Understanding Treatment-Resistant HIV

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Reprinted from *The PRN Notebook*® | June 2003 | Dr. James F. Braun, Editor-in-Chief | Tim Horn, Executive Editor. | Published in New York City by the Physicians' Research Network, Inc.®
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Resistance of HIV to antiretroviral drugs is one of the most common causes for therapeutic failure in people infected with HIV. Sadly, the emergence of drug-resistant HIV variants is usually an inevitable occurrence—even under the best of circumstances—given that no antiretroviral drug combination is completely effective in shutting down viral replication. And there is no shortage of data indicating that the emergence of HIV drug resistance is clearly associated with adverse treatment outcomes.

Fortunately, the availability of drug-resistance testing has substantially improved the ability of clinicians to deal knowledgeably with HIV drug resistance head on. Therapy can now be individualized based on the results of drug-resistance testing, by looking for drug-resistant mutants that are transmitted at the time of infection, by ferreting out ineffective individual drugs that are being used as part of a larger antiretroviral regimen, by capitalizing on the presence of mutants that lead to hypersusceptibility to certain antiretroviral drugs, or by maximizing the activity of a new antiretroviral drug through combination with an optimized background of older drugs using resistance testing as a guide. These evolving areas of research and practice were clearly explained in presentations by Drs. Veronica Miller, Richard Haubrich, and Daniel Kuritzkes at a recent PRN meeting moderated by Dr. Sheldon Brown, Chief of Infectious Diseases of the Bronx Veteran Affairs Medical Center. What follows are highlights from the state-of-the-art lectures delivered by these prominent experts in the field of HIV drug resistance.

Transmission of HIV Drug Resistance

Drug-resistance testing has come to be recognized as an important tool in tracking the growing and troublesome prevalence of transmission of drug resistant virus—a problem that, Dr. Miller says, is "here to stay." Recent studies have suggested that approximately 3% of newly diagnosed HIV cases involve strains resistant to at least one antiretroviral drug, whereas others suggest that the rate is closer to 22%. It is important to recognize that reported rates of drug resistance in primary HIV infection vary considerably and are dependent on the locations and size of the cohorts being studied and the definitions and assessments of resistance. Moreover, the ability to detect resistance may decrease as a function of time from initial infection. "There is generally a shift to wild-type over time, but this is not the case for all mutations," Dr. Miller said. For example, there is a growing number of antiretroviral-naive patients who have detectable HIV mutants with C, D, E, N, A, V, and S substitutions at codon 215 in the reverse transcriptase gene, long past the time of infection. These "revertant" mutations are indicative of transmitted strains that at one point carried the T215Y/F mutations, which are associated with resistance to thymidine analogues, and will rapidly revert back to resistant mutants if drug pressure is reapplied.

Three studies reported at the XI International HIV Drug Resistance Workshop, held last summer in Seville, looked at treatment outcomes among patients infected with drug-resistant HIV. Two of these studies failed to demonstrate any discernable effects (Chaix, 2002, Derdelinckx, 2002), whereas the third study demonstrated a decreased antiviral response in these patients (Balotta, 2002). In this third study, 13/40 (32%) antiretroviral-naive patients were found to be harboring one of the several 215 codon revertant mutations described above. Approximately 77% of the patients initiated therapy with a zidovudine (Retrovir)-containing regimen, and the remaining 23% initiated therapy with a stavudine (Zerit)-containing regimen. A multivariable Cox regression analysis demonstrated that patients carrying these mutations had an increased risk of experiencing virologic failure, compared to those not carrying such mutations, with a relative hazard of 2.44. Of the 13 patients who carried the 215 variants, nine experienced virologic failure. In blood samples collected around the time of failure in five of these patients, the 215 revertants were replaced by the T215Y mutation.

There's still a great deal to be learned about therapeutic outcomes in patients with drug-resistant mutants prior to receiving their first dose of antiretroviral treatment or changing to a new treatment regimen. "There have been studies demonstrating an association between baseline resistance and virologic outcome," Dr. Miller explained. "However, CD4+ cell responses in patients with baseline mutations have not been addressed systematically. Nor have there been any data to systematically address clinical progression as a function of baseline resistance."

Beyond the potential clinical consequences for individuals infected with drug-resistant HIV mutants, there are larger public health factors to consider. "What I really want to stress here," Dr. Miller commented, "is that we're seeing a lot of transmitted HIV drug resistance. Assuming that these mutants are coming from treated patients and not from intermediary patients, it really reflects the treatment and prevention practices in that area. The transmission of drug-resistant viruses really is an indication of prevention failure. Here you have patients, who are on treatment, and somehow engaging in practices that result in the transmission of drug-resistant virus to others. Clearly, the prevention messages are not getting across to these patients."

Dr. Miller stressed the importance of stepping up prevention campaigns to reflect the very real risk of transmitting drug-resistant virus strains. "This is definitely true in populations with widespread access to treatment and prevention counseling," she said. "This is also true in resource-poor settings, where both horizontal and vertical transmission of

drug-resistant HIV are very real threats. The spread of resistance to one drug classs, for example NNRTIs, may destroy the potential utility of simple drug regimens that are proposed to be used—or the only ones available—in these settings. Hence, treatment and prevention counseling is greatly needed in places where treatment options are extremely limited."

Resistance and Replication Capacity Assays

Resistance Assays and Interpretation

UNDERSTANDING THE PREMISE BEHIND DRUG-RESISTANCE TESTING BEGINS with two fundamental terms: genotype and phenotype. HIV's genotype refers to the nucleotide sequences and corresponding amino acids that make up its genetic structure. The phenotype of HIV, on the other hand, refers to the overall characteristics or behavior of the virus (e.g., its growth characteristics or sensitivity to antiretroviral drugs in cell cultures). Genotypic and phenotypic assays serve as the technological backbones of the drug-resistance tests being used today.

Genotypic Testing. Genotypic resistance tests amplify the protease and reverse transcriptase genes using PCR technology. The amplicons of these genes are then subjected to automated DNA sequencing by a variety of techniques. Available genotypic resistance tests include both sequencing assays (e.g., chain-termination and microchip assays produced by Visible Genetics, ABI/Perkin Elmer, Affymetrix, Virco, and ViroLogic) and point mutation assays (differential hybridization and line-probe assays manufactured by Innogenetics and Chiron). Sequencing assays scan the complete sequence of the amplified gene, whereas point-mutation assays detect only key minority species.

For several years, the Drug Resistance Mutations Group—a panel of 12 HIV-drug resistance experts from around the world—of the International AIDS Society-USA (IAS-USA) has been reviewing evolving data on HIV drug resistance and maintaining a verified list of mutations associated with clinical resistance to antiretrovirals. The most recent list, which is broken down by drug class and illustrated in mutations figures, was reported in the November/December 2002 issue of *Topics in HIV Medicine*, published by the IAS-USA (see sidebar beginning on page 20) (D'Aquila, 2003). Included in the group's most recent review of drug resistance mutations were data reported at the 11th International HIV Drug Resistance Workshop in Seville—held in June 2002—and other recent conferences. These figures are accessible through IAS-USA's Web site (www.iasusa.org) and are available on pocket-size folding cards that can be ordered directly from the organization.

While it is generally believed that there is a high level of interlaboratory reproducibility using the various genotypic tests—meaning that testing the same sample using different genotypic assays would likely yield similar mutation profiles—there are variations in the interpretations provided by different laboratories. Both Drs. Miller and Haubrich pointed out that clinicians are largely dependent on these interpretations—algorithms designed to help predict clinical response—given that it would be an insurmountable task for an individual health-care provider to stay on top of each mutation associated with drug resistance and its clinical significance. Just as it would be frustrating if pathology laboratories were to use different criteria for reporting the results of a biopsy, it can be equally frustrating when laboratories use different algorithms to interpret the results of genotypic testing results.

