Minority HIV-1 Drug Resistance Mutations and the Risk of NNRTI-based Antiretroviral Treatment Failure: A Systematic Review and Pooled Analysis

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Abstract

Context—Evidence regarding the impact of minority, or low frequency, HIV-1 drug-resistant variants on the effectiveness of first-line antiretroviral treatment (ART) is conflicting.

Objective—To evaluate the association of pre-existing HIV-1 minority drug-resistant variants with risk of first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral virologic failure.

Data Sources—We searched published and unpublished studies in MEDLINE (1966 through December, 2010), EMBASE (1974 through December, 2010), conference abstracts, and article references.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 population sequencing. 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. Minority HIV-1 drug resistance mutations were associated with an increased risk of virologic failure (HR 2.3 [95% CI, 1.7–3.3], P<0.001) after controlling for medication adherence, ethnicity, baseline CD4 cell count and plasma HIV-1 RNA levels. The 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<0.001). Among participants from the cohort studies, 35% of those with detectable minority variants experienced virologic failure as compared to 15% of those without minority variants. The presence of minority variants was associated with 2.5–3 times the risk of virologic failure at either ≥95% or <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 this pooled analysis, minority HIV-1 resistance mutations, particularly involving NNRTI-resistance, were significantly associated with a dose-dependent increased risk of virologic failure with first-line ART.

Keywords

INTRODUCTION

Genotypic tests for HIV-1 drug resistance employ PCR amplification and population sequencing techniques that detect resistance-associated mutations present at ≥15–25% 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 resistance mutations present as minority variants within the population of HIV-1 quasispecies in an infected individual. A number of ultra-sensitive assays, including allele-specific PCR and deep sequencing, can detect mutations at a far lower frequency than standard population sequencing[5–7]. Presence of these minority, or low frequency, variants may adversely affect the response to antiretroviral treatment (ART), but their clinical significance continues to be the subject of considerable debate and uncertainty.

Non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens are the most popular first-line HIV treatment regimen both in the United States and world-wide[8, 9]. Although success rates are high, further improvements would avoid the costs associated with treatment failure and accumulating additional drug resistance mutations. A number of studies have been undertaken to evaluate the impact of baseline NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations on rates of initial ART treatment failure. Results of these studies have been mixed, with some showing that minority drug-resistant variants significantly increase the risk of treatment failure and others showing no significant effect. In contrast, a small number of studies that evaluated the importance of minority resistance mutations on integrase and protease inhibitor-based treatment have generally failed to find a significant association with increased risk of treatment failure[10–15].

We performed a systematic review of the literature and performed a pooled analysis to examine the relationship between baseline minority HIV-1 drug resistance mutations and the risk of initial NNRTI-based virologic failure.

METHODS

Data Sources, Study and Participant Selection

A computerized literature search was conducted of 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 were reviewed, and abstracts from the International HIV Drug Resistance Workshop and the Conference on Retroviruses and Opportunistic Infections (2007–2010) were searched for additional studies. The inclusion criteria included cohort or case-control studies that evaluated the impact of minority HIV-1 NRTI- and NNRTI-resistance mutations on the rate of virologic failure in treatment-naïve 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 were cross-sectional studies. To assess evidence of publication bias, a funnel plot using study-specific definitions of minority variants and virologic failure was created (RevMan 5.0, Copenhagen). 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 because they lacked treatment outcome data (e.g., cross-sectional

study only), had no comparison group (i.e., small case series), or on the basis of the study population (e.g., not on NNRTI-based regimen). In addition, one 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 two of the authors (J.Z.L. and R.P.). Investigators from all 10 studies agreed to provide patient-level data (e.g., demographic, laboratory, minority resistance, and adherence data) and to participate in this pooled analysis (Table 1)[14–23]. Individual patients were excluded with any pre-treatment 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).

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 minority drug resistance mutations were classified as harboring a minority NNRTI-resistant HIV-1 variant; those with M184V or K65R were classified as having a minority NRTI-resistant variant. In one study, three patients were found to have one of three additional minority mutations associated with NNRTI resistance (G190A, K101E, and P225H) and were included in the analysis as harboring a minority NNRTI-resistant variant[14]. Minority 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 three studies, which in aggregate contributed 78% of the patients used for the primary analysis. ART adherence measurements were based on pill counts[19], 4-day self-report[20], or 7-day self-report[14] 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%.

