When Do Minority Drug-Resistant HIV-1 Variants Have a Major Clinical Impact?

Walid Heneine
Laboratory Branch, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

(See the article by Paredes et al, on pages 662–671, and the article by Halvas et al, on pages 672–680.)

As our understanding of the virologic and clinical significance of drug-resistant human immunodeficiency virus type 1 (HIV-1) continues to expand, attention is now turning to detecting and assessing the clinical impact of minority (low frequency/abundance) drug-resistant HIV-1, the variants in the viral population present below the ~20% detection threshold of conventional bulk sequencing. The interest in studying minority resistance has been fueled by improved diagnostic technologies that allow detection of drug-resistant variants below the 1% threshold, and by the recognition that drug-resistant viral populations often exist as a complex mixture of genetic populations in which only the dominant viral sequence is detected by standard sequencing. Three common approaches are used to detect minority resistance: point mutations assays, clonal sequencing, and ultra deep sequencing. Point mutations assays selectively amplify resistance or wild-type alleles at a drug resistance position and can have detection limits as low as 0.0001% on cloned mutant templates, although such detection thresholds are not always achieved in clinical samples [1–4]. These assays are so sensitive that they are able to detect naturally occurring mutations in the viral quasispecies of samples from the pre-antiretroviral drug era but can be adjusted to cutoffs above which the detection of any mutation would reflect the presence of drug-selected viruses [1]. Clonal sequencing uses the standard sequencing method on a large number of variants cloned from polymerase chain reaction (PCR)-amplified viral RNA from a plasma sample or generated by limiting-dilution PCR such as in single-genome sequencing methods [5]. Ultra-deep sequencing refers to more recently developed high-throughput sequencing technologies like pyrosequencing that allow sequencing large numbers of individual DNA molecules in a sample [6]. Two articles in the current issue of the Journal by Halvas et al [7] and Paredes et al [8] use the first 2 approaches to examine the clinical impact of minority nonnucleoside reverse-transcriptase inhibitor (NNRTI) mutations in treatment-naive and treatment-experienced patients. NNRTIs are essential components of antiretroviral therapy. Treatment guidelines recommend NNRTIs with a combination of nucleoside or nucleotide reverse-transcriptase inhibitors for initial therapy [9]. NNRTIs have a low genetic barrier to resistance often requiring only 1 mutation to cause resistance to the commonly used first-generation NNRTIs efavirenz and nevirapine. The K103N mutation in reverse transcriptase is the most frequently seen NNRTI mutation in patients who experience failure of NNRTI-based regimens, although other mutations such as Y181C are also prevalent [10].

In patients who have received antiretroviral therapy, a few studies have reported minority resistant variants to either NNRTIs or protease inhibitors [11, 12]. These variants often expanded clinical cross-resistance to other drugs in the same class and predicted failure of second regimens containing such drugs [13, 14]. The study of Halvas et al [7] in this issue provides direct evidence that minority NNRTI-resistant variants not detected by bulk sequencing are common among patients with prior exposure to NNRTIs (nevirapine or delavirdine) and, importantly, contribute to failure of antiretroviral regimens containing the NNRTI efavirenz. The authors retrospectively analyzed samples for minority NNRTI resistance by single-genome sequencing and allele-specific PCR from patients enrolled in the AIDS Clinical Trials Group (ACTG) study 398. They show that these minority mutations were detected by both methods.
at study entry more frequently in NNRTI-experienced than in NNRTI-naive patients. For instance, an average of 46 sequences generated for each sample by single-genome sequencing showed that sequences with NNRTI resistance mutations were detected in 8 of 12 NNRTI-experienced patients, compared with 3 of 15 NNRTI-naive patients \( (P = .022) \). The fraction of these sequences was higher in the NNRTI-experienced group than in the NNRTI-naive group. Because the mean duration after discontinuation of prior NNRTI therapy in the NNRTI-experienced patients was \( \sim 1 \) year, these findings also reflect the persistently long and ongoing replication of these NNRTI-resistant mutants. There are at least 2 ways by which minority resistant variants present before therapy contribute to virologic failure. They can be selected directly with minimal evolution by the regimen, outgrowing wild-type variants to become the dominant mutant virus population during virologic failure. Data provided by Halvas et al [7] support this possibility. The authors phylogenetically analyzed NNRTI-resistant and wild-type sequences at entry and at the time of virologic failure and demonstrated tight clustering of NNRTI-resistant sequences in 2 of 11 patients. Although limited sequence sampling and recombination may explain the lack of clustering in some patients, it is also possible that minority drug-resistant variants contribute to virologic failure through a second mechanism that provides a replicating virus population from which more-resistant viruses emerge. The authors also quantified the prevalence of K103N and Y181C by allele-specific PCR in 95 additional patients and confirmed that baseline samples from NNRTI-experienced patients had more Y181C or K103N mutations than those from NNRTI-naive patients. Importantly, K103N at frequencies of 0.5%–1%, but not \( \leq 0.5\% \), was strongly associated with decreased virologic response to efavirenz-containing regimens. By contrast, Y181C minority variants, whether at frequencies \( \leq 1\% \) or \( > 1\% \), were not associated with virologic failure, a finding likely reflecting the higher level of resistance of K103N variants to efavirenz, compared with Y181C variants [15]. These data are important because they point to a threshold above which minority mutants become clinically relevant.

