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## Insight into HIV-1 viral fitness

Kara Nyberg, PhD

The clinical progression of HIV-1-infected individuals varies widely. Host factors have clearly been shown to be associated with differences in disease progression but do not explain all of the variability. More specifically, genetic differences (eg, human leukocyte antigen differences, chemokine receptor polymorphisms, and chemokine levels) between individuals play a crucial part in the course of viremia and disease progression, but the fitness of the virus may also play a significant role in the natural history of HIV-1 disease. Indeed, preliminary data are emerging that suggest some factors influencing viral fitness may also influence disease progression in both treated and untreated individuals.

A variety of methods have been used to assess aspects of viral fitness, but all come with limitations. Regardless, these assays are now being utilized to define how viral fitness may influence HIV disease progression in those who are and are not taking antiretroviral therapy (ART). This *Navigating HIV Resistance* newsletter article reviews these methods and addresses their possible application in clinical practice.

## I. Fitness concepts

*Defining fitness*


HIV-1 fitness refers to the ability of the virus to adapt and compete within a specific environment. The overall fitness of a given viral strain is defined by intrinsic properties of the virus, defined by multiple parts of the viral genome as well as factors present *in vivo* such as host immune responses, antiretroviral (ARV) drug pressure, and target cell availability. Measuring the *in vivo*

fitness of the innumerable different HIV strains within an individual is virtually impossible, and most work has focused on the use of various *in vitro* assays that measure particular aspects of fitness, as will be described later in this review.

*Drug resistance effects on fitness*

HIV-1-infected individuals on ART often develop drug-resistance mutations; hence, understanding how these mutations might alter viral fitness could provide insight into the course of viremia and possible treatment options for those failing therapy. HIV-1 drug resistance occurs in the molecular targets of ART, thereby causing the viral proteins to adopt non-native conformations that nearly always result in some decrease in their ability to replicate. Evidence supporting this includes the *in vivo* observation that upon discontinuing therapy, drug-susceptible viruses present at low frequencies often rapidly replace drug-resistant strains.<sup>12</sup> Moreover, increases in plasma HIV-1 RNA levels typically accompany this phenomenon. Conversely, virologic rebound due to drug resistance is often incomplete, with virus levels failing to return to baseline despite high-level resistance when therapy is continued. In addition, a large number of *in vitro* studies have also demonstrated that drug-resistant viruses replicate more slowly under laboratory conditions and are out-competed when mixed with wild-type virus.

Under *in vitro* conditions, resistance mutations to protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitor (NRTI) mutations appear to impair HIV-1 replication to a greater degree than nonnucleoside reverse



transcriptase inhibitor (NNRTI) mutations. The degree of fitness impairment associated with different resistance mutations varies widely. For example, PI mutations in the protease active site cause a greater decrease in fitness than those outside of the active site.<sup>3</sup> In the reverse transcriptase (RT), the K65R, L74V, and M184V mutations cause greater decreases in fitness than thymidine analog mutations (TAMs).<sup>4,5</sup> NNRTI mutations occur in a hydrophobic pocket of the RT enzyme rather than in the active site and therefore appear to be less detrimental than PI and NRTI mutations.<sup>6</sup>


As a general rule, drug-resistance variants found *in vivo* replicate better than lab strains engineered with drug-resistance mutations, because of compensatory mutations that the strains develop *in vivo*. These mutations do not directly alter the drug-resistance profile of the strain in which they reside; rather, they help to ameliorate any decreases in fitness caused by drug-resistance mutations. For example, protease compensatory changes can occur in one or more of the nine cleavage sites on which the protease acts.<sup>7</sup> Specific compensatory changes in the *gag* gene have been especially well documented for mutations in the protease substrate cleft (eg, at positions 50 and 82). Genetic changes outside of the RT and protease sequences may also compensate for decreased replication associated with mutations in these proteins. These compensatory changes may affect the activity of the protease and RT proteins, or they may be more nonspecific, functioning to increase the replication of the virus or its spread from cell to cell. Indeed, changes in the viral envelope may also have profound effects on HIV replication *in vivo*.<sup>8</sup>

### Measuring fitness

*In vivo* assessment of viral fitness would be ideal, but such measures cannot be practically performed. Instead, other fitness measures have been devised that focus on the intrinsic property of the virus to replicate *in vitro*. Some of these assays analyze the replication capacity (RC) of the whole virus, whereas others assess just select portions of the viral genome.

