The response to antiretroviral therapy in human immunodeficiency virus (HIV)-infected patients is limited by the emergence of drug resistance. This resistance is a consequence of the high rate of HIV mutation, the high rate of viral replication (especially when potent multidrug therapies are not used or taken reliably), and the selective effect of these drugs, which favors emergence of mutations that can establish clinical drug resistance. The introduction of highly active antiretroviral therapy (HAART), which typically includes at least 2 nucleoside reverse transcriptase inhibitors (RTIs) and a protease inhibitor or a nonnucleoside RTI, for most treatment-naive patients results in a reduction of viral load below the limit of detection determined by currently available HIV RNA assays. It is this marked reduction that results in durable viral suppression, usually only possible by the simultaneous use of 3 or 4 drugs. The RTI components of HAART are crucial for these benefits of combination therapy. Specific amino acid changes are associated with resistance to several RTIs, but new mutation complexes have been observed that can confer broad cross-resistance within this class. Genotypic and phenotypic resistance assays to measure drug resistance are being developed, but refinements in both methodology and our ability to interpret results of these assays are necessary before they are introduced into widespread clinical use.

The first 5 drugs approved for treatment of HIV infection were reverse transcriptase inhibitors (RTIs), all of which are nucleoside RTIs. More recently, nonnucleoside RTIs have become available, and nucleotide RTIs are being developed. Unfortunately, because these drugs were gradually introduced one at a time (starting with zidovudine in March 1987), monotherapies were routinely employed until 1994. When response was lost to 1 drug in a patient, treatments were changed to a different monotherapy. The response to the second therapy, however, was not as good as when the same drug was given to a treatment-naive patient [1, 2]. Even before the genetic mutations associated with drug resistance were identified, it seemed likely that resistance to 1 RTI diminished the response to subsequent ones.

Treatment Advances with Better Understanding of HIV Disease

In early 1995, the studies of Ho et al. [3] and Wei et al. [4] revolutionized our concepts of the virology of AIDS. Before these reports, the long period of clinical latency in HIV-infected patients was thought to be associated with a virologically silent period, with increases in viral replication seen only at the late stages of disease. These reports, coupled with our ability to sensitively and quantitatively detect HIV in plasma, revealed that HIV replication is exuberant at all stages of disease, with high levels of daily virus production and rapid turnover. This high replication rate, coupled with a high rate of viral mutation (about 1 mutation for each cycle of viral replication), indicated why earlier monotherapies were unsuccessful. Effective antiretroviral therapy results in decreases in the plasma HIV RNA level (viral load [VL]) over the first few weeks, usually associated with increases in CD4+ T lymphocyte counts. With monotherapies of low potency, there may still be substantial viral replication. Random mutations result in the appearance of new virus strains, and resistant virus strains rapidly emerge with the selective effect of antiretroviral therapy.

Combination therapies that use 2 RTIs were introduced in 1994 [5–7]. By use of 2 drugs in initial therapy, VL reduction was greater and more durable. The use of sequential therapy (i.e., adding 1 drug to previous monotherapy after benefits are lost) results in a much lower virologic response than if 2 drugs are used in the initial therapy. When a new drug is added to a regimen that is not adequately suppressing viral replication, the only active agent may be the single new drug. The concept of greater VL reduction with more-intensive therapies was cemented in our treatment policies by the landmark studies that showed that 3- or 4-drug combination therapies that include a protease inhibitor (PI) or a nonnucleoside RTI are able to reduce VL to below levels of detection [8–15]. These intensive 3- or 4-drug therapy regimens have been termed highly active antiretroviral therapy (HAART).

