Nucleoside Reverse Transcriptase Inhibitors: Resistance, Cross-Resistance, and Resistance Testing

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With so much emphasis being placed on protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) in recent years, it's no wonder that key research involving the "old reliables"—the nucleoside reverse transcriptase inhibitors (NRTIs)—is often overlooked while thumbing through the pages of conference abstract books and peer-reviewed medical journals. Yet, just as there are remaining questions regarding how best to use PIs and NNRTIs, there are similar issues facing the NRTIs that have yet to be fully addressed—most notably, how best to sequence their use in light of their individual drug-resistance profiles. Ironically, this particular issue is becoming more complex as time goes on, given recent data suggesting that resistance within this class of drugs may not be as agent-specific as was originally suspected.

This article stems from a sweeping review of the most recent trends in clinical research regarding antiretroviral drug resistance, delivered by Dr. Daniel Kuritzkes at the December 2001 meeting of PRN. We have limited this review to his discussion of resistance and cross-resistance among the NRTIs, a weighty topic that has not been covered in any significant detail in *The PRN Notebook*. Some of the other issues raised by Dr. Kuritzkes, including the advantages and limitations of drug-resistance testing, have been reviewed in previous issues of the *Notebook* (see: "HIV Drug Resistance and Drug-Resistance Testing in the March 1999 issue, based on a PRN lecture delivered by Scott Hammer, MD) and are also discussed in the most recent set of drug-resistance testing recommendations produced by the International AIDS Society-USA, (Hirsch, 2000).

Phenotypic Cutoffs: Defining Resistance and Virologic Outcomes

In order to appreciate the finer discussion points regarding resistance and cross-resistance among the NRTIs, it is first necessary to elucidate some of the more recent discussions regarding phenotypic resistance testing, most notably what is considered "sensitive" virus and what is considered "resistant" virus. 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 typically 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., a 2.5-fold to fourfold increase in IC_{50}).

"The problem is," commented Dr. Kuritzkes, "laboratory reference viruses are not necessarily representative of viruses in the general population. If we were to sample 100 or 1,000 antiretroviral-naive patients, we would likely find a natural variation in the susceptibility of wild-type virus. Just as reference laboratories are constantly assessing drug susceptibility of staphylococcus and *E. coli* in the general population, we need to be doing the same thing with HIV if we are to determine what we can realistically expect from treatment."

In order to develop more meaningful "biological" cutoff values for each drug—including the NRTIs—investigators at Tibotec-Virco measured 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. In other words, a test result falling above the cutoff can be said to be above the normal susceptible range with 97.5% confidence.

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.

The use of the new cutoffs will change the amount of resistance being reported, at least with the Antivirogram test (See Table 1). For example, the new cutoff values for the dideoxynucleoside analogues are lower than before and, in a study of 5,000 random clinical samples, revealed a higher and more realistic incidence of resistance. Conversely, the cutoffs for the non-nucleoside reverse transcriptase inhibitors are higher than before.

Tibotec-Virco now uses biological cutoff

TABLE 1. Drug-Specific Cutoffs for Tibotec-Virco's Antivirogram, ViroLogic's Phenosense, and VIRalliance's Phenoscript

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

Source: ViroLogic

values in their lab reports. "This has definitely been a step in the right direction," added Dr. Kuritzkes. "We're moving away from laboratory-defined drug resistance and into a more realistic era of population-defined resistance."

Cutoffs and Virologic Outcomes

RESISTANCE TO ANTIRETROVIRAL THERAPY IS hardly a black-and-white issue. For example, the new biological cutoff for zidovudine using Antivirogram is a fourfold increase in its IC_{50}. Anything below this value suggests that a given isolate is sensitive to the agent, whereas a value above this cutoff suggests resistance. "Resistance is really a continuum," Dr. Kuritzkes pointed out. "It's not as if an HIV variant with a fourfold increase has completely lost sensitivity to zidovudine. Sensitivity and the likelihood of virologic success decrease as the fold change increases. Unfortunately, we don't have much in the way of data to determine what we can realistically expect from an individual agent as the fold change in IC_{50} increases. Is an eightfold increase significantly worse than a fivefold increase? Is there any antiviral benefit, even if an eightfold increase in IC_{50} is found? Does a sixfold increase in resistance for one drug mean the same thing clinically for another drug?" In other words, relying on technical or biological cutoffs will not help predict the likely utility of a drug. For this, it is necessary to evaluate clinical trial data correlating the results of resistance testing with virologic outcome data.

