Perspective

Incomplete Viral Suppression Under Potent Antiretroviral Therapy: Determinants of Treatment Outcome

At the International AIDS Society–USA course in Los Angeles in March 2002, Steven G. Deeks, MD, discussed recent findings regarding the effects of antiretroviral therapy on HIV replicative capacity and the potential implications of these findings for treatment strategies.

Virologic failure of potent antiretroviral therapy remains common in clinical practice, and is often associated with the emergence of a drug-resistant HIV. One recent population-based study demonstrated that approximately 78% of viremic patients in clinical care harbor drug-resistant HIV (Richman et al, ICAAC, 2001). Many factors are known to contribute to the inability of potent antiretroviral therapy to completely suppress viral replication, including preexisting resistance, limited drug potency, nonadherence, altered drug metabolism, tissue compartmentalization, and, for unclear reasons, advanced HIV disease.

Although much is known about the causes of virologic failure, much remains unknown about its long-term clinical consequences. Patients in whom virologic failure occurs often continue to respond to their combination treatment, in that plasma HIV-1 RNA level does not return to pretreatment levels and CD4+ cell counts remain elevated for a prolonged duration. CD4+ T-cell count changes were recently studied in a cohort of 291 patients experiencing continuous virologic failure of protease inhibitor therapy, with most patients being on a second "failing" regimen (Deeks et al, AIDS, 2002). The majority of patients maintained some gain in CD4+ cell count above pretreatment levels after more than 3 years of continuous virologic failure (Figure 1). The change in plasma HIV-1 RNA levels below the pretreatment baseline level was the single most important predictor of CD4+ T-cell outcomes in this cohort. Specifically, sustained CD4+ T-cell gains were observed as long as patients maintained a change in HIV-1 RNA level of at least 0.7 $\log_{10}$ copies/mL below baseline.

Still, it is clear that virologic failure ultimately results in immunologic decline and clinical progression. A recent report from the Swiss HIV Cohort showed that the relative risk for progression to a new AIDS event or death was markedly increased according to increments in average plasma HIV-1 RNA level over time from the start of potent antiretroviral therapy. This result indicates that residual viral replication during therapy is predictive of disease progression (Egger et al, 9th CROI, 2002). In other words, although some patients do well for years with low to moderate levels of viremia, it is clearly preferable to achieve and maintain a plasma HIV-1 RNA level as low as possible.

Why Does Viral Load Often Remain Below Pretreatment Levels After Emergence of High-Level Resistance?

Plasma HIV-1 RNA levels often remain below pretreatment baseline levels after emergence of high-level drug resistance. Factors that may contribute to this phenomenon include viral replicative capacity or fitness, characteristics of the HIV-specific cellular immune response, and persistent activity of some drugs against resistant variants. The role of viral replicative fitness in viral replication under antiretroviral drug pressure has recently been investigated.

In an attempt to identify factors in the prolonged maintenance of relatively low and stable plasma HIV-1 RNA level despite the likelihood of significant antiretroviral resistance, investigations were performed (Deeks et al, *N Engl J Med*, 2001) in a group of patients with virologic failure who underwent a structured treatment interruption. All pa-

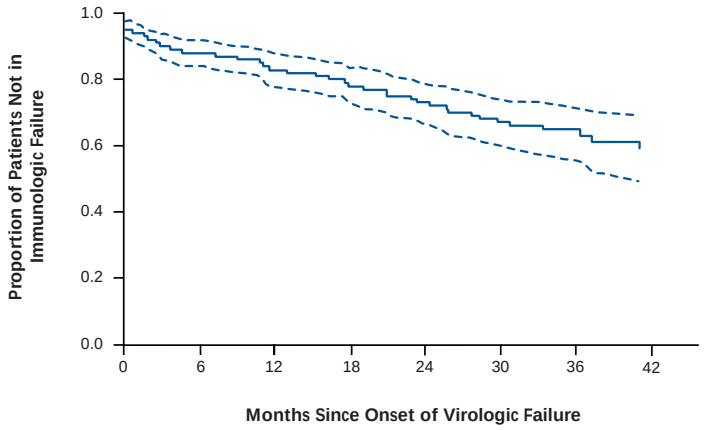

Figure **1.** Proportion of 291 patients maintained on failing protease inhibitor-containing regimen in whom CD4+ cell count remained above pretreatment baseline level (ie, not in immunologic failure) for more than 3 years. Adapted with permission from Deeks et al, *AIDS*, 2002.

Dr Deeks is Associate Clinical Professor at the University of California San Francisco.