RTIs Are Part of HAART

Although the use of PIs in multidrug regimens may provide most of the impact of HAART, combination therapies that
include RTIs have resulted in greater viral suppression than those that use PIs alone [8, 16]. The use of ≥3 drugs initiated in unison allows suppression of the virus to undetectable levels at 1 year in most treatment-naive HIV-infected patients. VL below the level of detection is generally accepted as the goal of antiretroviral therapy. When PIs are used as monotherapy, usually only half of treated patients have VL reductions to undetectable levels [8, 16]. The response rate of 50% does not increase even when RTIs are added to treatment subsequently. Substantial reduction of VL to undetectable levels usually requires combinations of at least 3 or 4 drugs taken reliably and may be even more difficult to achieve and sustain in patients with advanced HIV disease [9].

Durable HIV Suppression Associated with Nondetectability of Plasma HIV RNA Levels

In combined retrospective analyses of 2 clinical trials that included 131 patients treated with ritonavir, the nadir of VL after treatment predicted the durability of response (defined as the time from initiation of therapy to the point where VL increased 0.6 log_{10} or 4-fold) from the nadir value [17]. Durability of response was not highly associated with either baseline VL or the magnitude of VL decline but was strongly associated with the nadir value (P < .01). With a VL decrease to a nadir of ~10,000 copies/mL, VL increased within weeks to months. This occurrence is similar to what is seen with monotherapy or 2-RTI therapy. The only patients with sustained viral suppression that extended to at least 1 year were those in whom VL was reduced to undetectable levels, which in this study was <200 copies/mL.

Evolution of viral sequences can still be observed in patients with good viral suppression, as seen by the emergence of the M184V mutation associated with lamivudine resistance in some patients with VLs <400 copies/mL during treatment with zidovudine, lamivudine, and indinavir [18]. VL reduction to even lower levels, as measured by ultrasensitive assays (detection threshold, 20–50 copies/mL), may provide greater durability than viral suppression below detection thresholds of standard assays (400–500 copies/mL). Analyses of response and outcomes in both the Agouron 511 study [19] (zidovudine, lamivudine, and nevirapine) and the INCAS (Italy, Netherlands, Canada, Australia study) trial [20, 21] (zidovudine, didanosine, and nevirapine) demonstrated that VL reduction to <20 copies/mL was associated with virologic relapse in only 10%–15% of patients for whom this treatment goal was achieved. Predictably, most patients who never had VL reductions of <400 copies/mL had increases in VL within 6 months. Surprisingly, in both of these studies, the durability of viral suppression in individuals whose nadir value was <400 copies/mL but who had VLs >20 copies/mL was not much different than that seen in those whose VLs never reached <400 copies/mL [12, 19, 21]. Thus, our previous standard of response (<400 copies/mL) does not appear to be sufficient for the durable response that we hope to see in our patients. The results of these studies support the current treatment guidelines of the US Department of Health and Human Services [22], which are designed to try to reduce VL to levels determined by sensitive assays. This goal, although difficult to achieve for some patients, is the only way to achieve a long-term benefit.

Resistance Mutations Seen with Nucleoside RTIs

Zidovudine has been more intensively studied and characterized in terms of resistance than any other RTI. Zidovudine was the only US Food and Drug Administration–approved antiretroviral agent from March 1987 to October 1991 and was consequently the focus of intensive investigation for several years. When monotherapy was the standard of care, zidovudine-resistant HIV type 1 (HIV-1) strains appeared within 1 year of treatment in many individuals [23, 24]. In many cases, HIV-1 strains develop gradual and stepwise resistance to zidovudine by accumulating mutations at 5 codons in reverse transcriptase (codons 41, 67, 70, 215, and 219 [25–27]; figure 1). The linked codon mutations 41 and 215 are commonly associated with high levels of zidovudine resistance, and their predominance in zidovudine-treated patients appears to reflect the selective growth advantage conferred by higher levels of drug resistance [30]. These mutations may also appear in patients who are receiving prolonged didanosine monotherapy, in the absence of prior or concomitant zidovudine treatment [31], which suggests that acquisition of these mutations may have consequences for other nucleoside RTI therapies.