Unfortunately, finding these clinical correlates is easier said than done. Most clinical trials of antiretroviral agents, at least in this day and age of viral load and drug-resistance testing, involve multidrug regimens. In turn, it has been extremely difficult to pinpoint with accuracy the virologic ramifications of resistance to one particular agent being used in a combination. However, such data do exist for some NRTIs, including abacavir and tenofovir DF (discussed below), as well as the dual-protease inhibitor lopinavir/ritonavir (Kaletra). Established clinical cutoffs, determined using ViroLogic's Phenosense assay and VIRalliance's Phenoscript assay, are listed in Table 1.

The Abacavir Experience

AT THE 8TH CONFERENCE ON RETROVIRUSES and Opportunistic Infections, held last year in Chicago, Dr. Randell Lanier of GlaxoSmithKline presented data from a retrospective analysis that helped define the various cutoffs associated with use of abacavir (Ziagen). This study, conducted in collaboration with ViroLogic, evaluated stored specimens from four clinical trials (CNA 2003, 3001, 3002, and 3009) in which abacavir was added to stable antiretroviral regimens in order to intensify treatment. In effect, any virologic response that occurred during the follow-up period could likely be attributed to the use of abacavir.

After 24 weeks, it was found that an HIV phenotype of less than 4.5-fold resistance to abacavir was associated with maximal response. Any phenotypic value above sevenfold was associated with "severely" limited or no response. Phenotypes between 4.5- and sevenfold were associated with a partial response.

"What these data show us," Dr. Kuritzkes explained, "is that, the more resistant the virus is to the drug, the less a response you can expect. It's not as though you cross some threshold and suddenly

there is no response at all. As one begins to move up the scale of resistance, you should expect diminishing returns in terms of antiviral activity. However, there may still be partial activity, which may really mean something significant for patients who are highly treatment experienced. We should keep in mind that assembling a regimen of several partially active drugs may enable us to create regimens that have reasonable antiviral activity. Chances are, we won't be able to create regimens that will achieve full viral suppression, but we can create respectable drug combinations that will translate into meaningful clinical benefits for our patients."

NRTIs and Cross-Resistance

WHILE THE PROTEASE INHIBITORS (PIS) HAVE essentially revolutionized the treatment of HIV infection, there has long been concern regarding the significant problem of cross-resistance within this potent class of drugs. In this way, NRTIs were considered to be the saving grace of antiretroviral therapy sequencing strategies. "For many years, we all believed that each of the NRTIs was unique and that cross-resistance wouldn't be a significant problem," Dr. Kuritzkes said. "Over the past two to three years, however, we've learned that this simply isn't the case. The more we learn about nucleoside resistance, the clearer it is that viruses with high-level nucleoside resistance are very likely to be pan-resistant to all of the NRTIs."

Mechanisms of Cross-Resistance

THERE ARE ESSENTIALLY TWO MECHANISMS BY which genotypic mutations can lead to reduced phenotypic sensitivity to antiretroviral drugs. The first involves mutations that occur at or near the drug-binding site of either the reverse transcriptase (RT) or protease genes, resulting in increased drug discrimination by these enzymes. This is the primary mechanism of genotypic resistance to all of the protease inhibitors, all of the non-nucleoside reverse transcriptase inhibitors (NNRTIs), and most of the NRTIs. For example, the primary mutations that can arise during therapy with one NNRTI (e.g., nevirapine) often impair the ability of subsequent NNRTIs (e.g., efavirenz) to bind with the RT gene and successfully halt viral replication.

The second mechanism that is of concern when discussing NRTIs involves key mutations that essentially work to undo the action of these drugs, even if they do manage to bind correctly with the RT gene. NRTIs exert a blocking effect by plugging a nucleoside monophosphate to the 3' end of the growing proviral DNA chain. This effectively terminates chain extension and, ultimately, inhibits replication of the virus. However, this process can be reversed by an RT reaction that removes the chain-terminating residue and reinstates an extendable primer. This reverse reaction of DNA polymerization, termed pyrophosphorolysis, enables reverse transcription and DNA synthesis to resume.

TABLE 2. TAMs* to Watch Out For

M41L	V118I‡
E44D/A‡	L210W
D67N	T215Y/F
K70R	K219Q/E/N

* TAMs = Thymidine analogue mutations in HIV reverse transcriptase.