This particular topic was a central theme at the 5th International Workshop on HIV Drug Resistance and Treatment Strategies, held in Scottsdale, Arizona, in June 2001. In one presentation, researchers representing the EuroGuidelines Group for HIV Resistance presented an overview of the similarities—and many differences—among 19 different interpretation systems that have been used by laboratories doing genotyping or published on the World Wide Web for educational purposes (Schapiro, 2001). Also of interest were data presented by Dr. Randall Lanier and his colleagues of GlaxoSmithKline, evaluating the predictive value of 16 genotype interpretation algorithms for determining abacavir (Ziagen) resistance, compared to responses seen in a 166-patient meta-analysis of four abacavir intensification trials (Lanier, 2001). Response in this context was defined as either a >0.5 log copies/mL reduction in viral load or a reduction to <400 copies/mL at week 4. Disparate results were obtained, with accuracy ranging from 35% to 80%, but many of the current algorithms showed a >75% overall accuracy score. However, even the best algorithms classified >25% of responders as having abacavir-resistant virus.

Phenotypic Testing. Phenotypic assays—including ViroLogic's PhenoSense-HIV, Virco's Antivirogram, and VIRALLIANCE's HIV-1 Phenoscript—measure the ability of HIV isolates to grow in the presence of an antiretroviral drug and are able to determine the degree of virus replication at different drug concentrations. Results are used to calculate the 50% or 90% inhibitory concentration (IC_{50} and IC_{90}), along with the fold-change in IC_{50} and IC_{90} for each isolate as compared with a drug-susceptible control strain. For technical reasons, these commercially available tests rely on the IC_{50} parameter.

Until recently, phenotypic resistance assays have used somewhat arbitrary cutoffs to define resistance, which loosely translates into the maximum level of reduced drug susceptibility at which an HIV-positive individual still has a high probability of successful response to treatment with a particular drug. Assay cutoffs have been based on the reproducibility of the tests themselves, using a standard laboratory-derived virus as the reference. By repeatedly running a test with the standard reference virus, the reproducibility of the test was measured and a cutoff was set at this level (e.g., 2.5-fold increase in IC_{50} for the ViroLogic and a fourfold increase in IC_{50} using the Virco assay).

One problem with this approach is that laboratory reference viruses are not necessarily representative of viruses in the general population. For example, if a laboratory were to sample 100 or 1,000 antiretroviral-naive patients, there would likely be a natural variation in the susceptibility of wild-type virus. Just as reference laboratories are constantly assessing drug susceptibility of other infectious organisms—such as staphylococcus and *E. coli*—in the general population, similar procedures are necessary to assess the sensitivity of HIV if clinicians are to know what they can realistically expect from treatment.

In order to develop more meaningful cutoff values for each drug, investigators at Virco used the Antivirogram assay to measure the IC_{50} values for 1,000 untreated patients as well as several thousand HIV samples with no resistance mutations (Harrigan, 2001). The average and the range of susceptibility were calculated for each drug. The cutoffs were then set at two standard deviations above the mean. Any values above this "biologic" cutoff were classified as having reduced susceptibility.

Since the susceptibility of untreated and unmutated virus varied considerably from drug to drug, the new cutoffs are different for each drug. All the cutoffs are above the reproducibility limit of the Antivirogram assay, which is approximately a twofold change in susceptibility. Over the past few years, Virco has been using these biologic cutoff values in their lab reports (see Table 1).

Another important advance has been the development of "clinical" cutoffs, which have been defined for two drugs using the Antivirogram assay and five drugs using the PhenoSense assay. Clinical cutoffs are defined as the fold change in IC_{50} beyond which the probability of a clinical response begins to decline. As fold change values increase beyond the cutoff, the likelihood of treatment success diminishes. Clinical cutoffs are established using rigorous analyses of patient outcome data from clinical trials to correlate phenotypic susceptibility at baseline with virologic outcome.

"What would be most helpful would be to have two clinical cutoffs," Dr. Haubrich said. "The first cutoff would be the fold change beyond which virologic response to a drug begins to decline. The second cutoff would be the fold change beyond which no virologic response to a drug can be expected." To date, the only antiretroviral that has been fully explored in this respect is abacavir. According to ViroLogic's PhenoSense assay, the only test that has determined both clinical cutoffs for this drug, decreased sensitivity to abacavir begins when there is a greater than 4.5-fold increase in the IC_{50}, with no antiviral activity expected when there is a greater than 6.5-fold increase in the IC_{50}.

At the present time, Virco has established the first of these two clinical cutoffs for two antiretrovirals (tenofovir [Viread] and the lopinavir in Kaletra), and ViroLogic has determined the first of these two clinical cutoffs for four antiretrovirals (didanosine [Videx/Videx EC], stavudine [Zerit], tenofovir, and lopinavir) (see Table 1). Both Virco and ViroLogic are actively engaged in multiple collaborations to establish clinical cutoffs for all antiretroviral drugs.

Virtual Phenotype Testing: Virtual phenotyping—branded and marketed as VirtualPhenotype by Virco—is a quantitative system for predicting phenotype from the genotype. To perform the test, a genotype is first derived from a patient isolate. The resulting sequence is then submitted and compared with the Virco database, which contains approximately 50,000 genotype/phenotype pairs from previously collected samples from other patients. The phenotypes from samples with matching genotypes are then retrieved from the database, and the IC_{50}s for each drug are calculated.

Virtual phenotype bypasses much of the guesswork needed to interpret standard genotypic test results. As discussed above, genotypic testing reveals the mutations that have occurred in the relevant sequence of the HIV genetic code. However, this information requires interpretation to predict to which drugs the virus will and will not be sensitive. The problem is that there are more than 100 mutations already known to be involved in the development of resistance, and more are being discovered all the time. Some mutations cause resistance to one drug, while some are associated with cross-resistance. Some mutations are sufficient on their own to cause resistance, while some need to combine with others. There are also mutations that effectively reverse resistance caused by other mutations. Given the complexity of genotypic testing, especially in patients with variants harboring a large number of mutations, interpreting results using subjective judgment or rule-based algorithms (determined by consensus panels or laboratories) is not without potential flaws.

VirtualPhenotype works by establishing the patient's mutational pattern and matching that pattern to as many patients in the Virco database as exist. The average phenotype for each drug is calculated. While the approach is consistent, there are limitations to the interpretations provided. "There's an almost endless number of possible mutation combinations," Dr. Haubrich explained. "Even though the genotypes and corresponding phenotypes for thousands of patients are in the Virco database, certain combinations of mutations may be limited. This can limit the results of virtual phenotyping." Rare combinations of mutations may not be represented in the database and thus be reported as "Uninterpretable." IC_{50}'s that are based on only a few database matches may not be as informative as when many matches are present. To ensure accurate predictions, genotypes and actual phenotypes from isolates continue to be entered into the database. This process should continue to enhance the usefulness of this method.

Table 1. **Drug-Specific Cutoffs for Virco's Antivirogram, ViroLogic's PhenoSense, and VIRalliance's Phenoscript**

	Biological Cutoffs		Clinical Cutoffs		
Drug	**Antivirogram**	**Phenosense**	**Antivirogram**	**Phenosense**	**Phenoscript**
Zidovudine	4.0	1.9			
Lamivudine	4.5			3.5	
Stavudine	3.0			1.7	3.0
Didanosine	3.5			1.7	2.5
Zalcitabine	3.5	1.7			
Abacavir	3.0			4.5	8.0
Nevirapine	8.0	2.5*			
Delavirdine	10.0	2.5*			
Efavirenz	6.0	2.5*			5.0
Saquinavir	2.5	1.7			11.0
Indinavir	3.0	2.1			20.0
Ritonavir	3.5	2.5*			
Nelfinavir	4.0	2.5*			
Amprenavir	2.5	2.0			7.0
Lopinavir	2.5		10.0	10.0	10.0
Tenofovir	3.0		4.0	1.4	

* Technical cutoff only; biological or clinical cutoff not yet determined.

Source: ViroLogic; Virco

Hypersusceptibility

DR. HAUBRICH CONSIDERS HYPERSUSCEPTIBILITY of HIV to antiretroviral agents to be a natural extension of the interpretation of phenotypic assays. He explained that hypersusceptibility is defined as a fold change (in IC_{50}) of less than 1 compared with the reference strain; in other words, the patient isolates require less drug to inhibit replication than does wild-type control virus. "The obvious question here is, are we dealing with some laboratory artifact or is there clinical significance behind hypersusceptibility," Dr. Haubrich asked. "Hypersusceptibility has been described in all three classes of agents—the nucleoside analogues, the NNRTIs, and the PIs, and now several clinical analyses have demonstrated the correlation with improved virologic response."

