Perspectives

Drug Resistance Testing in the Management of Antiretroviral Therapy

Daniel R. Kuritzkes, MD, discussed aspects of genotypic and phenotypic resistance testing, and virologic results achieved with treatment guided by testing in clinical studies, at the International AIDS Society–USA course in Boston in March.

Antiretroviral resistance due to HIV-1 mutations develops differently among different drug classes and can evolve via different pathways for specific drugs. For some drugs, such as lamivudine and the nonnucleoside reverse transcriptase inhibitors (NNRTIs), single point mutations can rapidly confer high-level resistance. For others, such as zidovudine and protease inhibitors, high-level resistance requires 3 or more mutations within a single genome. Continued use of drugs in a failing regimen results in an accumulation of mutations in addition to those initially conferring resistance, indicating continued viral adaptation to growth in drug presence and resulting in greater levels of resistance and confinement of increased cross-resistance to other members of the drug class.

Mutational pathways to resistance are difficult to predict and are associated with different resistance patterns. For zidovudine, for example, resistance mediated by the codon 215 mutation (in conjunction with other mutations) also confers resistance to the thymidine analogue stavudine, resistance mediated by the Q151M mutation confers multinucleoside resistance excluding the investigational nucleoside reverse transcriptase inhibitor (nRTI) tenofovir, and resistance mediated by the insertional mutation at codon 69 confers multinucleoside resistance excluding the investigational nucleoside reverse transcriptase inhibitor (nRTI) DAPD. Although it was once thought that evolution of nel-

finavir resistance was relatively straightforward, it is now known that whereas the more commonly observed D30N mutation is associated with narrow nel-

finavir resistance, resistance mediated by the L90M mutation (observed in some 10% to 15% of cases) is associated with resistance to saquinavir, indinavir, and other protease inhibitors. Similarly, narrow resistance to amprenavir is conferred by the I50V mutation (often accompanied by the M46I and I47V mutations), whereas multidrug resistance among protease inhibitors is conferred by the I84V amprenavir-associated resistance mutation.

Along with difficulty in predicting mutation pathways, the increasing frequency of transmission of drug-resistant virus is an important factor motivating clinical use of resistance testing. Data on phenotypic resistance (defined as >10-fold resistance on phenotypic assay) of virus from recently infected patients at a number of centers in 1999 to 2000 indicate that compared with sensitivities in 1995 to 1998, rates of any antiretroviral resistance increased from 3.5% to 14% (P = .001). This included increases in nRTI resistance from 2.7% to 8.2% (P < .03), in NNRTI resistance from 1.3% to 7.1% (P = .007), in protease inhibitor resistance from 0.4% to 8.2% (P = .001), and in resistance to 2 or more drugs from 0.4% to 5.8% (P = .002; Little et al, 8th CROI, 2001).

Assays for Drug Resistance

Drug resistance can be assessed by genotypic assay or phenotypic assay. Currently, genotypic assays determine the presence or absence of specific changes in HIV-1 protease and reverse transcriptase genes, since these enzymes are the targets of currently available drugs. Assays will eventually have to incorporate viral envelope genes as fusion inhibiting drugs make their way into clinical use. Genotyping is widely available and is performed by several different methods. Since geno-

typic assays simply indicate whether mutations are present or not and do not indicate how the virus examined behaves in the presence of drug, resistance is inferred by the presence of known resistance mutations, use in clinical practice thus presupposes knowledge of important resistance mutations for particular drugs.

Phenotypic assays measure the 50% or 90% inhibitory concentration (IC50, IC90) for a drug by recombinant virus assay. These assays are currently performed by only 2 laboratories. They can reliably detect changes in susceptibility of as small as 2.5-fold. In brief, the assays are performed by extracting HIV-1 RNA from a plasma sample, which is then converted to complementary DNA by reverse transcription in the test tube. The DNA is then amplified by polymerase chain reaction (PCR), the proximity of the reverse transcriptase and protease genes allows performance of a single PCR reaction generating a single amplicon. This material can be sequenced for reverse transcriptase and protease genotype. For phenotype analysis, the pooled amplicons, reflecting the diversity of genetic sequences present in circulating virus, are introduced into plasmids by recombination or site-specific cloning to form infectious HIV-1 clones that contain the same envelope and regulatory sequences for each patient specimen. Infectious virus generated by these procedures is then tested for drug susceptibility in an automated assay format, yielding inhibition curves from which inhibitory concentrations can be calculated.