‡ Additional TAMs that can reduce susceptibility to lamivudine in the absence of the M184V mutation (but always in the presence of other TAMs).

Pyrophosphorolysis can be enhanced by key mutations, most notably those selected by zidovudine (Retrovir) and stavudine (Zerit). These mutations are sometimes referred to as thymidine analogue mutations (TAMs), as both zidovudine and stavudine are thymidine analogues and appear to select—and are hobbled by—many of the same mutations in RT that confer drug resistance (see Table 2). While pyrophosphorolysis is believed to be the primary mechanism of resistance to zidovudine and stavudine, the process is not drug-specific in the way that discriminatory mutations tend to be. Consequently, these pyrophosphorolysis-enhancing mutations can confer reduced susceptibility to all of the NRTIs. As summarized by Dr. Kuritzkes: "Mutations that are selected under pressure of one drug can lead to resistance to one or more drugs not yet tried, either because of overlapping mutational profiles or, as in the case of pyrophosphorolysis-enhancing mutations, because the mutation profiles of some drugs can reduce the response to other drugs that do not themselves select for them."

Mutations Conferring Broad Cross-Class Resistance

WITHOUT A DOUBT, THE MOST SEVERE FORM of resistance is cross-class resistance, in which mutations that arise during therapy with one particular agent lead to marked reductions in susceptibility to any other member of the same class. This is most likely to occur with any of the NNRTIs and less likely to occur with the protease inhibitors. As for the NRTIs, two patterns of mutations have been found to confer high-level cross-class resistance.

The first, relatively well-known pattern is a cluster of RT mutations associated with the dreaded Q151M substitution, which alone confers partial resistance to all NRTIs (but not tenofovir DF). With the addition of key mutations at positions 62, 75, 77 and 116, both the severity of cross-resistance and the replicative fitness of the HIV variant increase.

More recently, a second pattern of resistance mutations has been found to yield significant cross-resistance among the NRTIs. The pattern involves a T69S insertion that incorporates two extra amino acids at that position. The first extra residue is another serine, while the second is usually a serine (T69SSS), alanine (T69SSA) or glycine (T69SSG). When this codon insertion is combined with the L210W and T215W mutations—which can arise during zidovudine therapy—profound cross-resistance among the NRTIs can occur.

Mutations Conferring Resistance to Lamivudine

IT IS COMMONLY KNOWN THAT THE M184V SUBstitution in the RT gene is associated with high-level resistance to lamivudine (Epivir), usually arising during treatment with the drug. However, key mutations associated with lamivudine resistance can also be selected by other NRTIs. Two mutations discussed by Dr. Kuritzkes were E44D/A and V118I, which confer moderate (15-fold) resistance to lamivudine. These mutations can arise during prolonged therapy with zidovudine and are usually found in association with pyrophosphorolysis-enhancing mutations, particularly T215Y. Neither E44D/A nor V118I are directly associated with reduced sensitivity to zidovudine; thus they are most likely compensatory changes that, once selected, render the virus less sensitive to lamivudine treatment.

Another point raised by Dr. Kuritzkes is

the need to sequence abacavir and lamivudine carefully. Both drugs select for the M184V mutation, which can spell trouble if it arises during therapy with an initial regimen containing abacavir but not lamivudine. "Lamivudine should be used either with or before abacavir. A number of mutations are needed to confer high-level resistance to abacavir, whereas you only need an M184V mutation to cause high-level resistance to lamivudine. If M184V occurs during lamivudine therapy, before abacavir has been started, abacavir will still likely have some effect once it is initiated. If lamivudine follows abacavir, lamivudine may not offer much in the way of an advantage."

Cross-Resistance Between Stavudine and Zidovudine

FOR MUCH OF ITS EIGHT YEARS OF COMMERCIAL availability, stavudine (Zerit) has been considered an anomaly when it comes to resistance. *In vitro*, mutations at RT positions 69, 75, and 151 are associated with resistance to the drug. *In vivo*, however, there has been little in the way of clarity regarding the precise mutations conferring decreased sensitivity to stavudine. "We've known all along that patients lose sensitivity to stavudine," commented Dr. Kuritzkes. "But it took some doing to figure out what, in fact, was going on with the virus. Unfortunately, much of what we have learned reveals a great deal of cross-resistance between stavudine and other NRTIs, most notably zidovudine."