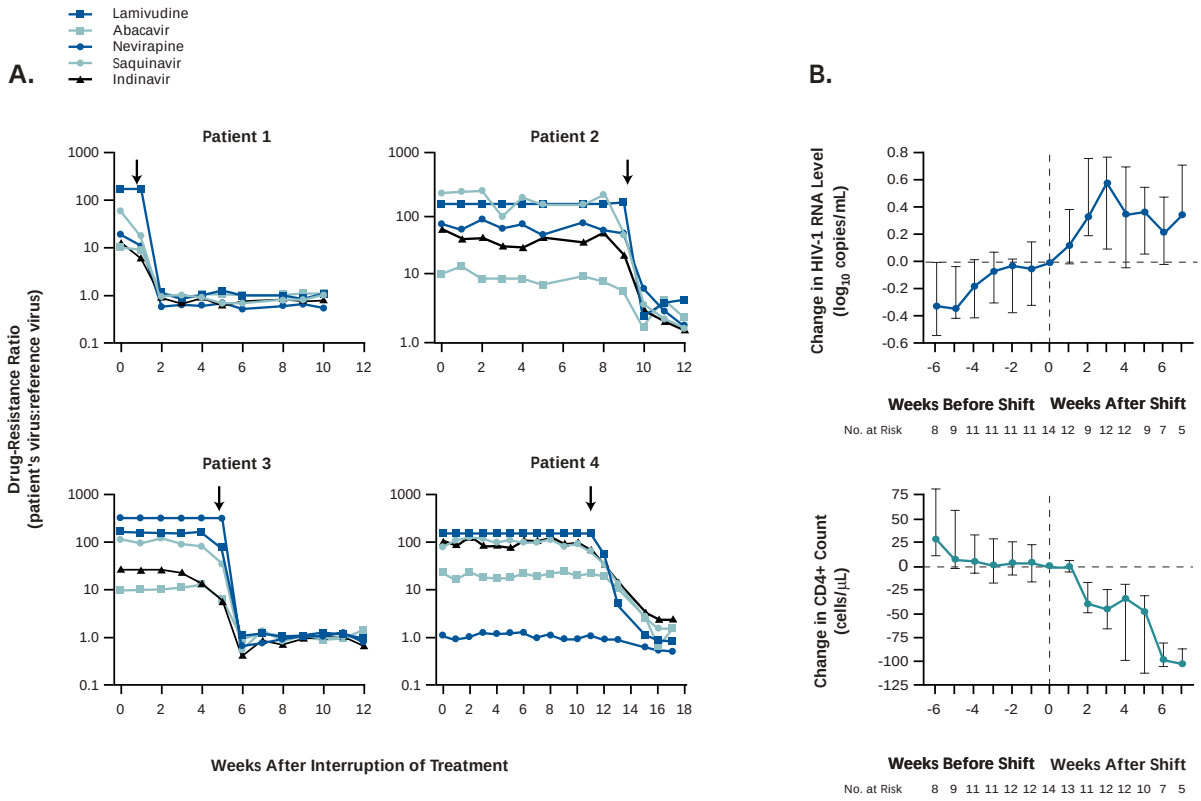

Figure **2A.** Changes in drug resistance ratios after treatment interruption in 4 patients with continuous virologic failure. Arrows indicate onset of rapid switch to susceptible phenotype for all drugs simultaneously and also show time 0 in Figure 2B. **B.** Change in plasma HIV-1 RNA level (top) and CD4+ cell count (bottom) from time of switch from resistant to susceptible phenotype according to time before and after switch. Median values and interquartile ranges are shown. Number of patients contributing data at each time point is shown in the "at risk" line. Adapted with permission from Deeks et al, *N Engl J Med,* 2001.

tients were on long-term protease inhibitor regimens, had detectable plasma viremia, and exhibited clear evidence of protease inhibitor resistance. All patients stopped antiretroviral drugs and were followed up weekly.

With regard to drug resistance characteristics, most patients exhibited a similar pattern, as shown by data from 4 representative patients (Figure 2A). Levels of phenotypic resistance to protease inhibitors remained relatively stable over a period of weeks following treatment interruption. At a time point that differed among patients, a rapid dramatic shift took place in viral phenotype from resistant to wild-type virus, with a complete shift occurring over a median of 2 weeks. This rapid shift in drug resistance generally occurred for all drugs simultaneously. Analysis of plasma HIV-1 RNA level and CD4+ cell count changes before and after the shift in viral phenotype showed that the shift was associated with a marked increase in the rate of HIV-1 RNA increase and the rate of CD4+ cell count decrease (Figure 2B).

The investigators hypothesized that the rapid increase in viremia in these patients during treatment interruption was due to the greater replicative fitness of the wild-type virus that rapidly emerged in this setting. To assess this hypothesis, they used a recently developed assay to measure viral replicative capacity. It should be noted that "replicative fitness" refers to the ability of a species or strain to compete in a defined environment with other species or strains (eg, the resistant virus is more fit than wild-type virus in an environment defined as a patient on potent antiretroviral therapy). Assays that measure viral fitness thus require direct competition between strains in vitro (mixing experiments) or in vivo; these traditional assays are laborious and expensive and can only be performed on a small number of samples. In contrast, the replicative capacity assay provides a noncompetitive measure of the inherent ability of a given strain to replicate. It can be used with a large number of specimens and requires only a small amount of plasma.

