HIV Resistance to Antiretroviral Drugs

Stefano Vella, MD, and Marco Floridia, MD

New insights into HIV pathogenesis, the development of quantitative plasma HIV RNA testing, and the increased availability of antiretroviral drugs have significantly changed our approach to treating HIV infection over the last year or two. Moreover, recent results of two large controlled trials (the European-Australian DELTA trial and the US AIDS Clinical Trials Group [ACTG] 175 study)—although different in design, pretreatment characteristics of the patients, and primary endpoints—provided the long-awaited proof of concept that a significant and sustained reduction of viral replication is associated with improved clinical outcome.

The emergence of HIV resistance has been described for all classes of antiretroviral drugs used in monotherapy or in dual combination regimens and has continued to be considered an inevitable factor that ultimately determines the loss of efficacy of antiretroviral treatment, even though treatment failure is complicated by the concomitant interaction of virologic factors other than resistance. In this article, the general mechanisms of the development of resistance to antiretroviral drugs are reviewed together with recent findings in the field, and the latest advancements that have been made toward overcoming resistance.

Virology of Drug Resistance

The high rate of viral replication found throughout the course of HIV infection and the high frequency of virus mutations occurring during each replication cycle (a phenomenon common to all single-stranded RNA viruses) due to the lack of proofreading mechanisms, are the basis for the emergence of drug-resistant variants under the selective pressure of antiretroviral drugs. In fact, a "Darwinian" model can be applied to HIV dynamics, with the continuous production of variants and the continuous selection of the "fittest" virus.

Unfortunately, with daily production of perhaps 10^8 to 10^{10} virions and a mutation rate of 3×10^{-5} nucleotides per replication cycle, it is likely that any single mutation already exists before any drug is introduced. The relative level of viral mutants at a given point in time is probably determined by the forward mutation frequency (the amount of copying errors at a particular codon) and the cost of the mutation to the replicative capability of the virus.

Drug resistance, which in reality, can be better described as altered drug susceptibility, is clearly a relative characteristic, and is defined as the alteration of the drug concentration needed to inhibit in vitro growth of the virus. Virus susceptibility is usually quantified in terms of the concentration of a drug that is needed to inhibit 50% or 90% of viral growth, which defines the IC_{50} or IC_{90}, respectively. If the IC_{50} (or IC_{90}) value that is characteristic of the so-called wild-type virus is known, the IC_{50} (or IC_{90}) value for a resistant virus will be X-fold greater. The increase in the IC_{50} value needed to define a virus as resistant to a particular drug is often empirically established. For example, a virus highly-resistant to zidovudine is assumed to have an IC_{50} value at least greater than 1.00 µM, while wild-virus generally have an IC_{50} value of about 0.01 µM to 0.05 µM.

Drug susceptibility of the virus harbored by an HIV-infected individual can be assessed by isolating the virus, preferably from plasma (which will better represent the actively growing virus population), in the presence of various concentrations of the drug under study (phenotypic analysis of resistance). However, because phenotypic resistance is the consequence of specific mutations in the genes for the target enzymes (ie, reverse transcriptase [RT] or protease), polymerase chain reaction (PCR) assays and gene sequencing methods have been developed to detect these mutations directly (genotypic analysis of resistance). The relationship between phenotypic and genotypic analyses of resistance is often direct, but can be altered in some cases by the fact that the emergence of resistance is a dynamic process, and multiple strains of virus with various susceptibilities frequently coexist in the patient.

Because resistant variants may exist before treatment is initiated and may evolve under selective pressure, therapy can address viral resistance in three ways: (a) maximizing the suppression of viral replication; (b) using drugs when multiple mutations are required for resistance; and (c) forcing the emergence of variants with attenuated replication or decreased virulence.

Resistance to RT Inhibitors

Resistance to nucleoside analogues. HIV variants with decreased susceptibility to zidovudine were first reported in 1989. Viral variants with different levels of drug resistance to other nucleoside analogues subsequently have been described (see Table 1).

In general, advanced disease-stage, baseline low CD4+ cell counts, and high plasma HIV RNA levels predict the development of resistance to zidovudine, and this is likely to be true for all other antiretroviral drugs.

Table 1. Some Genotypic Changes Associated with the In Vivo Use of Antiretroviral Drugs.