Several small studies reported over the past three years have demonstrated that stavudine displays a resistance profile that is almost identical to that of zidovudine (Ross, 1999; Pellegrin, 1999; Coakley, 1999; Calvez, 1999, 2000; Pozniak, 2000). One of the largest data sets discussed by Dr. Kuritzkes was a retrospective analysis of ACTG 370, a randomized trial comparing continued lamivudine or a switch to delavirdine in more than 300 HIV-positive patients receiving one of three dual-NRTI combinations: zidovudine/lamivudine, stavudine/lamivudine, or didanosine/lamivudine (Johnson, 2001). At the time of randomization, all patients in the study also added indinavir. "We simply went back and looked at the samples collected prior to the switch and the addition of the protease inhibitor," Dr. Kuritzkes explained. "The study volunteers had been receiving dual-nucleoside therapy for approximately one and a half years before enrolling in the study."

Among volunteers who had been taking zidovudine and lamivudine prior to entering the study, 50% had one or more RT mutations associated with zidovudine resistance. In the stavudine/lamivudine group, approximately 40% had one or more mutations typically associated with zidovudine resistance. The difference between these two groups was not statistically significant. "If we had a larger population of patients, we suspect that we'd have found a statistically significant difference," Dr. Kuritzkes added. "Although these two drugs select for the same mutations, we suspect that zidovudine does so more vigorously. It may be selecting these mutations more quickly than stavudine does, at least in the context of old-fashioned dual-nucleoside therapy. Of course, much of this goes out the window when we talk about HAART. But we definitely have evidence to suggest that TAMs result in decreased sensitivity to both drugs."

Dr. Kuritzkes also reviewed data from a retrospective analysis of ACTG 302, a study involving patients heavily pretreated with zidovudine monotherapy who switched to stavudine monotherapy. Thirty-one patients were included in this analysis. Eight patients were dubbed responders—defined as a 0.3 log or greater decrease in HIV-RNA upon switching—and 23 were considered nonresponders. Seven of the eight responders had only the K70R RT mutation at baseline. Among the nonresponders, three had only the K70R mutation, compared to 15 who had multiple TAMs. As for the remaining five who had no genotypic evidence of TAMs at baseline, the general consensus was that zidovudine/stavudine failure in these individuals was likely because of poor drug absorption or poor adherence.

"The really important thing about this and other studies," commented Dr. Kuritzkes, "is the close association between zidovudine and stavudine. The lesson here for clinicians is simple. We now know that susceptibility of the virus to stavudine is very similar to that of zidovudine. Instead of using phenotypic assays to determine if a patient's sample is sensitive to stavudine, we can look at what happens in terms of sensitivity to zidovudine. If we see high-level resistance to zidovudine, we're pretty much seeing what we can expect upon switching to stavudine."

Tenofovir Resistance

TENOFOVIR DF (VIREAD) IS THE MOST RECENT addition to the RT-inhibitor family. It is a nucleotide analogue manufactured by Gilead Sciences. *In vitro*, tenofovir selects for the RT mutation K65R, which confers a threefold reduced susceptibility to the drug. "We don't really know what this threefold value means," Dr. Kuritzkes said. "We might consider this to be the biological cutoff, but we don't know what it means in terms of clinical responses to tenofovir."

In one comprehensive poster presentation at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, held last year in Chicago, investigators at Tibotec-Virco and Gilead Sciences assessed the distribution of tenofovir susceptibility in nearly 1000 treatment-naive HIV-infected individuals using the Antivirogram assay (Harrigan, 2001a). In addition, phenotypic susceptibility to tenofovir and other RT inhibitors was determined in a panel of nearly 5000 consecutive clinical isolates.

More than 97.5% of treatment-naive individuals had tenofovir susceptibility less than threefold above the wild-type control. Therefore, a biological cutoff for tenofovir was confirmed at threefold for the Antivirogram assay. The clinically derived panel of 5000 samples showed a broad range of drug susceptibilities, including 69% having a greater than tenfold decreased susceptibility to at least one antiretroviral drug class, 43% to two classes, and 16% to all three classes. Large decreases in tenofovir susceptibility were rare, with 4% and 1% of samples having greater than fivefold or greater than tenfold decreases in tenofovir susceptibility, respectively. More than 88% of the 5000 clinically derived samples were within the threefold normal range for tenofovir. Among samples with reduced susceptibility to lamivudine, zidovudine, stavudine, didanosine, or abacavir, the percentage of samples that remained within the normal range for tenofovir was 85%, 71%, 62%, 72%, and 76%, respectively.