*In vitro* assays analyzing the entire genomes of different HIV-1 strains provide an assessment of the relative fitness of each strain using either non-competitive or competitive assays. In a non-competitive assay, cell culture systems are individually infected with one viral strain, and the growth kinetics of the strains in each cell system are compared to estimate their relative fitness. Alternatively, in a competition assay, a cell culture system is inoculated with multiple viral strains, and the rate at which one strain outgrows the others indicates their relative fitness. These whole-virus *in vitro* methods provide some understanding of how quickly viruses may grow in human cell populations, but they are limited in that they do not represent the many selective forces found in the *in vivo* host environment.

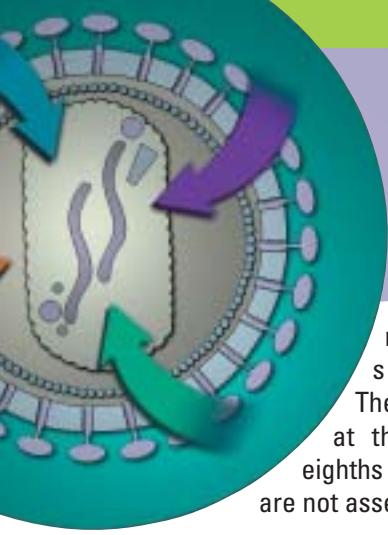
The best data on the relationship between viral fitness and clinical disease progression employed competition assays, and demonstrated that the relative fitness of viruses from those with rapid disease progression is more fit than the wild-type reference strain, whereas long-term non-progressors were shown to have viruses less fit than the reference strain.<sup>9</sup> Fitness was also correlated with viral load levels, although the study size was small. In a more recent study, the same research group again used competition assays to measure the fitness of HIV-1 primary isolates



and *env* gene recombinant viruses to determine the contribution of wild-type *env* sequences to overall HIV-1 fitness.<sup>10</sup> The results indicated that the envelope does reflect overall fitness for some viruses, whereas for others it did not, suggesting that other viral components are relevant in determining the level of fitness.

Because of the techniques and research equipment involved, the *in vitro* non-competitive and competitive assays are limited to select research laboratories, and they are not high-throughput measures of fitness. As a result, the need exists for other assays that can potentially be exploited in larger studies to define the relevance of select measures of HIV-1 fitness, and also used in clinical practice. In answer to this need, ViroLogic has developed a reproducible, high-throughput *in vitro* method to measure viral RC.

In ViroLogic's assay, which is automatically performed as a control whenever phenotypic susceptibility is measured by the PhenoSense™ assay, the patient-derived protease and RT sequences are cloned into a reporter construct containing the gene for luciferase. The population of cloned viruses undergoes one round of replication in the absence of drug-selection pressure, and the amount of light produced by the luciferase protein indicates the RC of the patient-derived virus compared against the median of many wild-type primary isolates after normalizing for virus input. Several studies have suggested that the results of this simple assay appear to correlate well with the more time-consuming, whole-virus *in vitro* tests.<sup>10,11</sup> However, it is important to realize that the ViroLogic assay measures the RC of only about one eighth of the total HIV genome—



the 3' portion of the *gag* gene, the protease sequence, and most of the RT sequence. Therefore, changes at the other seven eighths of the genome are not assessed.

## II. Can RC serve as a measure of fitness?

For those treating HIV-1-infected individuals, a pertinent question is whether select measures of fitness, such as viral RC, are clinically relevant. Although it has limitations, the ViroLogic RC assay is currently the only reproducible, high-throughput measure that can be used to further investigate the clinical relevance of at least this unique measure of fitness and its role in HIV disease progression. Promising data obtained from the application of the ViroLogic RC assay provide the impetus for considering future studies to determine how this method might be used in clinical practice.

