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Drug Resistance Testing: Time to be Used in Clinical Practice?

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Abstract

The objective of this study is to review the clinical utility of drug resistance testing for management of antiretroviral therapy in HIV-1 infection.
Published reports in English of original research and meeting abstracts have been consulted. As a result, technological advances have led to improved assays for phenotyping and genotyping HIV-1 directly from plasma samples, making it feasible to perform drug resistance testing in real time. A correlation between emergence of drug resistance and virologic failure can be demonstrated for most antiretroviral agents. Preliminary results from retrospective studies suggest that drug resistance testing can identify which patients are likely to respond to particular treatment regimens.
We can conclude that drug resistance testing may be useful in guiding the choice of initial antiretroviral therapy, explaining and managing treatment failure, and tracking the transmission of drug-resistant isolates. Ongoing clinical trials will help to determine whether a treatment strategy that incorporates results of resistance testing leads to improved clinical outcome. Uniform criteria for interpretation and quality control are needed in order to standardize assay results across the various methods being applied. Despite these concerns, resistance testing will become a valuable tool in the clinical management of HIV-1 infection.

Key words

Drug resistance. Mutations. Antiretroviral drugs.

The emergence of drug-resistant variants of human immunodeficiency virus type 1 (HIV-1) is responsible, at least in part, for the failure of antiretroviral therapy in a significant number of infected individuals. In the past, the cumbersome and time-consuming nature of most resistance assays limited testing of HIV-1 isolates for drug resistance to a small number of research laboratories. The development of automated methods for phenotyping and genotyping HIV-1 directly from plasma samples has made resistance testing more widely available. Preliminary results from retrospective studies suggest that resistance assays can identify which patients are likely to respond (or not) to particular treatment regimens. Although a number of questions remain unanswered, these assays are becoming more widely used to help guide the choice of antiretroviral therapy in patients experiencing failure of their current regimen. The growing prevalence of primary drug resistance in HIV-1 isolates obtained from individuals with newly acquired infection provides an additional rationale for considering the use of resistance testing in the management of antiretroviral

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therapy. This article reviews the kinds of assays available for HIV-1 drug resistance testing and summarizes the results of recent studies regarding their clinical utility.

Factors contributing to drug resistance in HIV-1

The high error rate of HIV-1 reverse transcriptase[1] and the rapid turnover of the virus population[2,3] result in the accumulation of a large number of variants (mutants) in the virus population, which is termed a quasispecies. Mutations that confer drug resistance are pre-existent in the quasispecies but remain at low levels in the population unless selective pressure (in the form of antiretroviral therapy) is applied[4]. Treatment with antiretroviral agents leads to the emergence of drug-resistant variants as the predominant species within weeks to months if therapy fails to suppress completely virus replication[3,5-8]. The time required for emergence of resistance depends on the prevalence of drug resistant variants in the population prior to treatment, the strength of the selective pressure applied by the drug (or regimen), and the relative growth advantage conferred by the mutations in question. For example, resistance to nevirapine and lamivudine emerges within weeks of initiating monotherapy due to point mutations that result in a thousand-fold reduction in susceptibility to those drugs. By contrast, resistance to dideoxynucleosides (eg., didanosine and zalcitabine) emerges slowly as a result of mutations that confer only modest levels of drug resistance. In the case of zidovudine and the protease inhibitors, high-level resistance occurs as the consequence of the accumulation of multiple mutations over time[8,10]. For a detailed discussion of resistance to individual antiretroviral agents the reader is referred to several recent reviews[11-15].

Although emergence of drug resistance over time is nearly universal in the setting of partially suppressive therapy, numerous factors in addition to drug resistance can contribute to treatment failure. These include low intrinsic potency of a drug or regimen, poor adherence, persistence of viral reservoirs in compartments into which drugs penetrate poorly, and the lack of appropriate drug activation in resting cells[16]. Taken together these factors combine to limit drug activity, resulting in ongoing virus replication and continued immunologic decline.

Testing for drug resistance

The emergence of drug-resistant variants can be demonstrated by assays of viral genotype or phenotype. Genotypic assays detect specific changes in the viral genes encoding the targets of drug action (ie., the viral polymerase or protease) whereas phenotypic assays determine the average behavior of the population in the presence of increasing drug concentrations. Each of these assays has potential advantages and disadvantages.