Patterns of genotypic resistance to some other nucleoside RTIs are less complex. For example, high levels of phenotypic resistance to lamivudine are conferred by a mutation that directs a methionine to valine change at position 184 (M184V). Single amino acid substitutions have also been shown to confer phenotypic resistance to other nucleoside RTIs (e.g., V75T for stavudine resistance, K65R and L74V for didanosine resistance, and K65R and T69D for dideoxycytidine resistance; figure 1). Although M184V rapidly emerges during lamivudine monotherapy and confers high levels of resistance to this agent, this mutation can also cause cross-resistance to the recently approved nucleoside RTI abacavir and in vitro may also cause cross-resistance to didanosine and dideoxycytidine. The clinical relevance of M184V for resistance to these drugs is controversial, since HIV-1 strains recovered from patients that encode M184V can be susceptible to didanosine and dideoxycytidine in phenotypic assays [32].

Four mutations (65, 74, 115, and 184) are associated with abacavir resistance. The presence of 1 or 2 of these resistance mutations only modestly increased HIV phenotypic resistance to abacavir in culture [33, 34]. Clinical resistance to abacavir in patients previously treated with zidovudine is related to the number of zidovudine resistance mutations detected. The mag-
Figure 1. Amino acid codon changes associated with resistance to reverse transcriptase inhibitor (RTI) monotherapy (data adapted from [28], [29]).


The magnitude of VL reduction and the percentage of patients with VL reduction below detection limits were similar for individuals with 0 or 1 zidovudine resistance mutation, with or without M184V, but were significantly less if there were ≥3 of these mutations [33]. Because none of the zidovudine resistance mutations are known to emerge from use of abacavir, either in vivo or in vitro, accumulation of zidovudine resistance mutations may be a surrogate for prior antiretroviral experience.

Prior nucleoside RTI therapy may induce clinical resistance by cellular mechanisms, which may include reduced phosphorylation of drugs into the active form, decreased drug uptake or use, or induction of drug metabolism.

Clinical resistance to stavudine, in particular, may not always involve the emergence of strains with specific genotypic mutations. Although the V75T mutation has been associated with modest increases in IC₅₀ of stavudine, this mutation is not detected in most patients with loss of viral suppression during stavudine treatment [35–37]. Studies of phenotypic resistance to stavudine, in which defined molecular clones were used, suggest that most other RTI resistance mutations do not have much effect on drug susceptibility. In particular, recombinant HIV strains that contain ≥1 of the zidovudine resistance mutations, including T215Y, still typically retain phenotypic susceptibility to stavudine [38]. Zidovudine resistance mutations are seen in zidovudine-naive patients who do not have virologic suppression on stavudine-containing therapy [39]. The appearance of zidovudine resistance mutations is also associated with low CD4⁺ T cell counts and high VL and may be a surrogate for host changes associated with prior therapy.

Multidrug Resistance

Recently, a complex mutation pattern was identified with a 2 amino acid insertion between codons 68 and 70 of the reverse transcriptase gene, together with other amino acid changes at codon 69. This most commonly results in the insertion of sequences that encode 2 serines just after position 69 (T69S-S-S) [40, 41]. It is not clear whether the insertion is before or after codon 69. When seen together with other RTI resistance mutations, this mutation complex can confer multinucleoside drug resistance to all nucleoside RTIs, including stavudine, for which specific mutations associated with resistance are infrequently demonstrated in patient samples. Another multinucleoside drug resistance mutation, Q151M, is also associated with high levels of resistance to multiple nucleoside RTIs, but accumulation of mutations that result in changes at other codons is required for
this broad resistance (62V, V75I, F77L, and F116Y) [42, 43]. Fortunately, these multidrug resistance mutations are rarely seen in patients [44, 45], presumably, in part, because the Q151M mutation and the insertion result in attenuated growth in culture. Accumulation of the additional mutations appears to improve replicative capacity.

