Clinical Use of Genotypic and Phenotypic Drug Resistance Testing to Monitor Antiretroviral Chemotherapy

George J. Hanna and Richard T. D’Aquila
Massachusetts General Hospital and Harvard Medical School, Boston

Assays that detect antiretroviral drug resistance in human immunodeficiency virus have recently become available to clinicians. Phenotypic assays measure the drug susceptibility of the virus by determining the concentration of drug that inhibits viral replication in tissue culture. Genotypic assays determine the presence of mutations that are known to confer decreased drug susceptibility. Although each type of assay has specific advantages, limitations associated with these tests often complicate the interpretation of results. Several retrospective clinical trials have suggested that resistance testing may be useful in the assessment of the success of salvage antiretroviral therapy. Prospective, controlled trials have demonstrated that resistance testing improves short-term virological response. Resistance testing is currently recommended to help guide the choice of new drugs for patients after treatment has failed and for pregnant women. Resistance testing should also be considered for treatment-naive patients, to detect transmission of resistant virus.

Although the number of available antiretroviral agents has markedly increased in the past few years, the selection of a combination regimen that maximally suppresses HIV type 1 (HIV-1) replication continues to be challenging because of the potential of HIV-1 to develop drug resistance. For patients who have undergone antiretroviral therapy (hereafter referred to as “antiretroviral-experienced patients”), resistance to previously used drugs and potential cross-resistance to other drugs have a significant impact on the efficacy of salvage regimens. Even in antiretroviral-naive patients, infection with drug-resistant virus may limit the virological and immunologic benefits of the initial combination regimen. Several methods of assessing antiretroviral resistance in HIV-1 are currently available to aid clinicians in the selection of combination regimens. In the present review, we describe these methods, and we highlight their advantages and limitations. We also review the limited clinical trials that have evaluated the role of drug resistance testing and make recommendations for clinical use of these assays.

METHODS OF DRUG RESISTANCE TESTING

Two types of assays are used to assess antiretroviral drug resistance. Phenotypic drug susceptibility assays determine the inhibitory concentration of an antiretroviral agent that reduces HIV-1 replication by 50% (IC50) in tissue culture. Genotypic assays determine the presence of mutations that are known to confer decreased drug susceptibility.

Phenotypic assays. A standardized assay is available for determination of drug susceptibilities of most HIV-1 isolates in peripheral blood mononuclear cells (PBMC) [1]. The method involves cocultivating patient PBMC with seronegative donor PBMC to generate a virus stock; titrating the virus stock to determine the virus infectivity of the stock, using a standardized inoculum (on the basis of the infectivity properties of the stock) to infect cultures at varying concentrations of an antiretroviral agent; and then calculating IC50 on the basis of the measure of infection (HIV-1 p24 antigen levels). Although the assay reproducibly determines phenotypic susceptibility, it has several limitations. It is labor intensive and time consuming, taking a minimum of 6 weeks from the time of initial specimen
collection to generation of data. Furthermore, the assay may impose significant in vitro selective pressures (varying infectivity of different lots of seronegative PBMC and a lack of antiretroviral drug pressure during lengthy stock generation) that may favor the outgrowth of certain virus isolates and not others from the original heterogeneous virus population. This method determines the susceptibility of virus present in a patient’s infected PBMC and does not assess plasma virus directly. Some studies have shown that detection of HIV-1 resistance mutations directly in the DNA of a patient’s infected PBMC can lag behind their detection in plasma HIV-1 RNA [2, 3]. However, recent studies have shown good concordance in resistance mutations between plasma HIV-1 RNA and viruses cultured from PBMC [4, 5].

