Perspective

Resistance and Replication Capacity Assays: Clinical Utility and Interpretation

Resistance testing has emerged as an important tool for antiretroviral management. Research continues to refine phenotypic susceptibility cut-offs and genotypic interpretation schemes that relate resistance mutations with antiretroviral drug effectiveness. Highly sensitive phenotypic assays have allowed for the recognition of drug hypersusceptibility in HIV, and other studies have related hypersusceptibility to resistance mutations; efforts are ongoing to use what is known about hypersusceptibility to optimize the benefits of antiretroviral therapy. Resistance-associated mutations in several viral genes result in viruses that exhibit reduced replication capacity; assays to measure replication capacity are being developed that may, in the future, be useful in guiding therapy to improve treatment outcomes. This article summarizes a presentation given by Richard H. Haubrich, MD, at the International AIDS Society–USA Sacramento course in November 2003.

Resistance testing is an important component of management of antiretroviral-experienced patients, assisting in selection of appropriate regimens in patients in whom treatment is failing due to resistant virus. Such testing is also becoming an increasingly important part of determining initial antiretroviral therapy given the high (and rising) rates of resistant virus transmission in a number of areas around the world. Current options for assessing HIV resistance are phenotypic and genotypic assays.

Phenotypic Assays

Phenotypic assays assess susceptibility of clinical HIV isolates to antiretroviral drugs by comparing the concentration of drug needed to inhibit the clinical isolates with that of wild-type reference strains. Figure 1 illustrates examples of a clinical isolate that is susceptible to the drug tested, showing 50% inhibitory concentrations (IC_{50}) identical to those of the reference strain, and an isolate that has a 100-fold reduced susceptibility to the drug tested. To be optimally useful, these tests should provide results that are predictive of virologic response to therapy. Biologic cut-offs for susceptibility have been obtained for different phenotypic assays by determining mean fold change in susceptibility compared with a reference strain among a large number of isolates from treatment-naive, HIV-infected patients. The standard interpretation is that the isolate is susceptible if it is within the mean plus 2 standard deviations. This biologic cut-off varies from drug to drug and from assay to assay according to performance characteristics of the particular phenotypic assay. Although the measure provides an indication of whether a clinical isolate can be considered susceptible to a particular drug, clinical cut-point values are still needed to correlate degree of susceptibility with virologic response of the patient. Clinical cut-points are derived from data relating resistance assays to clinical response, usually by determining a phenotypic cut-point at which a drug starts to show a decreased virologic response (eg, smaller reduction in plasma HIV RNA levels). Ideally, 2 phenotypic cut-points should be derived for each drug. The first cut-point would be defined as the fold change at which there is any reduction in antiviral activity, and the second cut-point would be that for which there is no drug activity.

Cut-points may differ for different assays (eg, Virco and ViroLogic assays). Examples of cut-points for reduced susceptibility that have been determined for the ViroLogic assay (expressed as susceptibility fold change) are 1.7 for didanosine, 1.7 for stavudine, 4.5 for abacavir (6.5 for loss of antiretroviral activity), 10.0 for lopinavir, and 1.4 for tenofovir.

