

Perspective

HIV Resistance: Frequency, Testing, Mechanisms

Recent data suggest that the prevalence of drug-resistant HIV in newly diagnosed patients may have leveled off at approximately 10% over the past several years. Further, there is some indication that the rates of emergence of resistance during long-term treatment have decreased in recent years with optimization of antiretroviral therapy. The importance of initial resistance testing is emphasized by findings indicating markedly increased risk of treatment failure in patients with resistance mutations at baseline. Genotypic and phenotypic testing are each associated with advantages and disadvantages in initial and subsequent resistance testing. Data on resistance patterns for new drugs and new drug classes are beginning to emerge. This article summarizes a presentation on HIV resistance made by Daniel R. Kuritzkes, MD, at an International AIDS Society–USA Continuing Medical Education course in San Francisco in May 2007. The original presentation is available as a Webcast at www.iasusa.org

Prevalence of Antiretroviral Resistance

The prevalence of antiretroviral-resistant virus in patients with newly diagnosed HIV infection according to Centers for Disease Control and Prevention (CDC) surveys is shown in Table 1. In the most recent survey, covering 2003 to 2006, 10.9% of patients exhibited resistance to any drug, with the majority having resistance to nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs) and 1.9% having resistance to at least 1 drug in numerous drug classes. At one point, data for this latter period indicated a prevalence rate of approximately 15% for resistance to any drug, and it is unclear whether a peak and decline in prevalence occurred or whether prevalence has indeed been stable at around 10% for the past 4 or 5 years. It should also be noted that very few of the patients with multiclass resistance exhibit resistance to multiple drugs or all drugs within multiple classes.

Data on long-term risk of developing resistance from the UK Collaborative HIV Cohort (CHIC) study in 4306 patients beginning antiretroviral therapy with 2 nucleoside analogue reverse

transcriptase inhibitors (nRTIs) and a third agent show that risk of accumulating resistance mutations to any drug over 6 years was 27%, in the context of an overall treatment failure rate of 38% (Phillips et al, *AIDS*, 2005).

As shown in Figure 1, resistance to 2 and 3 drug classes occurred in approximately 20% and 5% of patients, respectively, after 6 years. However, these data were first reported in 2004 and may reflect antiretroviral therapy practices from as early as 1998. With use of newer, better-tolerated, and

more potent regimens in recent years (including fixed-dose nRTI combinations, once-daily regimens, ritonavir-boosted protease inhibitors [PIs], and efavirenz-based regimens), virologic failure rates and resistance rates are likely lower than those reflected in this study. This impression is supported by some data, including those from a chart review study in the University of North Carolina HIV Cohort Study (n = 1466; Napravnik et al, *Antivir Ther*, 2006). Overall, 8% of patients had triple-class resistance, with independent predictors of such resistance consisting of prior use of antiretrovirals (odds ratio [OR], 1.7) and use of a nonantiretroviral regimen as the first treatment regimen (OR, 1.7). Of 24 patients with triple-class resistance whose first regimen was potent antiretroviral therapy, the regimen included an unboosted PI in 21 (87.5%) and nelfinavir in 15. These findings suggest that triple-class resistance is indeed likely to become less common in patients initiating antiretroviral therapy as currently practiced.

Based in part on evidence that resistance testing before treatment is cost-effective when the prevalence of resistance in untreated patients is greater

Table 1. Centers for Disease Control and Prevention Surveys: Drug-resistant HIV Among Newly Diagnosed Patients

	Prevalence of Drug Resistance (%)			
	1998 (n = 257)	1999 (n = 239)	2000 (n = 299)	2003 – 2006 (n = 3130)
Any drug	5.5	8.8	10.7	10.4
nRTI	5.1	7.1	7.7	3.6
NNRTI	0.4	2.1	1.7	6.9
PI	0	0.8	3.0	2.4
≥2 drug classes	0	1.3	1.3	1.9

nRTI indicates nucleoside analogue reverse transcriptase inhibitor; NNRTI, nonnucleoside analogue reverse transcriptase inhibitor; PI, protease inhibitor. Adapted from Bennett et al, 9th CROI, 2002; Bennett et al, 12th CROI 2005; Wheeler et al, 14th CROI, 2007.

