonnucleoside reverse transcriptase inhibitor (NNRTI)–based regimens are the most popular first-line HIV-1 treatment regimens, both in the United States and worldwide.[8-10] Although success rates are high, further improvements in tailoring regimens to resistance genotypes would avoid the costs associated with treatment failure and the accumulation of additional drug resistance mutations. A number of studies have been undertaken to evaluate the effects of baseline low-frequency NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations on the rates of treatment failure associated with the initial ART regimen. Results of these studies have been mixed, with some showing that drug-resistant minority variants significantly increase the risk of treatment failure and others showing no significant effect. In contrast, the small number of studies that evaluated the importance of low-frequency HIV-1 drug resistance mutations on integrase- and protease inhibitor–based treatment regimens have generally failed to find a significant association with increased risk of treatment failure.[11-16]

We performed a systematic review of the literature and a pooled analysis to examine the relationship between the presence of baseline low-frequency HIV-1 drug resistance mutations and the risk of virologic failure with NNRTI-based regimens in treatment-naive adults.

METHODS

Data Sources, Study and Participant Selection

A computerized literature search was conducted in PubMed (1966 through December 2010) and EMBASE (1974 through December 2010) using the search terms *HIV Infections*[mesh] OR *HIV*[mesh] OR *HIV*[tiab] OR *Acquired Immune Deficiency Syndrome Virus*[tiab] OR *Human Immunodeficiency Virus*[tiab] OR *Human Immunodeficiency Viruses*[tiab] OR *AIDS virus*[tiab] AND (*minor*[tiab] OR *minority*[tiab] OR *low abundance*[tiab] OR *low frequency* OR *minorities*[tiab]) AND (*variants*[tiab] OR *variant*[tiab] OR *mutation*[tiab] OR *mutations*[tiab] OR *mutant*[tiab] OR *mutants*[tiab] OR *quasispecies*[tiab]) AND (*Drug Resistance, Viral*[mesh] OR *Treatment Failure*[mesh] OR *treatment failure*[tiab] OR *resistance*[tiab] OR *resistant*[tiab]). In addition, experts in the field were contacted, reference lists reviewed, and abstracts from the International HIV Drug Resistance Workshop and the Conference on Retroviruses and Opportunistic Infections (2007-2010) searched for additional studies.

The inclusion criteria included cohort or case-control studies that evaluated the effects of low-frequency HIV-1 NRTI and NNRTI resistance mutations on the rate of virologic failure in treatment-naive adults receiving an initial NNRTI-based antiretroviral regimen. Studies were excluded if they had no comparison group, did not have treatment outcome data, focused solely on primary infection, or had a cross-sectional design. To assess evidence of publication bias, a funnel plot using study-specific definitions of minority variants and virologic failure was created (RevMan 5.0; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). Heterogeneity of the minority variant effect across studies was evaluated with a test of interaction between the presence of minority variants and study.

Of the 347 citations obtained from PubMed and 376 citations from EMBASE, 25 full-text articles were identified as potentially relevant and screened for inclusion (FIGURE 1). Of these, 16 were excluded on the basis of the study population (eg, not receiving an NNRTI-based regimen) or because they lacked treatment outcome data (eg, cross-sectional study only) or had no comparison group (ie, small case series). In addition, 1 previously unpublished study was identified that matched the inclusion and exclusion criteria. The literature search and review of full-text articles were independently performed by 2 authors (J.Z.L., R.P.).

Investigators from all 10 studies meeting inclusion and exclusion criteria agreed to provide patient-level (eg, demographic, laboratory, drug-resistant minority variant, and adherence) data and to participate in this pooled analysis (TABLE 1).[15-24] Individual patients with any pretreatment evidence of reduced NRTI or NNRTI drug susceptibility by standard genotyping based on the Stanford Resistance DB mutation scoring system (score ≥10 for any antiretroviral medication) were excluded.