The most direct evidence that the ViroLogic measurement of RC is indeed assessing a clinically relevant factor comes from data obtained by three different research groups. First, Stephen Deeks, MD (University of California, San Francisco), led a study in which patients exhibiting virologic failure discontinued ART.<sup>1</sup> These patients showed an increase in viral RC concomitant with a shift in prevalence from drug-resistant to wild-type virus, an increase in HIV-1 RNA levels, and a decline in CD4 counts. Second, Eric S. Daar, MD (Harbor-UCLA Medical Center, Los Angeles), presented data at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in which his research group performed a retrospective analysis of factors predictive of disease progression in untreated or minimally treated (zidovudine [ZDV] monotherapy) hemophilic patients.<sup>13</sup> This study demon-

strated that a single measure of RC was inversely correlated with CD4 counts and tended to be directly related to HIV-1 RNA measures. More importantly, RC values at baseline predicted increased rates of CD4 count decline independent of HIV-1 RNA, CD4 count, and cellular tropism, as well as progression to clinical AIDS, even when adjusted for HIV-1 RNA or CD4 count. Third, Jason Barbour, PhD, MHS (University of California, San Francisco), *et al* found that individuals infected with a virus with a low RC ( $\leq 43\%$  of the reference virus) had significantly higher CD4 counts at study entry and over time, both before and while on ART.<sup>14</sup>

It is worth mentioning that, surprisingly, RC has been reported to remain stable over time following the development of drug resistance, in another report from Barbour's group.<sup>15</sup> This result defies everything currently known about HIV-1, which suggests that RC would increase during ongoing HIV-1 replication due to the accrual of compensatory mutations. This may suggest that changes are perhaps occurring outside the RT and protease regions that were not detected by the ViroLogic RC assay.

## III. Conclusion

The available data on viral fitness as measured by the ViroLogic RC assay suggest that it is clinically relevant both in those with and without drug resistance. The unanswered question is how it can be used. Although measures of HIV-1 RC have some prognostic value for disease progression, it is probably of limited value for those in which treatment can produce undetectable levels of HIV-1 RNA. Ultimately, RC measures may prove to be most useful in managing those with limited treatment options—for example, those exhibiting incomplete virologic suppression while maintaining stable CD4 counts while on ART. This is particularly true if researchers and clinicians can define

how HIV-1 can be manipulated by ARV drugs to maintain low RC levels (and thus delay progression) while minimizing the number of drugs needed to sustain immunologic stability in those who cannot be fully suppressed. Consequently, promising preliminary RC data need additional confirmation, followed then by further research to define how this measure might be used in clinical practice.

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# Veteran HIV docs mull resistance puzzles

Mark Mascolini

Resistance to antiretrovirals remains among the most devilish concerns for clinicians planning therapy. It is no coincidence that the organizers of the annual IAPAC Sessions-USA clinical symposia picked resistance as one of four topics explored for three years running. Other hot topics come and go; resistance resists transition.

What resistance issues most perplex the most experienced HIV clinicians? This year's IAPAC Sessions-USA, held May 20-21, 2004, in Chicago, afforded an opportunity to find out. These symposia give IAPAC member clinicians leave from their front-line duty for two days of dialogue and debate with experts on four issues. This year's resistance adepts were Daniel Kuritzkes, MD (Brigham and Women's Hospital, Boston), David Katzenstein, MD (Stanford University), and Richard Haubrich, MD (University of California, San Diego). Calvin Cohen, MD, MSc (Community Research Initiative of New England, Boston) also joined the discussion. The following issues arose in the question-and-answer time that followed the resistance presentations:

*Can clinicians take advantage of resistance research to refine the sequencing of antiretroviral regimens?*

For Katzenstein, the short answer is "yes." But whether planning a sequence of regimens is the most important consideration when starting therapy remains an open question. The first issue clinicians should address is devising the strongest, most convenient, and least expensive regimen for each patient. That combination may or may not be the ideal first regimen in a sequence.