Tenofovir resistance data are also available from GS 902, a phase II, placebo-controlled, double-blinded study that evaluated three doses of tenofovir added to stable HAART regimens in 189 treatment-experienced patients (Miller, 2001). Approximately 94% of pts had NRTI resistance mutations at baseline, yet there was a statistically significant mean HIV-RNA reduction of 0.58

Table 3. Tenofovir DF HIV-RNA Responses by Baseline Resistance Mutations

Baseline Mutation Group	Mean $DAVG_{24}$ (n)		Net Treatment Effect
	Placebo	Tenofovir	
All Patients	-0.03 (110)	-0.59 (222)	-0.56
No M184V	+0.08 (40)	-0.42 (73)	-0.50
M184V	-0.08 (70)	-0.67 (149)	-0.59
M184V / No TAM[1]	-0.12 (20)	-0.96 (51)	-0.84
No TAM[1]	-0.11 (29)	-0.80 (68)	-0.69
TAM[1]	0.00 (81)	-0.50 (154)	-0.50
TAM[1]/ No M184V	+0.13 (3)	-0.45 (56)	-0.58
TAM[1] + M184V	-0.08 (50)	-0.52 (98)	-0.60
T215Y/F	+0.03 (53)	-0.35 (106)	-0.38
T215Y/F /No M184V	+0.14 (24)	-0.37 (44)	-0.51
T215Y/F + M184V	-0.06 (29)	-0.34 (62)	-0.28
T69D/N	-0.08 (19)	-0.48 (25)	-0.56
L74V/I	+0.11 (16)	-0.17 (18)	-0.28
K65R	0	-0.01 (6)	-0.01
Q151M	+0.05 (2)	+0.38 (2)	+0.33
T69S Insertions	0	+0.29 (2)	+0.29
NNRTI-R[2]	+0.06 (53)	-0.50 (97)	-0.56
PI-R[3]	0 (70)	-0.52 (129)	-0.52

* Patients included in these subgroups may have other TAMs or mutations in addition to the baseline TAM listed

[1] TAM = M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N (Also known as TAMs)

[2] NNRTI-associated resistance mutations = K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E or P263L

[3] PI-associated resistance mutations = D30N, V32I, G48V, I50V, V82A/F/T/S, I84V or L90M

log with the 300 mg tenofovir dose that was durable through week 48.

Using the Antivirogram assay, baseline phenotypes from 53 of the 54 patients who received the 300 mg tenofovir dose revealed a mean reduced susceptibility of 1.9-fold over wild-type for tenofovir and 13.8-fold for zidovudine. Only four patients had greater than fourfold reduced susceptibility to tenofovir at baseline. In regression analyses, the HIV-RNA response to tenofovir correlated significantly with baseline susceptibility to tenofovir and zidovudine but not to other NRTIs.

At week 48, 29 patients had sufficient HIV-RNA for phenotypic analyses. Compared to baseline, 15 patients showed increased susceptibility to tenofovir and 14 patients showed decreased susceptibility; six of these 14 showed greater than 2.5-fold decreases, which included Gilead studies 902 and 907 included plans to prospectively evaluate HIV-RNA responses according to the presence of baseline mutations. Primary analysis plans specified the evaluation of various thymidine analogue mutations (TAMs) and the M184V mutation. This table summarizes a detailed analysis by the U.S. Food and Drug Administration (FDA), conducted as a part of the agency's review of Gilead Science's new drug application (NDA) for tenofovir. A second analysis conducted by the manufacturer regarding the effects of baseline NRTI-associated mutations was presented at the 9th Conference on Retroviruses and Opportunistic Infections (CROI) in February.

The FDA's initial review of studies 902 and 907 demonstrated that TAMs M41L, L210W or T215Y/F were associated with reduced viral susceptibility to tenofovir. Further analyses by the FDA and Gilead Sciences demonstrated that the diminished response noted in patients with the T215Y/F mutation at baseline was likely due to the presence of M41L or L210W mutation, not the T215Y/F mutation itself. As for the other TAMs, D67D, K70R and K219Q/E/N do not appear to have a negative effect on virologic responses to tenofovir.