Minority Variant and Adherence Information, End Points, and Data Compilation

The most commonly examined mutations across studies included K103N, Y181C, M184V, and K65R (TABLE 2). For each study, patients with K103N or Y181C low-frequency drug resistance mutations were classified as harboring an NNRTI-resistant minority variant; those with M184V or K65R were classified as having an NRTI-resistant minority variant. In 1 study, 3 patients were found to have 1 of 3 additional minority variants associated with NNRTI resistance (G190A, K101E, and P225H) and were included in the analysis as harboring an NNRTI-resistant minority variant.[15] Mi-

Table 1. Baseline Characteristics of Studies Included in the Pooled Analysis

Characteristic	Peuchant et al,[16] 2008	Simen et al,[15] 2009	Balduin et al,[17] 2009	Jakobsen et al,[18] 2010	Metzner et al,[19] 2011	Goodman et al,[20] 2011	Paredes et al,[21] 2010	Johnson et al,[22] 2008	Geretti et al,[23] 2009	Metzner et al,[24] 2009	Total
Study design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case-control	Case-control	Case-control	
Virologic failure, No.	2	45	7	1	1	44	150	52	14	3	315
Total participants, No.	13	70	54	20	56	423	280	240	89	18	1263
Age, mean (SD), y	38 (16.8)	37 (8.8)	41 (11.7)	43 (12.3)	42 (11.1)	38 (9.4)	37 (9.6)	37 (9.5)	38 (8.5)	43 (9.5)	38 (9.8)
Men, No. (%)	12 (92)	56 (80)	41 (76)	19 (95)	45 (80)	365 (86)	227 (81)	196 (82)	78 (88)	13 (72)	1052 (83)
Race/ethnicity, No. (%) Participants, No.	13	70	52	NR	NR	422	279	240	89	17	1182
White	12 (92)	16 (23)	39 (75)			253 (6)	110 (39)	132 (55)	78 (88)	14 (82)	654 (55)
Black	1 (8)	38 (54)	11 (21)			94 (22)	110 (39)	61 (25)	10 (11)	3 (18)	328 (28)
Hispanic	0	14 (20)	0			61 (14)	54 (19)	42 (18)	0	0	171 (14)
Other	0	2 (3)	2 (4)			14 (3)	5 (2)	5 (2)	1 (1)	0	29 (2)
CD4 cell count, median (IQR), cells/mm^3	426 (303-522)	247 (38-344)	251 (196-326)	200 (48-278)	279 (191-368)	227 (127-319)	202 (69-331)	243 (145-327)	222 (126-299)	222 (59-249)	229 (125-324)
$\log_{10}$ HIV RNA, median (IQR), copies/mL	4.4 (4.2-5.3)	5.3 (4.9-5.8)	4.7 (4.0-4.9)	5.1 (4.6-5.8)	4.9 (4.5-5.3)	5.0 (4.6-5.4)	4.8 (4.4-5.4)	5.1 (4.5-5.5)	5.2 (4.9-5.5)	5.4 (4.9-5.9)	5.0 (4.6-5.4)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NR, not reported.

Table 2. Characteristics of Minority Variants by Study[a]

Characteristic	Peuchant et al,[16] 2008	Simen et al,[15] 2009	Balduin et al,[17] 2009	Jakobsen et al,[18] 2010	Metzner et al,[19] 2011	Goodman et al,[20] 2011	Paredes et al,[21] 2010	Johnson et al,[22] 2008	Geretti et al,[23] 2009	Metzner et al,[24] 2009	Total for Cohort Studies[b]
Study design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case-control	Case-control	Case-control	
Method of detection[c]	AS-PCR	454	AS-PCR	SNaPshot	AS-PCR	AS-PCR	AS-PCR	AS-PCR	AS-PCR	AS-PCR	
Limit of detection (% of viral population)											
K103N	0.4	1.0	0.2	2.0	0.01	0.5	0.003	0.9	0.9	0.01	
Y181C		1.0		2.0			0.03	1.0	1.0	0.2	
M184V	0.3	1.0		2.0	0.2			0.5	0.5	0.2	
K65R		1.0		2.0	0.4				0.3	0.4	
Other NNRTI[d]		1.0		2.0					0.9		
No. with MVs and VF/total No. with MVs[e]											
K103N	1/3	1/1	3/13	1/2	0/2	5/14	27/39	1/1	3/3	1/1	17/53
Y181C		0/0		0/0			83/123	1/1	0/0	1/1	25/65
M184V	0/3	0/0		1/1	0/3			1/1	0/0	2/2	1/7
K65R		0/0		0/0	0/2				0/0	0/0	0/2
Other NNRTI[d]		3/3		0/0					0/0		3/3