The definition of virologic failure was standardized for all patients to a plasma HIV-1 RNA level of ≥200 copies/ml at two consecutive time points at least 16 weeks after treatment initiation. Patients were also counted as virologic failures if the last available HIV-1 RNA level was ≥200 copies/ml 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 versus 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, non-randomly sampled controls (non-failures) were excluded and non-randomly sampled cases (virologic failures) contributed to the Cox proportional hazard models only at their time of failure. The resulting analysis framework may be considered analogous to a Prentice weighted analysis for a case-cohort study[24, 25]. For the same reason, Kaplan-Meier failure time distributions were estimated using only patients from randomly sampled cohorts (including analysis of randomized controlled trials and the random subcohort analysis of A5095)[14–20]. To assess the robustness of the findings, sensitivity analyses were performed using cohort studies[14–17, 19, 20, 26], largest cohort studies[14, 19, 20], and excluding the study contributing the largest number of participants[20]. 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 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 dataset 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[22, 23]. The number needed to screen was calculated based on the prevalence of K103N and/or Y181C minority variants detected using the most sensitive resistance test[20] and overall virologic failure rates for patients with and without minority NNRTI-resistant variants.

Analysis of minority variant copy numbers excluded three studies using assays that could not provide a percentage[17, 21, 22]. For the minority variant 1% threshold analysis, one study was excluded due to a limit of detection of 2% for the assay[17] and only NNRTI minority variants were evaluated for two studies due to incompatible limits of detection for the NRTI minority variants[21, 22]. Four studies were excluded from the minority variant analysis using a 0.5% threshold due to higher limits of minority variant detection[14, 17, 21, 22]. Statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC) and PASW Statistics 18 (IBM SPSS, Chicago, IL). Findings with a P-value <0.05 were considered to be statistically significant.

RESULTS

Systematic Review and Baseline Characteristics

In total, 10 studies met the inclusion and exclusion criteria[14–17, 19–23, 26]. The qualifying studies included six cohort studies[14–17, 19, 26], three case-control studies[21–23], and one case-cohort study[20] (Table 1). Of 1263 patients, 985 were included in the primary Cox proportional hazard analysis. At baseline, the average age of the entire study population was 38 years and 83% were men. The median CD4 cell count was 229 [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; one study used the HIV-SNaPshot assay[17] and one used deep sequencing (Roche/454 Life Sciences)[14]. The study that used deep sequencing detected additional minority NNRTI-resistant HIV-1 variants (G190A, K101E, and P225H) in three 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 one of the allele-specific PCR assays (Table 2). The assays for three studies were unable to quantify the percentage of minority variants present[17, 21, 22]. No significant heterogeneity was seen among studies (P=0.77), but there was evidence of limited publication bias (Figure S1).

Minority drug-resistant variants were found in 187 participants including 14% (117/808) of patients in the cohort studies[14–17, 19, 20, 26]. Patients with minority variants had a baseline median HIV-1 RNA level of 4.79 [IQR 4.4–5.4] $\log_{10}$ copies/mL as compared to 4.95 [IQR 4.6–5.4] $\log_{10}$ copies/mL for those without detectable minority variants (P=0.49). Patients with minority resistance variants had lower CD4 cell counts than those in whom these variants were not detected (median 208 [IQR 50–330] versus 234 [IQR 134–329] cells/mm^3, respectively; P=0.03). 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] versus 5.0 [IQR 4.6–5.4] $\log_{10}$ copies/mL, respectively; P=0.90) or CD4 cell counts (median 222 [IQR 87–325] versus 235 [135–324] cells/mm^3, respectively; P=0.47). Among participants

in the cohort studies, the proportion of those harboring HIV drug-resistant minority variants did not differ significantly by ethnicity (P=0.13).