*We all worry about resistant virus, but we all have patients with multidrug-resistant virus who remain healthy for years. Why do these people keep doing so well on incompletely suppressive regimens?*

Both clinical experience and research show that some suppression is better than none, Cohen noted. There appears to be a subset of patients who can maintain a reasonable CD4 count and avoid opportunists for a long time with a low, though detectable, viral load.

**Whether planning a sequence of regimens is the most important consideration when starting therapy remains an open question. The first issue clinicians should address is devising the strongest, most convenient, and least expensive regimen for each patient.**

Katzenstein added that perhaps the most revealing work on this question was a study of the EuroSIDA cohort. Veronica Miller, PhD (then at JW Goethe University, Frankfurt) *et al* found a consistent correlation between treatment with up to five drugs and a lower risk of progression or death in 1,106 people with resistant virus and CD4 counts <50 cells/mm<sup>3</sup>.<sup>1</sup>

Some non-progressors may be people who have less immune activation despite ongoing low-level viremia, Haubrich suggested. Research done years ago by the late Janis Giorgi, PhD (formerly at the

University of California, Los Angeles) singled out markers of immune activation as independent predictors of death.<sup>2</sup>

*As a first PI regimen, is boosted atazanavir (ATV) as good as—or better than—lopinavir/ritonavir (LPV/RTV)?*

Katzenstein has been impressed so far with boosted ATV as up-front therapy. He thinks boosted ATV makes sense as a first protease inhibitor (PI) because he believes LPV/RTV has built a strong track record as the best PI after failure with other PIs. But we still need resistance results following ATV failure. How susceptible will virus be to other PIs if ATV's signature I50L mutation emerges? We don't know. In addition, there are no data from clinical trials with ATV/RTV in PI-naïve patients. We have no data telling us that people will fail ATV/RTV with the I50L mutation that is seen so far with unboosted ATV.

*When will we see the second-generation nonnucleoside reverse transcriptase inhibitors (NNRTIs) such as capravirine and the efavirenz (EFV) follow-ons that Bristol-Myers Squibb inherited from Dupont Pharmaceuticals? Will they fulfill the promise of controlling virus resistant to EFV or nevirapine (NVP)?*

Capravirine, Agouron/Pfizer's investigational NNRTI, has inched into phase 3 trials, Kuritzkes noted. But we've heard little recently about the Bristol-Myers Squibb's NNRTIs, labeled DPC-961 and DPC-963 when Dupont Pharmaceuticals was

developing them. Kuritzkes evinced scant optimism about faster development of these drugs or, indeed, about how much they may offer clinically. He saw two problems:

- These drugs will prove difficult to meld into rescue regimens because they are potent inducers of the CYP3A4 enzyme. Inducing this critical metabolic mediator will speed the elimination of protease inhibitors and other CYP3A4 substrates. Dose adjustments in multidrug rescue regimens could prove challenging.
- Although these agents show activity against many single- and double-mutant viruses resistant to EFV or NVP, they may be just another two mutations away from further resistance. Notorious for its evolutionary virtuosity, HIV seems unlikely to be long daunted by the second-generation NNRTIs.

If they are licensed, Kuritzkes suggested, the second-generation NNRTIs may find a role in powerful salvage regimens for people who will also respond to agents in other classes, such as Roche Laboratories' fusion inhibitor enfuvirtide (ENF) and RTV-boosted tipranavir (TPV), Boehringer Ingelheim's investigational PI.

#### *When should we use ENF?*

In the Boston area, Kuritzkes said, most clinicians use ENF in third-, fourth-, or fifth-line regimens. These are patients

with highly resistant HIV who have the opportunity to start at least one new drug to which their virus remains susceptible. For example, when RTV-boosted TPV expanded access began, many people started that boosted PI with ENF. Kuritzkes' advice mirrored guidance from a panel of nine experts<sup>3</sup>:

- "The optimal time to initiate [ENF] is when a strong and sustained virological response can be predicted on the basis of treatment history, resistance data and viro-immunological parameters."
- "Maximum response may be best achieved in patients whose virus retains at least partial activity to at least two drugs, ideally from different classes."
- "These conditions are most likely to apply to triple-class experienced patients looking to start a third or fourth regimen."
- "While residual activity to two or more drugs is preferable... the availability of only a single agent deemed active by resistance testing should not be considered a contraindication for [ENF] use."