The need for more-rapid assays that directly assess the virus in plasma led to the development of several assays based on recombinant viruses [6–10]. These assays are based on direct amplification of the patient’s virus–derived gene sequence of interest (protease, reverse transcriptase [RT], or both) from viral RNA in plasma, by means of reverse transcription–PCR. The PCR product is inserted into a modified HIV-1 vector that lacks the analogous sequence. A recombinant virus is produced and then used in rapid drug susceptibility studies. Currently, 2 highly automated recombinant virus–based phenotypic assays are available to clinicians. The Antivirogram assay (Virco) requires plasma with ≥1000 HIV-1 RNA copies/mL and includes protease and most of RT (codons 1–482) [8]. Results are reported as “sensitive” (<4-fold increase in IC₅₀ compared with that for wild-type virus), “intermediate” (4–10-fold increase in IC₅₀), or “resistant” (>10-fold increase in IC₅₀). The PhenoSense assay (ViroLogic) can be done on plasma with ≥500 HIV-1 RNA copies/mL, and it spans several protease cleavage sites in gag, all of protease, and the RT region to codon 313 [10]. Results of replicate assays vary by <2.5-fold [10]. A >2.5-fold increase in IC₅₀ is reported as “decreasing susceptibility.” Both tests are generally completed in 8–10 days, and the practical turnaround time to clinicians, including shipping, is 2–4 weeks.

A limitation of current phenotypic resistance testing is the lack of consensus on what increase in the IC₅₀ of a drug is clinically significant. This increase is likely to be different for antiretroviral agents and is related to the blood levels of the drug. For instance, a 4-fold increase in the IC₅₀ of a nucleoside RT inhibitor (NRTI) or a single protease inhibitor (PI) may well be associated with a lack of clinical efficacy of the drug. However, the same increase in the IC₅₀ of a non–nucleoside RT inhibitor (NNRTI) may not necessarily compromise the efficacy of the drug.

**Genotypic assays.** Genotypic assays are based on determination of the nucleotide sequence of regions that confer phenotypic resistance. Dideoxynucleotide sequencing has been the standard method of sequencing the HIV-1 protease and RT genes, and current applications rely on automated sequencers. Sequence alignment and editing, followed by mutation detection and interpretation, require further operator input. These latter functions have been increasingly automated with recently introduced HIV-1–specific kits and software (TrueGene HIV-1 genotyping kit and OpenGene DNA sequencing system [Visible Genetics]; ViroSeq HIV-1 genotyping system [PE Biosystems]). This automation has reduced the time from receipt of a specimen to final report of data to as few as 2 days, and practical turnaround time to clinicians ranges from 3 to 21 days.

Other sequencing methods, which are based on determination of the sequence through hybridization to defined oligonucleotide probes, are also available. The entire protease and most of the RT sequence can be determined through sequencing by hybridization to miniaturized high-density arrays of oligonucleotide probes, followed by automated nucleotide base calling and mutation detection (GeneChip; Affymetrix) [11]. More-limited sequence information can be obtained by use of a hybridization-based line probe assay (LiPA; InnoGenetics), which rapidly and simultaneously determines the presence of preselected drug resistance mutations in some, but not all, codons [12].

The comparative performance of cycle sequencing and sequencing done on the basis of hybridization has been the subject of several studies. A study of 29 clinical specimens from 12 antiretroviral–experienced patients found a nucleotide concordance rate of 98.8% between cycle sequencing and GeneChip [13]. Another study of 49 virus isolates from 22 antiretroviral–experienced patients showed an overall amino acid concordance rate of 97.4% between the 2 methods [14]. However, a significantly higher rate of discordance (3.9%) was observed among resistance–associated RT codon 67, with cycle sequencing more commonly calling a known resistance–associated amino acid than hybridization sequencing. These studies suggested that the presence of genetic mixtures at the resistance–associated position, or nucleotide sequence polymorphisms in the vicinity of those codons, influenced population–based hybridization sequencing.

A study of 24 plasma samples from 22 mostly antiretroviral–experienced patients showed a similar rate of discordance (3.4%) among resistance–associated codons when GeneChip was compared with cycle sequencing [15]. This study also compared the performance of LiPA with that of both GeneChip and cycle sequencing, and it found a discordance rate of 8% between LiPA and cycle sequencing as well as between LiPA and GeneChip. Problems with hybridization sequencing technology have also been noted with the use of GeneChip for analysis of non–clade B HIV-1 isolates and clade B HIV-1 isolates with insertion mutations (such as the insertion mutations in the vicinity of RT codon 69 that are associated with multinucleoside resistance).
specimen’s genotype to a database of experts and testing laboratories, a strategy that compares a virus and improve on the different interpretations from different genotypic assays often requires expert opinion. Zidovudine resistance mutations. The interpretation of data to HIV-1 to zidovudine, even in the context of several associated with nevirapine resistance) can restore the susceptibility of HIV-1 RNA per milliliter. Testing of plasma specimens resistance to other drugs [17]. For instance, several mutations (RT L74V, which is associated with didanosine resistance; RT M184V, associated with lamivudine resistance; and RT Y181C, associated with nevirapine resistance) can restore the susceptibility of HIV-1 to zidovudine, even in the context of several zidovudine resistance mutations. The interpretation of data from genotypic assays often requires expert opinion.