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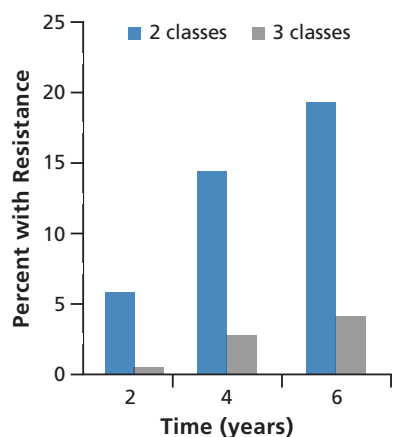


Figure 1. Risk of developing multiclass antiretroviral resistance over 6 years among 4306 patients starting therapy with triple-drug therapy including 2 nucleoside analogue reverse transcriptase inhibitors. Adapted from Phillips et al, *AIDS*, 2005.

than 1%, pretreatment testing is recommended by Department of Health and Human Services and International AIDS Society–USA guidelines (Hammer et al, *JAMA*, 2006; Hirsch et al, *Clin Infect Dis*, 2003). Genotypic testing is preferred for initial testing, because it at least identifies minority variants that constitute more than 20% of the viral pool in a patient, whereas reduced susceptibility might not yet be evident on phenotypic testing. Resistance testing should be performed at the first evaluation after diagnosis of infection.

Whether repeat testing should be performed in patients for whom there is a considerable duration between initial testing and the start of antiretroviral therapy is unresolved. There is a small chance that an individual might acquire superinfection with a resistant strain after initial testing. However, given the low likelihood of this occurrence, it is rational to begin antiretroviral therapy selected on the basis of the initial resistance test, assess HIV RNA level at 2 and 4 weeks, and perform resistance testing again if the expected viral response is not observed.

Dr Kuritzkes and colleagues recently performed a case-cohort study in the population of AIDS Clinical Trial Group (ACTG) 5095 study that provides information on risk of treatment failure in

patients with pre-existing antiretroviral mutations at the time of first antiretroviral therapy (Kuritzkes et al, *J Infect Dis*, in press). The ACTG 5095 study results showed that there was no improvement in virologic response or risk of treatment failure with the addition of abacavir to efavirenz plus zidovudine/lamivudine after a median of 144 weeks. The case-cohort study was performed because assessment of baseline samples in patients in whom therapy was failing in ACTG 5095 showed an appreciable frequency of resistance at baseline.

Patients in whom therapy had failed and in whom baseline genotypic testing had subsequently been performed were pooled with a randomly selected subpopulation in the trial. Overall, baseline genotypic testing was available for 191 treatment failures and 151 nonfailures. Baseline efavirenz resistance was present in 8% of patients with treatment failure versus 2% of nonfailures; lamivudine resistance was present in 1% versus 0%; and PI resistance was present in 3% versus 2%. Overall, the risk of treatment failure was markedly higher for patients with efavirenz resistance at baseline versus no efavirenz resistance (relative hazard, 2.27). Each of the 3 patients with baseline efavirenz resistance who did not have treatment failure had received the 4-drug regimen.

Testing Methods

Genotypic and phenotypic testing each have advantages and disadvantages. Advantages of genotypic testing include that it is less expensive and faster than phenotypic testing. In addition, some idea of potential resistance to a new drug in an established class can be gained from knowing the resistance mutations for other members of the class. Genotypic testing also permits determination of viral subtype and ruling out of contamination or sample mix-up by phylogenetic analysis. A disadvantage of genotypic testing is its reliance on algorithms for interpretation, given the fact that algorithms rely on expert opinion and thus emerge and are updated slowly. It is also difficult

to predict the net effect of mutational interactions (eg, resistance mutations for a particular drug may increase or decrease susceptibility to another drug) solely on the basis of genotyping. Because this problem is fairly common with PI mutations, phenotypic testing may be more useful when attempting to identify resistance or sensitivity of virus to individual PIs and in highly treatment-experienced patients.

Advantages of phenotypic testing include the fact that the amount of drug needed to inhibit the virus (at least in the laboratory) is specified, a measurement that includes the net effect of multiple mutations, if present. For this reason, as noted, phenotypic testing results may be more helpful for decisions regarding PIs. Phenotypic testing also detects resistance that arises from previously unidentified resistance mutations. Disadvantages of the assays include their high cost and lengthy turnaround time, and the fact that values in the susceptible range may be reported when there are resistant minority variants present, with identification of the latter requiring genotypic testing. If sequencing is not provided, phenotypic testing also does not include viral subtyping or provide the ability to rule out sample contamination or mix-up. Further, the reliability of clinical cut-off values reported for some drugs is uncertain, because the values are close to assay precision limits. Owing to regulatory issues, phenotypic testing of patients with regard to new drugs is frequently not available until after US Food and Drug Administration (FDA) approval of the agents.