Use of resistance assays is complicated by a number of factors. In general, plasma samples with more than 500 to 1000 HIV-1 RNA copies/mL are needed to generate results. In addition, virus species constituting less than 20% to 30% (even as high as 50%) of the amplified product may not be detected. The

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results from the predominant species may thus not reflect important minority populations. Moreover, false-positive and false-negative results can be generated during PCR due to contamination from prior samples or random polymerase errors. With regard to genotypic assays, interpretation of results is limited by incomplete knowledge of the mutations associated with resistance and by lag in availability of data coordinating particular mutations with resistance. A number of sources provide interpretations of genotypic findings that are updated at variable intervals, and interpretation at any given time may thus rely on outdated data. With regard to phenotypic assays, changes in susceptibility do not necessarily imply resistance or predict clinical response to a given drug; resistance is best considered as a combination of the intrinsic susceptibility of the virus to the drug, the achievable concentration of the drug in the host, and the interaction of pharmacologic and virologic factors that lead to clinical response or lack thereof. Clinically relevant breakpoints for most drugs have yet to be established.

Some of the limitations of the resistance assays are demonstrated by cases arising in clinical practice where genotypic and phenotypic data are at apparent odds. In one case described by Dr Kuritzkes, genotypic analysis suggested the presence of didanosine resistance, based on a codon 74 mutation, lamivudine susceptibility, based on absence of the codon 184 mutation, and stavudine susceptibility. Phenotypic assay results showed borderline susceptibility to didanosine and resistance to both lamivudine and stavudine. In this case, interpretation of genotypic findings by the laboratory performing the assay was based on data that did not yet reflect recognition of mutations arising in the context of long-term didanosine treatment that can confer cross-resistance to lamivudine. In another case, virus judged to be phenotypically sensitive to zidovudine was found to have all 5 of the major zidovudine-resistance mutations. However, the virus also contained the 184V resistance mutation to lamivudine, which resulted in reversion to phenotypic zidovudine susceptibility.

Work is ongoing to refine interpretation of findings on both types of assays. Until recently, any virus showing a higher than 2.5-fold variation in susceptibility compared to wild-type reference strain was considered resistant, although it was recognized that there must be naturally occurring interstrain differences in susceptibility. Data have been collected using large numbers of drug-naive patient samples that show the magnitude of phenotypic variation for different drugs. These data have allowed new cut-off values for phenotypic resistance to be formulated. As shown in Figure 1, the range of variability for NNRTIs was found to be quite large compared with nRTIs and protease inhibitors (Graham et al, 8th CROI, 2001).

More important than the establishment of cut-off values based on phenotypic variation is the correlation of phenotypic resistance with virologic response. An attempt has been made to identify clinical breakpoints for some agents. Analysis of the effects of the addition of abacavir as a single drug to existing regimens in early trials of the drug showed that in the majority of patients, there was a more than 0.5 log-decrease in plasma HIV-1 RNA in the context of 0- to 4.5-fold abacavir phenotypic resistance (Lanier et al, 8th CROI, 2001). Fewer patients exhibited a decrease of this magnitude at 4.5- to 6.5-fold resistance, with very few achieving such a reduction at more than 6.5-fold resistance. Based on these findings, a 4.5-fold change in susceptibility was selected as the susceptibility breakpoint.

Analysis of the effects of adding lopinavir and an NNRTI to failing protease inhibitor-based regimens in multiple protease inhibitor-experienced patients showed virologic response (<400 HIV-1 RNA copies/mL) in 93% of patients with less than 10-fold phenotypic resistance to lopinavir at baseline (Kempf et al, 4th Int Workshop HIV Drug Resistance Treatment Strategies, 2000). Although this level of resistance was selected as the clinically relevant cut-off for susceptibility, patients still exhibited response at higher-fold resistance levels, with the degree of contribution of the NNRTI to this response being unclear. The abacavir and lopinavir data serve to support the notion that there is not likely to be a rigid cut-off value defining resistance to any single drug, but rather a gradient of likelihood of response that is proportional to the level of resistance.

