HIV-1 Drug-Resistant Minority Variants: Sweating the Small Stuff

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Keywords. HIV; minority variants; virologic failure.

Within each human immunodeficiency virus (HIV)–infected individual, there exists a remarkably heterogeneous collection of viruses. Over the course of a typical HIV infection, a starting population of relatively homogeneous virus may undergo substantial diversification, such that mutations may be detected at >10% of nucleotide positions [1]. This diversity is driven both by the error-prone nature of HIV reverse transcription and the high rate of viral replication. The HIV reverse transcriptase enzyme lacks a proofreading mechanism, and it is estimated that up to 5 mutations may be introduced during each replication cycle [2, 3]. In addition, the virus is thought to undergo 10–100 million rounds of replication daily, leading to the production of more than a billion new virions [4, 5].

This degree of viral diversity poses a significant challenge for current HIV genotypic drug resistance tests [6]. These assays use a population (Sanger) sequencing technique that cannot reliably detect drug resistance mutations present below 15%–20% of the viral population within an HIV-infected patient [7, 8]. These drug-resistant minority variants arise from 2 sources: drug-resistant viruses transmitted at the time of initial infection or de novo–generated variants that arise spontaneously. HIV harboring resistance mutations is generally less fit than wild-type viruses and in the absence of drug selective pressure, the frequency of HIV harboring drug resistance mutations will generally decay over time until these variants become a minority population no longer detectable by conventional resistance testing [9, 10]. HIV drug-resistant minority variants may also arise spontaneously as a result of the extensive viral diversification that occurs during the course of infection and may be present at low levels during chronic HIV infection even in the absence of drug exposure [11].

The presence of HIV drug-resistant minority variants can be detected by a number of ultrasensitive assays, such as allele-specific polymerase chain reaction (PCR) and next-generation sequencing [6]. Using these platforms, low-frequency HIV drug-resistant minority variants have been detected in a substantial proportion of both treatment-naive and treatment-experienced individuals. In one pooled analysis of treatment-naive patients, baseline low-frequency nucleoside reverse transcriptase inhibitor (NRTI) or nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations were detected in 14% of participants [12]. The use of ultrasensitive assays has also increased the detection of HIV drug-resistant minority variants that confer resistance to protease inhibitors [13, 14], integrase inhibitors [15, 16], and CCR5 antagonists [17, 18]. The prevalence of detectable minority variants will vary depending on the limit of detection of the assay as well as the number of resistance mutations evaluated by the assay.

There is strong evidence that HIV drug-resistant minority variants increase the risk of treatment failure under 3 clinical scenarios. First, several studies have shown that HIV minority variants can confer resistance to the CCR5 antagonists (eg, maraviroc). The mechanism is either through the utilization of drug-bound CCR5 coreceptor or the usage of the alternate CXCR4 coreceptor [19, 20]. In the second scenario, HIV-infected women who have been exposed to single-dose nevirapine (sdNVP) to prevent mother-to-child HIV transmission are at high risk of developing NNRTI resistance mutations, which are often present as minority variants not detectable by conventional resistance testing [21, 22]. The OCTANE/A5208 study was comprised of 2 concurrent, randomized trials evaluating the efficacy of a nevirapine vs ritonavir-boosted lopinavir combination antiretroviral therapy (cART) regimen. Trial 1 participants included 243 women who had previously received sdNVP and trial 2 enrolled participants without prior

Received and accepted 18 November 2013; electronically published 23 November 2013.

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The Journal of Infectious Diseases 2014;209:639–41

DOI: 10.1093/infdis/jit656

sdNVP exposure. In the analysis of the OCTANE/A5208 trial 1, Boltz et al showed that sdNVP-exposed participants with detectable NNRTI-resistant minority variants had 2.7 times the risk of virologic failure or death when treated with a nevirapine-based cART regimen [23]. Finally, a pooled analysis of 10 studies demonstrated that NNRTI-resistant minority variants increase the risk of virologic failure for treatment-naive patients initiating an NNRTI-based cART regimen [12], even after taking into account medication adherence and other factors [24]. However, it was not clear whether the results of this study extended to resource-limited settings, as participants were only enrolled from North America and Europe.