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; MV, minority variant; NNRTI, nonnucleoside reverse transcriptase inhibitor; VF, virologic failure.
[a] Empty cells indicate population not tested for that variant.
[b] Totals only reflect participants of the cohort studies including the random subcohort from case-cohort analysis of A5095 by Paredes et al.
[c] HIV-SNaPshot is named for a multiplex primer-extension assay for detecting HIV minority variants[18]; 454 indicates 454 ultradeep pyrosequencing.
[d] Other NNRTI-resistant minority variants evaluated include G190A (Geretti et al), G190A/S/E (Jakobsen et al), and multiple (Simen et al). Three patients in Simen et al were found to have other low-frequency NNRTI resistance mutations (G190A, 2.1%; K101E, 3%; and P225H, 3.4%) and were included in the analysis as having a low-frequency NNRTI resistance mutation.
[e] All participants from the cohort studies and cases (participants with virologic failure) from the case-control studies were used in the primary Cox proportional hazard model and are described here. Data for Paredes et al include entire case-cohort study (participants in random subcohort and additional virologic failures).

nority variant copy numbers were calculated by multiplying the percentage of the minority variant by the plasma HIV-1 RNA level at the time of minority variant measurement. In the analysis of minority variant percentage or copy number, if multiple resistance mutations were present, the minority variant with the highest percentage or copy number was used.

Data on ART adherence were available from 3 studies, which in aggregate contributed 78% of the patients used for the primary analysis. Adherence measurements were based on pill counts,[20] 4-day self-report,[21] or 7-day self-report[15] and were averaged over the course of the study until the time of virologic failure or censoring. The lower of the NRTI and NNRTI adherence measurements was used as the overall medication adherence rate. Overall adherence was classified as high if the adherence rate was 95% or greater.

The definition of virologic failure was standardized for all patients to a plasma HIV-1 RNA level of 200 copies/mL or greater at 2 consecutive points at least 16 weeks after treatment initiation. Patients were also counted as having experienced virologic failure if the last available HIV-1 RNA level was 200 copies/mL or greater without a confirmatory measurement.

Statistical Analysis

Cox proportional hazard models stratified by study were used to estimate the risk of virologic failure across multiple factors: with and without minority variants (overall, NNRTI, NRTI), ART regimens (efavirenz vs nevirapine), adherence classifications, and minority variant percentage and copy number categories; tests of interactions were evaluated as appropriate. To avoid bias induced by targeted sampling, nonrandomly sampled controls (no virologic failure) were excluded and nonrandomly sampled cases (virologic failure) contributed to the Cox proportional hazard models only at the time of failure. The resulting analysis framework may be considered analogous to a Prentice weighted analysis for a case-cohort study.[25,26] For the same reason, Kaplan-Meier failure-time distributions were estimated using only patient data from randomly sampled cohorts (including the random subcohort analysis of A5095).[15-21]

To assess the robustness of the findings, sensitivity analyses were performed 3 ways: using only the largest cohort studies,[15,20,21] using all cohort studies,[15-21] and excluding the study contributing the largest number of participants.[21] Stratified Wilcoxon rank-sum tests were used to compare the distributions of HIV-1 RNA levels and CD4 cell counts between patients with and without minority variants or virologic failure.[27] The Cochran-Mantel-Haenszel test was used to compare the racial/ethnic distributions between patients with and without minority variants. Only patients from the cohort studies were included in group comparisons by minority variants; the entire data set was used to compare participants with or without virologic failure, with the exception of case-control studies that matched controls based on viral load or CD4 cell count.[23,24] The number needed to screen was calculated based on the prevalence of K103N or Y181C minority variants detected using the most sensitive resistance test[21] and overall virologic failure rates for patients with and without NNRTI-resistant minority variants.

Analysis of minority variant copy numbers excluded 3 studies using assays that could not provide a percentage.[18,22,23] For the minority variant 1% threshold analysis, 1 study was excluded because of a limit of detection of 2% for the assay,[18] and only NNRTI-resistant minority variants were evaluated for 2 studies because of incompatible limits of detection for the NRTI-resistant minority variants.[22,23] Four studies were excluded from the minority variant analysis using a 0.5% threshold because of higher limits of minority variant detection.[15,18,22,23]

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina) and PASW Statistics version 18 (IBM SPSS, Chicago, Illinois). Findings with $P < .05$ were considered statistically significant.

RESULTS

Systematic Review and Baseline Characteristics

In total, 10 studies met the inclusion and exclusion criteria.[15-24] The qualifying studies included 6 cohort studies,[15-20] 3 case-control studies,[22-24] and 1 case-cohort study[21] (Table 1). Of 1263 patients, 985 were included in the primary Cox proportional hazard analysis. At baseline, the mean age of the entire study population was 38 years, and 83% were men. The median CD4 cell count was 229 (interquartile range, [IQR], 125-324) cells/mm^3, and median plasma HIV-1 RNA level was 5.0 (IQR, 4.6-5.4) $\log_{10}$ copies/mL.