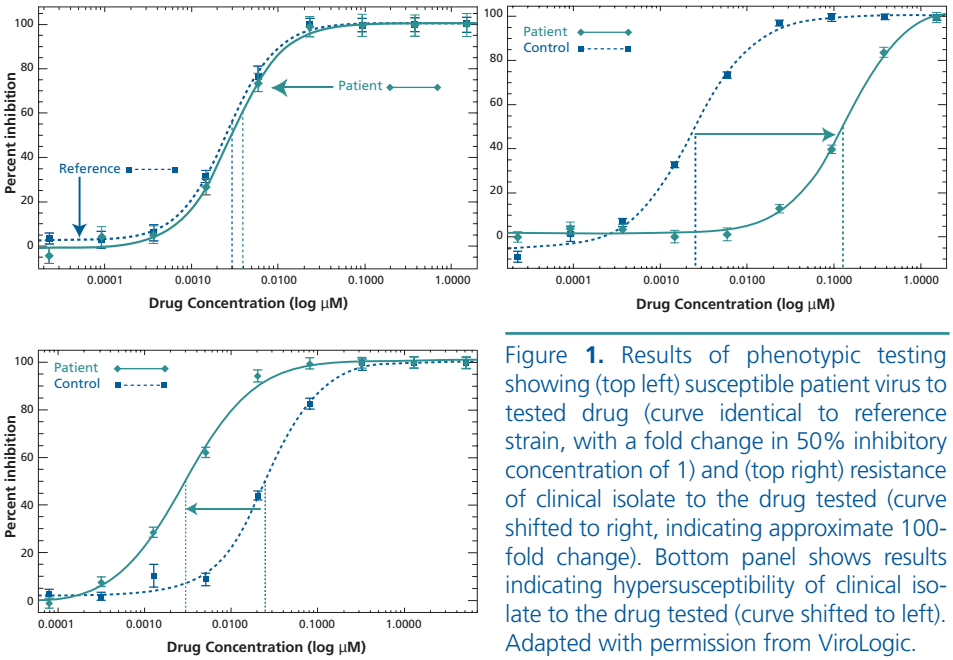

Figure **1.** Results of phenotypic testing showing (top left) susceptible patient virus to tested drug (curve identical to reference strain, with a fold change in 50% inhibitory concentration of 1) and (top right) resistance of clinical isolate to the drug tested (curve shifted to right, indicating approximate 100-fold change). Bottom panel shows results indicating hypersusceptibility of clinical isolate to the drug tested (curve shifted to left). Adapted with permission from ViroLogic.

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Drug		Matches in database	Proportion of matched samples: within normal susceptible range [2]; above normal susceptible range [2]; above normal susceptible range but below clinical cut-off [2, 3, 4]	Fold change in IC_{50} (Cut-off for normal susceptible range)	Ref.
Trade name	**Generic name**		25 50 75 (%)		
NRTI					
Retrovir®	Zidovudine	2,600		4.0 (4.0)	
Epivir®	Lamivudine	3,396		48.1 (4.5)	
Videx®	Didanosine	2,092		1.4 (2.0)	
Hivid®	Zalcitabine	1,524		1.7 (2.0)	
Zerit®	Stavudine	3,220		1.0 (1.75)	
Ziagen®	Abacavir	1,083		2.6 (3.0)	
NtRTI					
Viread™	Tenofovir DF	1,094		1.3 (3.0)	4
NNRTI					
Viramune®	Nevirapine	12,574		1.4 (8.0)	
Rescriptor®	Delavirdine	11,723		1.6 (10.0)	
Sustiva®, Stocrin®	Efavirenz	11,517		1.1 (6.0)	
PI					
Crixivan®	Indinavir	1,671		9.3 (3.0)	
Norvir®	Ritonavir	1,783		10.8 (3.5)	
Viracept®	Nelfinavir	2,050		24.1 (4.0)	
Invirase®, Fortovase®	Saquinavir	1,975		4.9 (2.5)	
Agenerase®	Amprenavir	1,689		2.3 (2.0)	
A component of Kaletra®	Lopinavir	1,026		2.5 (2.5)	3

Figure **2.** Example of virtual phenotypic output, showing predicted fold change in 50% inhibitory concentrations (IC_{50}) for drugs based on relating genotype to phenotype matches in the database. Reprinted with permission from Virco.

Genotypic Assays

Genotypic assays identify mutations in HIV genes (currently, those for reverse transcriptase and protease) that are associated with viral resistance. Interpretation of genotypes is difficult for a number of reasons, including the complexity of determining the effects of interactions among numerous resistance mutations that may be present in clinical HIV strains. There are a number of clinical tools that assist clinicians in interpreting genotype assays. Many compilations of resistance mutations, such as that maintained by the International AIDS Society–USA (Johnson et al, *Top HIV Med*, 2003) are continuously updated to provide profiles and interpretation of resistance mutations for specific antiretroviral agents (www.iasusa.org/resistance_mutations/mutations_figures.pdf).