Reverse transcriptase inhibitor	Codon mutations in the reverse transcriptase gene	Protease inhibitor	Codon mutations in the protease gene
Nucleoside			
Zidovudine (ZDV)	41, 67, 70, 215, 219	Saquinavir	10, 48, 63, 71, 90
Didanosine (ddI)	65, 74, 184	Ritonavir	20, 33, 36, 46, 54, 71, 82, 84, 90
Zalcitabine (ddC)	65, 69, 184	Indinavir	10, 20, 24, 46, 54, 63, 64, 82, 84, 90
Lamivudine (3TC)	184		
Stavudine (d4T)	75 (rare)		
Nonnucleoside			
Nevirapine	103, 106, 108, 181, 188, 190		
Loviride	103, 181		
Delavirdine	181, 236		

Although resistance to zidovudine appeared to be related to the ordered emergence of viral variants with mutations at RT codons 70, 215, 41, 67, and 219, high-level resistance to didanosine has not been reported to date; also, diminished susceptibility to zalcitabine and stavudine in vivo has not been well documented. There is not a clear explanation for these findings, although it has been hypothesized that it may be related to the greater similarity of these compounds to the natural substrate. The pattern of viral mutations associated with didanosine has been further investigated by several groups, and their results substantially confirm that susceptibility is reduced when strains with one or more of the L74V, K65R, and M184V mutations emerge. One exception to the above statements are the recently described isolates that are highly cross-resistant to zidovudine, didanosine, zalcitabine, and stavudine on the basis of a distinctive set of mutations in the RT, the most important being at codon 151. This multiple resistance associated with unique genetic patterns has also been observed in HIV strains isolated from patients given combination therapy with nucleoside analogues, especially zidovudine and didanosine.

Cross-resistance has been reported between zidovudine and other 3'-azido-nucleosides. Reduced susceptibility has been described among zalcitabine, didanosine, and lamivudine (involving the K65R, V75T, and M184V mutations) and between didanosine and zalcitabine (L74V). Although zidovudine resistance was shown to predict more rapid progression of HIV disease, the clinical significance of resistance to the dideoxynucleosides and the clinical correlates of in vitro cross-resistance within this class are still not completely defined.

Resistance to lamivudine occurs rapidly in vivo, and is associated with substitution at codon 184. This mutation leads to high-level resistance to lamivudine, as well as to some cross-resistance to didanosine and zalcitabine. This mutation antagonizes zidovudine resistance mediated through the 215 (and 41) mutations, restoring phenotypic sensitivity to zidovudine.

The genetic profile of resistance to the new carbocyclic nucleoside 1592U89 has also been investigated in patients in early clinical trials. Some mutations common to other nucleosides have been observed (M184V, K65R, L74V), but their role has not yet been fully established.

Resistance to NNRTIs. The rapid development of reduced susceptibility to nonnucleoside reverse transcriptase inhibitors (NNRTIs) when used in monotherapy or in dual combination regimens initially suggested a limited use for these drugs in clinical practice. However, very interesting results were obtained using one drug in this class, nevirapine, as part of triple combination regimens in antiretroviral-naive patients. Effects on CD4+ cell counts and plasma HIV RNA levels were sustained for at least one year in many of the patients. Interim results of the Boehringer Ingelheim 1046 trial in antiretroviral-naive patients given either zidovudine/didanosine, zidovudine/nevirapine, or zidovudine/didanosine/nevirapine demonstrated that in the triple combination arm the majority of isolates obtained from patients who were compliant with all three of the medications remained sensitive to nevirapine. Patients who were poorly compliant with the medications had detectable nevirapine-resistant isolates. These findings again prove the concept that resistance occurs as a direct consequence of viral replication. When the regimens used do not completely suppress HIV, resistance emerges in the presence of plasma levels of the drug.

Treatment with another NNRTI, delavirdine, has induced the emergence of variant strains with K103N and Y181C mutations and occasionally with the P236L mutation. The pattern of resistance may vary according to the extent of the genetic pressure of the drug, with some mutations being preferentially selected by more gradual increases in drug concentrations (V106I, G190T) and others being selected by greater increases in drug concentrations with every subsequent passage (G190E).

Cross-resistance is a common phenomenon with NNRTIs. In vitro studies have revealed several mutations common to different compounds: A98G (pyridinones); L100I (TIBOs, pyridinones, BHAPs); K103N (TIBOs, pyridinones, nevirapine, BHAPs); V106A (TIBOs, BHAPs, HEPTs, nevirapine); V108I (nevirapine, pyridinones); Y181C (nevirapine, TIBOs, pyridinones, BHAPs); Y188H (nevirapine, TIBOs, BHAPs); and Y188C (nevirapine, HEPTs). Most of these mutations also have been observed in vivo following treatment with these drugs as monotherapy.