Genotypic assays

The specific sequence of nucleosides that constitute the genes of HIV-1 defines the viral genotype. Genotypic assays for drug resistance are those that seek to determine the nucleotide sequence (and by inference the predicted amino acid sequence) of the genes that encode reverse transcriptase [RT] and protease [PR]. All such assays depend upon amplification of HIV-1 PR and RT genes from viral RNA in plasma (by means of reverse transcriptase-coupled polymerase chain reaction [RT-PCR]) or from proviral DNA (by PCR). The PR-RT amplicons can then be subjected to automated DNA sequencing by a variety of techniques, probed by hybridization-based assays such as the line probe assay (LiPA)[17], tested by further PCR using selective priming (ARMS[18]) or selective nucleotide addition (point mutation assay)[19]. Automated sequencing usually provides comprehensive data regarding the entire PR and RT genes, which may be more information than is required in most clinical situations. Assays such as the LiPA, selective PCR or point mutation assay provide a narrower spectrum of data limited to the specific codons implicated in drug resistance.

Phenotypic assays

The characteristics and growth properties of a viral isolate are referred to as the viral phenotype. In the context of drug resistance, phenotype refers to the susceptibility of HIV-1 to inhibition by a particular drug. Drug susceptibility is defined by determining the amount of drug required to inhibit virus production *in vitro* by 50%, 90%, or 95% (IC_{50}, IC_{90}, or IC_{95}, respectively). The data suggest that each of these parameters is similarly useful in identifying susceptible and resistant viruses, but for technical reasons the IC_{50} usually can be determined with the greatest precision.

Initial drug susceptibility assays for HIV-1 required the preparation of high-titer stocks of primary virus isolates[20]. This procedure was laborious and time-consuming, requiring six to eight weeks to generate a result. Because primary clinical isolates grow best in peripheral blood mononuclear cells (PBMC), these assays made use of lectin-stimulated PBMC from seronegative donors. However, PBMC from different donors vary in their ability to support the growth of HIV-1, leading to significant interassay variation.

Many of these problems have been overcome by the development of recombinant virus assays, in which the PR and RT genes are amplified by RT-PCR from plasma HIV-1 RNA and inserted into a molecular clone of HIV-1[21,22]. The resultant recombinant viruses have common envelope and accessory genes (eg., tat, vif, nef), but carry PR and RT from the patient's virus. Because this approach bypasses the need to obtain a primary isolate the time required to generate a result is shortened considerably. Moreover, the common genetic backbone of the recombinant viruses minimizes interassay varia-

Table 1. *Advantages and disadvantages of drug resistance tests for HIV-1.*

Genotypic Assays	
Advantages	Disadvantages
– Relatively simple to perform	– Insensitive to presence of minor variants
– Widely available	– Interpretation requires prior knowledge of genetic determinants of resistance
– Rapid turn-around time	– Cannot predict affect on phenotype of mutational interactions
– Allow detection of sentinel mutations prior to change in phenotype	
Phenotypic Assays	
Advantages	Disadvantages
– Assess "net" affect of mutations on drug susceptibility	– Insensitive to presence of minor variants
– Provide data on cross-resistance	– Time-consuming and expensive to perform
	– Complexity of the assays limits availability outside a small number of laboratories
	– Slow turn-around time

Table 2. *Potential clinical application of drug resistance testing in HIV-1 infection.*

– Guide selection of initial antiretroviral regimen.
– Explain and manage treatment failure.
– Track prevalence of drug resistance in primary HIV-1 infection.

tion. In general, IC_{50}'s that are >4-fold higher than control isolates suggest resistance to the drug in question. Recombinant virus assays are amenable to automation, resulting in a substantial reduction in the amount of the labor required.

Advantages and disadvantages of resistance assays

Genotypic and phenotypic resistance assays provide complimentary information. Both approaches have distinct advantages and disadvantages, but all assays share certain limitations (Table 1). For example, currently available assays are relatively insensitive to the presence of minority species in the virus population. Therefore, resistant variants may not be detected by most genotypic and phenotypic assays until they constitute ≥20% of the quasispecies. In addition, technical limitations in the RT-PCR step required to amplify PR and RT genes make it difficult to obtain reliable results when the plasma HIV-1 RNA level is <1000 copies/mL.

Genotypic assays have the relative advantage of being faster and easier to perform, resulting in quicker turn-around times and lower cost than most phenotypic assays. In addition, sentinel mutations may be detectable by genotypic assay before a shift in drug susceptibility becomes apparent. Comprehensive sequencing is of great value in identifying novel mutations that confer resistance to a particular drug, but is only clinically useful once this information is known. Likewise, more selective genotypic assays presuppose that the genetic basis of resistance has been determined.

A major limitation of genotypic assays is the difficulty in predicting the consequences of mutational interactions on phenotype. This situation is illustrated best by the variable effect of the lamivudine resistance mutation (M184V) on resistance to zidovudine[23,24]. Polymorphisms and mutations at numerous loci not directly involved in drug resistance appear to modulate the expression of dual resistance to ZDV and 3TC[25,26]. Similarly, the extent of cross-resistance among drugs within a class (eg., protease inhibitors) can be difficult to predict on the basis of genotype alone.

