

## Perspective

# Transmitted Minority Drug-Resistant HIV Variants: A New Epidemic?

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Despite high rates of viral turnover and viral evolution, HIV has proven to be surprisingly easy to suppress with combination antiretroviral therapy (ART) in the regions of the world where such treatment is available. Recent reports indicate that the vast majority of patients initiating ART should be able to achieve durable if not indefinite viral suppression [1]. Given that there are now over 20 antiretroviral drugs from six unique classes, even if one regimen fails, others are often readily available. The emerging consensus among clinicians and clinical investigators is that fewer and fewer patients will generate highly resistant HIV during the course of their treatment.

## Transmitted Antiretroviral Drug Resistance

As the prevalence of drug resistance among long-term treated patients wanes, concerns are now turning to those treatment-naïve patients who may have acquired drug resistance from their partners. There are two ways in which they can acquire such resistance—either at the time of primary infection or (less commonly) after a “superinfection” event (i.e., a person with HIV becomes infected with a second, drug-resistant HIV strain) [2,3]. Using standard “bulk sequencing” genotypic assays, researchers have found that the percentage of treatment-naïve patients with detectable levels of antiretroviral drug resistance has been stable in the 5% to 15% range [4–8]. Given this high prevalence of resistance, most guideline panels now recommend the use of genotypic resistance testing prior to the introduction of ART.

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## Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine*:

Johnson JA, Li J-F, Wei X, Lipscomb J, Irlbeck D, et al. (2008) Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naïve populations and associate with reduced treatment efficacy. *PLoS Med* 5(7): e158. doi:10.1371/journal.pmed.0050158

Using real-time PCR to detect HIV resistance mutations present at low levels, Jeffrey Johnson and colleagues investigate prevalence and clinical implications of minority transmitted mutations.

Several lines of evidence suggest that the reported prevalence of drug resistance among treatment-naïve individuals under-represents the true scope of the issue. In the absence of selective pressure exerted by antiretroviral drugs, drug resistance mutations often wane to low levels, presumably as these mutations negatively affect viral replicative fitness [9,10]. Hence, it is likely that by the time individuals present clinically, resistance mutations may have declined below readily detectable levels, although they will persist indefinitely in the “latent reservoir.” Also, acute HIV infection is often associated with the transmission of multiple distinct variants, some of which may persist at very low levels [11].

To determine if low-level (or “minority”) drug-resistant variants of HIV exist in untreated individuals, Jeffrey Johnson and colleagues developed and validated a highly sensitive real-time polymerase chain reaction assay that can detect certain drug resistance mutations, even when such mutations are present in less than 1% of the plasma virus population [12].

This assay was so sensitive that it risked detecting mutations that emerge as a consequence of natural (unselected) variation [13]. In an elegant series of studies aimed at validating their assay, reported in *PLoS ONE*, Johnson et al. applied this assay to a cohort of individuals from the pre-antiretroviral drug era and established specific levels above which the detection of any mutation would likely reflect the presence of a drug-selected virus population [12].

As reported in the current issue of *PLoS Medicine*, Johnson and colleagues next applied this assay to several distinct cohorts [14]. Two novel and important findings were reported. First, among a cohort of 205 HIV-infected, treatment-naïve individuals in Los Angeles and Chicago who lacked any evidence of drug resistance using conventional assays, 34 (17%) harbored a minority variant containing at least one clinically relevant drug resistance mutation. As these assays can only detect a subset of the known drug resistance mutations, the true

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**Abbreviations:** ART, antiretroviral therapy

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prevalence of transmitted drug resistance may have been even greater.

The second and perhaps more worrisome finding comes from a retrospective case-control analysis of treatment-naïve individuals who enrolled in one of two clinical trials. All individuals received a standard regimen of two nucleoside analogues and efavirenz, a non-nucleoside reverse transcriptase inhibitor. Of the approximately 1,400 individuals who received therapy and who lacked readily detectable drug resistance at baseline, 95 eventually met a standard definition of virologic failure (these 95 patients were selected as the cases in the case-control study). Of the remaining individuals whose treatment did not fail, 221 were selected as controls. The frequency of low-level drug-resistant variants was higher in the cases (seven of 95, or 7.4%) than the controls (two of 221, or 0.9%) ( $p = 0.004$ ). In a logistic regression analysis, the presence of low-frequency drug resistance mutations was independently predictive of subsequent failure, although the small number of controls with low-level resistance did not permit precise estimates of risk.

### Clinical Implications and Unresolved Issues

Assuming that further clinical evidence confirms this novel association between minority resistance mutations and HIV treatment failure, should we be screening treatment-naïve individuals for minority resistance variants, and if so, will it be feasible to develop commercially viable assays? Although the concept of screening everyone who starts ART for pre-existing drug resistance seems logical, a close look at the data might suggest otherwise. Of the over 1,400 treatment-naïve patients who received a standard regimen of two nucleoside analogues and efavirenz, only seven subsequent failures would have been avoided by screening for the key drug resistance mutations (K103N, M184V, or Y181C). Also, if one reasonably assumes that the prevalence of minority drug-resistant variants was the same in all of the treatment successes as was present in the subset tested, then more treatment successes than failures had low levels of drug-resistant HIV before starting therapy. In terms of identifying patients likely to experience treatment failure,

the positive predictive value of this test might therefore be expected to be low. Given the considerable expense associated with the various methods to detect low-level variants, and given the challenges of routinely accessing other complex but clinically useful assays (e.g., assays used to determine which coreceptor HIV uses for cell entry), it seems unlikely that approaches to detect minority variants will become widely available in the near future.

The work of Johnson and colleagues also provides important insights into HIV pathogenesis, or at least opens up new avenues for future research. The current accepted wisdom is that once resistance is present, it becomes permanently integrated in the human genome, and will re-emerge under subsequent drug pressure. If this is so, then it is difficult to explain why individuals with detectable resistance to efavirenz had a robust response to a regimen containing this drug. This counterintuitive outcome was even more dramatic in a recent analysis of efavirenz-treated individuals enrolled in clinical trial ACTG 5095 [15]. Using a very sensitive allele-specific PCR assay, researchers found low levels of certain efavirenz-associated mutations in a large subset of patients. Most of the study participants harboring a resistant variant responded well to therapy (the assay used in this study was very sensitive, and likely detected variants that appeared as a consequence of natural variation). Similarly, many pregnant women administered a single dose of nevirapine to prevent HIV transmission show transient evidence of high-level nevirapine resistance [16], yet exposure to single-dose nevirapine is not associated with failure in subsequent regimens, as long as these regimens are initiated at least six months after nevirapine exposure [17,18]. Collectively, these observations challenge the assumption that once resistance emerges, it is permanently archived in a manner that compromises future therapeutic options.

In summary, a sizable proportion of treatment-naïve HIV-infected individuals harbor a minority population of drug-resistant HIV. In some individuals, these infrequent variants may reflect natural variability and are unlikely to compromise treatment, while in the remaining individuals these variants likely reflect

transmission of a virus population that has been exposed to suboptimal drug pressures. This latter viral population can clearly compromise future responses in some but not all individuals. It remains to be determined if the prevalence of these presumably transmitted mutations will wane with time (as might be expected given the general reduction in drug resistance among the chronically infected population). It also remains to be determined whether assays for the detection of low-level variants can be developed for patient management. ■

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