All studies evaluated the presence of K103N (Table 2). Other commonly evaluated minority variants included Y181C (n=435) and the NRTI resistance mutations M184V (n=228) and K65R (n=163). Most studies used allele-specific real-time PCR to detect minority variants; 1 study used the HIV-SNaPshot assay,[18] and 1 used ultradeep pyrosequencing (Roche/454 Life Sciences, Branford, Connecticut).[15] The study that used deep sequencing detected additional NNRTI-resistant minority variants (G190A, K101E, and P225H) in 3 patients, who were also included in the analysis. The lower limit of detection of minority variants differed widely between assays, with an upper range of 2% for the HIV-SNaPshot assay and a lower range of 0.003% for 1 of the allele-specific PCR assays (Table 2). The assays for 3 studies were unable to quantify the percentage of minority variants present.[18,22,23] No significant heterogeneity was seen among studies (P=.77), but there was evidence of limited publication bias (eFigure, available at http://www.jama.com).

Drug-resistant minority variants were found in 187 participants, including 117 of 808 patients (14%) in the cohort studies.[15-21] Patients with minority variants had a baseline median HIV-1 RNA level of 4.79 (IQR, 4.4-5.4) $\log_{10}$ copies/mL compared to 4.95 (IQR, 4.6-5.4) $\log_{10}$ copies/mL for those without detectable minority variants (P=.49). Patients with drug-resistant minority

variants had lower CD4 cell counts than those in whom these variants were not detected (median, 208 [IQR, 50-330] cells/mm^3 vs 234 [IQR, 134-329] cells/mm^3, respectively; $P=.03$). Patients with or without virologic failure had no significant differences in either baseline plasma HIV-1 RNA levels (median, 5.0 [IQR, 4.6-5.5] $\log_{10}$ copies/mL vs 5.0 [IQR, 4.6-5.4] $\log_{10}$ copies/mL, respectively; $P=.90$) or CD4 cell counts (median, 222 [IQR, 87-325] cells/mm^3 vs 235 [IQR, 135-324] cells/mm^3, respectively; $P=.47$). Among participants in the cohort studies, the proportion of those harboring drug-resistant HIV minority variants did not differ significantly by race/ethnicity ($P=.13$).

Drug-Resistant HIV-1 Minority Variants and Increased Risk of Virologic Failure

The presence of any NNRTI- or NRTI-resistant minority variant was associated with an increased risk of virologic failure (hazard ratio [HR], 2.6 [95% confidence interval {CI}, 1.9-3.5]; $P<.001$). This result was still apparent when the study contributing the largest number of patients with virologic failure[21] was excluded (HR, 3.6 [95% CI, 1.9-6.9]; $P<.001$) and when the analysis was restricted to include only participants from cohort studies (HR, 3.7 [95% CI, 2.3-5.9]; $P<.001$) (FIGURE 2). Specifically, among the 808 participants from cohort studies, 35% of those with detectable minority variants experienced virologic failure compared with 15% of those without minority variants. A sensitivity analysis that included only the largest cohort studies[15,20,21] gave similar results, with a virologic failure rate of 40% in participants with minority variants vs 17% in those without (HR, 3.9 [95% CI, 2.3-6.4]; $P<.001$ [n=665]).

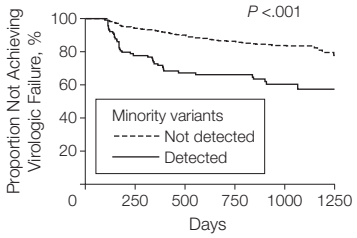
The increased risk of virologic failure was most strongly associated with NNRTI-resistant minority variants (HR, 2.6 [95% CI, 1.9-3.5]; $P<.001$) (FIGURE 3). The presence of only NRTI-resistant minority variants was not associated with a significantly increased risk of virologic failure (HR, 1.6 [95% CI, 0.1-17.7]), but only 9 participants fell into this category. In participants with NNRTI-resistant minority variants, the overall failure rate among those in the cohort studies was 37% compared to 15% in those without detectable minority variants (HR, 3.8 [95% CI, 2.4-6.1]; $P<.001$). No significant difference was found for the effect of NNRTI-resistant minority variants on the risk of virologic failure with efavirenz- vs nevirapine-based regimens ($P=.90$ for interaction) (Figure 3). There also was no significant difference in the rate of virologic failure between participants with K103N compared with Y181C minority variants (HR, 0.7 [95% CI, 0.4-1.4]; $P=.34$) among the subset of patients in whom testing for both mutations was performed (n=432).