One attempt currently being made to improve utility of genotypic findings is the development of an interpretive system based on derivation of a virtual phenotype from the genetic sequences in patient samples. The systems currently used for interpreting genotype data can be considered rules-based, using databases that require frequent updating as new data become available. Attempts are now being made to devise “intelligent” systems using neural networks that provide data-driven correlations between genotypes and phenotypes that can be updated in real time. In this virtual phenotype approach, samples from a large database (currently

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**Figure 1.** Comparative phenotypic variation among antiretrovirals using drug-naive patient samples. SD indicates standard deviation. Adapted with permission from Graham et al, 8th CROI, 2001. Courtesy of Virco, Mechelen, Belgium.
approximately 20,000 samples) of paired phenotypes and genotypes are queried for “matches” to a patient sample genetic sequence. An average phenotype is calculated based on the actual phenotypes of the matching genetic sequences identified in the database. Based on this virtual phenotype, the test genetic sequence is characterized as susceptible or resistant, with the number of matches and the distribution of susceptible and resistant isolates among the matching set being reported. The results of this analysis thus basically provide a probability of susceptibility or resistance.

For example, for a clinical sample with multiple zidovudine resistance mutations and no 184V lamivudine resistance mutation, results indicated that the virus was likely to be zidovudine-resistant, with an average of 30-fold resistance among the matching set, although 10% of the matches were categorized as zidovudine-susceptible. The average resistance to lamivudine was 3.3-fold, with 55% of matches being resistant and 45% susceptible. This virtual phenotype approach is associated with a number of advantages, including reduction of the complex genotypic data to simple categories, interpretation based on actual data from a growing set of samples, and the provision of a measure of robustness of the data. However, the simplicity of results may be misleading, particularly in cases in which the matching set is small, and strength of correlations from the matched sample pool will be weaker for new drugs and for rare viral variants.

with the predictive effect being significant in most of the individual studies, and (2) prediction of drug resistance was an independent risk factor for treatment failure.

Among prospective studies, the VIRADAPT study (Durant et al, *Lancet*, 1999) in patients in whom a protease inhibitor-containing 3-drug regimen was failing showed that 32.3% of patients having genotype analysis versus 14.0% of those receiving standard care had virologic response (viral load <200 copies/mL) at 6 months. Although this difference fell short of statistical significance, overall reduction in viral load was significantly greater in the genotyping group. In the GART study in patients in whom 3-drug combination regimens were failing, genotyping plus expert advice resulted in significant reductions in viral load over 12 weeks compared with usual care (Baxter et al, AIDS, 2000). The proportion of patients with virologic response (HIV-1 RNA <500 copies/mL) was also significantly greater in the genotyping plus advice group. In the recently reported Havana trial, patients in whom antiretroviral therapy was failing were assigned in factorial fashion to genotyping or no genotyping with or without expert advice. Intent-to-treat analysis showed that the genotyping/advice group had a significantly greater reduction in viral load at 24 weeks (1.3-log reduction, P = 0.015) than did the other groups (Figure 2). The reduction in the genotype/no advice group (1.0 log) was greater than those in the no genotype/advice and no genotype/no advice groups (0.8 log in both; Tural et al, 40th ICAAC, 2000).

A recent analysis of cost-effectiveness of genotypic resistance testing showed that such testing was highly cost effective when it produced reductions in virologic failure rates of the magnitude observed in the VIRADAPT and GART studies (25%-38%; Weinstein et al, *Ann Intern Med*, 2001). In fact, given the high cost of antiretroviral therapy and the cost-savings that would result from sparing use of ineffective drugs, it was calculated that genotypic testing would still be cost-effective at a cost of $10,000 per test.