Phenotypic assays can provide susceptibility data even if the genetic basis of resistance to a particular drug has not yet been worked out. Most clinicians are more familiar with interpreting data expressed as IC_{50}'s or IC_{90}'s as compared to genotypic data. However, specific break-points for classifying isolates as sensitive or resistant have not been established or clinically validated for most antiretroviral drugs. Phenotypic assays also have the advantage of determining the net affect of different mutations on drug susceptibility and cross-resistance. A major disadvantage of phenotypic assays is their relatively limited availability (only a few laboratories offer HIV-1 drug susceptibility testing as a commercial service) and greater cost as compared to genotypic assays. Another disadvantage of phenotypic assays is the time required to generate a result, which remains several weeks despite the advances introduced by recombinant virus assays.

Correlation of drug resistance and disease progression

Because treatment failure can occur in the absence of drug resistance, for many years it was uncertain whether drug resistance was a cause of

treatment failure or merely a consequence of disease progression and ongoing virus replication. Although several studies indicated that emergence of resistance mutations preceded a decline in CD4+ lymphocyte count, they did not determine whether presence of drug resistance contributed to disease progression independent of other prognostic markers such as CD4+ lymphocyte count, syncytium-inducing phenotype, and antiretroviral therapy[27,28]. Ultimately, studies showed that ZDV resistance confers a significantly increased risk of disease progression and death in ZDV-treated patients independently of other risk factors[29-31]. With the advent of plasma HIV-1 RNA monitoring, the relationship between drug resistance and virologic failure has become clearer. Such a relationship has been demonstrated for nearly every antiretroviral agent in clinical use.

Recent studies document the occurrence of virologic failure without emergence of PI resistance in the setting of good treatment adherence. In a study of induction/maintenance therapy (ACTG 343) patients were treated with ZDV/3TC/IDV for six months and subsequently randomized to ZDV/3TC, IDV monotherapy, or continued triple therapy[32]. No evidence of IDV resistance was found in isolates obtained at the time of first virologic failure from patients randomized to IDV monotherapy or to continued ZDV/3TC/IDV. By contrast, the M184V 3TC resistance mutation was detected in all isolates from patients failing ZDV/3TC/IDV[33]. Similar findings have since then been reported in the Trilège study[34].

Several hypotheses have been suggested to explain this finding: 1) 3TC-resistant variants arise more rapidly than IDV-resistant variants due to a greater selective advantage (in the presence of drug) conferred by the M184V mutation than any single protease mutation; 2) the maintenance regimens, and in some cases the induction regimen, lacked sufficient potency to maintain virus suppression in some patients even in the absence of PI resistance; 3) an increase in available target cells (activated CD4+ lymphocytes) led to increased virus replication and virologic breakthrough. This latter explanation is supported by results of mathematical modeling that predicted that patients with the greatest increase in CD4 lymphocyte counts during the induction phase would be at greatest risk of virologic failure during the maintenance phase[35]. These results have important implications for interpreting the results of resistance testing in patients with apparent virologic failure of PI-containing regimens.

Prognostic value of drug resistance testing

Although resistance assays can accurately identify the presence of drug-resistant viruses and help explain the causes of drug failure, scant data are available on the clinical utility of resistance testing for guiding treatment choices. Results of several retrospective studies provide preliminary data to support the use of resistance testing in certain settings. One study analyzed genotypic predictors of virologic response in patients who had failed at least one protease inhibitor-containing regimen. All patients received ritonavir (RTV) plus saquinavir (SQV) plus two nucleoside RT inhibitors (NRTIs), usually stavudine (d4T) or ZDV in combination with 3TC. Approximately 40% had a complete response, but one-third of patients failed to respond to the salvage regimen. Disease stage, CD4+ lymphocyte count and plasma HIV-1 RNA level at baseline were predictors of response ($P<0.03$), as were the number of prior NRTIs and protease inhibitors (PIs) and the duration of prior therapy ($P<0.05$). However, linear regression models also showed a strong association between the number of protease resistance mutations and antiviral response ($P<0.001$). Stepwise regression models showed that any combination of three or more mutations at codons 30, 46, 54, 82, 84, or 90 was highly predictive of treatment failure[36].

Another study demonstrated the potential clinical utility of phenotypic susceptibility testing. In this open-label study, 18 patients failing indinavir therapy received regimens that included abacavir (ABC), nelfinavir (NFV), saquinavir soft-gel capsules (SQVsgc), and nevirapine (NVP) or ABC, NFV, SQVsgc, and another NRTI. Baseline plasma HIV-1 samples were tested retrospectively by a recombinant virus assay[22]. Patients with HIV-1 that remained sensitive to two or three drugs in the rescue regimen were significantly more likely to have a virologic response as compared to those whose virus was sensitive to one or no drugs in the salvage regimen ($P=0.007$)[37].