*What do you think about using ENF in an earlier rescue regimen—for that extra antiviral push—then withdrawing it?*

Kuritzkes noted that some clinicians involved in phase 3 ENF trials endorse this approach. But it will remain an empiric strategy because it is nearly

impossible to test in a randomized trial. Such a trial would be difficult to enroll because almost everyone who needs ENF is already taking it, except for patients who depend on AIDS Drug Assistance Programs (ADAPs) in states that won't pay for this expensive drug.

*Work by Haubrich and others shows that HIV resistant to nucleoside reverse transcriptase inhibitors (NRTIs) can be hypersusceptible to NNRTIs.<sup>4</sup> Can we take advantage of this trait in planning therapy?*

Hypersensitivity to NNRTIs may bolster NNRTI activity in a second-line or later regimen, Haubrich confirmed. But treatment trends now favor NNRTIs as first-line therapy, when HIV is not resistant to NRTIs unless a patient has been infected with NRTI-resistant virus. So opportunities to profit from this hypersusceptibility are becoming rarer.

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# Nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance occurs at lower levels of adherence than protease inhibitor (PI) resistance

Bangsberg DR, Guzman D, Riley ED, *et al.*

XV International AIDS Conference. July 11-16, 2004. Bangkok. [Abstract WePeB5820]

## OBJECTIVES

While resistance to protease inhibitor (PI)-based ARV therapy occurs in highly adherent patients with incomplete viral suppression, the association between adherence and resistance to nonnucleoside reverse transcriptase inhibitor (NNRTI)-based therapy has not been characterized. We hypothesized that low levels of adherence place patients at greater risk for NNRTI than PI resistance.

## METHODS

PI- or NNRTI-treated patients were recruited from the REACH study, a cohort of homeless and marginally housed HIV-positive individuals in San Francisco. Mean adherence was calculated from monthly, unannounced pill counts conducted at the participant's usual place of residence over one year. Genotypic drug resistance was measured at the last detectable viral load (>50 copies/ml) on a stable NNRTI or PI regimen during adherence monitoring. Patients with one or more primary drug resistance mutations were classified as resistant. Patients with suppressed virus were classified as sensitive. The association between adherence quartile and proportion of individuals resistant was tested with chi square for trend and logistic regression.

## RESULTS

One hundred and two people were eligible for analysis. Sixty-one percent were injection drug users, 59% were non-white, and 21% were living on the street or a shelter. Forty-three individuals were on an NNRTI regimen, 59 were on a PI regimen. Forty-four percent had an average viral load (VL) <50 copies/ml. In the lowest adherence quartile (0-55 percent), the prevalence of NNRTI resistance was significantly higher than the prevalence of PI resistance (67% versus 13%;  $p=0.01$ ). Lower adherence was also associated with higher prevalence of NNRTI resistance ( $p=0.02$ ), but not PI resistance, by chi square for trend, and in a logistic model controlling for treatment duration, prior nucleoside exposure, and baseline CD4 ( $p=0.01$ ).

## CONCLUSIONS

Low levels of adherence are associated with higher prevalence of NNRTI resistance than PI resistance. Whereas low-pill-burden NNRTI regimens are often advocated for patients with low levels of adherence, these data suggest that patients with low levels of adherence may be at higher risk for resistance on NNRTI-based therapy than PI-based therapy.