NRTI Hyperusceptibility: The M184V mutation, which usually arises during therapy with Epivir, is one of the most intriguing mutations in terms of both hypersusceptibility and decreased sensitivity. The NRTI class can essentially be divided into two groups, based on the effect of the M184V mutation on susceptibility to a given NRTI. The first group comprises zidovudine, stavudine, and tenofovir—the M184V mutation has been documented to increase susceptibility to these drugs. In the second group is lamivudine (Epivir), didanosine, zalcitabine (Hivid), and abacavir—the M184V mutation increases resistance to these drugs. "In our clinical practice in San Diego, it's quite common for us to include lamivudine or reintroduce lamivudine when we have tenofovir in a salvage setting, presuming that we'll get some degree of phenotypic shift and improve the response to therapy."

NNRTI Hypersusceptibility: HIV hypersusceptibility to NNRTIs has been an interesting recent discovery. In one study, ViroLogic turned to its database containing phenotypic drug susceptibility and genotypic sequence results involving numerous patient isolates (Whitcomb, 2002). Hypersusceptibility to delavirdine (Rescriptor), efavirenz (Sustiva), and nevirapine (Viramune) was detected in 10.7%, 10.8%, and 8.0%, respectively, of more than 17,000 consecutive plasma samples submitted for phenotypic susceptibility testing. In analyses limited to a subset of viruses derived from patients with known treatment histories, NNRTI hypersusceptibility was more likely to be demonstrated by viruses from NRTI-experienced/NNRTI-naive patients. Of 447 patients who were NRTI-experienced and NNRTI-naive, 29% were hypersusceptible to delavirdine, 26% were hypersusceptible to efavirenz, and 21% were hypersusceptible to nevirapine. In contrast, hypersusceptibility was less likely to be seen in patients who were naive to both NRTIs and NNRTIs. Of 331 patients who fit this category, 5% were hypersusceptible to delavirdine, 9% were hypersusceptible to efavirenz, and 11% were hypersusceptible to nevirapine. "There have been a number of different analyses indicating that various NRTI-associated mutations, including M184V, increase virus susceptibility to NNRTIs," Dr. Haubrich said. "This has been a very important discovery."

There have been a number of clinical trials indicating that hypersusceptibility to NNRTIs is associated with improved clinical outcomes. In one study conducted by the California Collaborative Trials Group (CCTG 575), led by Dr. Haubrich, NRTI-experienced patients were switched to an NNRTI-containing regimen and followed for 12 months (Haubrich, 2002). Approximately 20% of baseline isolates collected from 177 patients showed hypersusceptibility to at least one of the approved NNRTIs. Patients who had hypersusceptibility and received an NNRTI-based regimen had sustained a significantly greater viral load reduction than those who did not have hypersusceptibility (-0.5 log copies/mL difference) after 12 months (see Figure 1). There was also a trend toward greater increases in CD4+ cell counts among patients with NNRTI hypersusceptibility, although the reported differences were not statistically significant. "This is something we're going to continue exploring in studies," Dr. Haubrich said. "NNRTI hypersusceptibility could play a role in the strategic management of HIV-infected patients."

PI Hypersusceptibility: As many as 20% of individuals with primary HIV infection have virus strains that are hypersusceptible to amprenavir (Agenerase) or ritonavir (Norvir). In antiretroviral-experienced patients, hypersusceptibility to amprenavir has been associated with N88S and I50L in the protease gene. Other studies have demonstrated that combinations of amino acids at several polymorphic sites can contribute to protease inhibitor hypersusceptibility, in both antiretroviral-naive and antiretroviral-experienced patients (Leigh Brown, 2003).

At the 10th Conference on Retroviruses and Opportunistic Infections, held this past winter in Boston, Dr. Chip Schooley and his colleagues presented the results of ESS40006, a GlaxoSmithKline-sponsored study designed to compare two regimens of amprenavir/ritonavir (600/100 mg and 900/100 mg BID) in patients failing their current PI-based antiretroviral regimen (Schooley, 2003). The analysis was restricted to those patients who were NNRTI-experienced and received a regimen of amprenavir/ritonavir, along with abacavir, tenofovir, and one other NRTI based on baseline phenotypic susceptibility. In a multivariate analysis amprenavir hypersusceptibility—defined as a fold change less than 0.66 compared to wild-type virus—and baseline viral load were the two strongest predictors of virologic response at 24 weeks. "The bottom line from this analysis is, if you look at factors that predict treatment response, hypersusceptibility was a predictor of response," Dr. Haubrich said. "Just as NNRTI hypersusceptibility may affect treatment outcomes, so might PI hypersusceptibility."

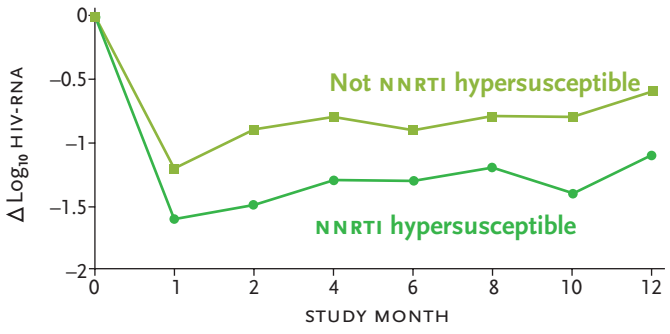
Replication Capacity

THERE HAS BEEN NO SHORTAGE OF CLINICAL STUDIES AND REAL-WORLD experience indicating that a sizeable percentage of patients experience a discordant response to therapy—loosely defined as a maintained CD4+ cell count increase despite the presence of a detectable virus. "Basically, we see a continued benefit of drug treatment in patients who have become resistant to the therapies they're taking," Dr. Haubrich said. "Some of this benefit can definitely be attributed to residual drug activity, but I think there's enough emerging evidence to suggest that impairment of replication capacity, caused by drug-resistance mutations, is a factor as well."

Technically speaking, HIV's "viral fitness" is defined as its ability to multiply in a given environment. Its "replication capacity" (RC) refers to the number of viral progeny produced (per round of infection or per unit time). And HIV's "virulence" involves its ability to cause disease (e.g., by destroying CD4+ cells). "I tend to use these terms interchangeably," warned Dr. Haubrich. To measure RC of a given patient's HIV, it is compared to a reference, usually wild-type virus. Decreased RC may be intrinsic to the virus strain or may result from mutations selected by pressure and resistance. There is an assumption that a virus with low RC will be less virulent—less likely to adversely impact the immune system.

ViroLogic has developed an assay for RC, using the PhenoSense testing technology as its platform. The ViroLogic RC assay has been used

Figure 1. **Change in HIV-RNA from Baseline—The Hypersusceptibility Advantage**



Not HS	83	35	60	66	57	54	55	48
HS	23	11	16	20	18	19	14	14
P		.1	.02	.04	.1	.1	.03	.2

Source: Richard Haubrich, MD

in a number of studies and is currently used on all samples submitted for phenotypic testing (RC of a patient's HIV is included in the lab report sent to clinicians by ViroLogic).

The ViroLogic RC assay is a multi-step process. First, recombinant viruses for RC testing are generated by introducing resistance test-vector DNA into cell cultures (transfection), along with an expression vector that produces a mouse retrovirus envelope protein. After transfection, virus is harvested from cells and the amount of virus produced is evaluated. The virus is then inoculated into new cell cultures and cells are incubated. After normalization for the amount of input virus, luciferase activity detected in the infected cells is determined and used as a direct measure of "infectivity," (i.e., the ability of the virus to infect target cells [dependent on protease activity] and to complete the reverse transcription and integrase steps of the life cycle [dependent on reverse transcriptase and integrase activity]). The relative RC is assessed by comparing the amount of luciferase activity produced by sample derived viruses to the amount of luciferase activity produced by a well-characterized reference virus (wild-type) derived from a molecular clone of HIV-1 (NL4-3). RC measurements are finally expressed as a percent of the wild-type reference virus (e.g., 70%).