In an effort to address the complexity of data interpretation and improve on the different interpretations from different experts and testing laboratories, a strategy that compares a virus specimen’s genotype to a database of >20,000 samples with known genotypes and phenotypes has been devised (Vircogen Virtual Phenotype assay; Virco). For each drug, matching is performed to all virus specimens that contain identical amino acids at previously defined key codons, and the likelihood that a specimen with the same genotype is susceptible, resistant, or intermediate (hereafter known as “intermediate”) is reported [18]. In a limited series of virus specimens, the prediction from this “virtual phenotype” matched the actual phenotype accurately in most cases predicted to be susceptible or resistant, with some discrepancies in cases predicted to be intermediate [18]. Prospective comparisons will help to demonstrate the clinical utility of this assay. HIV-1 exhibits considerable genetic diversity in regions not identified as key mutations matched to the database, and these variations may influence the phenotypic expression of a resistance mutation. In addition, new patterns of resistance mutations continue to be defined and will require continued reliance on phenotypic assays to keep the database updated.

Limitations of current assays for drug resistance testing. Several limitations apply to all currently available drug resistance assays. All methods require plasma with ≥500–1000 copies of HIV-1 RNA per milliliter. Testing of plasma specimens with lower levels of viremia does not yield consistent results, but the assay technology is improving. Detection of clinically significant minority species remains challenging, particularly in patients who were previously heavily treated and who have changed therapy. Within weeks of discontinuation of a drug, the predominant HIV-1 population may shift from a virus with drug-specific resistance mutations to a virus that lacks these mutations and appears to be susceptible to the discontinued drug [19]. However, resistance will quickly emerge again when this drug is reintroduced. Phenotypic assays yield the average IC₅₀ for the population of viruses in a specimen. Although the presence of a significantly resistant population (one that may be detected at levels of ~10%–50% in a virus population that otherwise is wild type) may be detected by the finding of an increased IC₅₀, resistant strains found at lower levels may be missed. Most genotypic assays can detect minority populations at levels as low as ~20%, and LiPA may detect them at levels as low as ~4% (although they may be detected more reliably at 10%–15%) [12]. However, minority populations at lower levels may also be clinically relevant and capable of rapidly dominating the population after introducing the selective effect of the relevant drug.

Problems with specimen mix-up, PCR contamination, and technical performance may compromise the quality of data from some clinical laboratories. A recent evaluation of 23 mainly academic laboratories for the detection of well-defined HIV-1 specimens that had wild-type RT or a homogeneous mutant population of RT, or that contained different mixtures of wild-type and mutant RT revealed large interlaboratory differences in the quality of results [20]. The ability of laboratories to detect mixtures also varied considerably. Although laboratories with greater expertise in drug resistance testing have improved performance [21], quality controls will clearly be important for this rapidly evolving and complex technology.

Finally, the impact of pharmacologically enhanced regimens on the interpretation of drug resistance testing for HIV-1 awaits further study. For example, blood levels of saquinavir, amprenavir, lopinavir, and indinavir are increased substantially when each drug is coadministered with ritonavir. Trough levels of PIs in some of these combinations exceeded the IC₅₀ for most PI-resistant viruses tested in one study, which suggests the utility of these combinations in treatment of infection with resistant viruses [22]. Therefore, the appropriate resistance assay criteria used to predict whether drugs used in a combination will produce a virological response may need to take pharmacokinetic considerations into account.