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

The presence of any minority NNRTI- or NRTI-resistant variant was associated with an increased risk of virologic failure (Hazard Ratio [HR] 2.6 [95% CI, 1.9–3.5], P<0.001). This result was still apparent when the study contributing the largest number of patients with virologic failure[20] was excluded (HR 3.6 [95% CI 1.9–6.9], P<0.001) and when the analysis was restricted to include only participants from cohort studies (HR 3.7 [95% CI 2.3–5.9], P<0.001; Figure 2). Specifically, among the 808 participants from cohort studies, 35% of those with detectable minority variants experienced virologic failure as compared to 15% of those without minority variants. A sensitivity analysis that included only the largest cohort studies[14, 19, 20] gave similar results, with a virologic failure rate of 40% in those with minority variants versus 17% in those without (HR 3.9 [95% CI 2.3–6.4], P<0.001, N=665).

The increased risk of virologic failure was most strongly associated with minority NNRTI-resistant variants (HR 2.6 [95% CI, 1.9–3.5], P<0.001; Figure 3). The presence of only minority NRTI-resistant variants was not associated with an elevated risk of virologic failure (HR 1.6 [95% CI 0.1–17.7]), but only nine participants fell into this category. In participants with minority NNRTI-resistant variants, the overall failure rate among those in the cohort studies was 37% as compared to 15% in those without detectable minority variants (HR 3.8 [95% CI 2.4–6.1], P<0.001). No significant difference was found on the effect of minority NNRTI-resistant variants on the risk of virologic failure with efavirenz- versus nevirapine-based regimens (Interaction P=0.90; Figure 3). There was also no significant difference in the rate of virologic failure between participants with K103N compared to those with Y181C minority variants (HR 0.7 [95% CI 0.4–1.4], P=0.34) among the subset of patients in whom testing for both mutations were performed (N=432).

Given the virologic failure rates for patients with and without minority NNRTI-resistant variants (37% and 15%, respectively over a median 31 month follow-up period) and using the most sensitive resistance test[20], approximately 11 patients would need to be screened prior to initiating an NNRTI-based ART regimen to avoid one case of virologic failure.

Medication Adherence and Minority Variants

Participants with minority drug-resistant variants and ≥95% medication adherence had a significantly lower risk of virologic failure compared to those with minority variants and <95% adherence (HR 0.3 [95% CI, 0.2–0.4], P<0.001). Compared to all participants without minority variants, individuals with minority variants and <95% medication adherence had 5.1 times the risk of virologic failure (95% CI 3.6–7.2, P<0.001). Those with minority variants and ≥95% adherence had 1.5-times the risk of virologic failure (95% CI 0.98–2.3, P=0.06; Figure 3). When compared to participants with ≥95% 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<0.001 and HR 3.1 [95% CI 1.9–5.0], P<0.001, respectively; Figure 3). The combined presence of suboptimal medication adherence and minority drug-resistant variants resulted in a substantially increased risk of virologic failure (HR 10.6 [95% CI 6.9–16.4], P<0.001). Furthermore, within each adherence category, the presence of minority variants was associated with an increased risk of virologic failure (≥95% adherence HR 3.1 [95% CI 1.9–5.0], P<0.001; <95% adherence HR 2.7 [95% CI 1.8–3.8], P<0.001).

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

To evaluate whether a threshold existed for the effect of minority drug-resistant variants, analyses were performed to explore the risk of virologic failure associated with different percentages or absolute numbers of minority drug-resistant variants. Compared to those without minority drug-resistant variants, an increased risk of virologic failure was found when minority drug-resistant variants were present at either <1% or ≥1% of the viral population (HR 2.2 [95% CI 1.6–3.1], P<0.001 and HR 5.0 [2.4–10.3], P<0.001, respectively; Figure 3). However, the presence of minority variants at ≥1% conferred a significantly higher risk of virologic failure as compared to minority variants present at <1% (HR 2.2 [1.0–4.9], P=0.048). Similar results were observed when the proportion of resistant variants in the virus population was stratified as <0.5% versus ≥0.5% (Figure 3, P=0.01 for comparison of <0.5% versus ≥0.5%). A dose-dependent effect on the risk of virologic failure was found when participants were categorized as having 0, 1–9, 10–99, 100–999, and ≥1000 copies of minority drug-resistant variants per mL of plasma (Figure 3). The effect on virologic failure was similar when the analysis was limited to only minority NNRTI-resistant variants (see supplemental data).