There have been a number studies looking at the impact of various mutations on viral fitness. Figure 2 reviews some of the effects of various reverse transcriptase and protease mutations of RC, documented using the ViroLogic RC assay. "I should point out that these results are similar to those that have been yielded by other laboratories," Dr. Haubrich explained. Mutations that appear to have the greatest impact on viral fitness include the D30N mutation in the protease gene and the M184V mutation in the reverse transcriptase gene. At the same time, there are lingering questions regarding the NNRTI mutations and their impact on RC.

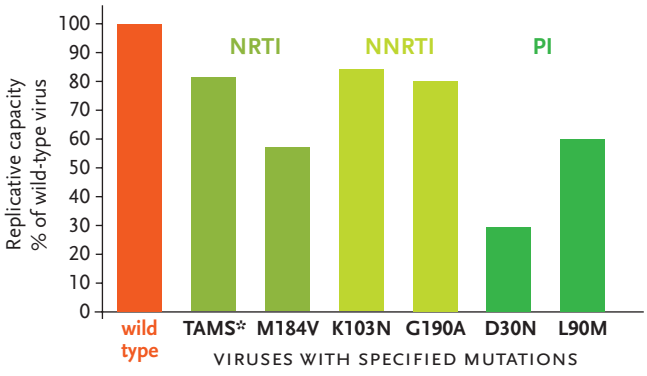
One study that has been reviewed on several occasions in *The PRN Notebook* was conducted by Dr. Steven Deeks and his colleagues, evaluating the virologic and immunologic consequences of discontinuing HAART in HIV-infected patients with detectable viremia (Deeks, 2001). The primary objective of this study was to determine whether antiretroviral therapy provides continued benefit—despite large reductions in drug susceptibility—and to identify the virologic mechanisms responsible for any continued benefit. A component of this objective was to measure the RC of the patients' drug-resistant HIV, using a modified version of the PhenoSense assay. At study entry, when there were high levels of drug resistance, RC was markedly diminished. After antiretroviral therapy was discontinued, drug-sensitive virus emerged and viral RC increased between baseline and week 12. This increase was greater in the patients who were randomly assigned to discontinue therapy than in those assigned to continue therapy, and there was a significant correlation between the change in HIV-RNA levels and the change in RC during the 12 weeks of evaluation. Although recombinant-virus assays cannot measure all aspects of viral fitness, these *in vitro* measurements of replicative capacity support the hypothesis that, in the absence of therapy, drug-sensitive virus has a greater ability to replicate than does drug-resistant virus.

A more recent study, designed to evaluate the predictive value of RC on CD4+ cell count maintenance and on HIV-RNA reduction—for those with detectable viral loads while receiving HAART—Dr. Haubrich and his colleagues analyzed phenotypic susceptibility testing data from CCTG 575, involving 207 patients with greater than six months of antiretroviral therapy experience, including up to two prior protease inhibitors and baseline (on-treatment) HIV-RNA levels in excess of 400 copies/mL (Haubrich, 2002a). All patients started a new baseline regimen, and 97 failed to suppress the viral load (HIV RNA > 400 at month 6). Baseline RC, treatment history, phenotypic susceptibility, and HIV-RNA levels were evaluated to determine their impact on the increase in CD4+ cells from the lowest CD4+ cell count ever recorded prior to study entry to the baseline value. ViroLogic responses from baseline to six months were evaluated in the 97 patients who failed to suppress viral load.

The mean CD4+ count nadir of 179 cells/mm^3 had increased to 312 cells/mm^3 at study baseline. The mean baseline HIV-RNA level was 4.2 log copies/mL. The median RC of the baseline isolates was 37%. Significant univariate associations of greater CD4+ cell count increase from nadir to baseline included longer duration of combination and PI therapy, lower baseline HIV-RNA levels, use of stavudine, and lower RC. In a multivariate linear regression model that included significant univariate predictors, only prior stavudine use and RC were associated with CD4+ cell count changes from nadir. Patients with a lower viral RC had an average increase from their nadir of 82 more CD4+ cells than those with higher viral RC. The association of lower viral RC with higher CD4+ cell counts persisted for six months after study entry.

For the 97 patients who failed to suppress HIV-RNA at month 6, there was a significant linear correlation between lower RC and more pronounced HIV-RNA reductions at month 6. Although the baseline HIV-RNA was the same, patients with lower RC had a mean HIV-RNA change of –0.54 log copies/mL at month 6, compared to +0.08 log copies/mL for those with higher RC. And in a multivariate model with RC, baseline HIV-RNA; CD4+ cell count; and the number of NRTIs, PIs, and NNRTIs to which the virus was susceptible, only the baseline HIV-RNA and RC were independent predictors of the HIV-RNA change at month 6. "When measured at the time of virologic failure of an ongoing regimen, the viral RC may be a marker of continued benefit of the regimen," Dr. Haubrich said. "Simply put, for patients who do not suppress viral load on a new regimen, reduced replication capacity of the virus is significantly associated with greater viral load reductions."

Figure 2. **Common RT/PR Mutations Have Different Effects on HIV Replicative Capacity**



*Virus containing 41+67+70+215+219 RT mutations

Source: Richard Haubrich, MD

New Antiretrovirals: Emerging Resistance Data

No discussion of HIV drug resistance is complete without a review of new antiretrovirals—including those recently approved and those in the late stages of clinical development—that are, perhaps, the greatest hope for patients failing current antiretroviral options. Presently, the spotlight is fixed on three potentially useful compounds: Bristol-Myers Squibb's atazanavir (Reyataz), Boehringer-Ingelheim's tipranavir, and Hoffmann LaRoche and Trimeris's enfuvirtide (Fuzeon). A review of the resistance profiles for these three drugs was provided by Dr. Kuritzkes. While much of the information presented by Dr. Kuritzkes was reported in an article reviewing up-and-coming experimental drugs in the last issue of the *Notebook*, a significant amount of additional information—along with some important commentaries—was provided by Dr. Kuritzkes and is reviewed here.

Atazanavir

Bristol-Myers Squibb's atazanavir (BMS-232,632) is a semi-symmetrical azapeptide protease inhibitor. On May 13th, the FDA's Antiviral Drug Advisory Committee recommended that atazanavir be approved (see "Atazanavir (Reyataz) Recommended for FDA Approval," on page 2). The drug is expected to be approved in June at a dose of 400mg—two 200 mg tablets once a day with food.

Atazanavir has had an optimistic showing in clinical trials reported to date. For starters, there have been a number of reports indicating that patients receiving atazanavir-based regimens in clinical trials have not experienced significant increases in triglyceride or cholesterol levels—an encouraging observation in light of the metabolic complications that have been seen in patients taking any of the currently approved protease inhibitors. In terms of its effectiveness, a pair of phase II clinical trials comparing atazanavir and two NRTIs to nelfinavir and two NRTIs found that both regimens yielded comparable results (Cahn, 2001; Sanne, 2001). And in a phase III study reviewed in the March 2003 issue of the *Notebook*, an atazanavir-based regimen was comparable to an efavirenz-based regimen in terms of HIV-RNA suppression and CD4+ cell count increases after 48 weeks (Squires, 2002).

Important data regarding atazanavir's resistance profile were reported by Dr. Richard Colonno of Bristol-Myers Squibb at the XI International HIV Drug Resistance Workshop (Colonno, 2002). Dr. Colonno's group reviewed data involving 76 isolates obtained from patients who were failing an atazanavir-based regimen in clinical trials. Seventeen of these patients had evidence of resistance to atazanavir, nine of whom were treatment-naive prior to receiving atazanavir. The remaining eight patients were antiretroviral-experienced and were combining atazanavir with another protease inhibitor, most notably saquinavir (Fortovase).

A novel mutation—I50L—was identified in 8/9 resistant isolates from the patients who were initially naive to antiretroviral therapy. Five of these eight patients also carried the A71V mutation. Interestingly, viruses carrying I50L remained susceptible or showed hypersusceptibility to other protease inhibitors, particularly amprenavir. It was also demonstrated that I50L substantially reduced viral RC and that the addition of the A71V mutation partially restored RC.