CLINICAL TRIALS

Several retrospective clinical studies have suggested that drug resistance testing may be useful in assessing the likelihood of success of salvage antiretroviral therapy. These studies have varied in methodology and end points. Twelve studies (some are summarized here; others were reported at meetings and are...
not yet published) were reanalyzed by use of a standard data analysis method and a primary end point of virological failure (plasma HIV-1 RNA level, ≥400 copies/mL) by week 24 of therapy [23]. Both baseline genotypic drug resistance and phenotypic drug resistance predicted virological failure, regardless of whether these factors were analyzed separately or were adjusted for other baseline confounding factors (such as plasma HIV-1 RNA level and the number and type of new antiretroviral agents in the regimen). In most of the reanalyzed studies, the odds of virological failure were reduced by approximately 2-fold for each additional drug in the regimen to which the patient’s virus was susceptible according to genotyping methods and by approximately 2- to 3-fold for each additional drug to which the virus was susceptible according to phenotyping.

**NRTI resistance.** Early studies that involved patients who received zidovudine monotherapy showed that the presence of zidovudine resistance mutations was associated with a worse CD4+ cell response [2] and an increased risk for disease progression or death, even when controlling for other factors predictive of disease progression [24]. Similarly, high-level zidovudine phenotypic resistance was associated with an increased risk for disease progression or death [25]. It is surprising that the deleterious effects of zidovudine resistance on CD4+ cell count, virus load, and clinical responses were even seen in patients who switched to didanosine monotherapy [24–27], despite the limited in vitro cross-resistance between zidovudine and didanosine [28]. In zidovudine-experienced patients who started receiving combination therapy with 2 NRTIs, the presence of zidovudine resistance mutations was associated with a significantly worse virological response among those who started receiving treatment with zidovudine and lamivudine [29] or with stavudine and lamivudine [27]. However, in zidovudine-experienced (but lamivudine- and PI–naïve) patients who started receiving treatment with a 3-drug combination of zidovudine, lamivudine, and ritonavir, the presence of zidovudine resistance mutations had no apparent effect on the probability of achieving plasma HIV-1 RNA levels of <100 copies/mL at week 48 [30].

A recent study examined the predictive value of baseline HIV-1 genotypic resistance mutations for failure of therapy that consists solely of NRTIs [31]. At 1–3 months and at 6 months after change in therapy, patients without preexisting resistance mutations to the new NRTI had a significantly better response in virus load than did patients with known preexisting resistance mutations to the new NRTI. The difference was particularly striking for patients whose treatment was modified with the addition of lamivudine. The presence of lamivudine resistance–associated RT M184V at baseline predicted a significantly worse virological response in these patients.

**NNRTI resistance.** It has been thought that failure of treatment with one currently available NNRTI would lead to broad cross-resistance to the entire class. Indeed, nevirapine and delavirdine share considerable cross-resistance patterns, and some of the mutants that emerge because of their selective effects are also cross-resistant to efavirenz. However, in vitro studies have suggested that several single resistance mutations (including RT V106A, RT Y181C, and RT G190A) that emerge because of nevirapine’s selective effect, do not confer high-level resistance to efavirenz [5, 32]. A study of individuals who were receiving incompletely suppressive therapy (therapy that resulted in a detectable plasma HIV-1 RNA of ≥200 copies/mL) with zidovudine, didanosine, and nevirapine showed considerable heterogeneity in the NNRTI resistance mutations [33]. The 2 single mutations known to confer clinically significant efavirenz resistance, RT K103N and RT Y188L, were observed at a frequency of only 27% early (8 weeks) and 33% late (48 weeks) during treatment failure. Another study of NNRTI-naïve individuals who started receiving salvage treatment with nevirapine, stavudine, and a PI showed a similar heterogeneity of NNRTI resistance mutations 6 months after initiation of nevirapine therapy [34]. Efavirenz resistance–associated mutations at RT codons 103 and 188 were seen in 45% of virus specimens at 6 months. At that time, phenotypic susceptibility showed that, although 93% of virus samples were resistant to nevirapine, 68% were resistant to efavirenz (including all specimens with RT K103N), 24% were susceptible to efavirenz, and 8% had intermediate levels of reduced susceptibility to efavirenz.