## ANALYSIS

Bangsberg *et al* presented data relating levels of adherence to virologic rebound and the development of drug resistance. Their abstract described 102 patients followed in the REACH study who received therapy as prescribed by their providers and had adherence assessed based upon unannounced pill counts conducted at the patient's usual place of residence. The authors describe data from patients treated with a variety of PIs (for the most part non-ritonavir (RTV)-boosted PIs) and NNRTIs. Levels of adherence, by quartile, were related to the type of therapy, percent with HIV RNA levels <50 copies/mL, and the likelihood of having drug resistance. Although the data set was small, the observations were striking, with a step-wise increase in the percent of patients having HIV RNA levels greater than 50 copies/mL as adherence declined below 95% for those on PI-based therapy. While the percent of patients with evidence of PI resistance also increased when adherence dropped below 95%, it remained relatively stable (approximately 20-30%) in the lower three adherence quartiles. In contrast, those receiving NNRTIs were more likely to have HIV RNA levels <50 copies/mL, and demonstrated little difference in the percent with viral suppression across the upper three quartiles of adherence. However, viral rebound was markedly increased in those in the lowest adherence quartile (<55%). Of note, the level of NNRTI resistance was higher than that for PIs across virtually all quartiles of adherence, with a marked increase seen in the lowest quartile.

This study complements previous reports of the relationship between adherence, viral rebound, and drug resistance. In fact, the outcome in the group on largely non-RTV-boosted PIs was very similar to the work published by Paterson *et al* in 2000,<sup>1</sup> and is interesting to view in light of work published by King *et al* from the Abbott 863 study.<sup>2</sup> Paterson *et al* showed that the likelihood of resistance to non-RTV-boosted PIs occurred when adherence declined to below 95%. This is in sharp contrast to the results from King *et al* demonstrating the lack of resistance emerging to a RTV-boosted PI, lopinavir (LPV). The current study by Bangsberg *et al* confirms the previous observations of non-RTV-boosted PIs, and expands the current database with the analysis of the NNRTI-treated patients. In fact, the study shows that viral rebound in NNRTI-treated patients is low until adherence levels decline dramatically, with the trade-off being the higher levels of resistance. These data nicely demonstrate what most would have expected: that NNRTIs which generally have long half-lives may be somewhat more forgiving of missed doses. However, unlike PI-based regimens where early viral rebound is usually to NRTIs, particularly lamivudine (3TC), the emergence of drug resistance in those taking NNRTIs is frequently to this class of drugs.

Eric S. Daar, MD

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# Early virological failure and occurrence of resistance in naive patients receiving tenofovir, didanosine, and efavirenz

Podzamczar D, Ferrer E, Gatell JM, *et al.*  
*Antiviral Therapy* 2004;9(4):S172.

## OBJECTIVES

To describe the occurrence of a high early virological failure (VF) rate and development of resistance mutations in patients receiving tenofovir (TDF), didanosine (ddl), and efavirenz (EFV).

## METHODS

HIV-infected naive patients were enrolled in a pilot, randomized, intensification trial of TDF + ddl (250 mg) + EFV with (arm A) or without (arm B) lopinavir (LPV) for 12 weeks. Viral loads were determined at 0, 1 and 3 months, and in a subgroup of patients additionally at days 3, 7, 14 and 21. In patients with VF, genotypic resistance tests were performed at baseline and at each time point. As several cases of early VF (a drop of <2 log at month 3, or a rebound of >1 log from the nadir) were detected in arm B, an unplanned interim analysis was performed and a DSMB recommended stopping enrollment.

## RESULTS

36 patients were enrolled, 19 in arm A and 17 in arm B. Baseline median CD4 counts were 185 (6-490) cells/ $\mu$ L and median viral load (VL) was 144,207 (29,752->500,000) copies/ml. Twenty-six of the 36 enrolled patients completed three months of therapy and were included in the interim analysis. Six of 14 (42.8%) patients in arm B developed VF versus zero of 12 in arm A ( $P=0.017$ ). Six of 6 VF patients had VL >100,000 copies/ml and an advanced stage of disease (CD4 <200 plus CDC stage C or B3) versus zero of eight non-VF patients ( $P<0.001$ ). T69D/N (one patient) and T69S (one patient) were the only relevant mutations at baseline. At failure, G190S/E alone or associated with K103N and other mutations were detected in five patients, K103N/L100I/V108I in one, L74V/I in four, and K65R in two cases. EFV mutations appeared as soon as day 14 or 21 to as late as month 3; L74V/I were detected between months 1 and 3; and K65R appeared at month 3.