Resistance to Protease Inhibitors

Resistance or reduced susceptibility has been reported with all available protease inhibitors, and appears to be associated with a loss of therapeutic effect. Results clearly indicate the frequent selection of strains exhibiting cross-resistance to different protease inhibitors following in vitro or in vivo drug exposure. The patterns of mutations for protease inhibitors, however, appear to be more complex than for RT inhibitors, with a greater number of sites involved, and greater variability in the temporal patterns and in the combinations of mutations leading to phenotypic resistance. This finding suggests that the protease gene may be able to adapt more easily than the RT gene under the genetic pressure induced by drugs. Although about 20 codons have been identified as sites of mutation, some changes appear to be more frequent and can therefore help to define subgroups of protease inhibitors according to different genetic profiles.

A phenomenon that has been frequently observed with protease inhibitors is that viruses selecting for a compromising mutation may survive under the pressure of protease inhibitors by selecting compensatory mutations that restore original levels of viral replication. In other words, two kinds of mutations are often seen during protease inhibitor therapy. The first type of mutations are active-site and non–active-site mutations that reduce inhibitor binding. The second type are nonspecific mutations that can restore impaired enzymatic activity.

The issue of resistance to protease inhibitors is also complicated by the fact that the protease enzyme cleaves itself out of the wider *gag-pol* precursor and that the replicative disadvantage conferred by specific mutations may be complemented by adaptive mutations in the *gag* gene whose sequence is recognized by the protease enzyme. This has been shown for the V82F/I84V mutations, whose significant negative impact on replication is apparently reversed by the presence of some mutations in the p17 *gag* region, such as E52Q and Q63E. Interestingly, these mutations do not necessarily involve known protease cleavage sites.

The V82T mutation has been confirmed as a leading viral mutant in reducing binding of the HIV protease to indinavir, although it seems that resistance to indinavir develops as a consequence of multiple changes in the protease gene (eg, V82A,F, or T, plus M46I,L) and the number of subsequent mutations correlates with the degree of resistance. Viral isolates from patients given indinavir showed a very high occurrence of cross-resistance to other protease inhibitors. Cross-resistance was invariably present between indinavir and ritonavir. A 60% to 80% rate of cross-resistance was observed between indinavir and saquinavir or VX478/141W94.

Resistance to ritonavir also occurs as a consequence of the stepwise accumulation of different mutations in the protease gene. Mutations in codon 82 appear to be the critical initial mutations, but additional changes in the protease gene are necessary for high-level resistance to develop. These mutants, including M46I and I84V, are also common to indinavir. The V82T mutation has been found to determine a reduction in replicative efficacy; however, this observation needs confirmation. Cross-resistance to indinavir and to a more limited extent to

saquinavir has been reported in samples from patients given long-term ritonavir.

With respect to saquinavir, the association of G48V and L90M has been confirmed as a double mutation with significant impact on the drug's activity–an impact that is greater than that of the two mutations alone. In patients given saquinavir, mutations at residues 10, 54, 63, and 71 may also contribute to resistance. The low incidence of resistance mutations in patients treated with saquinavir (approximately 50% at one year) may be an inherent attribute of the drug; however, it may also be the result of the low selective pressure exerted on the virus by saquinavir in its present formulation.

During in vitro selection with VX478/141W94, the I50V mutant is associated with an eightyfold reduction in affinity to VX478/141W94, but with only a minor reduction in affinity to saquinavir and indinavir.

Nelfinavir is another protease inhibitor that seems to be characterized by some distinct genetic pattern of mutations; the D30N mutation is associated with a sevenfold reduction in sensitivity in vitro (after 22 passages at increasing drug concentrations). The same mutation has also been observed in samples from patients given nelfinavir in clinical trials. Other less common mutations include M36I, L63P, V77I, and N88D. The L90M mutation has rarely been observed with nelfinavir, and other mutations, such as G48V, V82A,F,T, and I84V, common to other protease inhibitors have not been detected. However, only results from preliminary studies are available.

Resistance in Antiretroviral-naive Patients

Pretreatment genotypic resistance in antiretroviral-naive patients in the DELTA1 and ACTG 175 trials suggests a low incidence of preexisting resistant virus (10 of 173[6%] patients in the DELTA1 study had mutations in codons 70 or 215; 13% of patients in the ACTG 175 study had mutations in codon 215). These results, which need to be confirmed by sequencing, may indicate an unreported previous use of zidovudine; however, they are consistent with data from seroconverters (a mutation in codon 215 was found in about 10% of patients who seroconverted), which indicates transmission of zidovudine-resistant strains.