Given the virologic failure rates for patients with and without NNRTI-resistant minority variants (37% and 15%, respectively, over a median 31-month follow-up period) and using the most sensitive resistance test,[21] approximately 11 patients would need to be screened prior to initiating an NNRTI-based ART regimen to avoid 1 case of virologic failure.

Medication Adherence and Minority Variants

Participants with drug-resistant minority variants and 95% or greater medication adherence had a significantly lower risk of virologic failure compared with those with minority variants and less than 95% adherence (HR, 0.3 [95% CI, 0.2-0.4]; $P<.001$). Compared with all participants without minority variants, individuals with minority variants and less than 95% medication adherence had 5.1 times the risk of virologic failure (95% CI, 3.6-7.2; $P<.001$). Those with minority variants and 95% or greater adherence had 1.5 times the risk of virologic failure (95% CI, 0.98-2.3; $P=.06$) (Figure 3). When compared with participants with 95% or greater adherence and no minority variants, both suboptimal adherence and the presence of minority variants were associated with similarly increased risks of virologic failure (HR, 4.0 [95% CI, 2.8-5.8]; $P<.001$ and HR, 3.1 [95% CI, 1.9-5.0]; $P<.001$, respectively) (Figure 3). The combined presence of suboptimal medication adherence and drug-resistant minority variants resulted in a substantially increased risk of virologic failure (HR, 10.6 [95% CI, 6.9-16.4]; $P<.001$). Furthermore, within each adherence category, the presence of minority variants was associated with an increased risk of virologic failure (HR for ≥95% adherence, 3.1 [95% CI, 1.9-5.0]; $P<.001$ and HR for <95% adherence, 2.7 [95% CI, 1.8-3.8]; $P<.001$).

Figure 2. Kaplan-Meier Curves for Proportion of Patients Without Virologic Failure by Presence of Drug-Resistant HIV-1 Minority Variants



No. at risk

Minority variants						
Not detected	691	620	455	398	344	46
Detected	117	86	60	53	37	7

Both nonnucleoside reverse transcriptase inhibitor– and nucleoside reverse transcriptase inhibitor–resistant minority variants (MVs) are included in this analysis. To avoid bias induced by targeted sampling in case-control studies, Kaplan-Meier failure time distributions were estimated using only data from cohort studies.[15-21] Curves only shown to 1250 days because of small sample sizes thereafter. *P* value comparison by Cox proportional hazard analysis. Median follow-up time, 31 (interquartile range, 13-34) months. HIV indicates human immunodeficiency virus.

Dose-Dependent Association of Drug-Resistant Minority Variants With Increased Risk of Virologic Failure

To evaluate whether a threshold existed for the effect of drug-resistant minority variants, analyses were performed to explore the risk of virologic failure associated with different percentages or absolute numbers of drug-resistant minority variants. Compared with participants without drug-resistant minority variants, an increased risk of virologic failure was found when drug-resistant minority variants were present at either

less than 1% or 1% or greater of the viral population (HR, 2.2 [95% CI, 1.6-3.1]; $P<.001$ and HR, 5.0 [95% CI, 2.4-10.3]; $P<.001$, respectively) (Figure 3). However, the presence of minority variants at 1% or greater conferred a significantly higher risk of virologic failure compared with minority variants present at less than 1% (HR, 2.2 [95% CI, 1.0-4.9]; $P=.048$). Similar results were observed when the proportion of resistance variants in the virus population was stratified as less than 0.5% vs 0.5% or greater ($P=.01$ for comparison of <0.5% vs ≥0.5%) (Figure 3). A dose-dependent effect on the risk of virologic failure was found when participants were categorized as having 0, 1 through 9, 10 through 99, 100 through 999, and 1000 or more copies of drug-resistant minority variants per milliliter of plasma (Figure 3). The effect on virologic failure was similar when the analysis was limited to only NNRTI-resistant minority variants (eAppendix).