In the antiretroviral-experienced patients who took atazanavir in combination with saquinavir, the I50L mutation was not observed. Instead, the I84V mutation was documented, which ended up conferring broad cross-resistance to almost all of the protease inhibitors (with the exception of amprenavir, which is not affected by the I84V mutation).

The observed mutation at codon 50 in the protease gene is intriguing. Patients taking amprenavir who develop a different mutation at the same codon—I50V—develop resistance to amprenavir but remain susceptible to other protease inhibitors, including atazanavir. Conversely, an I50L mutation that arises during therapy with atazanavir results in hypersusceptibility to amprenavir. "We're talking about a tiny difference between a leucine substitution and a valine substitution," Dr. Kuritzkes explained. "It appears that atazanavir and amprenavir select for drug resistance mutations by mutually exclusive pathways. There's a dichotomous relationship at work here."

Tipranavir

Tipranavir is a nonpeptidic dihydropyrone, a new class of protease inhibitors believed to have greater flexibility in conforming to enzyme variants resistant to current protease inhibitors. The compound was originally developed by Pharmacia & Upjohn and has since been taken over by Boehringer Ingelheim. In February, BI announced the launch of the Phase III RESIST clinical trial program designed to further study the efficacy and safety of tipranavir as a component of HAART. The RESIST 1 and 2 trials—along with their accompanying companion studies—will evaluate tipranavir in antiretroviral-experienced patients in more than 280 clinical trial sites worldwide.

An initial glimpse into the *in vitro* activity of tipranavir against multiple-protease inhibitor-resistant HIV strains was published three years ago by Dr. Brendan Larder and his colleagues (Larder, 2000). Studied by Dr. Larder's team were 134 clinical viral isolates documented to be highly cross-resistant to currently available protease inhibitors. Of 105 isolates with more than tenfold resistance to three or four protease inhibitors—with an average of 6.1 key protease mutations per sample—95 (90%) were susceptible to tipranavir; eight (8%) had four- to tenfold resistance to tipranavir, and only two (2%) had more than tenfold resistance.

Data presented at the 9th CROI helped shed some light on baseline susceptibility to tipranavir in the setting of various protease mutations (Schwartz, 2002). In the reported analysis, the genotypic patterns of 41 protease inhibitor-experienced patients participating in a dose-finding study of tipranavir (BI 1182.2) were analyzed. At the start of the study, all patients had HIV-RNA levels above 5,000 and had failed two previous protease inhibitor-based regimens.

At baseline, 40/41 (97%) clinical isolates were considered to be susceptible to tipranavir—defined as a less than tenfold reduction in IC_{50}—despite decreases in susceptibility to a mean average of 2.9 currently available protease inhibitors. There was no association between the number of protease mutations at baseline and the magnitude of viral load reduction. For example, individuals with fewer than five baseline protease mutations experienced reductions in viral load of –2.39 log at week 48, compared to a reduction of –2.24 in patients with more than five protease mutations at baseline. Decreased tipranavir susceptibility was associated with a mean of 16 mutations including two or three universal protease inhibitor-associated mutations (UPAMs)—mutations that commonly arise during therapy with current protease inhibitors and are often associated with broad cross resistance—at positions L33I/V/F, V82F/L/T, I84V, and L90M.

Investigators have recently taken a closer look at baseline phenotypic and genotypic sensitivity to tipranavir in patients with multiple protease inhibitor experience (Cooper, 2003). In a phase IIa dose-optimization study of tipranavir (BI 1182.52), patients who had tried at least two protease inhibitors in the past and had strains of HIV harboring at least one UPAM

were randomized to receive one of three tipranavir doses in combination with ritonavir (500/100 mg, 500/200 mg, and 750/200 mg). According to phenotypic analyses of 157 isolates collected at the start of the study (216 patients were enrolled), the median fold increases in IC_{50} ranged from 7.0 to 94.2 for all of the currently approved protease inhibitors, compared to a 1.1-fold increase in the tipranavir IC_{50} against these highly resistant isolates. Tipranavir's IC_{50} increase was onefold or less in 42% of the isolates, between onefold and twofold in 27% of the isolates, between twofold and fourfold in 18%, and greater than fourfold in 12%. Among patients harboring HIV strains with twofold or less resistance to tipranavir, viral load decreased, on average, by 1.23 log copies/mL during the first month of the study. Among patients with greater than twofold resistance to tipranavir, median viral load decreases were less than 0.25 log copies/mL. In other words, a greater than twofold increase in tipranavir's IC_{50} appeared to be a breakpoint for the drug.

Also of interest are data analyzing the number of UPAMs at baseline and viral load responses after 14 days of tipranavir therapy. Looking at the patients who received 500 mg tipranavir plus 200 mg ritonavir—the dose that is currently being explored in phase III clinical trials—a median viral load reduction of 1.15 log copies/mL was seen in patients with one UPAM, a viral load reduction of 1.40 was seen in patients carrying virus with two UPAMs, and a viral load reduction of 0.33 was seen in patients with three UPAMs. "In the phenotypic analysis, patients with three UPAMs had a 2.2-fold increase in tipranavir IC_{50}, which doesn't appear to be much of a shift," Dr. Kuritzkes said. "However, when looking at the impact of these UPAMs on viral load, we see that there is a loss of activity when three UPAMs are present."

Enfuvirtide (Fuzeon)

ENFUVIRTIDE (FUZEON), DEVELOPED BY TRIMERIS AND HOFFMANN-LA-Roche, was approved by the U.S. Food and Drug Administration on March 13, 2003, and is now available, on a limited basis, through Roche and Statscript pharmacy. The dose is a 90 mg subcutaneous injection administered twice a day.

Briefly reviewed by Dr. Kuritzkes were the results of TORO 1 and TORO 2—with TORO standing for T-20 vs. Optimized Regimen Only—two pivotal phase III clinical trials conducted by Trimeris and Roche. Patients in the trials were treatment-experienced and/or had documented resistance to each of the three classes of available antiretrovirals. At entry, an optimized background regimen—consisting of three to five drugs, including up to two newly approved or investigational drugs, if appropriate—was chosen for each patient based on treatment history and the results of baseline phenotypic and genotypic resistance testing.

In TORO 1, conducted in North America and Brazil, patients who received enfuvirtide as part of their combination regimen achieved a mean reduction in HIV-RNA of 1.70 log copies/mL after 24 weeks of treatment, compared to 0.76 log copies/mL among those in the control arm. In TORO 2, conducted in Europe and Australia, patients who received enfuvirtide as part of their combination regimen achieved a mean reduction in HIV levels of 1.43 log copies/mL after 24 weeks of therapy, compared to a mean of 0.65 log copies/mL for those in the control arm.

The success of the enfuvirtide in this study was dependent on HIV's sensitivity to the optimized regimens used. No matter how powerful a single antiretroviral agent is in sequestering viral replication, prolonged suppression requires that a combination of antiretroviral agents be used. And for a combination of antiretrovirals to be effective, HIV must have at least some degree of sensitivity to the drugs being used.

An analysis of TORO 1 used genotypic sensitivity scoring (GSS) and phenotypic sensitivity scoring (PSS) to evaluate the effectiveness of enfuvirtide-based regimens on 24-week virologic results. A patient with either a phenotypic or genotypic score of zero had high-level resistance to all of the antiretrovirals used in their optimized regimen. Patients with a GSS or PSS of one or two had virus sensitive to one or two of the drugs used in their optimized regimen, and so on.

Patients with a PSS of one or two in the enfuvirtide group saw their viral loads drop by approximately 1.8 log copies/mL after 24 weeks, compared to a 0.8 log copies/mL drop in the optimized regimen group. Similarly, patients with a PSS of three or four in the enfuvirtide group saw their viral loads drop by approximately 2.4 log copies/mL after 24 weeks, compared to a 1.5 log copies/mL drop in the optimized regimen group. These data were similar in the analysis looking at GSS. "What we have here is compelling evidence showing that resistance testing really can provide a great deal of information for patients who have a great deal of treatment experience," Dr. Kuritzkes said. "Using both phenotypic and genotypic testing, it's possible to predict what we can expect, even if we're adding a single new drug to an optimized regimen."