In addition to the multinucleoside drug resistance mutations, complex new mutation patterns are emerging with combination RTI therapy that were not seen during the era of RTI monotherapy. One of the best examples is the interaction between zidovudine and lamivudine resistance mutations. Although the M184V mutation results in high levels of lamivudine resistance, when this mutation is seen in the presence of 1 or 2 zidovudine resistance mutations, the resulting virus strain has susceptibility to zidovudine restored. Until recently, this compensation was due to facilitation of dual resistance to both lamivudine and zidovudine in the presence of the 184 mutation [47]. The emergence of this simple mutation that can contribute to resistance to >1 drug is evidence that the high rate of mutation and the remarkable flexibility of viral enzymes allow HIV to tolerate marked genetic changes without a significant decrease in replication competence. The simplest and most efficient way to avoid drug inhibition will emerge with virus evolution, and even the attenuated strains that occur in multinucleoside drug resistance can still result in clinical progression, although perhaps not as rapidly as with wild-type virus.

Recent work on the mechanism of resistance in nucleoside RTIs may help to explain the high prevalence of zidovudine and lamivudine resistance [48, 49]. The most straightforward explanation for nucleoside RTI resistance is that changes in the catalytic pocket of the enzyme are modified by resistance mutations so that the analogues no longer bind well, although the enzyme is still able to function with cellular nucleotides. An alternative hypothesis for zidovudine resistance has recently been offered, in which zidovudine resistance leads to removal of zidovudine monophosphate, allowing for continued transcription [48]. In the presence of ATP, zidovudine-resistant mutant reverse transcriptase showed significantly greater completion of DNA synthesis, which may, in part, explain the increased ability of mutant reverse transcriptase to replicate in the presence of zidovudine. Compared with wild-type virus RT, zidovudine-resistant virus RT showed increased pyrophosphorylation (an alternative pathway that leads to rescue of DNA synthesis), but the lamivudine resistance M184V mutation resulted in marked reduction in this activity [49]. This finding may explain why zidovudine and lamivudine resistance mutation patterns are not compatible and why zidovudine susceptibility can be restored in the presence of the 184 mutation.

Support for Specific Drug Sequencing Strategies Based on Cross-Resistance

On the basis of patterns of resistance mutations and RTI cross-resistance associated with the M184V mutation, it is possible to make an argument that lamivudine should be used in the second drug regimen. This argument is based on concerns that the development of the mutation at position 184 contributes to resistance of several other RTIs. Use of didanosine in an initial drug regimen typically does not have a selection effect on the M184V mutation and will still leave lamivudine and abacavir available for subsequent treatment regimens. This proposed treatment strategy is entirely theoretical; however, there are several studies that will specifically examine the use of sequential pairs of RTIs in clinical trials, in particular the AIDS Clinical Trials Group Study 384, a 6-arm investigation including treatment-naïve patients in which there are reciprocal cross-overs of RTIs in the 2 subsequent treatment changes. Of even greater concern is the uncertainty of RTI resistance relationships in the context of triple-drug therapy with a PI. Confusing results have been obtained from AIDS Clinical Trials Group Study 343, in which patients who initiated zidovudine, lamivudine, and indinavir therapy were more likely to have viral rebound when therapy was reduced to maintenance doses of either indinavir alone or zidovudine plus lamivudine than when triple-drug therapy was continued [50]. Although perhaps it is not surprising that 1 or 2 drugs did not suppress viral replication as well as 3 drugs, it was remarkable that failure of triple-drug therapy was uniformly associated with the M184V mutation only; PI resistance mutations were found. It is possible that loss of response during less intensive antiretroviral regimens may be due to pharmacokinetic or cellular factors and not just to drug resistance.