These data suggest that drug resistance testing will be necessary to identify which mutations emerge when therapy with a combination that includes nevirapine is failing. In contrast to findings of earlier reports [35, 36], concomitant drugs (i.e., presence or absence of zidovudine in the combination) do not reliably predict the patterns of mutations that emerge because of nevirapine’s selective effect during failure of therapy with a 3-drug combination. However, it remains unknown whether some patients for whom nevirapine therapy fails without identifiable cross-resistance can benefit from efavirenz as a component of a salvage treatment regimen. A recent study examined determinants of virological response in antiretroviral–experienced patients who started receiving salvage treatment with regimens that contained efavirenz and adefovir [37]. Many patients had been previously treated with other NNRTIs, and all had undergone nucleoside analog therapy (therefore, only 1 or 2 active drugs may have been part of the salvage treatment regimen for many). NNRTI experience was a significant predictor of virological failure. However, patients whose virus had the baseline NNRTI resistance mutation RT Y181C or G190A alone had a significantly greater virological response to efavirenz (−1.32 log_{10}) at 4 weeks than did those with virus that contained RT K103N (−0.01 log_{10}); by 8 weeks, the differences were no longer statistically significant.

Similarly, other studies have shown that, in general, NNRTI
experience predicted a poorer virological response to efavirenz in suboptimal salvage treatment regimens (combinations that do not include 3 new, fully active antiretrovirals) [38, 39]. Nevertheless, the lack of efavirenz-specific resistance revealed by either phenotypic or genotypic assays may be associated with improved virological response in some patients [40, 41]. It remains unknown whether a more durable virological response can be seen if efavirenz is combined with 2 new, fully active antiretroviral agents in patients with virus that has single NNRTI resistance mutations other than RT K103N or Y188L, or in NNRTI-experienced patients with virus that retains in vitro efavirenz susceptibility. Testing for NNRTI resistance mutations or susceptibility phenotype will potentially be more useful when newer NNRTIs that have resistance profiles different from those of currently available drugs are introduced (such as capravirine, which has in vitro activity against RT K103N mutants [42]).

Pls. Several studies have focused on predictors of response to salvage therapy with a regimen of ritonavir plus saquinavir. One study examined the influence of baseline saquinavir phenotypic resistance and genotypic resistance on patients with antiretroviral experience (including ritonavir or indinavir but not saquinavir or NNRTIs) who were switched to treatment with a salvage regimen of ritonavir, saquinavir, efavirenz, and 2 NRTIs [43]. Patients with virus that exhibited phenotypic resistance to saquinavir at baseline had a significantly worse virological response (−0.91 log10) at week 24 of therapy than did those with susceptible virus strains (−1.52 log10). Baseline genotypic resistance to saquinavir (defined in the study exclusively as the presence of protease L90M or G48V) was not predictive of virological failure. However, the influence of other primary PI resistance mutations (I46L and V82A/F/S, which were present in as many baseline isolates as those with codon 48 and codon 90 mutations) was not assessed.

A second study analyzed the influence of resistance mutations on the virological response in PI-experienced patients who were switched to treatment with a salvage regimen that included saquinavir and ritonavir and who were followed up for ≤26 weeks [44]. Multivariate regression models showed that protease mutations present at the initiation of combination therapy with saquinavir and ritonavir were the strongest predictors of virological response. Mutations at protease codons 10, 46, 48, 54, 71, 82, and 90 were associated with poorer response, whereas mutations at codons 19 and 30 were associated with better response. A third study determined whether baseline drug resistance, determined either phenotypically (Antivirogram assay) or genotypically (Vircogen Virtual Phenotype assay), could help to predict failure of treatment with the combination of ritonavir and saquinavir for treatment-experienced patients [45]. Baseline drug resistance to saquinavir or ritonavir, determined by either assay, was predictive of poor virological response to treatment with this dual-PI combination, despite the confounding effects of other antiretroviral agents used in the salvage regimen.

Other studies have suggested that drug resistance testing may be useful for determination of salvage treatment regimens that include other PIs. One study examined saquinavir-experienced patients who were treated with a nelfinavir-based salvage regimen and were followed up for 16 weeks [46]. Rapid treatment failure was associated with the presence of the protease mutation L90M. Another study included PI-experienced (but nelfinavir-naïve) patients who were switched to salvage treatment with a regimen that included nelfinavir and who were followed up for 4–12 weeks [47]. Univariate analysis disclosed that virological response to salvage therapy was associated with several variables, including the number of RT inhibitor resistance mutations, number of primary and secondary PI resistance mutations, and history of PI use (duration of use and number of drugs taken). After adjustment for all variables, the number of RT inhibitor plus PI resistance mutations was the only independent predictor.