While it's safe to conclude that mutations that arise during therapy with NRTIs, NNRTIs, and PIs do not have an impact on HIV's susceptibility to enfuvirtide, investigators have found a broad range of susceptibility to Fuzeon among patients starting the drug for the first time (Sista, 2002). In one study reviewed by Dr. Kuritzkes, researchers at Trimeris analyzed isolates collected from 118 patients participating in a phase II study of enfuvirtide and reported a broad range of susceptibility (Lu, 2002). The geometric mean IC_{50} was 0.020 mcg/mL, with a range of 0.001 mcg/mL to 0.48 mcg/mL. "This was a much broader range than we see for other antiretrovirals," Dr. Kuritzkes commented. "Looking at the 24-week virologic results, it appears as if patients within two standard deviations above or below the geometric mean faired equally well."

As for enfuvirtide's resistance profile, both *in vitro* and *in vivo* data indicate that HIV resistance can and does occur. HIV variants with decreased sensitivity to enfuvirtide contain mutations in the HR1 region of the gp41 complex. The most significant mutations are those found in the GIVQQQNNLL sequence, located near the N-terminus of the HR1 region, most notably amino acid positions 36 through 45, the key enfuvirtide binding area. Preliminary data suggest that key mutations to look for—numerous genotypic assays now include HR1 region mutations in their reporting—include G36D/S, I37V, V38A/M, Q39R, N42T, and N43D.

While there is still much to be learned about the clinical relevance of these (and possible other) mutations in the HR1 region, we do know that these mutations are associated with some degree of virologic failure. In analysis stemming from TORO 1 and TORO 2, among the patients with genotypic data at the time genotypic failure was detected (within 24 weeks of treatment), 94% had substitutions at amino acid positions 36 through 45. Moreover, at the time virologic failure was detected, 98.9% of patients with greater than fourfold change from baseline in enfuvirtide phenotypic susceptibility had mutations at amino acid positions 36 through 45.

According to a study reported at the XI International HIV Drug Resistance Workshop, single mutations at codons 36 through 45 in the HR1 region conferred an average 33-fold increase in the geometric mean IC50, whereas double mutations conferred an average 220-fold increase compared with baseline (Greenberg, 2002). "It's likely that other mutations, in other regions of HIV's envelope, will affect the virus's susceptibility to enfuvirtide."

At the University of Colorado, using a home-brew RC assay, a research team that included Dr. Kuritzkes demonstrated that enfuvirtide resistance mutations result in a sharp reduction in viral fitness com-

pared with wild-type baseline isolates. "Mutations at amino acid positions 36 through 45 in the HR1 region resulted in a significant drop in the relative fitness of HIV isolates," he confirmed. "What we saw is on a par with what is typically seen in isolates containing the M184V mutation. Whether or not this reduction in viral fitness contributes to the sustained virologic and immunologic improvements seen in patients taking enfuvirtide has yet to be demonstrated."

Looking Forward

GENOTYPIC AND PHENOTYPIC DRUG-RESISTANCE ASSAYS HAVE RAPIDLY become mainstay tools in the clinical care of HIV-infected patients. Considerable data from retrospective and prospective studies support the use of both genotypic and/or phenotypic testing, and there is certainly no shortage of commercially available assays or laboratories prepared to meet the demands of clinicians. However, it is important to note that drug-resistance testing is an evolving science. Additional data are required to define the optimal strategy to interpret genotypic tests and to assign appropriate cut points for phenotypic tests. "The current challenge,' Dr. Miller said, "is to assess the predictive genotype pattern or phenotype cut-point that would predict virologic response for an individual drug in a patient population with diverse background treatment histories, diverse 'current' treatments, diverse tolerance, and diverse pharmacokinetic environments that is generally applicable to all patients."

The best approach to addressing this important issue remains unclear since the underlying clinical context is also changing quickly. The treatment of HIV itself is rapidly evolving and treatment experience is made increasingly complex as clinicians exercise options to minimize toxicities and improve adherence. Is it most appropriate to refine genotypic and phenotypic interpretation by using one super-large data set or several smaller data sets? Should studies focus on specific patient populations or include all patients in their analyses? Different definitions have been used to evaluate clinical outcomes in studies evaluating HIV susceptibility testing making comparisons across studies difficult. Some used a 1 log copies/mL reduction in viral load as an endpoint, whereas others have used an undetectable viral load as an endpoint. Can standardized definitions of response and failure by developed for studies analyzing the clinical utility of drug-resistance testing? Similarly, what should the durability of response be? Some studies followed patients for eight weeks, whereas others followed patients for 24 weeks or longer. Both Drs. Miller and Haubrich agree: difficulties in deriving clinically relevant definitions of resistance may be overcome by collaborative efforts.

Finally, it is encouraging to see that treatment-experienced patients will likely benefit from newer antiretroviral drugs making their way through the drug-development pipeline. It is hoped that drug-resistance testing will refine clinicians' abilities to prescribe new drugs effectively by helping to optimize their efficacy in combination with background therapy. "Of course," Dr. Kuritzkes said, "novel mutations or mutational patterns will continue to be discovered as new drugs are introduced into practice. This is why it will continue to be necessary to evaluate, in detail, the relationship between the genotype, the phenotype, and treatment outcome using drug-resistance testing to figure out how best to use these agents in salvage therapy." prn