Nucleotide RTIs

A novel class of compounds that are analogues of nucleotides rather than nucleosides have been developed for treating HIV infection, as well as hepatitis B. The 2 prototype compounds adefovir dipivoxil and the newer compound 9-[2-(R-phosphonomethoxy)propyl]adenine, or PMPA, have been shown to be effective inhibitors of HIV in vitro, as well as in preliminary clinical trials [51–53]. Resistance to adefovir dipivoxil is associated with a novel mutation K70E, as well as the K65R mutation associated with resistance to didanosine and dideoxycytidine [54, 55]. It is interesting that increased adefovir dipivoxil susceptibility and attenuated viral growth have been observed, along with the development of the RTI resistance M184V mutation because of concomitant lamivudine use [46]. Also, clinical trials with larger numbers of patients have demonstrated dose-dependent renal toxicity that may unfortunately limit the use of this class of drugs [53].
Nonnucleoside RTIs

Nonnucleoside RTIs have established an important role in combination therapy for HIV infection [15, 20]. Use of these drugs was slow to be established, since their use as monotherapy results in rapid emergence of drug-resistant strains [56–58]. In vitro studies demonstrated the emergence of single nucleotide changes at positions 103, 106, and 181 that were associated with high levels of resistance to this class of drugs, and in addition, mutations that conferred changes at residues 108, 188, and 190 have also been found in patient strains. These changes are different from the codons important for nucleoside RTIs, since these drugs do not bind at the catalytic site (figure 2).

Despite the rapid emergence of resistance when used as monotherapy, nonnucleoside RTIs are effective when used as part of a multidrug regimen that is intended to result in viral suppression below detection limits [13, 14, 19, 20]. It has not yet been determined whether RTI plus nonnucleoside RTI regimens will be as durable as those containing PI. The K103N mutation appears to be critical for all 3 of the approved nonnucleoside RTIs. The Y181C mutation, which efficiently emerges with the use of delavirdine, also results in resistance to nevirapine but appears to have variable effects on susceptibility to efavirenz [59–61]. Unfortunately, the nonnucleoside RTI resistance mutations often appear in combination, and with the presence of ≥2 amino acid codon changes associated with nonnucleoside RTI resistance, little response can be expected from the approved agents in this class of drugs.

Resistance Monitoring to Guide Antiretroviral Therapy

Just as VL is used extensively to determine when to initiate and change therapy, it would be desirable to have resistance assays to ascertain which therapies to avoid because of preexisting drug resistance [28]. In this scenario, patient strains would be tested for resistance at the onset of therapy, and drugs to which resistance is demonstrated would be avoided, if possible. If viral suppression is lost, patient strains would again be tested, and results of this testing would guide future therapy. Although drug resistance testing is routine for antibacterial drugs, there are several problems with this new approach to clinical monitoring of HIV resistance, particularly the technical rigor required and the difficulties associated with culture of HIV in the clinical laboratory.

Resistance assays can be classified as genotypic (analysis of specific genetic resistance mutations, by either direct sequencing or hybridization methodologies) or phenotypic (a more classical measure of resistance that uses culture of the pathogen in the presence of various drug concentrations). The genotypic assays,
based on either hybridization or direct sequencing, are appealing, since they are more rapid, less technically cumbersome for decentralized laboratories, and, at least currently, less expensive than phenotypic assays. Hybridization assays have the disadvantage of needing to specifically target mutations and would not detect novel mutations, particularly insertions or deletions. Sequence analysis can assess both the reverse transcriptase and protease genes in the same sample, and the assay is potentially very sensitive, since many of the available assays are based on PCR amplification. Some limitations of current genotypic assays are listed in table 1. These assays usually provide only 1 sequence, and minor sequence types are not detected. In order for a given sequence to be reliably detected, it must comprise at least 25% of the virus population, although a recent performance panel that tested known mixtures of virus in 35 laboratories suggested that detection of resistance mutations in a 50:50 mix was inconsistent and only 50%–70% reliable [62]. The sequence prevalence usually depends on selective effects of drugs; therefore, it will be difficult to assess genotypic resistance to drugs that patients are not currently taking. In addition, the patterns of mutations have not been fully defined for combination therapies, and, even more importantly, the genotypic patterns seen do not always correlate with the phenotype.