Multivariate Analysis

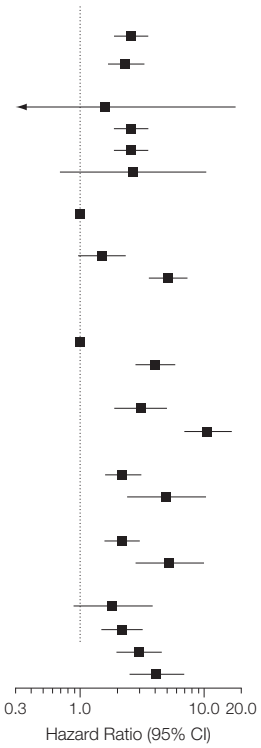
In a multivariate Cox proportional hazard model, the presence of a drug-resistant minority variant (HR, 2.3 [95% CI, 1.7-3.3]; $P<.001$) (Figure 3), overall medication adherence (HR, 0.86 per 5% higher adherence [95% CI, 0.83-0.88]; $P<.001$), and race/ethnicity were all significant independent predictors of virologic failure. Compared with white participants, those of black, Hispanic, and other races/ethnicities all had an increased risk of virologic failure (HR, 2.8 [95% CI, 2.0-3.8]; $P<.001$; HR, 2.1 [95% CI, 1.4-3.1]; $P<.001$; and HR, 2.6 [95% CI, 1.0-6.5]; $P=.045$, respectively). Associations with baseline CD4 cell count and plasma HIV-1 RNA levels were not detected ($P=.59$ and $P=.88$, respectively).

Time to Virologic Suppression

The effect of drug-resistant minority variants on viral decay dynamics was evaluated using 2 studies with frequent plasma HIV-1 RNA determinations after ART initiation (n=581).[20,21] The proportion of participants who

Figure 3. Effect of Minority Variants and Antiretroviral Therapy Adherence on Virologic Failure

Group vs No Minority Variant[c]	Virologic Failure, No.[a]		Total No.		HR (95% CI)[b]
	Minority Variant	No Minority Variant	Minority Variant	No Minority Variant	
Any minority variant	111	208	187	798	2.6 (1.9-3.5)
Any minority variant (multivariate)[d]	98	138	152	617	2.3 (1.7-3.3)
Minority variant type					
NRTI-resistant	2	104	9	202	1.6 (0.1-17.7)
NNRTI-resistant	109	210	178	807	2.6 (1.9-3.5)
Efavirenz-resistant	105	188	165	723	2.6 (1.9-3.5)
Nevirapine-resistant	4	22	13	84	2.7 (0.7-10.3)
Minority variant and adherence					
No minority variant and any adherence					1 [Reference]
Any minority variant					
Adherence ≥95%	35	138	73	617	1.5 (0.98-2.3)
Adherence <95%	63	138	79	617	5.1 (3.6-7.2)
No minority variant					
Adherence ≥95%					1 [Reference]
Adherence <95%		43	231	386	4.0 (2.8-5.8)
Any minority variant					
Adherence ≥95%	35	43	73	386	3.1 (1.9-5.0)
Adherence <95%	63	43	79	386	10.6 (6.9-16.4)
Minority variant, %					
<1	91	209	154	781	2.2 (1.6-3.1)
≥1	18	209	30	781	5.0 (2.4-10.3)
<0.5	86	107	143	654	2.2 (1.6-3.0)
≥0.5	14	107	32	654	5.2 (2.8-9.8)
Minority variant copies, No.					
1-9	8	148	15	720	1.8 (0.9-3.8)
10-99	41	148	71	720	2.2 (1.5-3.2)
100-999	35	148	55	720	3.0 (2.0-4.5)
≥1000	20	148	38	720	4.1 (2.5-6.8)



NNRTI indicates nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.
[a] Participant numbers include additional virologic failure cases from the case-control and case-cohort studies.[21-24]
[b] Cox proportional hazard ratios shown are in comparison to those without minority variants unless otherwise noted.
[c] Three studies contributed to the adherence analysis.[15,20,21] One study was excluded from the minority variant 1% threshold analysis because of a limit of detection of 2% for the assay,[18] and only NNRTI-resistant minority variants were evaluated for 2 studies because of incompatible limits of detection for NRTI-resistant minority variants.[22,23] Four studies were excluded from the 0.5% threshold analysis because of higher limits of minority variant detection.[15,18,22,23] Analysis of minority variant copy numbers excluded 3 studies using assays that could not provide a percentage.[18,22,23]
[d] Multivariate Cox regression analysis included adherence, race/ethnicity, baseline CD4 cell count, and HIV-1 RNA levels.

never reached a plasma HIV-1 RNA level of 200 copies/mL or less was significantly higher in the group with drug-resistant minority variants compared with those without detectable minority variants (9% vs 1%, respectively; $P<.001$). However, among participants who eventually demonstrated suppressed viral replication, there was no difference in the median number of days to virologic suppression (57 vs 57 days, respectively) (FIGURE 4).