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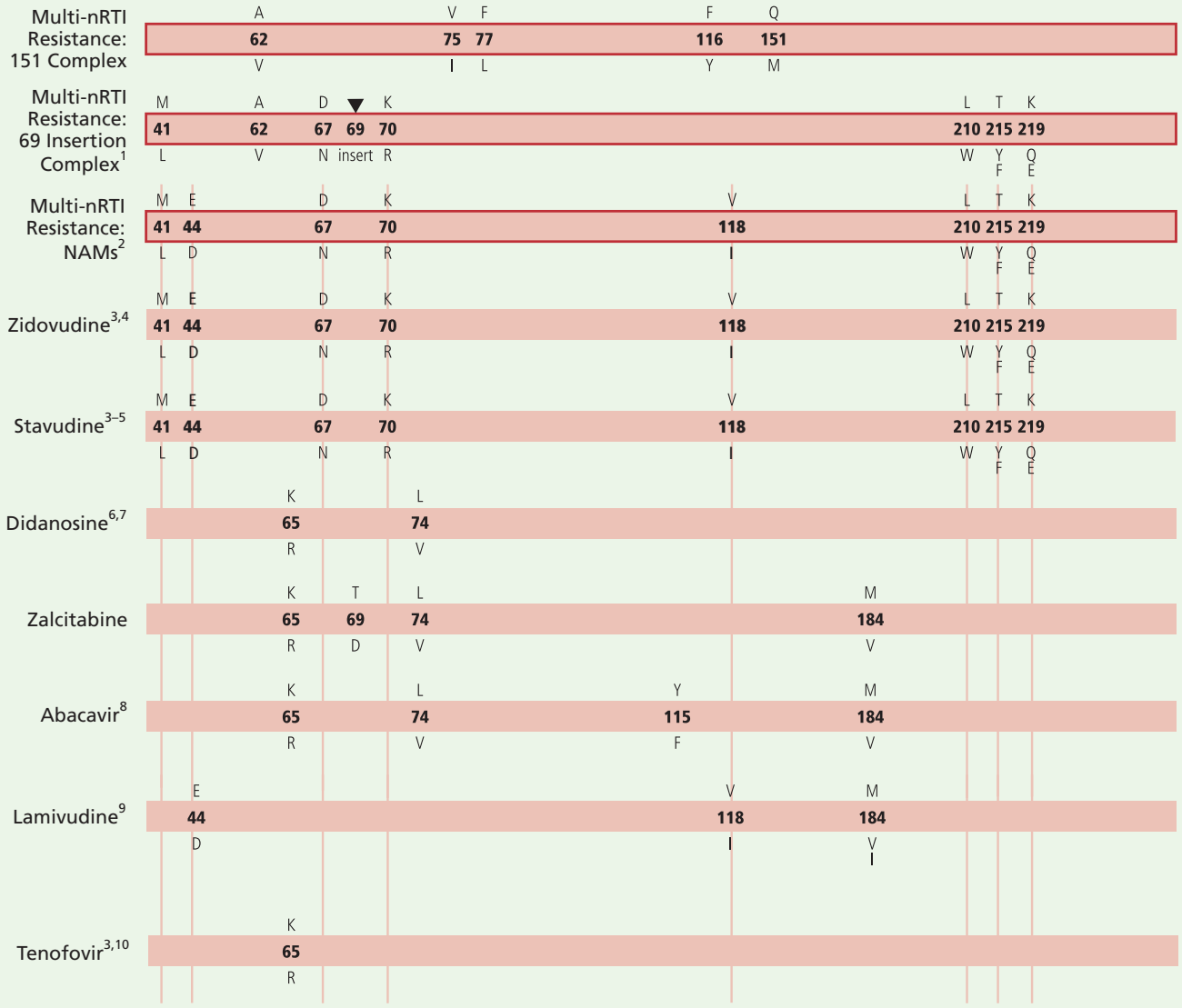
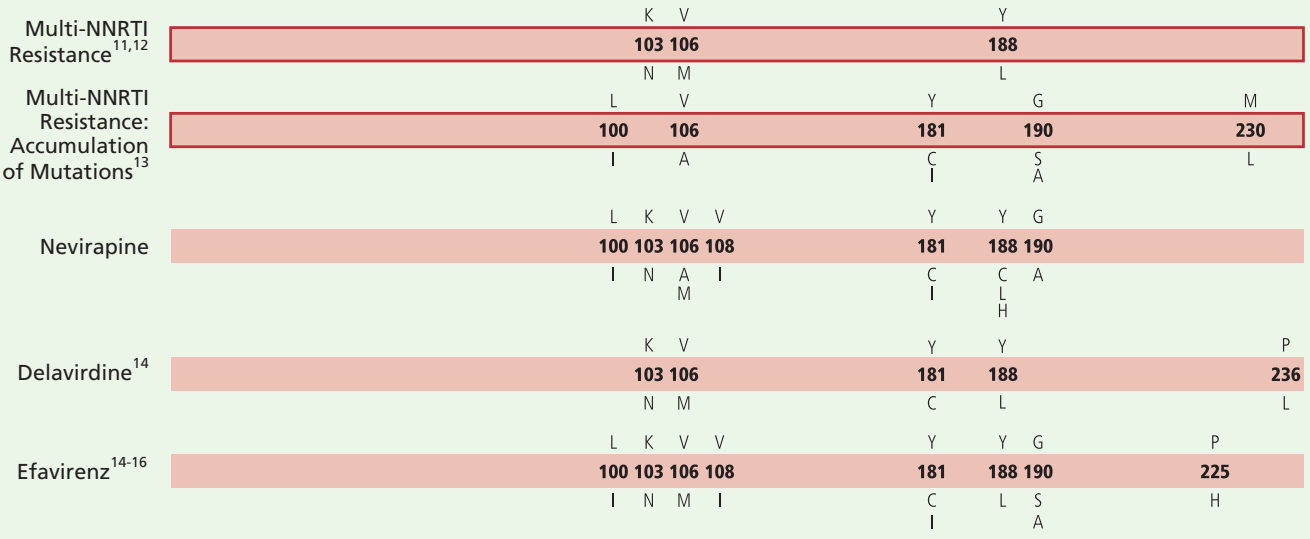
Drug Resistance Mutations in HIV-1*

MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors



Nonnucleoside Reverse Transcriptase Inhibitors



* See accompanying User Notes on page 22

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS

Protease Inhibitors[17]

Multi-PI Resistance: Accumulation of Mutations[18]

Wild-type	L	M	I	V	I	L
Position	10	**46**	54	**82**	**84**	**90**
Substitution	F, I, R, V	I, L	V, M, L	A, F, T, S	V	M

Indinavir[19]

Wild-type	L	K	L	V	M	M	I	A	G	V	V	I	L
Position	10	20	24	32	36	**46**	54	71	73	77	**82**	**84**	90
Substitution	I, R, V	M, R	I	I	I	I, L	V	V, T	S, A	I	A, F, T	V	M

Ritonavir

Wild-type	L	K	V	L	M	M	I	A	V	V	I	L
Position	10	20	32	33	36	46	54	71	77	**82**	**84**	90
Substitution	F, I, R, V	M, R	I	F	I	I, L	V, L	V, T	I	A, F, T, S	V	M

Saquinavir

Wild-type	L	G	I	A	G	V	V	I	L
Position	10	**48**	54	71	73	77	82	84	**90**
Substitution	I, R, V	V	V, L	V, T	S	I	A	V	M

Nelfinavir

Wild-type	L	D	M	M	A	V	V	I	N	L
Position	10	**30**	36	46	71	77	82	84	88	**90**
Substitution	F, I	N	I	I, L	V, T	I	A, F, T, S	V	D, S	M

Amprenavir

Wild-type	L	V	M	I	I	I	G	I	L
Position	10	32	46	47	**50**	54	73	**84**	90
Substitution	F, I, R, V	I	I, L	V	V	L, V, M	S	V	M

Lopinavir/ Ritonavir[20,21]

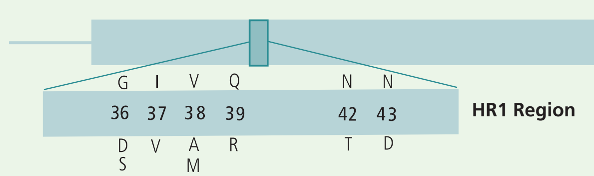
Wild-type	L	K	L	V	L	M	I	I	F	I	L	A	G	V	I	L
Position	**10**	**20**	**24**	**32**	**33**	**46**	**47**	**50**	**53**	**54**	**(63)**	**71**	**73**	**82**	**84**	**90**
Substitution	F, I, R, V	M, R	I	I	F	I, L	V	V	L	V, L	P	V, T	S	A, F, T, S	V	M

Atazanavir[22] (expanded access)

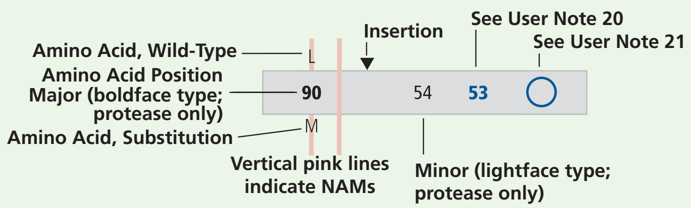
Wild-type	V	M	I	I	A	V	I	N	L
Position	32	46	**50**	54	71	82	84	88	90
Substitution	I	I	L	L	V	A	V	S	M

MUTATIONS IN THE GP41 ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

Enfuvirtide[23]



MUTATIONS



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User Notes

The IAS–USA Drug Resistance Mutations Group reviews new data on HIV drug resistance in order to maintain a current list of mutations associated with clinical resistance to HIV. This list, presented as the IAS–USA Mutations Figures, includes mutations that may contribute to a reduced virologic response to a drug. These mutations have been identified by one or more of the following criteria: (1) *in vitro* passage experiments; (2) susceptibility testing of laboratory or clinical isolates; (3) genetic sequencing of viruses from patients in whom the drug is failing; (4) correlation studies between genotype at baseline and virologic response in patients exposed to the drug. Drugs that have been approved by the US Food and Drug Administration (FDA) or are available through expanded access protocols are included. Additional information on the mutations is provided, where necessary, in these user notes.

1. The 69 insertion complex, consisting of a mutation at codon 69 (typically T69S) and followed by an insertion of 2 or more amino acids (S-S, S-A, S-G, or others), is associated with resistance to all FDA-approved NRTIs. The 69 insertion complex is often accompanied by mutations at other sites. Some other amino acid changes from the wild-type T in codon 69 without the insertion may also be associated with broad NRTI resistance.

2. The NRTI-associated mutations (NAMs), including M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F, and K219Q/E, are associated with cross-resistance to NRTIs and are represented by vertical pink lines. Zidovudine and stavudine select for these mutations, and as such, the positions and mutations are indicated on the bars along with the pink lines. For other NRTIs, the NAMs are not commonly selected by those drugs, but the presence of the NAMs confers cross-resistance to the drugs. This is represented by pink lines only at the positions.