Additional analyses conducted by the FDA and Gilead Sciences suggested that the number—and more importantly the type—of TAMs present at baseline might affect tenofovir activity. Tenofovir efficacy was diminished in patients with greater than three TAMs, in the presence of mutations M41L and L210L. If three or more TAMs are present, but do not include these two key mutations, susceptibility to tenofovir will not necessarily be diminished.

Also discussed in the FDA's analysis was the relevance of the L74V/I mutation, which confers resistance to abacavir, didanosine, and zalcitabine. Patients with this mutation had a stunted response to tenofovir: the mean $DAVG_{24}$ for those with this mutation was -0.17 log. As to not jump to the same premature conclusion that was made for the T215Y/F mutation, additional analyses were conducted to determine if the diminished response in the setting of L74V/I was attributed to the presence of other NRTI mutations, specifically the TAMs. Response rates were similar (-0.12 to -0.19 log) regardless of whether the M41L or L210W mutations were present. This finding suggests the potential for cross-resistance between tenofovir and didanosine; however, more data from patients with this mutation are needed to make any definitive conclusions.

As for phenotypic susceptibility, data reviewed at the 9th CROI confirm that HIV-RNA responses to tenofovir correlate significantly with baseline susceptibility to the drug. Response to tenofovir therapy ranged from -0.72 to -0.46 log for patients with <1, 1-2, 2-3, and 3-4-fold changes in baseline tenofovir susceptibility. Patients with greater than fourfold reductions in tenofovir susceptibility at baseline showed diminished responses to tenofovir therapy (-0.12 log $DAVG_{24}$). Recursive partitioning analysis confirmed a breakpoint of fourfold for response to tenofovir therapy. Genotypic analysis of HIV with greater than fourfold reduced susceptibility to tenofovir revealed the K65R mutation, a T69 double insertion, or multiple TAMs—including the M41L and L210W mutations—to be the most likely culprits.

Source: U.S. Food and Drug Administration. Background Package for NDA 21-356: Viread (tenofovir disoproxil fumarate). Accessed at: http://www.fda.gov/ohrms/dockets/ac/01/briefing/3792b1_03_FDA-tenofovir.htm; Miller MD, Margot NA, Lu B. **Effect of baseline nucleoside-associated resistance on response to tenofovir DF (TDF) therapy: integrated analyses of studies 902 and 907** [Abstract 43]. 9th Conference on Retroviruses and Opportunistic Infections, Seattle, 2002.

two patients who developed K65R. Analysis of the other two patients who developed K65R also showed threefold to fourfold reductions in tenofovir susceptibility. None of these four patients had evidence of viral load rebound associated with K65R.

Additional data pertaining to the relevance of RT mutations that exist prior to the initiation of tenofovir therapy are discussed in Table 2.

"As with abacavir, we're looking at a continuous relationship between susceptibility and responsiveness to tenofovir," Dr. Kuritzkes pointed out. "We don't yet have exact cutoffs for clinical response, but it looks as if sensitivity begins to decrease if the value is above onefold and sensitivity is markedly reduced above fourfold. Again, it's not like tenofovir either will or will not be effective. There will simply be varying levels of responsiveness, depending on the level of resistance present."

Conclusion

NRTIS HAVE LONG BEEN THE BACKBONE OF antiretroviral therapy. Once the only class of drugs available to treat HIV, they are now incorporated into virtually all antiretroviral drug combinations. Very often, the choice of which two (or more) NRTIS to use in a initial HAART regimen is taken for granted, given the general sentiment that cross-resistance is not a significant problem among this class of antiretroviral agents. But appearances can be deceiving. As discussed by Dr. Kurtizkes, there is substantially more cross-resistance among the thymidine analogues than was previously perceived. And in the setting of multiple NRTI mutations, HIV can lose sensitivity to abacavir and tenofovir—two of the most potent inhibitors of reverse transcriptase available.

Clearly, clinicians and patients cannot afford to be cavalier about their selection of NRTIS, whether it's in a first-line HAART regimen or subsequent combinations. Much like selecting a protease inhibitor or non-nucleoside reverse transcriptase, it's important to remember that NRTIS remain a vital component of antiretroviral therapy and that they must be sequenced judiciously—with the help of genotypic and/or phenotypic drug-resistance testing—to reap maximal and durable benefits. *prn*

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