The VIRA3001 study examined the utility of phenotypic resistance testing in patients in whom the first protease inhibitor-containing regimen was failing. Study results showed that patients receiving salvage treatment based on phenotypic assay results had a significantly greater reduction in viral load at week 16 compared with those receiving standard care (1.27 log versus 0.75 log, P = .005) (Cohen et al, XIII Int AIDS Conf, 2000). A significantly greater proportion of the patients in the phenotype group had a reduction in viral load to less than 400 copies/mL. In the NARVAL study, patients in whom a 3-drug protease inhibitor-containing regimen was failing received standard care, treatment based on genotypic testing, or treatment based on phenotypic testing. On final analysis, there were no significant differences among groups at 12 weeks in

Use of Resistance Data in Clinical Studies

Despite the limitations of the resistance assays, retrospective studies have shown that pretreatment genotype or phenotype is significantly predictive of virologic response and prospective studies have shown that treatment based on assay data is associated with improved virologic outcome. A meta-analysis of retrospective genotype and phenotype studies by the Resistance Collaborative Group (DeGruttola et al, *Antivir Ther*, 2000) showed that (1) the likelihood of virologic failure was reduced by 30% to 50% for each drug in salvage regimens to which the assay predicted susceptibility.

![Figure 2](image-url) Changes in plasma HIV-1 RNA level in HAVANA study according to whether patients had genotyping or not with or without expert advice. Adapted with permission from Tural et al, 40th ICAAC, 2000. Courtesy of B. Clotet, MD, PhD.
terms of proportions of patients with viral load reduced to below 200 copies/mL. However, an exploratory secondary analysis showed a significant difference among groups with regard to proportions of patients achieving such response at both 12 and 24 weeks, with the proportion of responders in the genotype group being significantly greater than that in the standard care group (29% vs 17%). Differences between these groups was more marked in patients with lower initial viral loads and in those with minimal prior treatment experience (Meynard et al, *Antivir Ther*, 2000).

The finding in the NARVAL study that resistance testing was not associated with any marked improvement in outcome in heavily pretreated patients is not surprising, given that such patients may have few or no treatment options available. In such cases, resistance testing may still prove useful, since it may provide guidance in removing drugs that are not working from the treatment regimen. However, it is important to note that there may still be advantages to continuing failing antiretroviral regimens in patients with few or no remaining options if partial viral suppression can be maintained.

The concept of inhibitory quotient—which characterizes the relationship between drug exposure and susceptibility of a pathogen—has begun to be applied to prediction of response to protease inhibitor therapy. Protease inhibitor therapy is a suitable candidate for such an endeavor, since protease inhibitor plasma levels can be altered by pharmacologic enhancement. With inhibitory quotient defined as trough drug plasma concentration divided by drug $IC_{50}$, the inhibitory quotient of lopinavir was found to predict response to lopinavir plus efavirenz and nRTI therapy in multiple protease inhibitor-experienced/NNRTI-naive patients. In another study, indinavir-experienced patients received indinavir plus ritonavir. Plasma HIV-1 RNA levels were maintained at less than 50 copies/mL at 48 weeks in 80% of patients with an indinavir inhibitory quotient of more than 2 (ie, trough concentration at least twice the $IC_{50}$) compared with 0% of patients with a quotient of less than 2 (Kempf et al, 8th CROI, 2001).

### Current Recommendations for Use of Resistance Testing

Available data provide a compelling rationale for use of resistance testing in managing antiretroviral therapy despite its acknowledged current limitations. Resistance testing currently is recommended in patients experiencing antiretroviral regimen failure and in pregnant women, in whom it should be used to assist in maximizing viral suppression. It should be considered for use in patients with primary infection and before starting therapy in patients in areas of high prevalence of transmission of resistant virus, including patients from such areas with chronic established infection.

**Presented in March 2001; reviewed and updated by Dr Kartikzes in July 2001.**

**Grant Support and Financial Disclosures:** Dr Kartikzes has received grant support or honoraria from or has served as a consultant to Abbott, Bristol-Myers Squibb, DaPont, Gilead, GlaxoSmithKline, Merck, Roche, Triangle, Trimeris, Virco, ViroLogic, and Visible Genetics.

### Suggested Reading


Brun-Vézinet F, Race E, Descamps D, et al. Differences between genotype and phenotype in the NARVAL trial, ANRS 088 *Antivir Ther* 2000;5(suppl 3) 78-79.