Virtual phenotypic testing is an approach to interpreting the genotype in which a patient's genotype is matched in a database correlating genotype with phenotype. Advantages include the fact that, as noted for the genotypic test, virtual phenotypic testing is less expensive and time-consuming to run than performing actual phenotypic testing, and it is convenient that the virtual phenotypic testing provides upper and lower cut-off values for most drugs. Disadvantages include the fact that the current version of the virtual phenotypic test has not been validated in clinical

trials and that it provides only an estimate of the likely phenotype. Further, the predictions can be only as good as the matches in the database; thus, for example, predictions made on the basis of mutation patterns that are not represented or are underrepresented in the database may have questionable accuracy.

Regarding which of these assays to use and when, Dr Kuritzkes' opinion is that genotypic testing may be most useful for initial evaluation and after failure of a first- or second-line regimen, at which points resistance patterns are more likely to have a straightforward interpretation. Phenotypic testing, usually in combination with genotypic testing, may be more useful in patients with a more complex history of treatment failure that is likely associated with more complex resistance patterns. The approach to testing is limited by access, which may be affected by what tests the payer is willing to support in particular settings.

Resistance to Newer Drugs

Tipranavir

Of some 21 mutations at 16 protease codons identified as contributing to tipranavir resistance, many (eg, at codons 13, 35, 43, 58, 74, and 83) have not been associated with resistance to other PIs. Major mutations associated with resistance to other PIs do not appear to contribute to the tipranavir mutation score (eg, D30N, G48V, N88D, and perhaps L90M). Initial analyses showed that response to tipranavir-containing regimens was inversely correlated with the number of mutations present from the set including L33F; V82L or T; I84V; or L90M. Subsequent analyses led to development of a more comprehensive tipranavir resistance score based on mutations at 16 positions in protease (L10V; I13V; K20M, R, or V; L33F; E35G; 36I; K43T; M46L; I47V; I54A, M, or V; Q58E; H69K; T74P; V82L or T, N83D; and I84V). An increasing number of these mutations was associated with decreased in vitro susceptibility of HIV-1 isolates and decreased virologic response to tipranavir in the phase II

and III clinical trials of this drug (Baxter et al, *J Virol*, 2006). Analysis of predictors of response to tipranavir/ritonavir in randomly selected samples from the Randomized Evaluation of Strategic Intervention in Multidrug Resistant Patients with Tipranavir (RESIST) 1 and 2 trials showed estimated effects on HIV RNA level at 24 weeks as follows: $-1.25 \log_{10}$ copies/mL for tipranavir/ritonavir use; $-0.91 \log_{10}$ copies/mL with enfuvirtide use; $-0.24 \log_{10}$ copies/mL for each active drug in optimized background therapy (OBT); and $+0.17 \log_{10}$ copies/mL per mutation in the tipranavir mutation score (each $P < .01$). The small effect of the active drugs in OBT suggests that resistance may have been misclassified using the genotypic or phenotypic mutation score.

The conclusions regarding tipranavir resistance are that decreased susceptibility is indicated by a 3-fold to less than 10-fold increase in the 50% inhibitory concentration (IC_{50}) value, and that resistance is indicated by a 10-fold or greater increase. For genotypic testing findings, a change of 0 to less than 3-fold in susceptibility at baseline is associated with a key mutation score (at codons 33, 82, 84, and 90) of 0 to 2 and a tipranavir score of 0 to 4; a change of 3-fold to less than 10-fold with scores of 3 and 5 to 7, respectively; and a change of 10-fold or more with scores of 4 and 8 or higher, respectively. The prominent emergent mutations with tipranavir are L33F, I, or V; V82T or L; and I84V.

Darunavir

In the Performance of TMC114/ritonavir When Evaluated in Treatment Experienced Patients with PI Resistance (POWER) 1, 2, and 3 studies, 11 mutations were associated with diminished response to darunavir (V11I; V32I; L33F; I47V; I50V; I54L or M; G73S; L76V; I84V; and L89V). Analysis of virologic response at 24 weeks showed reductions of $2.08 \log_{10}$ copies/mL when baseline change in darunavir susceptibility was 10-fold or less, $1.08 \log_{10}$ copies/mL with greater than 10- to 40-fold or less change, and $0.76 \log_{10}$ copies/mL with a greater than 40-fold change;

proportions of patients with reduction in viral load to less than 50 copies/mL were 50%, 25%, and 13%, respectively (De Meyer et al, *Antivir Ther*, 2006). Regression analysis indicated estimated fold-changes in darunavir susceptibility of less than 2 with mutations V11I, I54L, G73S, and L89V; 2- to 3-fold with V32I, L33F, and I47V; 3- to 4-fold with I54M, L76V, and I84V; and greater than 4-fold with I50V. Proportions of patients with viral load reductions to less than 50 copies/mL were 64% with no mutations, 50% with 1 mutation, 42% with 2 mutations, 22% with 3 mutations, and 10% with 4 mutations. Darunavir and amprenavir n -fold changes are highly correlated in patient samples, reflecting the sharing of the I50V mutation and other key amprenavir mutations. Darunavir and tipranavir n -fold changes exhibit no clear correlation (Elston et al, 14th CROI, 2007).