Multivariate Analysis

In a multivariate Cox proportional hazard model, the presence of a minority drug-resistant variant (HR 2.3 [95% CI 1.7–3.3], P<0.001; Figure 3), overall medication adherence (HR 0.86 per 5% higher adherence, [95% CI 0.83–0.88], P<0.001), and ethnicity were all significant independent predictors of virologic failure. Compared to whites, participants of black, Hispanic, and other ethnicities all had an increased risk of virologic failure (HR 2.8 [2.0–3.8], P<0.001; HR 2.1 [1.4–3.1], P<0.001; and HR 2.6 [1.0–6.5], P=0.045, respectively). Associations with baseline CD4 cell count and plasma HIV-1 RNA levels were not detected (P=0.59 and P=0.88, respectively).

Time to Virologic Suppression

The effect of minority drug-resistant variants on viral decay dynamics was evaluated using two studies with frequent plasma HIV-1 RNA determinations after ART initiation (N=581)[19, 20]. The proportion of participants who never reached a plasma HIV-1 RNA level ≤200 copies/mL was significantly higher in the group with minority drug-resistant variants compared to those without detectable minority variants (9% versus 1%, respectively; P<0.001). However, among participants who eventually became suppressed, there was no difference in the median number of days to virologic suppression (57 versus 57 days, respectively; Figure 4).

DISCUSSION

In this pooled analysis, we found that the presence of minority HIV-1 drug resistant 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, ethnicity, baseline CD4 cell count, and HIV-1 viral load. The presence of minority variants was associated with 2.5–3 times the risk of virologic failure at either high or low levels of medication adherence. The association of minority variants with virologic failure was dose-dependent and most prominent in those 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 minority drug-resistant variants was similar to that conferred by suboptimal medication adherence. Patients

with both minority drug-resistant variants and suboptimal medication adherence had a 10-fold risk of virologic failure when compared to 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 minority drug-resistant HIV-1 variants.

Interestingly, ethnicity was found to be a significant predictor of virologic failure and in particular, white participants had a lower risk of virologic failure compared to black and Hispanic participants. This risk differential was not due to differing rates of minority variant detection. While some studies have shown no association of race or HIV-1 subtype with initial treatment response[31], a secondary analysis of the A5095 trial uncovered an interaction between ethnicity and adherence, and found a greater effect of non-adherence on virologic failure in black participants[32]. It is interesting to note that the effect of ethnicity on virologic failure seen in our analysis was present even after adjusting for the level of medication adherence. The relationship between ethnicity and virologic failure most likely is 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-specific manner[33].

Minority drug-resistant 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 resulted from multivariant transmission[35, 36], whereas mutations present at extremely low frequencies (much less than 1% of the viral population) could be due 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 minority drug-resistance mutations has been described in HIV samples collected in the pre-ART drug era[7]. It has been proposed that minority drug-resistant variants present at extremely low levels may not have a significant clinical impact. While we found a dose-dependent effect of minority drug-resistant variants on risk of virologic failure, this increased risk was significant 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 ≥2,000 copies/ml of K103N-containing HIV-1 whereas patients with <2,000 copies/ml of K103N did not show an increased risk of virologic failure[19]. 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 minority drug-resistant variants of 0.5% of the virus population and therefore identified only a limited number of participants with resistant 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 minority drug-resistant variants are identified will experience virologic failure and a frequency-dependent effect of the minority drug-resistant population is clearly evident from the current pooled analysis. Further research is needed to identify additional factors that contribute to the risk of virologic failure.