COMMENT

In this pooled analysis, we found that the presence of drug-resistant HIV-1 minority variants was associated with more than twice the risk of virologic failure in patients receiving an initial NNRTI-based ART regimen in an analysis that controlled for medication adherence, race/ethnicity, baseline CD4 cell count, and baseline HIV-1 viral load. The presence of minority variants was associated with 2.5 to 3 times the risk of virologic failure at high and low levels of medication adherence. The association of minority variants with virologic failure was dose-dependent and most prominent in participants with NNRTI resistance mutations.

Multiple factors contribute to the risk of ART failure. Adherence to antiretroviral therapy is a major predictor of viral suppression and disease progression.[28-30] In this analysis, we found that the risk of virologic failure associated with the presence of drug-resistant minority variants was similar to that conferred by suboptimal medication adherence. Patients with drug-resistant minority variants as well as suboptimal medication adherence had a 10-fold risk of virologic failure compared with those with wild-type virus and excellent adherence. However, optimal medication adherence did not completely compensate for the higher risk of virologic failure in the presence of drug-resistant HIV-1 minority variants.

Interestingly, race/ethnicity was found to be a significant predictor of virologic failure; in particular, white participants had a lower risk of virologic failure compared with black and Hispanic participants. This risk differential was not attributable to differing rates of minority variant detection. While some studies have shown no association of race/ethnicity or HIV-1 subtype with initial treatment response,[31] a secondary analysis of the A5095 trial uncovered an interaction between race/ethnicity and adherence and found a greater effect of nonadherence on virologic failure in black participants.[32]

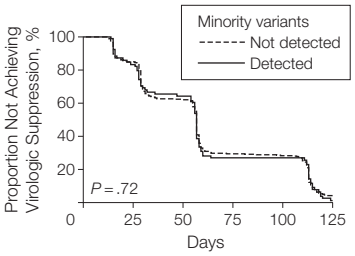
The effect of race/ethnicity on virologic failure seen in our analysis was present even after adjusting for the level of medication adherence. The relationship between race/ethnicity and virologic failure may be mediated by factors such as socioeconomic status, drug and alcohol use, or other factors not accounted for here that may correlate with adherence and could contribute to residual confounding. Another potential explanation for these findings could be related to the recent report that cytochrome P450 polymorphisms affect NNRTI pharmacokinetics and treatment outcome in a race/ethnicity–specific manner.[33]

Drug-resistant minority variants detected by ultrasensitive assays could arise from a few sources. Those found at higher proportions may represent transmitted drug resistance that have been replaced by wild-type revertants over time[34] or have resulted from multivariant transmission,[35,36] whereas mutations present at extremely low frequencies (much less than 1% of the viral population) could be attributable to de novo mutations resulting from errors introduced during viral replication[37] or laboratory artifacts from reverse transcription and PCR amplification. The presence of spontaneously appearing low-frequency drug resistance mutations has been described in HIV-1 samples collected in the pre-ART drug era.[7]

It has been proposed that drug-resistant minority variants present at extremely low levels may not have significant clinical effects. While we found a dose-dependent effect of drug-resistant minority variants on risk of virologic failure, an increased risk was detected even at very low minority variant frequencies (<0.5% and 10-99 copies/mL). A recent study reported a strong correlation between virologic failure and the presence of 2000 copies/mL or more of K103N-containing HIV-1, whereas patients with fewer than 2000 copies/mL of K103N did not show an increased risk of virologic failure.[20]

One explanation for the difference between these results and those of the current analysis is that the earlier study used an assay with a limit of detection for drug-resistant minority variants of 0.5% of the virus population and therefore identified only a limited number of participants with resistance variants present at low copy numbers. Other possible explanations include the lack of Y181C measurement in that study and differences between studies of the NRTI component of the regimen. Nevertheless, it is clear that not all patients in whom drug-resistant minority variants are identified will experience virologic failure, and a frequency-dependent effect of the population with such variants is clearly evident from the current pooled analysis. Further research is needed to iden-

Figure 4. Time to HIV-1 RNA Level Less Than 200 Copies/mL in Patients Achieving Virologic Suppression



No. at risk

Minority variants						
Not detected	503	427	313	148	143	21
Detected	78	65	50	21	21	1

Two studies with frequent human immunodeficiency virus 1 (HIV-1) RNA monitoring[20,21] were used to determine the time to HIV-1 RNA less than 200 copies/mL among individuals who experienced virologic suppression. *P* value comparison by Cox proportional hazard analysis. Median time to virologic suppression was 57 (interquartile range, 28-112) days for those with minority variants and 57 (interquartile range, 27-111) days for those without.

tify additional factors that contribute to the risk of virologic failure.