One method of improving the interpretation of a genotypic test uses the correlation of the genetic sequences in particular clinical strains with phenotypes in a large database. With this method of genotypic interpretation, the genetic sequence from a clinical isolate is entered into a database consisting of a large number of clinical isolate genotypes and corresponding phenotypic profiles. An average phenotype for the strain of interest is then defined by "matching" the genotype of the particular strain with similar genetic sequences in the database. An example of a virtual phenotype report is shown in Figure 2. The average phenotype for the genotypic matches is displayed as the fold change for the patient's genotype. The virtual phenotypic results are dependent on the number of matches of the patient's genotype found in the database; when few matching genotypes are found, a rules-based result is given.

Interpretations of resistance mutations can also be obtained from such online databases as the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu), which provides an interpretation of likely drug resistance based on entry of single or numerous resistance mutations. The interpretations reflect what is currently known about the effect of interaction of resistance mutations on drug susceptibility. Currently, most companies or institutions that provide genotypic analysis supply an interpretation of the results designed to be useful in guiding clinical decision making. However, methods for arriving at interpretations are not well standardized and may differ according to the method used and the database employed. Caution is nec-

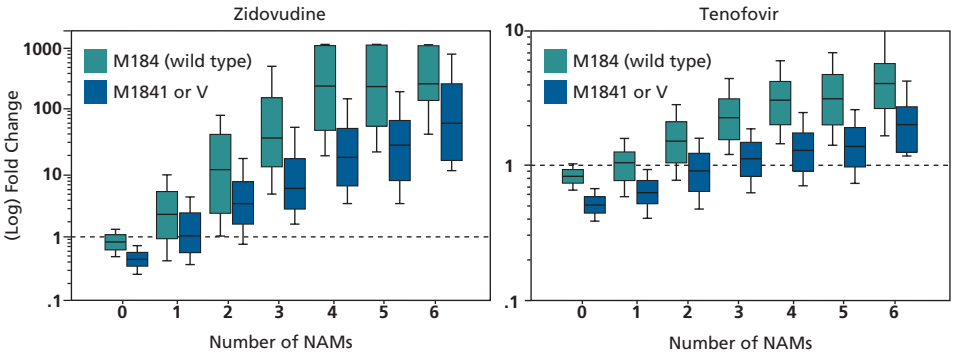


Figure **3.** Effect of the M184V mutation (blue) on susceptibility to zidovudine and tenofovir according to number of other nRTI-associated mutations (NAMs) present. NAMs include M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N. Adapted from Whitcomb et al, *J Infect Dis,* 2003.

essary in basing clinical decisions on interpretations, unless it is known that the interpretation comes from a reliable source utilizing continuously updated data. Use of several sources (ie, the International AIDS Society–USA Mutations tables as well as the Stanford Web site), may increase the clinician's confidence in the interpretation.

Hypersusceptibility

Phenotypic hypersusceptibility has recently been recognized and the clinical implications are being clarified. In phenotypic analysis, hypersusceptibility is shown by a shift in the inhibition-concentration curve to the left of the reference strain curve (Figure 1, bottom), and is expressed as a fold change of less than 1.0. For these isolates, the IC_{50} of the patient's virus is less than the IC_{50} of the control so that it might take lower concentrations of the antiretroviral to inhibit the viral strain than it would for a wild-type virus. The mechanism of hypersusceptibility is unknown, but it likely results from an interaction of mutations, in which mutations that lead to resistance to one drug lead to increased susceptibility to another drug. This has best been demonstrated for the nucleoside reverse transcriptase inhibitors (nRTIs). The nRTIs can be divided into 2 groups based on the effect of the nRTI resistance mutation M184V on the particular nRTI agent. That is, the presence of the mutation: (1) *increases* virus susceptibility to zidovudine, stavudine, and tenofovir; and (2) *decreases* susceptibility to lamivudine, zalcitabine, didanosine, and abacavir. Figure 3 shows that although susceptibility to zidovudine and tenofovir decreases with increasing number of nRTI resistance mutations (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N), the presence of the M184V mutation results in increased susceptibility compared with virus lacking the mutation. A practical implication of this phenomenon is that patients may benefit from maintaining the M184V mutation through drug pressure, since the phenotypic switch may be associated with improved response to treatment.