This analysis has several limitations. In order 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 only those patients with virologic failure and using a stratified Cox proportional hazard model in which virologic failure patients outside of the cohort studies were only counted at the time of failure. Although this approach has been validated in previous studies[24, 25], we confirmed the robustness of our findings in sensitivity analyses limited to data obtained only 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%)[17], 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 utilized the most sensitive assay[20]. As expected, patients from that study made up the greatest proportion of those with minority drug-resistant variants (72%). Nevertheless, the increased risk of virologic failure associated with presence of minority drug-resistant variants persisted even when this study was removed from the analysis. 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 minority drug-resistant variants during the early treatment period. Another limitation involves the types of 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 minority 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 minority NNRTI-resistant variants on virologic failure as 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 minority HIV-1 drug resistance mutations, and NNRTI resistance mutations in particular, confer a greater than 2-fold risk of virologic failure for individuals on 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 one case of virologic failure. These data provide a rationale for developing standardized clinical assays for the detection of minority NNRTI-resistant variants. As NNRTI-based regimens are the most commonly prescribed first-line antiretroviral therapy, the clinical use of ultra-sensitive HIV drug resistance screening could help identify individuals at greatest risk of virologic failure and allow ART to be tailored appropriately.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abstracts from CROI and Resistance Workshop (2007 – 2010)	347 Citations from Pubmed	376 Citations from EMBASE

290 Duplicates
408 Excluded after review of abstract[a]

1 Research group contacted	25 Full-text articles obtained for full review

16 Excluded after review:

4 Non-NNRTI based regimen
4 Treatment experienced
2 Cross-sectional study only
2 No control group
2 No virologic failure data
1 Review paper
1 Pediatrics

1 eligible study	9 eligible studies

Figure 1.
Study Selection Flow Diagram
[a]Common reasons for exclusion include: does not involve low-frequency resistance variants, review article, epidemiological study, and treatment experienced patient population only.
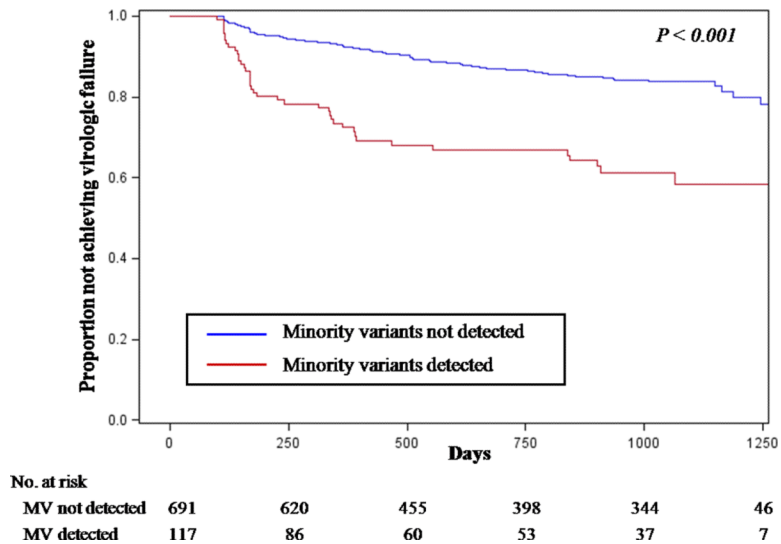
Figure 2.
Kaplan-Meier Curves for the Proportion of Patients without Virologic Failure by the Presence of Minority HIV-1 Drug-Resistant Variants

Abbreviations: MV, minority variants

Both NNRTI- and NRTI-resistant minority variants 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 date from cohort studies[14–20]. Kaplan-Meier curves only shown up to 1,250 days due to the small sample sizes thereafter. P-value comparison by Cox proportional hazard analysis. Median follow-up time is 31 months [IQR 12–34 months].