In this recombinant virus assay, which was devised by modifying a phenotypic assay, vectors containing patient-derived HIV reverse transcriptase or protease undergo a single round of replication. The vector contains a luciferase gene that permits quantitation of replication that is then compared to the level of replication of a wild-type HIV reference strain. The result is provided as the ratio of study-strain replication to reference-strain replication. One potential drawback is that the assay measures only reverse transcriptase and protease activity, and thus does not account for other potential compensatory mechanisms in the virus that could affect replicative ability.

Use of the replicative capacity assay in samples from patients in the treat-

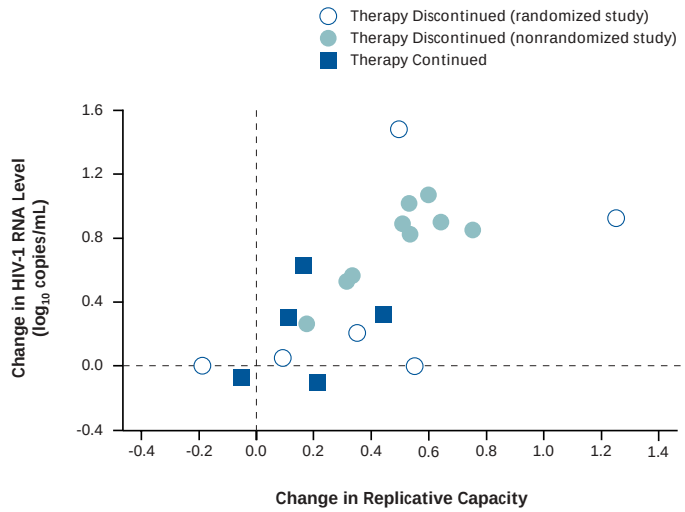

Figure **3.** Association of change in plasma HIV-1 RNA level with change in replicative capacity after switch to wild-type phenotype in patients in whom therapy was discontinued (circles) and with continued therapy in patients with continuous virologic failure (squares). Adapted with permission from Deeks et al, *N Engl J Med,* 2001.

ment interruption study showed that change in replicative capacity from baseline to 12 weeks after treatment interruption was strongly correlated with change in plasma HIV-1 RNA level over this period (Figure 3). The relative fitness of the resistant and wild-type strains was assessed by quantitating relative proportions of the resistant and wild-type phenotypes in weekly samples from each patient, allowing calculation of the slopes of decrease in drug-resistant virus and increase in wild-type virus. These studies showed that the fitness differences between the wild-type and resistant virus averaged more than 50% per generation. The findings using this gold-standard measure of fitness correlated very strongly with findings on the replicative capacity assay, suggesting that the latter may provide a good indication of what is occurring in vivo. Overall, these findings indicate that the benefit observed in patients with persistent viremia on protease inhibitor regimens is at least in part a reflection of maintenance of a viral population with reduced replicative capacity.

Is There a Role for Viral Fitness Measurements in Clinical Management?

If measurement of viral fitness is to have a role in clinical management of HIV disease, it needs to provide information that is useful in predicting outcome in patients in whom virologic failure has occurred. To assess the predictive capability of fitness measurements, Barbour and colleagues (9th CROI, 2002) investigated viral evolution parameters in a separate cohort of 20 patients who remained on protease inhibitor regimens despite incomplete viral suppression. Plasma HIV-1 RNA level remained at less than 10,000 copies/mL over 24 months on the failing regimen in more than half of the patients studied. Ongoing viral replication in the presence of drug was associated with increasing protease inhibitor and nRTI resistance in most patients. Increased resistance was observed in some patients even as the plasma HIV-1 RNA level remained stable and low. Replicative capacity was low and remained low in most patients. Achievement of the steady-state level of viremia was characterized by a substantial reduction in viral evolution, and the drug-resistant variants remained the primary virus population at the end of follow-up.

These data suggest that it may be possible to monitor viral fitness over time and to predict long-term treatment outcome based on replicative capacity and level of drug resistance. However, there are other factors involved in maintenance of treatment benefit despite virologic failure, with additional data suggesting that although reduced replicative capacity may be a necessary condition for durable benefit, it is not a sufficient condition. For example, it has previously been observed that immunologic and virologic benefit might not be achievable in patients with advanced disease despite presence of virus with extremely low replicative capacity. As with untreated patients, numerous factors are likely important determinants of long-term outcome in partially treated patients, and more than one such factor might be needed to ensure durable treatment benefit in the presence of drug-resistant HIV.