Phenotypic assays come with their own set of advantages and disadvantages. An advantage is that the actual susceptibility to various agents can be directly determined from culture. These phenotypic assays are necessary to interpret genotypic changes, since the complexities of genetic patterns cannot be used reliably to discern properties of viral resistance. Phenotypic assays can also determine mutational interactions (i.e., the effects of different patterns of mutations on viral growth in the presence of drug). Because it is not practical to obtain virus isolates by culture of patient blood, commercial recombinant virus phenotypic assays have been developed. In these assays, the PCR-amplified fragment that encodes reverse transcriptase and protease is cotransfected with an HIV vector deleted in these genes, to give rise to a recombinant virus that can be tested in culture assays. There are great technical challenges, however, that restrict these assays to specialized laboratories (table 2). Such phenotypic assays generally are expensive and time-consuming and, more importantly, require propagation of infectious virus in the clinical laboratory. As is the case with genotypic assays, recombinant virus phenotypic assays generally assess only 1 sequence type, which may not be the virus strain that is most relevant in patients. The in vitro assays of necessity use either cells from another donor or cell lines, which may not reflect the virus-cell interactions that are specific for a given patient. Because these assays need to be standardized for widespread application, they need to be developed with defined breakpoint values for resistance and with good interlaboratory reproducibility.

Despite their shortcomings, it is certain that these assays will continue to be developed and ultimately will improve the ability of physicians to guide treatment of HIV infection. Prospective trials have been completed in both France [63] and the United States [64], to assess the utility of genotypic resistance testing for selecting antiretroviral therapy. The Viradapt study in France [63] was a community-based trial that included patients with VLs >10,000 copies/mL who would be changing therapy. The patient population was treatment-experienced, had VLs of ~150,000 copies/mL, and was randomized to a treatment group for which genotype testing was performed, and interpretation and treatment recommendations were provided or to a treatment group for which current clinical standards of care were applied (control group). At 3 months, there was significantly greater VL reduction in the group that received genotype information and treatment recommendations (~0.5 log greater VL reduction than in the control group), but it was no longer significant at 6 months. Approximately twice as many patients in the genotype group than in the control group had VL reduction to undetectable levels.

The GART study [64] in the United States included treatment-experienced patients with VL increases of 0.5 log during therapy. Although baseline VLs were ~25,000 copies/mL, this study had essentially the same results with significantly greater VL reduction at 1 and 2 months in the group for which genotype testing was done and a diminished difference at 3 months. In this study, results of genotypic drug resistance testing allowed for the selection of more drugs likely to be active, which was associated with greater VL reduction. In a follow-up study of the French trial, control patients underwent genotype testing at 6 months, and VL reduction was similar to that seen for patients in the group for which genotype testing was performed before the initial treatment decision [65]. The results of these studies provide strong support for genotype testing to help improve selection of antiretroviral drugs. Resistance testing will provide an incremental benefit to drug selection but will not have the impact on guiding therapy that the introduction of VL testing had.

Table 1. Limitations of genotypic resistance assays for HIV-1.

<table>
<thead>
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<th>Limitation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Sequence frequency must be &gt;25% for detection</td>
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<tr>
<td>Predominant sequence is dependent on the selective effect of current</td>
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<td>treatment</td>
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<td>Unreliable detection of resistance mutations relevant for potential</td>
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<td>new therapies</td>
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<td>Assays may preferentially detect some sequences</td>
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<td>May not correlate with phenotype</td>
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<td>Expert interpretation is required</td>
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Table 2. Limitations of phenotypic resistance assays for HIV-1.

<table>
<thead>
<tr>
<th>Limitation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Longer time to results (weeks)</td>
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<tr>
<td>Usually only assesses a single sequence from the viral swarm</td>
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<td>Detection of resistant virus may not preclude a clinical response</td>
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<td>Target cell not the same as in the patient; may affect viral growth</td>
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<td>properties</td>
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<td>Poor reproducibility in primary cell systems</td>
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