Another study investigated patients who had virological failure during treatment with a regimen that included indinavir or ritonavir and who were switched to salvage therapy with regimens that included nelfinavir, saquinavir, abacavir, and either another nucleoside analog or nevirapine [48]. Baseline phenotypic drug susceptibility was strongly correlated with outcome. At week 24 after the start of salvage treatment, subjects with baseline virus that was phenotypically susceptible to 2 or 3 drugs in the salvage regimen had a significantly greater reduction in virus load (−2.24 log10) than did those with baseline virus that was susceptible to no or 1 drug (−0.35 log10).

Virological failure of treatment with 3-drug regimens that included PIs may not necessarily be associated with PI resistance at the time of the initial virus load rebound. For 24 drug-naïve patients who had virus load rebound during combination treatment with zidovudine, lamivudine, and indinavir, no PI resistance mutation was seen at the time of the initial rebound [49]. Another study examined the resistance profile for drug-naïve patients who were treated with the combination of zidovudine, lamivudine, and indinavir [50]. In the 17 subjects with virus load rebound, indinavir phenotypic resistance was not detected in viruses despite plasma HIV RNA levels that ranged from 10^1 to 10^6 copies/mL. Only 1 subject’s virus developed PI resistance mutation (M46L). The lack of PI resistance in both studies is in contrast to the finding of the lamivudine resistance mutation RT M184V in virus isolates from >80% of those patients with viral rebound in the same studies.

In a study of 29 drug-naïve patients who were treated with stavudine, zalcitabine, and saquinavir for 24 weeks, 10 had virus load rebound to >200 copies/mL, but only 2 patients had viruses that developed PI resistance mutations (both with L90M) [51]. However, there was no significant increase in the saquinavir IC50.
for any virus isolate, with or without the L90M mutation. Similarly, of 7 patients with virus load rebound during treatment with zidovudine, lamivudine, and amprenavir, only 1 had evidence of PI resistance mutations, but 4 were infected with viruses with lamivudine resistance–associated RT M184V [52]. These studies suggest that virus load rebound during early failure of treatment with PIs may be caused by factors other than viral resistance (for instance, decreased drug levels in blood or cells). Drug resistance testing may be helpful in determining whether part of a failing treatment combination may be recycled later in a successful salvage regimen.

Prospective trials of drug resistance assays. Several recently completed or ongoing prospective trials of drug resistance assays should help define the clinical utility of these tests. Two reported prospective studies have examined the clinical utility of genotypic resistance assays. The first study randomly assigned 108 treatment-experienced patients (with ≥1 NRTIs and ≥1 PIs) either to receive treatment that is based on the results of a genotypic resistance assay (TrueGene HIV-1 genotyping kit) or to receive standard care [53]. By month 3, the mean change in the plasma HIV-1 RNA level was significantly greater in the genotypic assay arm of the study (−1.04 log10) than in the standard care arm of the study (−0.46 log10). In addition, HIV-1 RNA was undetectable (<200 copies/mL) in significantly more patients in the genotypic assay arm than in the standard care arm (29% vs. 14% of patients, respectively). Similar differences were maintained at month 6 (change in virus load, −1.15 vs. −0.67 log10; proportion of patients with undetectable virus load, 32% vs. 14%, respectively).

Another study assessed the short-term effects of genotypic resistance testing with expert advice in the management of patients for whom treatment with a PI and 2 NRTIs was failing [54]. The study randomized 153 subjects to either a genotypic-assay study arm, in which genotype interpretation and suggested treatment regimens were provided to clinicians, or to a control arm, in which treatment choices were made without such input. The change in the plasma HIV-1 RNA level (averaged at 4 and 8 weeks after the start of salvage treatment) was significantly better for the patients in the genotypic-assay study arm (−1.19 log10) than it was for the controls (−0.61 log10). The proportion of patients with undetectable virus loads (<500 copies/mL) was significantly higher in the genotypic-assay study arm than in the control study arm at week 4 (45% vs. 23%, respectively) and week 8 (55% vs. 25%, respectively), but it was no longer statistically significant at week 12 (34% vs. 22%, respectively). A third recently reported trial showed similar advantages of genotypic testing 3 months after genotyping was performed [55].