The conclusions regarding darunavir resistance in the POWER studies are that baseline darunavir susceptibility is the strongest predictor of virologic response and that clinical cut-off values for intermediate susceptibility and resistance are a 10-fold increase and a 40-fold increase in IC_{50} value, respectively. Darunavir resistance mutations generally occur along with a large number of other PI resistance mutations, and there is diminished response to darunavir when 3 or more of these mutations are present. Resistance data remain to be developed in the treatment-naïve and early treatment-failure settings.

Etravirine

Week 24 data from the TMC-225-C223 trial showed a $1\text{-}\log_{10}$ copies/mL greater reduction in viral load in the etravirine arm versus the control arm. An analysis of the effect of presence of the NNRTI mutations K103N and Y181C in patients receiving etravirine showed no effect of the K103N mutation; presence of the mutation was associated with a 1.70-fold change in susceptibility at baseline and a reduction in viral load of $1.43 \log_{10}$ copies/mL at 24 weeks compared with a 1.95-fold change and a reduction of 1.40

\log_{10} copies/mL in the absence of the mutation (Vingerhoets et al, 15th IHDRW, 2006). However, presence of the Y181C mutation was associated with a 4.50-fold change in baseline susceptibility and a reduction in viral load of 0.86 \log_{10} copies/mL compared with a 1.10-fold change and reduction in viral load of 1.70 \log_{10} copies/mL when the mutation was absent. Analysis of data from the phase III trials of etravirine identified 13 etravirine resistance-associated mutations at 9 codons in reverse transcriptase: V90I; A98G; L100I; K101E or P; V106I; V179D or F; Y181C, I, or V; G190A or S (Vingerhoets et al, *Antivir Ther*, 2007). The IC_{50} value for etravirine increased and virologic response decreased with the presence of increasing numbers of etravirine-associated mutations. When 3 or more of these mutations were present, the response to etravirine was no different from the response to placebo.

Integrase Inhibitors

In vivo data from phase III studies of the integrase inhibitor raltegravir indicate that there are 2 mutually exclusive pathways to resistance: (1) N155H as the major mutation, with minor mutations of E92Q, V151I, T97A, G163K, and L64M; and (2) Q148K, R, or H as the major mutation, with minor mutations of G140S or A and E138K (Cooper et al, 14th CROI, 2007; Steigbigel et al, 14th CROI, 2007). Resistance to the investigational drug elvitegravir was analyzed in a phase IIb trial. The most common integrase mutations identified were E92Q; E138K; Q148R, K, or H; N155H (each observed in 39%); S147G (32%); and T66I, A, or K (18%) (McColl et al, *Antivir Ther*, 2007). Viruses from subjects experiencing virologic failure in this study showed a mean increase in IC_{50} value of greater than 151-fold for elvitegravir and greater than 28-fold for raltegravir compared with control, providing evidence for cross-resistance between these 2 integrase inhibitors. Further confirmation of cross-resistance came from a pilot study in which 2 patients with elvitegravir-resistant virus were treated with raltegravir; virologic failure occurred

promptly in both patients (DeJesus et al, IAS, 2007).

CCR5 Antagonists

A unique feature of the pattern of resistance being observed with the chemokine receptor R5 (CCR5) antagonist fusion inhibitors (eg, maraviroc and the investigational agent vicriviroc) is the flattening of the percent inhibition versus drug concentration curve, compared with the typical scenario in which the curve is displaced to the right. That is, instead of reduced susceptibility being indicated by the need for a higher concentration of drug to produce a given percent inhibition of virus, a plateau degree of inhibition is reached despite increasing CCR5 antagonist concentration. The mechanisms of resistance include mutations in HIV gp120 that allow recognition of and binding to the drug-bound form of CCR5, resulting in noncompetitive inhibition of the antagonist (Mosley et al, 13th CROI, 2006; Westby et al, *J Virol*, 2007; Pugach et al, *Virology*, 2007). One implication of these findings is that susceptibility and resistance will need to be defined in terms of changes in maximal achievable suppression rather than changes in IC_{50} value.

Overall, CCR5 antagonist resistance appears to emerge slowly and involves mutations in the V3 and other regions of the viral envelope. Limited data on cross-resistance among these agents are available. Failure is more frequently attributable to emergence and outgrowth of CXC chemokine receptor 4 (CXCR4)-using viruses that preexisted as minority populations before the initiation of CCR5 antagonist therapy.

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Suggested Reading

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