Altered Pathogenicity of Drug-Resistant HIV

It is clear that plasma HIV-1 RNA levels can remain partially suppressed in some patients despite the emergence of drug-resistant variants, and that this partial suppression is related to several factors. It is also clear that CD4+ T-cell counts can remain elevated in such patients, and that this sustained CD4+ T-cell benefit is partially explained by the treatment-mediated decrease in plasma HIV-1 RNA level. What remains unclear is whether sustained CD4+ T-cell gains occur for other reasons that are independent of the level of partial viral suppression. In other words, is there any evidence that for any given level of viremia, immunologic outcomes are improved for patients with drug-resistant HIV versus wild-type HIV?

The relative virulence or pathogenicity of the protease inhibitor-resistant virus was addressed in a study that examined the immunologic mechanisms underlying sustained increases in CD4+ cell count in patients with protease inhibitor-resistant viremia (Deeks et al, *J Infect Dis*, 2002). The investigators observed CD4+ cell turnover in vivo in untreated patients with wild-type virus, in patients with virologic failure with drug-resistant virus maintained on stable antiretroviral therapy, and in patients with plasma HIV-1 RNA below detection limits on potent therapy. The median fractional replacement rate of the total peripheral CD4+ T-cell population in patients with drug-resistant virus was significantly lower than that

observed in the untreated group (P<.001). However, the difference between the virologic failure group and the group with undetectable HIV-1 RNA levels was not significant (P=.32). Based on these fractional replacement rates, the estimated median CD4+ T-cell half-lives for the 3 groups were 68 days (virologic failure group), 22 days (untreated group), and 82 days (virologic "success" group). Similar trends were observed in the CD8+ T-cell population. These data indicate that the drug-resistant virus may be less virulent (ie, cause less CD4+ T-cell turnover) in vivo than the wild-type virus, even after controlling for the level of plasma viremia. Further research is needed to elucidate the complex dynamic between the host immune system and characteristics of the virus. In summary, some patients experience durable virologic and immunologic benefit despite incomplete viral suppression and the emergence of drug-resistant HIV. The mechanisms underlying this benefit are likely multifactorial and interrelated.

Host Genetics as Predictor of Treatment Outcome

Host genetic factors likely have a major influence on both HIV disease course and response to treatment. One intriguing strand of research in this regard involves the role of human leukocyte antigen (HLA) haplotypes in determining treatment outcome. Class I HLA molecules occur on all cells and present peptides to receptors on CD8+ T cells, whereas class II HLA molecules occur on antigen-presenting cells and present peptides to receptors on CD4+ T cells. HLA haplotypes vary widely among individuals, and it is likely that in every individual infected with HIV, only some components of the virus are presented to the immune system. Given the strong influence of genetic polymorphisms within HLA and the chemokine receptors on disease progression in untreated HIV infection, it is reasonable to assume that such polymorphisms are likely to predict the response to potent antiretroviral therapy. However, data supporting this hypothesis have been inconsistent (O'Brien et al, AIDS, 2000; Malhotra et al, *J Clin Invest*, 2001).

In a recent study to assess effects of viral and host genetics on drug resistance (Moore et al, 9th CROI, 2002), viral genotype and presence of specific host haplotypes were determined in a large cohort of patients with continued detectable viremia while receiving potent antiretroviral therapy. The V82A protease mutation associated with indinavir resistance was, as would be expected, significantly more common in patients receiving indinavir than in patients not receiving indinavir (odds ratio, 4.3). However, the presence of the HLA-A2 haplotype, which recognizes a portion of HIV protease overlapping the V82 amino acid residue, had an independent effect in predicting the presence of the V82A mutation (odds ratio, 5.4).

Such findings indicate that there are strong independent effects of drug and host immune pressure on viral evolution. Similar studies may help explain why, for example, the majority of patients who develop nelfinavir resistance do so via the D30N resistance mutation rather than the L90N pathway. Continued research in this area may ultimately allow prediction of viral evolutionary pathways in individual patients, enabling selection of drug treatment to maximize response and to avoid pathways that lead to cross-resistance. It is likely that the near future will bring a number of retrospective analyses of defined patient cohorts that shed additional light on the influence of host genetics on treatment response.

Conclusions

Maintenance of viral suppression to levels below 50 HIV-1 RNA copies/mL is not feasible in many patients with current antiretroviral therapy. Thus, a thorough understanding of the determinants of viral evolution and disease progression under potent antiretroviral therapy is needed to define better treatment strategies. In addition to using resistance testing, therapeutic drug monitoring, and adherence monitoring to optimize treatment outcome, clinical management may ultimately also include measures of viral replicative fitness, viral pathogenicity, and assessment of host genetics.

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Suggested Reading

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