Hypersusceptibility to nonnucleoside reverse transcriptase inhibitors (NNRTIs) is observed in viral samples from patients who have never received nRTIs or NNRTIs. However, the prevalence of hypersusceptibility is greatly increased in samples from patients with prior nRTI treatment, indicating that nRTI resistance mutations in the reverse transcriptase enzyme can result in increased susceptibility to NNRTIs. Whitcomb and colleagues (*AIDS,* 2002) recently reported that among 331 nRTI/NNRTI-naive patients, hypersusceptibility to delavirdine was present in 5% of samples, and hypersusceptibility was present in 9% and 11% of samples in efavirenz and nevirapine, respectively. Among 447 nRTI-experienced, NNRTI-naive patients, hypersusceptibility to delavirdine was present in 29% of samples, to efavirenz in 26%, and to nevirapine in 21%. There is accumulating evidence that such hypersusceptibility results in improved treatment response, with 5 separate trials demonstrating improved outcomes in the presence of NNRTI hypersusceptibility (Haubrich, *AIDS*, 2002; Shulman, *AIDS,* 2001; Albrecht, 9th CROI, 2002; Mellors, 9th CROI, 2002; Haubrich, 11th CROI, 2004). For example, Haubrich and colleagues (*AIDS,* 2002) found that reduction in HIV-1 RNA in patients initiating NNRTI treatment was approximately 0.5 $\log_{10}$ RNA copies/mL greater in patients with hypersusceptible virus than in those without such virus (Figure 4).

Hypersusceptibility has also been observed for protease inhibitors (PIs). With these drugs, hypersusceptibility has been associated with improved virologic outcome. In a study by Schooley and colleagues (10th CROI, 2003), for example, the effects of amprenavir treatment were assessed in PI-experienced patients who were amprenavir-sensitive (maximum fold change in IC_{50} of 4.0 on phenotypic assay at baseline). Independent predictors of response to fewer than 200 plasma HIV RNA copies/mL at week 24 consisted of baseline viral load, number of prior PIs received, fold change in amprenavir at baseline, and baseline hypersusceptibility to amprenavir (defined as fold change in IC_{50} < 0.66).

Impact of Drug Concentrations on Resistance

Pharmacokinetic boosting of PIs with ritonavir to raise drug concentrations in the blood has been found to improve therapeutic response, and it may also curtail emergence of resistance from patients in whom their first antiretroviral regimen failed. Such reduction in resistance was perhaps first demonstrated in a clinical trial comparing lopinavir/ritonavir-based therapy with nelfinavir-based therapy for the treatment of antiretroviral-naive patients. In this trial, nelfinavir resistance was observed in 30% of patients with virologic failure of the nelfinavir regimen, but lopinavir resistance was not found in any patients with failure of the lopinavir/ritonavir regimen. Confirming data have now been reported in a study of the amprenavir prodrug fosamprenavir (Macmanus, 10th CROI, 2003). In this cross-study comparison, resistance to unboosted fosamprenavir was pres-

ent at first virologic failure in 8 (28%) of 29 patients receiving unboosted drug (mutations I54M, M46I, and V32I + I47V), whereas no resistance was found at first failure in 32 patients receiving fosamprenavir/ritonavir. By comparison, nelfinavir resistance was found at first failure in 35 (44%) of 80 patients receiving nelfinavir. It thus appears that maintaining elevated levels of PIs in a treatment regimen can reduce PI resistance for patients in whom the initial regimen is failing. The consequences of this apparent reduction in resistance in predicting augmented responses to the next PI-based regimen as compared with failure of an unboosted PI have not been demonstrated.