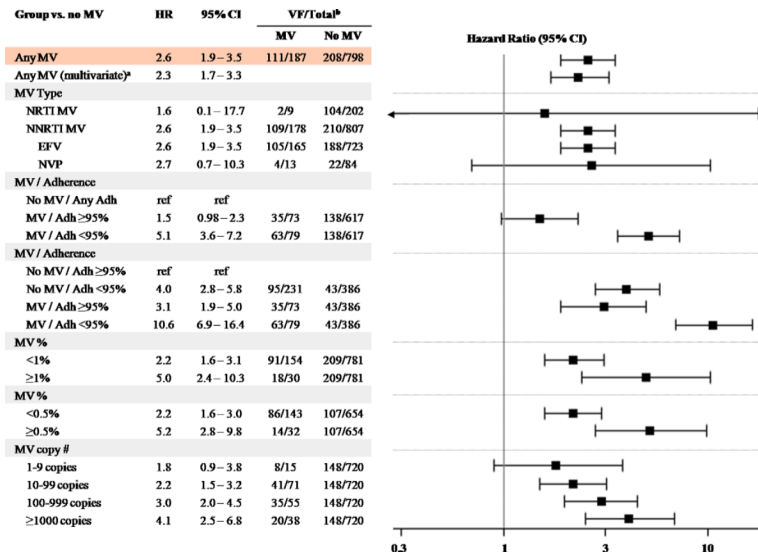| Group vs. no MV	HR	95% CI	VF/Total[b] MV	VF/Total[b] No MV
Any MV	2.6	1.9 − 3.5	111/187	208/798
Any MV (multivariate)[a]	2.3	1.7 − 3.3		
MV Type				
NRTI MV	1.6	0.1 − 17.7	2/9	104/202
NNRTI MV	2.6	1.9 − 3.5	109/178	210/807
EFV	2.6	1.9 − 3.5	105/165	188/723
NVP	2.7	0.7 − 10.3	4/13	22/84
MV / Adherence				
No MV / Any Adh	ref	ref		
MV / Adh ≥95%	1.5	0.98 − 2.3	35/73	138/617
MV / Adh <95%	5.1	3.6 − 7.2	63/79	138/617
MV / Adherence				
No MV / Adh ≥95%	ref	ref		
No MV / Adh <95%	4.0	2.8 − 5.8	95/231	43/386
MV / Adh ≥95%	3.1	1.9 − 5.0	35/73	43/386
MV / Adh <95%	10.6	6.9 − 16.4	63/79	43/386
MV %				
<1%	2.2	1.6 − 3.1	91/154	209/781
≥1%	5.0	2.4 − 10.3	18/30	209/781
MV %				
<0.5%	2.2	1.6 − 3.0	86/143	107/654
≥0.5%	5.2	2.8 − 9.8	14/32	107/654
MV copy #				
1-9 copies	1.8	0.9 − 3.8	8/15	148/720
10-99 copies	2.2	1.5 − 3.2	41/71	148/720
100-999 copies	3.0	2.0 − 4.5	35/55	148/720
≥1000 copies	4.1	2.5 − 6.8	20/38	148/720

Figure 3.
Effect of Minority Variants and Antiretroviral Therapy Adherence on Virologic Failure
Abbreviations: MV, Minority Variants; VF, Virologic Failure
[a]Multivariate Cox regression analysis included adherence, ethnicity, baseline CD4 cell count, and HIV-1 RNA levels.
[b]Number of participants with virologic failure / total participants categorized by those with and without detectable minority variants. Participant numbers include additional virologic failure cases from the case-control and case cohort studies[20–23].
Cox proportional hazard rations shown are in comparison to those without minority variants unless otherwise noted. Three studies contributed to the adherence analysis[14, 19, 20].
Analysis of MV copy numbers excluded three studies using assays that could not provide a percentage[17, 21, 22]. For the MV 1% threshold analysis, one study was excluded due to a limit of detection of 2% for the assay[17] and only NNRT1 MVs were evaluated for two studies due to incompatible limits of detection fon the NRTI MVs[21, 22]. Four studies were excluded from the 0.5% threshold analysis due to higher limits of MV detection[14, 17, 21, 22].
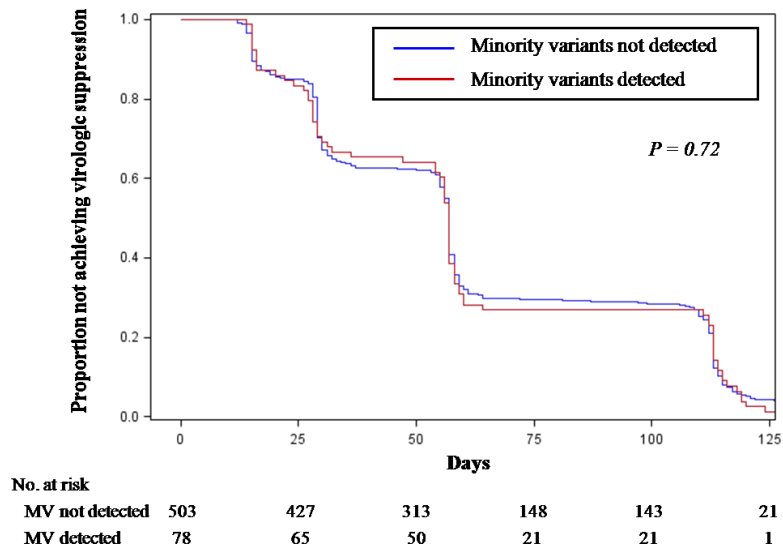
Figure 4.
Time to HIV-1 RNA <200 copies/ml in Patients Achieving Virologic Suppression
Abbreviations: MV, minority variants
Two studies with frequent HIV-1 RNA monitoring[19,20] were used to determine flie time to
HIV-1 RNA <200 copies/ml among individuals who became virologically suppressed. P-
value comparison by Cox proportional hazard analysis. Median time to virologic
suppression was 57 days [IQR 28–112 days] for those with minority variants and 57 days
[IQR 27–111 days] for those without.