Genotype was also predictive of the response to nelfinavir in heavily pre-treated patients who received nelfinavir through the U.S. expanded access program. Patients had received a mean of 4.5 RT inhibitors and more than 80% had previously received at least two protease inhibitors. Genotypic analysis of PR performed on HIV-1 sequences from 28 patients demonstrated PI resistance mutations at codons 48, 82, 84, and/or 90. A statistically significant relationship was observed between the number of such mutations and virologic response ($P=0.013$)[38].

Both genotype and phenotype were highly predictive of virologic outcome in a cohort of 84 NRTI-experienced patients who received treatment with ritonavir plus saquinavir. Patients with susceptible virus prior to treatment were 12 times more likely to achieve a complete virologic response (<500 copies HIV-1 RNA/mL) ($P<0.05$). Similarly, patients with genotypically wild-type isolates were four times more likely to achieve a complete virologic response[39]. Genotype and phenotype were likewise predictive of the response to abacavir in nucleoside-experienced patients[40].

Although these results are encouraging, several caveats should be kept in mind:1) because of their retrospective design, none of these studies assigned treatment on the basis of the results of resistance testing; 2) virologic response was variably defined; 3) follow-up was relatively brief (as little as 12 weeks in some cases); and 4) the sample sizes

in some studies was relatively small. Results from a number of randomized trials that prospectively test the use of resistant assays in patient management are expected soon.

Clinical uses of HIV-1 drug susceptibility testing

Assays for HIV-1 drug resistance potentially could be useful in guiding antiretroviral therapy in several ways (Table 2). These include choice of initial treatment regimen, explaining and managing treatment failure, and tracking the prevalence of primary (i.e., transmitted) drug resistance. Recent data suggest that drug resistance mutations are present in HIV-1 from 10-15% of newly infected individuals[41,42], although the prevalence of resistance in treatment-naïve individuals varies by region. Transmission of multiply resistant HIV-1 also has been documented[43]. Screening for the presence of drug resistance prior to initiating antiretroviral therapy is sensible, particular in areas of high prevalence. In the absence of antiretroviral therapy resistant variants that are transmitted could be overgrown by wild-type revertants. Resistant variants would persist as minor species but go undetected by currently available assays. Nevertheless, these variants would emerge rapidly once antiretroviral therapy is initiated. Therefore, resistance testing will be most sensitive for detecting transmission of drug-resistant HIV-1 if performed as close as possible to the time of transmission.

The emergence of HIV-1 variants with altered drug susceptibility or carrying known resistance mutations in the context of a rising plasma HIV-1 RNA level is evidence that the drug(s) in question no longer is effective. Conversely, the complete absence of drug resistance markers in a patient with a rising plasma HIV-1 RNA level suggests poor adherence or inadequate potency of the regimen. In either case, a change in regimen is warranted. In the case of poor adherence, factors such as toxicity or complexity of the regimen that complicate adherence should be identified and alternative agents selected, if available. Treatment intensification may be necessary in the case of the adherent patient unable to achieve complete viral suppression despite the absence of drug resistance.

For patients with drug-resistant HIV-1, results of resistance testing may help guide the choice of salvage therapy. Drug resistance testing is likely to be most useful in patients failing an initial treatment regimen, and in identifying the presence of resistance to drugs in the currently failing regimen. Resistance to drugs with which the patient has been treated in the past may go undetected as a result of rapid shifts in the HIV-1 quasispecies. Thus, resistance testing will be most useful in identifying drugs to be avoided, but the absence of apparent drug resistance is no guarantee of therapeutic success. Additional caveats regarding the use of drug resistance testing in this situation are discussed in the recent consensus statement on antiretroviral drug resistance testing in HIV-infected adults[15].

Conclusions

Advances in genotypic and phenotypic tests for detecting drug resistance in HIV-1 make it feasible to apply these assays in the management of antiretroviral therapy for infected individuals. Although a number of questions remain unanswered, preliminary results suggest that use of these tests may be beneficial in guiding the choice of treatment regimen in patients failing current therapy. Whether a treatment strategy that incorporates results of resistance testing leads to improved clinical outcome is the subject of several ongoing clinical trials. Uniform criteria for interpretation and quality control are needed in order to standardize assay results across the various methods being applied. Despite these concerns, it now seems likely that resistance testing will become a routine part of clinical management of HIV-1 infection in the near future.

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