

# The Role of HIV-1 Drug-Resistant Minority Variants in Treatment Failure

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Human immunodeficiency virus type 1 (HIV-1) drug resistance genotyping is recommended to help in the selection of antiretroviral therapy and to prevent virologic failure. There are several ultrasensitive assays able to detect HIV-1 drug-resistance minority variants (DRMVs) not detectable by standard population sequencing-based HIV genotyping assays. Presence of these DRMVs has been shown to be clinically relevant, but its impact does not appear to be uniform across drug classes. In this review, we summarize key evidence for the clinical impact of DRMVs across drug classes for both antiretroviral treatment-naïve and antiretroviral treatment-experienced patients, and highlight areas where more supporting evidence is needed.

**Keywords.** HIV-1 drug resistance; drug resistant minority variants; treatment failure.

Human immunodeficiency virus type 1 (HIV-1) drug resistance genotyping is recommended to help in the selection of antiretroviral therapy (ART) and to prevent virologic failure (VF). Genotypic tests for HIV drug resistance that are based on standard population sequencing fail to detect drug-resistant minority variants (DRMVs) present in less than 15%–25% of the total viral population [1, 2]. More sensitive techniques have been developed, such as allele-specific polymerase chain reaction (ASPCR) [3], oligonucleotide ligation assay [4], SNaPshot assay [5], and ultra-deep sequencing (UDS) [6, 7], to detect and quantify DRMVs. The lower limit of detection of minority variants differs widely between assays, with an upper range of 2%–5% for the HIV-SNaPshot assay [5] and certain UDS assays [8], and a lower range of <0.01% has been reported for ASPCR [9].

DRMVs have been shown to be clinically relevant, but their impact does not appear to be uniform across drug classes. The clinical relevance of DRMVs is related to the genetic barrier to resistance to specific drugs and can be classified based on 3 levels of evidence. The best evidence that DRMVs may adversely affect response to ART lies in the assessment of resistance mutations active against the nonnucleoside reverse transcriptase inhibitors (NNRTIs) and CCR5 antagonists. These drugs have a low genetic barrier to resistance and a single mutation can dramatically impact drug susceptibility. A moderate amount of evidence has accumulated that DRMVs against nucleoside

reverse transcriptase inhibitors (NRTIs) and the integrase strand transfer inhibitor (INSTI) raltegravir may affect their clinical efficacy. Finally, in the case of the protease inhibitors (PIs), elvitegravir and dolutegravir, there is little evidence so far that DRMVs increase the risk of VF, although few studies have been performed and more research is needed. In this review, we review key evidence for both ART-naïve and ART-experienced patients and highlight areas where more supporting evidence is needed.