## CONCLUSIONS

A high early virological failure rate was found in a group of naive patients with advanced disease and high VL, treated with TDF + ddl + EFV. A peculiar resistance pattern was detected including G190E/S and L74V/I in most patients. It should be elucidated if interference between TDF and ddl, or other mechanisms, are involved.

## ANALYSIS

Podzamczar *et al* presented the above small randomized controlled, double-blinded study at the XIII International HIV Drug Resistance Workshop comparing TDF + ddl + EFV with or without LPV for 12 weeks in previously untreated patients. Surprisingly, six of 14 patients receiving TDF + ddl + EFV developed early virologic failure and drug resistance, compared with zero of 12 receiving the same drugs in combination with LPV. Each of the six virologic failures had a baseline RNA level >100,000 copies/ml and a CD4 count <200 cells/ $\mu$ L. At virologic failure, the nucleoside reverse transcriptase inhibitor (NRTI)-resistance mutations L74V/I and K65R were present in four and two patients, respectively, and the nonnucleoside reverse transcriptase inhibitor (NNRTI)-resistance mutations G190S/E and K103N (+L100I) were present in five patients and one patient, respectively.

This study suggests that not all once-daily dual NRTI/NNRTI regimens are equivalent for initial HIV treatment. Once-daily combinations of TDF + lamivudine (3TC), TDF + emtricitabine (FTC), abacavir (ABC) + 3TC, ddl + 3TC, and ddl + FTC have been shown to be highly effective in combination with EFV and are among the regimens recommended in the US DHHS "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents" (March 23, 2004). The poor response to TDF + ddl + EFV was surprising to many because this regimen is not used that infrequently. Some Resistance Workshop attendees speculated that the high RNA levels may have played a role (four patients had levels of 300,000 copies/ml). Some suggested that the regimen may have a low genetic barrier to resistance and recalled a past paper suggesting that mutations at positions 74 and 190 may act synergistically to confer resistance to both NRTIs and NNRTIs.<sup>1</sup> Others suggested that a dual NRTI/NNRTI combination lacking either 3TC or FTC is insufficiently potent to suppress HIV in all patients.

Another recently published study raised a different question about the combination of tenofovir and ddl. Negrodo *et al* recently described a study in which 169 patients with virologic suppression on a twice-daily NNRTI- or protease inhibitor (PI)-containing antiretroviral regimen were randomized to continued therapy versus simplification with once-daily TDF + ddl + nevirapine (NVP).<sup>2</sup> At week 48, the patients randomized to simplification maintained virologic suppression as well as the control group but, in contrast to the control group, experienced a 95-cell decrease in CD4 count. This study was done before the interaction between TDF and ddl was known and patients were receiving the standard rather than the recommended reduced dose of ddl.

Two months ago, I described a case in which the combination of TDF + ddl + EFV suppressed virus to undetectable levels in a patient with virologic failure during treatment with zidovudine (ZDV) + 3TC + nelfinavir (NFV). However, in light of these two studies, retrospective analyses of the performance of this regimen in patients with varying levels of baseline plasma HIV RNA are now urgently needed.

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1. Kleim JP, Rosner M, Wingler I, *et al.* Selective pressure from a quinoxaline nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) on HIV-1 replication results in the emergence of nucleoside RT-inhibitor-specific (RT Leu-74—<Val or Ile and Val-75—<Leu or Ile) HIV-1 mutants. *Proc Natl Acad Sci USA* 1996;93:34-38.  
2. Negrodo E, Molto J, Burger D, *et al.* Unexpected CD4 cell count decline in patients receiving Didanosine and tenofovir-based regimens despite undetectable viral load. *AIDS* 2004;18:459-463.