This analysis has several limitations. To combine patient-level data from studies with different study designs, statistical adjustments were required such as limiting the inclusion of patients from case-control studies to include only those patients with virologic failure and using a stratified Cox proportional hazard model in which patients with virologic failure outside of the cohort studies were only included at the time of failure. This approach has been validated in prior studies,[25,26] but we also confirmed the robustness of our findings in sensitivity analyses limited to data obtained from the cohort studies.

In addition, studies that contributed data to this analysis had differences with regard to assay methodology, sensitivity, and resistance mutations detected. The assay with the highest limit of detection was the HIV-SNaPshot assay (2%),[18] whereas allele-specific PCR assays had lower limits of detection (down to 0.003%). The study that contributed the second-largest number of participants and the largest proportion of virologic failures used the most sensitive assay.[21] As expected, patients from that study made up the greatest proportion of those with drug-resistant minority variants (72%). Nevertheless, reanalysis of the data excluding the contribution from this study found that the increased risk of virologic failure associated with presence of drug-resistant minority variants persisted. Visual inspection of the Kaplan-Meier curves (Figure 2) suggests that the increased risk of virologic failure associated with minority variants may be most prominent early in the course of treatment. Such a result would not be unexpected and would mean that the hazard ratios presented (which represent the average hazard ratio over the entire study period) may underestimate the effect of drug-resistant minority variants during the early treatment period.

Another limitation relates to the specific drug resistance mutations studied. All studies measured the levels of K103N, but only 6 studies evaluated the presence of Y181C (44% of total patients), and only a small proportion of the total study population was tested for the presence of M184V (23%) or K65R (17%). Consequently, our ability to detect a significant association of NRTI resistance mutations and risk of virologic failure or a difference in effect between K103N and Y181C minority variants was limited. Because only a subset of participants were tested for the presence of other NNRTI resistance mutations, our results most likely underestimate the effect of NNRTI-resistant minority variants on virologic failure, because a significant proportion of those categorized as having no detectable minority variants may have had unmeasured Y181C or other NNRTI resistance mutations.

The findings of this pooled analysis demonstrate that low-frequency HIV-1 drug resistance mutations, and NNRTI resistance mutations in particular, confer a greater than 2-fold risk of virologic failure in treatment-naive individuals initiating a first-line NNRTI-containing ART regimen. Using the most sensitive test for NNRTI resistance mutations, approximately 11 patients would need to be screened prior to initiating an NNRTI based ART regimen to avoid 1 case of virologic failure. These data provide a rationale for developing standardized clinical assays for the detection of NNRTI-resistant minority variants. Because NNRTI-based regimens are the most commonly prescribed first-line antiretroviral therapy, the clinical use of ultrasensitive screening for drug-resistant HIV could help identify individuals at greatest risk of virologic failure and allow ART to be tailored appropriately.

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Author Contributions: Dr. Li had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Li, Paredes, Ribaudo, Svarovskaia, Geretti, Masquelier, Miller, Kuritzkes.
Acquisition of data: Li, Ribaudo, Svarovskaia, Metzner, Kozal, Huppler Hullsiek, Balduin, Jakobsen, Geretti, Thiebaut, Ostergaard, Masquelier, Johnson.
Analysis and interpretation of data: Li, Paredes, Ribaudo, Svarovskaia, Metzner, Kozal, Huppler Hullsiek, Balduin, Jakobsen, Geretti, Masquelier, Johnson, Miller, Kuritzkes.
Drafting of the manuscript: Li, Geretti, Kuritzkes.
Critical revision of the manuscript for important intellectual content: Li, Paredes, Ribaudo, Svarovskaia, Metzner, Kozal, Huppler Hullsiek, Balduin, Jakobsen, Geretti, Thiebaut, Ostergaard, Masquelier, Johnson, Miller, Kuritzkes.
Statistical analysis: Li, Paredes, Ribaudo, Svarovskaia, Hupler Hullsiek, Geretti.
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Study supervision: Paredes, Geretti, Miller, Kuritzkes.
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