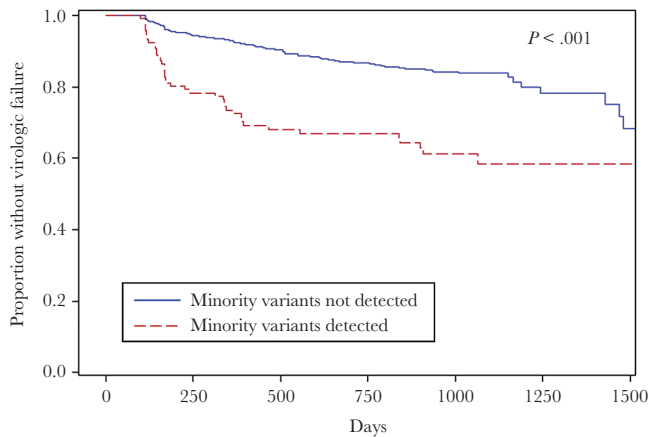
## NNRTIs AND NRTIs

There have been several studies evaluating the effects of baseline low-frequency NNRTI and NRTI resistance mutations on the rates of treatment failure for ART-naïve individuals. A pooled analysis was performed of 10 studies involving 985 ART-naïve participants and included only individuals with no detectable pre-ART NNRTI and NRTI resistance by standard genotyping [10]. The pooled analysis showed that 14% of participants harbored either NNRTI or NRTI DRMVs by ultrasensitive assays and the presence of DRMVs at baseline was associated with more than twice the risk of VF. The increased risk of VF was most evident early after therapy initiation (Figure 1) and was mediated primarily by NNRTI-resistant minority variants (hazard ratio [HR], 2.6). The effect of the minority variants was detectable even after controlling for medication adherence [11]. In addition, the risk of VF was detected in a dose-dependent manner and even at some of the lowest DRMV frequencies (<0.5% and 10–99 mutant copies/mL) [10]. These results are supported by several subsequent studies. In a Europe-wide case-control study of 260 participants initiating an NNRTI-based regimen, DRMVs were detected by UDS in 21% of subjects and the presence of DRMVs was associated with an increased risk of VF (odds ratio [OR], 2.75) [12]. The strength of the minority

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**Figure 1.** Kaplan-Meier curves for the proportion of patients without virologic failure on first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)-based combination antiretroviral therapy regimen by the presence of HIV-1 nucleoside reverse transcriptase inhibitor and NNRTI-resistant minority variants. Adapted from [10].

variant (MV) effect was similar between DRMVs to NNRTIs (OR, 2.41) or NRTIs (OR, 2.27), but the effect of the NRTI-resistant MVs did not reach statistical significance, possibly due to the smaller numbers of NRTI MVs detected. In addition, a direct dose-effect relationship between the mutational load of NNRTI DRMVs and risk of VF was found.

There is also strong evidence that NNRTI-resistant MVs are commonly found in those failing an NNRTI-based ART regimen and that these mutations increase in the risk of VF. In the AIDS Clinical Trials Group (ACTG) study 398, NNRTI-resistant MVs were more commonly detected in treatment-experienced individuals and were associated with an increased risk of virologic failure [13]. In addition, a number of other studies have demonstrated the detection of NNRTI and NRTI DRMVs in treatment-experienced individuals that may affect downstream treatment efficacy [14–19].

### NNRTIs IN AFRICAN STUDIES

Several studies have now demonstrated the selection of DRMVs in African patients treated with single-dose nevirapine (sdNVP) [20–22]. One of the largest studies was the Optimal Combination Therapy After Nevirapine Exposure (OCTANE) Trial 1 of 232 women treated with sdNVP with DRMVs evaluated by ASPCR. Exposure to sdNVP was associated with an increased detection of NNRTI DRMVs and these MVs were associated with a significantly increased risk of VF (HR, 2.71) after restarting an NNRTI-based regimen [23]. The risk of VF appeared to be mitigated by a longer interval between sdNVP exposure and start of combination ART [24]. This finding is likely due to the continued decay of the proportion of drug-resistant variants after sdNVP in light of the fact that DRMV frequency was associated with the extent of risk for the primary endpoint (VF or death) [23]. Interestingly,

in the parallel OCTANE Trial 2 of African women without prior sdNVP exposure, no significant association was detected between NNRTI DRMV detection and risk of treatment failure [25]. A subsequent study by the ANRS team also showed that while an ultrasensitive assay could detect more DRMVs in treatment-naive African patients, these mutations were not associated with an increased risk of VF [26]. The differential effects of DRMVs seen in these African patients could be related to differences in their NRTI backbone, HIV subtype differences, or extent of multiple linked mutations. However, these hypotheses deserve further exploration.