Multidrug Resistance

The patterns of mutations observed during combination therapy may be substantially different from those observed using the same drugs as monotherapy. Assessment of the genotype of HIV strains from patients given different antiretrovirals has also shown that although still rare, multidrug resistance can occur. The genetic basis of this phenomenon is currently being investigated. Some sets of mutations responsible for multiple high-level resistance to dideoxynucleosides–particularly to zidovudine, didanosine, zalcitabine, and stavudine–have been described, including V75I, F77L, F116Y, A62V, and Q151M. The Q151M mutation, which appears to be the critical first mutation in this set, is somewhat uncommon in untreated patients, and seems to arise via a two-step process–through a Q-L change that precedes the L-M change. The prevalence of this mutation appears to be about 5% in patients treated with combinations of zidovudine and didanosine.

Reversal, Delay, and Suppression of Drug Resistance

As a general rule, drugs that rapidly induce high-level resistance have been expected to have limited clinical use. However, at least two issues have recently modified this view: (a) the theoretical possibility that higher drug dosages can partially overcome resistance (provided that the drug has a very high therapeutic index); and (b) the possibility that the selection of resistance mutations can become a positive tool, if the resistance pattern of one drug can restore or increase the sensitivity to another drug.

Several in vitro experiments have shown that depending on the combinations of mutations, both multiple resistance and restored sensitivity are possible. Most of these studies have been conducted in zidovudine-resistant virus (specifically, with the T215Y mutation alone or in association with the M41L mutation). Interestingly, in these studies, the effect of a second drug-induced mutation was potentially different, according to the genetic pattern of zidovudine resistance. The L74V mutation, associated with didanosine exposure, has been shown to restore zidovudine sensitivity in the presence of the T215Y mutation alone; resistance to both zidovudine and didanosine was induced when both the T215Y and the M41L mutations were present. The addition of the V106A mutation to this set (V106A being associated with exposure to nevirapine and other NNRTIs) conferred added resistance to nevirapine. In contrast, the Y181C mutation confers added resistance to nevirapine, but restores zidovudine sensitivity in the presence of the T215Y mutation.

Zidovudine/lamivudine has become an attractive combination, based on the observed suppression of zidovudine resistance in lamivudine-resistant strains with the M184I/V mutations. In the antiretroviral-naive population of the NUCB3001 trial, the combination of zidovudine/lamivudine induced significant CD4+ increases and viral load reductions, with both responses being greater in magnitude than would be expected with this nucleoside combination. The rapid emergence of variant strains with the M184V mutation was observed in almost all patients, indicating rapid selection of lamivudine-resistant strains. At week 24, the proportion of patients who maintained wild-type strains with respect to zidovudine-associated mutations was significantly greater in the zidovudine/lamivudine arm than in the monotherapy arm (75% vs 31%, respectively). This finding suggests that a delay in zidovudine resistance may contribute to the sustained antiretroviral effects of this combination. In this trial, the possibility of reversing zidovudine resistance by adding lamivudine could not be evaluated, because the study population was zidovudine-naive. This important issue has been investigated in other trials of zidovudine-pretreated individuals. Preliminary results, although confirming that resensitization to zidovudine also can occur in vivo, have shown that resistance to both zidovudine and lamivudine can occur with the loss of activity of the combination.

Mutually counteracting mutations have been detected also among NNRTIs. The P236L mutation, associated with the BHAP compounds delavirdine and atevirdine, apparently reverses resistance to nevirapine, TIBOs, and L697,661 conferred by the Y181C mutation. Unfortunately, the Y181C mutation confers resistance to delavirdine and atevirdine as well, so the combined use of NNRTIs selects for virus with mutations conferring resistance to all of the components of the combination.

As discussed above, there was enough evidence only a few months ago to assume that resistance would occur with any effective antiretroviral drug, even when given in combination regimens. However, results, although anecdotal, emerged from the recently reported preliminary analyses of trials of zidovudine/lamivudine/indinavir and zidovudine/didanosine/nevirapine to suggest that a sustained high-level suppression of viral load can significantly delay or even prevent the emergence of resistance.

As a consequence of these experiments, it has been hypothesized that after some years of theoretical "zero viral replication," HIV infection may even "burnout" as chronically infected cells die off (described as viral eradication). However, reducing viral load to below the level of detection of the assays does not necessarily mean that HIV is absent from the body. Although it is generally accepted that an undetectable viral load level is the optimal target, this target cannot be achieved in all patients, even with highly effective regimens. Also, resistance can occur theoretically even at plasma HIV RNA levels below the current limits of detection, if some level of

replication occurs in "reservoir" tissues. Further studies are clearly needed to investigate these issues.