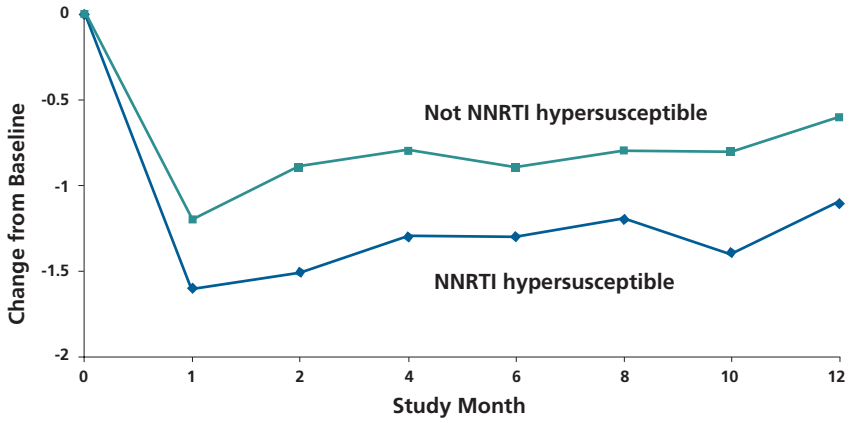
Replication Capacity

Viral replication capacity is defined as the ability of virus to multiply, in a given environment, usually compared with a reference or control (wild-type) virus. Differences in replication capacity may be intrinsic to the virus strain or may result from mutations selected by drug pressure. Determination of viral replication capacity is of clinical interest since it is hoped that virus with low replication capacity will be less pathogenic.

Available assays of replication capacity compare HIV reverse transcriptase and protease sequences in clinical and reference strains using modified phenotypic assays. Changes in other viral components, particularly the viral envelope, are also likely to be important determinants of viral replicative fitness, but are not measured in the commercially available assays. Replication capacity of a particular viral sample is expressed as a percentage of quantified replication of the reference strain. A number of reverse transcriptase and protease mutations affect the replication capacity of a virus. The nRTI resistance mutation M184V and the PI mutation D30N, for example, are single mutations that are associated with reduced viral replication compared with wild-type virus, suggesting that the "cost" to the virus of acquiring such resistance is reduced ability to replicate in the presence of the drug that selected for the resistance mutation. Such a phenomenon may help explain the ongoing benefits of antiretroviral therapy despite the presence of resistance. In a recent study, Haubrich and colleagues (XI Drug Resistance Workshop, 2002) examined CD4+ cell count changes from nadir counts in patients with detectable viral load and phenotypic resistance. Gains in CD4+ cell count from the nadir were significantly higher in those patients in whom virus had lower replication capacity, indicating that reduced capacity is indeed associated with better clinical course.

In another study, Daar and colleagues (*Antivir Ther,* 2003) evaluated stored clinical samples from HIV-infected patients with hemophilia who had received nRTI monotherapy or dual therapy prior to the potent antiretroviral therapy era to determine whether viral replication capacity was predictive of clinical progression. It was found that: (1) replication capacity was correlated with CD4+ cell count ($P = 0.025$) and plasma HIV RNA level ($P = 0.062$); (2) replication capacity ($P < 0.0001$) and viral load ($P = 0.008$) were independently predictive of CD4+ cell count decline; and (3) decreases in replication capacity were associated with delayed progression to clinical HIV disease even after controlling for viral load and CD4+ cell count. These findings indicate that replication capacity is a marker of viral fitness that independently influences HIV disease progression.

An effect of a resistance mutation to the HIV fusion inhibitor enfuvirtide on replication capacity also has been observed recently. Enfuvirtide has been used in patients in whom there are few treatment options remaining; in many, an initial robust reduction in viral load is followed by rebound, and it remains unclear whether to discontinue enfuvirtide treatment in such patients. Lu and colleagues (XI Drug Resistance Workshop, 2002) found that wild-type virus outcompeted virus with the enfuvirtide-resistance mutation in the absence of enfuvirtide and that the mutant virus became the predominant species in the presence of enfuvirtide. Such findings suggest that continued benefit of enfuvirtide may occur despite resistance in those who harbor virus with enfuvirtide-associated mutations.



No. subjects								
Not HS	83	35	60	66	57	54	55	48
HS	23	11	16	20	18	19	14	14
P value		*0.1*	*0.02*	*0.04*	*0.1*	*0.1*	*0.03*	*0.2*

Figure **4.** Effect of NNRTI hypersusceptibility on virologic response ($\log_{10}$ HIV RNA copies/mL) to initiation of NNRTI therapy. Adapted with permission from Haubrich, *AIDS,* 2002. NNRTI indicates nonnucleoside reverse transcriptase inhibitor; HS, hypersusceptible.

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Suggested Reading

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