### CCR5 ANTAGONIST

HIV-1 requires the use of either CCR5 or CXCR4 as a co-receptor to enter target cells. Maraviroc, a CCR5 antagonist that blocks HIV-1 entry, was approved for clinical use against R5-tropic virus. Several phenotypic and genotypic assays have been developed to assess HIV-1 co-receptor tropism [27]. Historically, a phenotypic HIV tropism assay has been used in the United States, but minority populations of CXCR4-using (R4-tropic) virus can be a cause of VF [28–31]. In a retrospective reanalysis of treatment-naive patients, UDS showed the same ability as an improved version of the phenotypic assay in predicting the response to Maraviroc [32]. A genotypic tropism assay with UDS is now available in the United States (HIV-1 Coreceptor Tropism with Reflex to Ultradeep Sequencing, Quest Diagnostics). This test includes initial population sequencing followed by UDS analysis of those samples that showed only R5-tropic virus by population sequencing. In a retrospective analysis of 327 treatment-experienced patients who received maraviroc in the Maraviroc versus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients and A4001029 trials, this test had greater sensitivity than population sequencing alone to detect minority non-R5 and was equivalent to the phenotypic test for predicting maraviroc responders from nonresponders [33].

### INTEGRASE INHIBITORS

Integrase strand transfer inhibitors (INSTIs) have become a key component of ART, but the clinical impact of INSTI DRMVs remains understudied. The concern surrounding raltegravir (RAL) DRMVs is two-fold. First, there have been several case reports of RAL DRMVs that led to subsequent treatment failure [34, 35]. In addition, RAL DRMVs can be found in a large subset of treatment-naive patients [36] and may be found at a higher rate in treatment-naive individuals [37]. However, those findings have not been replicated in other studies and RAL DRMVs have not yet been found to definitively impact VF rates in larger studies of treatment-naive [6, 38, 39] or treatment-experienced individuals [40]. The clinical impact of DRMVs on elvitegravir (EVG) and dolutegravir (DTG) has not been carefully studied.

However, there is evidence that ultrasensitive assays can detect EVG and DTG DRMVs that would affect the predicted susceptibility profiles for these drugs, especially in INSTI-experienced patients [41, 42].

## PROTEASE INHIBITORS

The use of more sensitive genotyping methods has significantly increased the number of PI DRMVs detected in treatment-naïve patients, but these DRMVs have not been shown to affect the efficacy of first-line PI therapy [43, 44]. For example, a retrospective analysis of 123 baseline clinical samples from the ADVANZ and ADVANZ-3 studies found that NNRTI DRMVs increased the risk of VF for an EFV-based regimen, but PI DRMVs had no significant effect on VF for a PI-based regimen [14]. For treatment-experienced patients, PI DRMVs have been detected in a high proportion of individuals, but these mutations have also not been clearly linked to increased risk of VF [43, 45]. Several factors can contribute to this lack of association. PIs have a high genetic barrier to resistance and thus, multiple mutations are required to confer significant resistance [46, 47]. In addition, the prevalence of transmitted PI-resistance mutations is low and viruses with multiple PI-resistance mutations are rarely transmitted [48, 49]. Of note, in an analysis of the CASTLE study, the presence of NRTI DRMVs was associated with virologic failure of the lopinavir-based ART regimen [44], although this effect was not found in other studies [50].

## CONCLUSIONS

The detection of DRMVs of HIV-1 has been shown to be clinically significant mainly in 3 settings: detection of NNRTI-resistant minority variant prior to the initiation of a first-line NNRTI-based regimen outside of Africa, detection of NNRTI-resistant minority variants after exposure to sdNVP, and detection of CXCR4-using variants prior to initiation of maraviroc-containing regimens. However, there are a number of scenarios where the clinical impact of DRMVs remains controversial and additional studies are needed. These include first-line NNRTI-based regimens in an African setting, as well as DRMVs against NRTIs, PIs, and INSTIs.

During the past decade, UDS technologies have continued to evolve and their sequencing costs have been greatly reduced, making it an increasingly cost-efficient technique, especially in regions where demand for HIV genotyping is high [8, 51]. Adoption of these platforms may improve access to HIV genotyping worldwide, and additional studies on the clinical impact of DRMVs are needed to guide the interpretation of these minority variants in the clinical setting.

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