The E44D and V118I mutations are listed as NAMs. In a recent study, the E44D and V118I mutations were more common in virus from patients treated with zidovudine and lamivudine, and were associated with higher-level resistance to zidovudine (Kuritzkes et al, *Antimicrob Agents Chemother,* in press). When present together with other NAMs, the E44D and V118I mutations confer resistance to lamivudine. Analysis from the AIDS Clinical Trials Group (ACTG) study 136 has shown that the V118I mutation is commonly selected by a zidovudine/didanosine regimen (Shafer et al, *J Infect Dis,* 1995). Findings from ACTG study 241 have shown that the E44D mutation is commonly selected by zidovudine/didanosine (Hanna et al, *J Infect Dis,* 2002) and that the E44D mutation is associated with a significantly worse response to treatment with zidovudine and didanosine, with or without nevirapine (Precious et al, *AIDS,* 2000). The significance of E44D or V118I when each occurs in isolation is unknown (Romano et al, *J Infect Dis,* 2002; Walter et al, *Antimicrob Agents Chemother,* 2002; Girouard et al, *Antivir Ther,* 2002).

3. The M184V mutation may enhance susceptibility to zidovudine, stavudine, or tenofovir. This effect may be overcome by an accumulation of NAMs or other mutations. The clinical significance of this effect is not known.

4. Recent data on revertant mutations in codon 215 indicate that the T215D/C/S/E/N/A/V substitutions confer increased risk of virologic failure of zidovudine and stavudine in antiretroviral-naive adults starting therapy with these drugs (Riva et al, *Antivir Ther,* 2002). *In vitro* studies and preliminary clinical studies suggest that the T215Y mutant may emerge quickly from these mutations in the presence of zidovudine or stavudine (Garcia-Lerma et al, *Proc Natl Acad Sci U S A,* 2001; Lanier et al, *Antivir Ther,* 2002; Riva et al, *Antivir Ther,* 2002).

5. Mutations at codon 75 (V75T/M/S/A) have been observed *in vitro* and may confer a low-level change in susceptibility to stavudine (Lacey et al, *Antimicrob Agents Chemother,* 1994).

6. The K65R mutation or the L74V mutation, alone or in combination with the NAMs and/or T69D/N can lead to didanosine resistance.

7. Based on preliminary, yet-unpublished data, the M184V mutation does not appear to have a negative impact on *in vivo* responses to didanosine, even though the mutation reduces susceptibility *in vitro* (Winters et al, *Antivir Ther,* 2002; Eron et al, *Antivir Ther,* 2002; Pozniak et al, *Antivir Ther*, 2002).

8. When present with NAMs, the M184V mutation contributes to reduced susceptibility to abacavir and is associated with impaired response *in vivo*. However, when present alone, the M184V mutation does not appear to be associated with a reduced virologic response to abacavir *in vivo* (Harrigan et al, *J Infect Dis,* 2000).

9. The E44D and V118I mutations were reported to confer low-level resistance to lamivudine when accompanied by several other NRTI-associated mutations (M41L, D67N, L210W, T215Y/F, K219Q/E) in the absence of a concurrent M184V mutation (Hertogs et al, *Antimicrob Agents Chemother,* 2000). Data presented but not yet published (D'Arminio-Monforte et al, 8th CROI, 2001), reported no association over the short term between E44D or V118I and virologic response to a lamivudine-containing combination regimen. (See also User Note 2.)

10. The accumulation of NAMs (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E [note: data here do not include E44D and V118I]) increases resistance to tenofovir. Mutations M41L and L210W contribute more than others. Therefore, the number and type of NAMs will determine the degree of reduced response. T69D/N/S may also contribute to a reduced response to tenofovir (Miller et al, *Antivir Ther,* 2002; Lu et al, *Antivir Ther,* 2002; Masquelier et al, *Antivir Ther,* 2002).

11. The K103N or Y188L mutation alone can substantially reduce the clinical utility of all currently approved NNRTIs.

12. The V106M mutation confers high-level resistance *in vitro* to nevirapine, delavirdine, and efavirenz (Brenner et al, *AIDS,* 2003). This mutation has been observed only in HIV clade C clinical isolates, although site-directed mutagenesis indicates that V106M confers cross-resistance to all NNRTIs in HIV clade B virus.

13. Accumulation of 2 or more of these mutations substantially reduces the clinical utility of all of the currently approved NNRTIs.

14. The prevalence of the Y318F mutation in clinical isolates along with mutations K103N, Y181C, or P236L was approximately 5%, 2%, and 15%, respectively (Kemp et al, *Antivir Ther,* 2001). *In vitro* this mutation confers resistance to nevirapine, delavirdine, and efavirenz.

15. The Y181C/I mutation is not selected by efavirenz, but its presence contributes to low-level cross-resistance to the drug. Clinical impact of this mutation may be overcome with a fully active antiretroviral combination regimen, although no clinical trial data yet address this question.

16. V108I and P225H each contribute to efavirenz resistance when present in combination with other NNRTI-associated mutations. Although V108I or P225H alone does not confer measurable resistance in laboratory strains of HIV-1, their presence in a clinical isolate may indicate prior selection for efavirenz-resistant variants.

17. Resistance mutations in the protease gene are classified as either "major" or "minor" (if known).

> Major: In general, major mutations are either (1) selected first in the presence of the drug; or (2) shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. By themselves, major mutations have an effect on phenotype. In general, these mutations tend to be the major contact residues for drug binding.
>
> Minor: In general, minor mutations appear later than major mutations, and by themselves do not have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of virus carrying major mutations.

18. Accumulation of 4 or more of these mutations is likely to cause multi-PI resistance (Palmer et al, *AIDS,* 1999; Shafer et al, *Ann Intern Med,* 1998).

19. For indinavir, the mutations listed as major may not be the first mutations selected, but they are present in most clinical isolates in combination with other mutations.

20. Major and minor mutations have not been designated for lopinavir/ritonavir-associated resistance since currently there are no clear data defining degrees of influence with this drug combination. The accumulation of 6 or more of these mutations is associated with a diminished response to lopinavir/ritonavir. The product information states that accumulation of 7 or 8 mutations confers resistance to the drug. However, recent data suggest as few as 4 mutations can be associated with such high-level resistance (Prado et al, *AIDS,* 2002). Further clinical experience and research are needed to better define the mutations that affect the clinical effectiveness of lopinavir/ritonavir. It is reasonable to consider phenotyping to assess this in individual cases.

21. Protease mutation L63P is common in viruses that have never been exposed to PIs (Kozal et al, *Nat Med,* 1996) and may be more prevalent in viruses from patients in whom a PI-containing regimen has failed. However, by itself, L63P does not cause any appreciable increase in the IC_{50} for any PI. L63P is listed for lopinavir/ritonavir (and not any other PI) because studies have shown that this mutation, when present with multiple other mutations, is associated with clinical failure.

22. Atazanavir is currently available through an expanded access protocol and is not approved by the US FDA. When administered to patients as the initial PI, atazanavir selects for the mutations I50L and A71V (Colonno et al, *Antivir Ther,* 2002). When used as a subsequent PI in combination with saquinavir, atazanavir selects for I54L and I84V (Colonno et al, *Antivir Ther,* 2002). *In vitro*, atazanavir selects for V32I, M46I, I84V, and N88S (Gong et al, *Antimicrob Agents Chemother,* 2000). Although other major mutations, such as V82A and L90M, have not been selected for by atazanavir either *in vitro* or *in vivo*, these mutations have been shown to confer cross-resistance to atazanavir, particularly when present in combination with each other or with other known PI resistance mutations (Colonno et al, *Antivir Ther*, 2000).

23. To date, resistance mutations in the gp41 envelope gene have been identified primarily at positions 36 to 45 of the first heptad repeat (HR1) region. These mutations have been identified in viruses from patients treated with the drug and have been shown to confer resistance or reduced susceptibility (Wei et al, *Antimicrob Agents Chemother,* 2002; Sista et al, *Antivir Ther,* 2002; Mink et al, *Antivir Ther,* 2002). It is important to note that wild-type viruses in this region show a 500-fold range in susceptibility, and mutations in other regions in the envelope may affect susceptibility to enfuvirtide. Further research is needed to evaluate the clinical relevance of these mutations.

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