Clinical Implications

The increased number of available anti-HIV compounds is opening new perspectives and new questions for the design of therapeutic strategies. With about 15 drugs now in clinical practice or in clinical trials, the potential number of combinations is remarkable, and rational criteria must be adopted to select the combinations with the greatest likelihood of efficacy.

The level and durability of antiretroviral activity (ie, viral load and CD4+ cell count changes) is undoubtedly a good criterion for selection of regimens, but other factors, including the resistance characteristics of the drugs, are also important considerations. Although further studies are necessary to assess the complex implications of the resistance patterns for the care of patients with HIV/AIDS, some of the information already available can be used in clinical practice. As a general rule, combinations of drugs that share clear cross-resistance properties should be avoided. Switching between such drugs should also be performed with caution, considering the possibility of a reduced efficacy with the new regimen.

Ideally, therapy with regimens able to induce and maintain maximal viral suppression must be started early to prevent the emergence of resistant strains. Because even complete inhibition of viral replication may not prevent the selection of resistant mutants in the long term if they exist at a sufficiently high frequency prior to therapy, there is a compelling rationale for treating the patient very early in the course of infection, when the virus is most homogeneous. There is current interest in whether determination of resistance patterns in patients' isolates would be useful in clinical practice. Such information might theoretically be useful for planning treatment on the basis of pretreatment susceptibility or of cross-resistance profiles.

However, a number of issues limit the use of resistance information as a longitudinal marker in clinical management, including: differences among the labor-intensive and time-consuming techniques used; difficulties in quantifying the drug-resistant virus by PCR assays; and the uncertain clinical meaning of drug resistance for many of the currently used anti-HIV drugs. It is also not known to what extent cross-resistance in vitro precludes sequential use of drugs characterized by common mutation profiles. Further clarification of these issues, together with the development of simpler tests that could be used in clinical practice, would be required before drug sensitivity information would have a role in planning and managing antiretroviral therapy for individual patients.

Summary

The possibility of preventing or counteracting the emergence of drug-resistant strains still represents a major issue in antiretroviral therapy. The mechanisms and patterns of resistance development in combination therapy appear to be more complex than those in monotherapy, and their clinical implications remain only partially known. Although dual combination treatment does not seem to avoid the emergence of resistance, at least in some cases it has been shown to limit or to delay it, or to lead to the selection of less pathogenic quasispecies, all of which may yield a positive impact on the progression of HIV disease. An issue currently being evaluated in clinical trials is the efficacy of combination regimens based on mutually counteracting, drug-induced mutations, which may convert the unavoidable selection of mutant viruses into an at least partially favorable phenomenon.

Finally, there is hope that the prevention of the emergence of resistance may be achieved by using combinations of drugs that completely inhibit viral replication. This strategy has been shown to be effective in vitro. Data emerging from triple combination regimens with enhanced antiretroviral potency suggest that the emergence of resistance is reduced when viral replication is effectively suppressed, with the subsequent hope of prolonging the antiretroviral effects of future therapeutic strategies.

Stefano Vella is Research Director and Head, Retrovirus Section of the Laboratory of Virology at the Istituto Superiore di Sanità in Rome, Italy.

Marco Floridia is Research Assistant at the Laboratory of Virology at the Istituto Superiore di Sanità in Rome, Italy.

Suggested Readings

Condra JH, Schleif WA, Blahy OM, et al. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature.* 1995;374:569–571.

De Clercq E. How to overcome resistance to HIV-1-specific reverse transcriptase inhibitors. *AIDS.* 1994;8:1020–1021.

Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine isolated during prolonged therapy. *Science.* 1989;243:1731–1734.

Larder BA, Kemp SD, Harrigan R. Potential mechanism for sustained antiretroviral efficacy of zidovudine-lamivudine combination therapy. *Science.* 1995;269:696–699.

Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* 1996;272:1167–1170.

Mellors JW, et al. Mutation in HIV-1 reverse transcriptase and protease associated with drug resistance. *Int Antiviral News.* 1995;3:8–15.

Richman DD. HIV therapeutics. *Science.* 1996;272:1886–1888.

Richman DD. Resistance, drug failure, and disease progression. *Aids Res Hum Retroviruses.* 1994;10:901–905.

Shirasaka T, Kavlick MF, Ueno T, et al. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. *Proc Natl Acad Sci USA.* 1995;92:2398–2402.