An open-label, randomized trial evaluated the clinical utility of phenotypic resistance testing by comparing the Antivirogram assay (Virco) with standard care [56]. A total of 273 antiretroviral-experienced subjects (those with ≥2 NRTIs and 1 PI) with plasma virus loads of >2000 copies/mL were randomized either to treatment that was based on the results of a phenotypic resistance assay or to standard care. At week 16, patients randomized to the phenotypic-assay study arm had a significantly greater decrease in virus load (−1.23 log10) than did patients randomized to the standard-care study arm (−0.87 log10). Significantly more subjects in the phenotypic-assay study arm had undetectable virus loads (<400 copies/mL) than did those in the standard-care study arm (59% vs. 42%, respectively). However, preliminary results of other trials of phenotyping were mixed. One trial, which also used the Antivirogram assay, showed improved virological response at week 4 of therapy in patients undergoing resistance testing, but no difference was found between the phenotypic-assay study arm and the standard-care study arm at week 16 [57]. Another study, which used a different assay, failed to demonstrate any difference in virological response between patients undergoing resistance testing and those treated with standard care at week 12 [58]. Other trials are under way, including some that use other phenotypic assays [59].

Other types of studies are also under way. Studies that compare phenotypic assays with genotypic assays will be important for the determination of whether one type of assay is clinically more useful in certain situations. Studies that compare different genotypic assays with each other (and also those that compare different phenotypic assays with each other) may help to assess whether any particular assay is better able to guide therapy. Finally, these assays are costly, with genotypic assays having a cost of ~$400 per specimen and phenotypic assays having a cost in the range of $750–$1000 per specimen. The extent to which these tests are cost-effective and improve long-term clinical outcomes is being studied.

**RECOMMENDATIONS FOR CLINICAL USE**

Recently, consensus recommendations were proposed by a panel of physician and virologist experts on HIV-1 drug resistance that was assembled by the International AIDS Society-USA [60]. These recommendations are similar to those issued by a panel convened by the US Department of Health and Human Services (Washington, DC) and the Henry J. Kaiser Family Foundation (Menlo Park, CA) [61]. Drug resistance testing is recommended to help guide the choice of new regimens after failure of treatment to document drugs to which there is resistance and to optimize the number of active drugs in the next regimen. The studies cited in this article form a compelling basis for this recommendation. Resistance testing ideally should be done when the selective effect of a drug is still present, to minimize the chance of missing important resistant minority populations. Expert interpretation should be
sought, given the complexity of results and assay limitations associated with currently available tests. Resistance testing is also recommended for HIV-1–infected pregnant women, to optimize maternal treatment and prophylaxis for the neonate.

Fewer data are currently available on the utility of drug resistance testing for treatment-naïve patients. Recent reports have documented that the prevalence of resistance among newly infected patients may be as high as 16% (one study of genotypic resistance [62]) and 26% (another study of phenotypic resistance [63]). However, the level of resistance was variable (high-level reduced susceptibility of >10-fold was seen for <5% of patients in one study [63]), and the clinical significance of these resistance patterns remains to be defined. Ancedotal reports have suggested that the virological and immunologic responses to therapy are poorer in treatment-naïve patients who start a treatment regimen to which their virus has preexisting resistance [64].

The International AIDS Society-USA panel recommends that drug resistance testing should be considered for treatment-naïve patients with established infection, to detect prior transmission of drug-resistant virus. This analysis may be difficult with the use of current tests, since initially acquired resistant strains may become a minority population after years of virus replication without the selective effects of drugs. However, further study is required by comparing the prevalence of resistance among patients presenting very soon after initial infection with that among patients presenting with established infection of uncertain duration. Testing is more strongly suggested for patients with acute HIV-1 infection, to detect transmission of drug-resistant virus and to modify therapy for optimization of virological and immune responses. However, initiation of therapy should not be delayed pending these test results. The rationale is that very early therapy may preserve CD4⁺ cellular immune responses to HIV-1 if the virus can be quickly suppressed by therapy [65].

### References