NNRTI mutations are also increasingly seen in patients who have not received antiretroviral therapy and have replaced thymidine analog mutations as the most prevalent mutations, accounting for at least one-half of all transmitted resistance among persons who are newly diagnosed with HIV-1 infection in the United States [16, 17]. Many recent studies have detected minority NNRTI resistant variants in this population and some evaluated their impact on treatment efficacy [6, 13, 18, 19]. In another article in this issue of the Journal, Paredes et al [8] examined the clinical relevance of minority K103N and Y181C among drug-naive patients. The authors used allele-specific PCR to screen baseline samples of patients in ACTG A5095 prior to initial antiretroviral therapy with an efavirenz-containing regimen. A total of 195 patients were evaluated, including a subcohort of 51 randomly selected individuals and 127 nonrandomly selected subjects who experienced virologic failure, in addition to 144 subjects who successfully suppressed virus. Using low interpretation thresholds for the K103N (0.001%–0.003%) and Y181C (0.03%) assays, minority K103N or Y181C mutations were detected in 4.4% and 29.5%, respectively, in the random subcohort, and 6% had both mutations. The levels of detected mutants were all \(< 1\% \); the median prevalence of Y181C was 0.06%, and the median prevalence of K103N alleles was 0.012% and 0.013%. The 29.5% prevalence of minority Y181C was a substantial increase over the 0.2% Y181C found by standard sequencing and raises the possibility that the Y181C assay used captures naturally occurring Y181C variants in the viral quasispecies of some patients. Nonetheless, the authors found that Y181C was associated with a 3.5-fold increased risk of virologic failure in the presence of good treatment adherence, but this relationship was not observed in nonadherent patients.

This virologic outcome is seen despite the lower levels of minority Y181C in this study, compared with that reported by Halvas et al [7]. Detection of minority K103N was not associated with virologic failure, although this conclusion may be limited by a small sample. Genotyping at the time of virologic failure of patients who had baseline minority Y181C showed that detection of minority Y181C did not predispose for Y181C emergence, because only 7% of these patients failed with Y181C mutants and 38% failed with K103N mutants. Consistent with the observations by Halvas et al [7], minority Y181C variants in the Paredes et al [8] study may have contributed to virologic failure by providing a replicating virus population from which more-resistant viruses with K103N and G190S mutations emerged. Thus, this study advances our understanding of the clinical impact of minority Y181C occurring in \(< 0.1\% \) of the viral population.

What are the implications of these studies? They both extend previous findings and demonstrate that minority NNRTI mutants increase the risk of virologic failure of efavirenz-based regimens [6, 18, 19]. Both studies indicate that the risk of virologic failure depends on both the individual NNRTI mutation and its frequency in the viral population. Other factors, such as treatment adherence, could also increase risks of treatment failure if mutations are present at very low levels. Both studies exemplify the advances in diagnostic drug resistance testing and illustrate how the clinical significance of new technologies can be assessed in retrospective studies of patients with well-defined virologic outcomes. However, future research should build on this work and focus on refining assay thresholds that identify patients in different treatment settings at greatest risk of experiencing poor outcomes. Answering such questions will require studies with large numbers of patients and standardized methods and can be facilitated by available low-cost point mutations assays. Collectively, these data
will inform decisions on when testing for minority drug resistance will be beneficial for patient management.

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References