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Table 1

Baseline Characteristics of Studies Included in the Pooled-Analysis

Characteristic	Peuchant[15] 2008	Simen[14] 2009	Balduin[16] 2009	Jakobsen[17] 2010	Metzner[18] 2010	Goodman[19] 2011	Paredes[20] 2010	Johnson[21] 2008	Geretti[22] 2009	Metzner[23] 2009	Total
Study Design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case control	Case control	Case control	
Virologic Failure (N)	2	45	7	1	1	44	150	52	14	3	315
Total Participants (N)	13	70	54	20	56	423	280	240	89	18	1263
Age, mean (yr)	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
Male Sex % (n/N)	92 (12/13)	80 (56/70)	76 (41/54)	95 (19/20)	80 (45/56)	86 (365/423)	81 (227/280)	82 (196/240)	88 (78/89)	72 (13/18)	83 (1052/1263)
Ethnicity % (n/N)											
White	92 (12/13)	23 (16/70)	75 (39/52)			60 (253/422)	39 (110/279)	55 (132/240)	88 (78/89)	82 (14/17)	55 (654/1182)
Black	8 (1/13)	54 (38/70)	21 (11/52)			22 (94/422)	39 (110/279)	25 (61/240)	11 (10/89)	18 (3/17)	28 (328/1182)
Hispanic	0 (0/13)	20 (14/70)	0 (0/52)			14 (61/422)	19 (54/279)	18 (42/240)	0 (0/89)	0 (0/17)	14 (171/1182)
Others	0 (0/13)	3 (2/70)	4 (2/52)			3 (14/422)	2 (5/279)	2 (5/240)	1 (1/89)	0 (0/17)	2 (29/1182)
CD4+ Count, median (IQR)	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 cp/ml, median (IQR)	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: IQR, Inter-quartile range

Table 2

Characteristics of Minority Variants by Study

Characteristic	Peuchant[15] 2008	Simen[14] 2009	Balduin[16] 2009	Jakobsen[17] 2010	Metzner[18] 2010	Goodman[19] 2011	Paredes[20] 2010	Johnson[21] 2008	Geretti[22] 2009	Metzner[23] 2009	Total for Cohort Studies[d]
Study Design	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Case cohort	Case control	Case control	Case control	
Method of Detection[a]	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[b]		1.0		2.0					0.9		
Participants with MVs and VF/MVs[c]											
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[b]	3/3	3/3		0/0	0/0				0/0		3/3

Abbreviations: MVs, Minority Variants; VF, Virologic Failure

[a] AS-PCR, allele-specific PCR; 454, 454 ultradeep pyrosequencing; HIV-SNaPshot is named for a multiplex primer-extension assay for detecting HIV minority variants[17].

[b] Other NNRTI minority variants evaluated include G190A (Geretti), G190A/S/E (Jakobsen), and multiple (Simen). Three patients in Simen et al. were found to have other NNRTI minority mutations (G190A 2.1%, K101E 3%, and P225H 3.4%) and were included in the analysis as having an NNRTI minority variant.

[c] Only 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. includes entire case cohort study (participants in random subcohort and additional virologic failures).

[d] Totals only reflect participants of the cohort studies including the random subcohort from Paredes et al.'s case cohort analysis of A5095.