In this issue of *The Journal of Infectious Diseases*, Boltz et al addressed this question by turning their attention to evaluating the impact of NNRTI-resistant minority variants in the OCTANE/A5208 trial 2 [25], which evaluated 500 treatment-naive African women from 7 countries who had no prior sdNVP exposure. In this analysis, the authors used an ultrasensitive HIV resistance assay (allele-specific PCR) to measure the levels of 3 key NNRTI resistance mutations (K103N, Y181C, and G190A) in 219 participants randomized to receive the nevirapine-based regimen. They compared the results to their previously published analysis of sdNVP-exposed participants in OCTANE/A5208 trial 1 [23]. As expected, the prevalence of baseline NNRTI-resistant minority variants was lower in treatment-naive participants compared to those with prior sdNVP exposure (18% vs 45%, respectively). However, participants of trial 2 with detectable NNRTI-resistant minority variants did not have a higher risk of virologic failure or death compared to those without detectable resistance (21% vs 17%).

The results of this study are surprising as they contrast sharply with both the findings of OCTANE/A5208 trial 1 in sdNVP-exposed women [23] and the results of the pooled analysis of treatment-naive individuals performed in North America and Europe [12]. How do we reconcile the discordant findings from these studies? The authors state that the differences seen between sdNVP-exposed and unexposed patients did not appear to be related to either the prevalence of the minority variant in the patient population or the frequencies of minority variants detected in the viral population. They postulate that the differences may be due to sdNVP exposure and the presence of other linked resistance mutations from prior ART exposure that were unmeasured in this study. Although this hypothesis has merit, unmeasured NRTI resistance mutations would only be expected in a small subset of OCTANE/A5208 trial 1 participants as only 10% had prior NRTI exposure, and HIV variants with >1 resistance mutation are highly unlikely to arise without drug selective pressure [11]. Alternatively, resistance mutations that developed in the setting of sdNVP may have seeded a larger proportion of the cellular HIV reservoir, as these mutations are generally found at high proportions before decaying because of fitness constraints. A larger reservoir of drug-resistant variants may increase the chances that suboptimal ART adherence or tissue penetration may lead to resistance emergence and virologic failure.

The authors also hypothesize that linked drug resistance mutations could also account for differences seen between this study and a pooled analysis of treatment-naive patients from North America and Europe [12]. Developed countries tend to have higher rates of transmitted drug resistance, which often involve the transmission of multiple, linked mutations. The authors speculate that fewer of the drug-resistant minority variants in this study will contain additional linked mutations, given the lower rates of transmitted drug resistance in Africa. They also noted differences in HIV subtypes between this study and those performed in North America and Europe. More difficult to explain, however, is the contrast of these results with a smaller study by Coovadia et al of 94 HIV-infected women in South Africa with prior sdNVP exposure and 60 women without sdNVP exposure [26]. In that study, the detection of the K103N minority variant was predictive of inadequate virologic response, regardless of sdNVP exposure history. The study reported by Boltz et al is larger, but there are many similarities to the 2 studies. Both studies were performed in Africa with largely subtype C HIV infections, and both studies used an ultrasensitive allele-specific PCR assay to detect the minority variants. Nonetheless, the results of the current study are intriguing and may suggest that the associations seen between NNRTI-resistant minority variants and the risk of virologic failure may not be as straightforward as originally thought. Additional studies are needed to fully explore the clinical significance of drug-resistant HIV minority variants, both in the developed and developing regions of the world.

Next-generation deep sequencing platforms, such as those offered by 454 Life Sciences and Illumina, have transformed the study of HIV minority variants. Unlike point-mutation assays such as allele-specific PCR, next-generation sequencing offers the advantage of evaluating all HIV resistance mutations within the sequenced regions. These platforms not only detect the presence of drug-resistant minority variants, but are also more cost-effective than current Sanger sequencing–based techniques [27]. Next-generation sequencing is already being offered as part of a commercial HIV tropism test [28], and will likely be adopted for clinical HIV resistance testing, especially for high-throughput centers performing large numbers of HIV resistance genotyping. In the near future, clinicians may be provided details of HIV minority variants detected by these assays, and additional studies will be needed to guide the clinical interpretation of such testing results.

Notes

Financial support. This work was supported in part by National Institutes of Health (NIH) grants K08 AI100699 and R01 AI098558.

Potential conflicts of interest. The author has served as a consultant for TherapyEdge, SeraCare Life Sciences, and Quest Diagnostics.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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