

Antiretroviral Drug Resistance Testing in Adult HIV-1 Infection

Recommendations of an International AIDS Society–USA Panel

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UNTIL RECENTLY, THE COMPLEXITY of human immunodeficiency virus type 1 (HIV-1) drug-resistance testing and the limited information on its clinical utility made routine clinical application impractical. The development of automated assay technology for rapid genotypic and phenotypic characterization of HIV in plasma samples now makes it feasible to use these assays in the management of antiretroviral therapy.

Preliminary recommendations regarding resistance testing suggested that it might be helpful in limited situations.¹ An increasing number of commercial laboratories now offer pheno-

For editorial comment see p 2442.

Objective Assays for drug resistance testing in human immunodeficiency virus type 1 (HIV-1) infection are now available and clinical studies suggest that viral drug resistance is correlated with poor virologic response to new therapy. The International AIDS Society–USA sought to update prior recommendations to provide guidance for clinicians regarding indications for HIV-1 resistance testing.

Participants An International AIDS Society–USA 13-member physician panel with expertise in basic science, clinical research, and patient care involving HIV resistance to antiretroviral drugs was reconvened to provide recommendations for the clinical use of drug resistance testing.

Evidence and Consensus Process The full panel met regularly between January and October 1999. Resistance and resistance testing data appearing in the last decade through April 2000 and presentations at national and international research conferences were reviewed. Recommendations and considerations were developed by 100% group consensus, acknowledging that definitive data to support final recommendations are not yet available.

Conclusions Emerging data indicate that despite limitations, resistance testing should be incorporated into patient management in some settings. Resistance testing is recommended to help guide the choice of new regimens after treatment failure and for guiding therapy for pregnant women. It should be considered in treatment-naive patients with established infection, but cannot be firmly recommended in this setting. Testing also should be considered prior to initiating therapy in patients with acute HIV infection, although therapy should not be delayed pending the results. Expert interpretation is recommended given the complexity of results and assay limitations.

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typic and genotypic testing. In addition, kits for genotyping the protease and reverse transcriptase coding regions of the HIV *pol* gene are available for investigational use.

Evidence suggesting the clinical utility of drug resistance testing is accumulating from retrospective²⁻⁹ and prospective intervention-based¹⁰⁻¹³ studies. A significant correlation between drug resistance and virologic response to a new treatment regimen when prior therapy has failed was observed in the studies shown in TABLE 1. Moreover, the presence of drug resistance is an independent risk factor for a poor virologic response after controlling for plasma HIV RNA level, CD4⁺ cell count, and treatment history. Virologic failure is not inevitably accompanied by resistance to all drugs in a treatment regimen.¹⁴⁻¹⁷ These data provide a rationale for resistance testing in patient management.

This article provides updated recommendations for the use and interpretation of HIV-1 drug-resistance tests in the management of antiretroviral therapy, prepared by a 13-member international panel of physician experts experienced in antiretroviral drug resistance research and treatment of HIV infection. Meeting regularly between January 1999 and October 1999, the panel considered all relevant available clinical and basic science data from published reports appearing in the last decade through April 2000 and conference presentations and expert opinion in constructing the recommendations, which were developed by consensus (recommendations reflect 100% agreement by members). Specific guidelines are qualified by the descriptors *recommend* or *consider*. *Recommend* indicates that the panel concluded that there is sufficient evidence, based on assess-

ment of relevant scientific data, and including experience as reflected in expert opinion, to warrant routine use of resistance testing in a certain clinical setting; *consider*, that preliminary data are suggestive but not convincing enough to warrant recommending routine use. Although definitive data are needed for ultimate guidance, resistance testing may currently enhance clinical management in certain settings.

ANTIRETROVIRAL RESISTANCE ASSAYS

Phenotype Assays

Drug susceptibility testing measures the ability of an HIV-1 isolate to grow in the presence of drug and is performed using assays in which the degree of virus replication inhibition at different drug concentrations is assessed. Results are used to calculate the 50% or 90% inhibitory concentration (IC₅₀ or IC₉₀) of

Table 1. Selected Studies Supporting the Clinical Utility of HIV Resistance Testing of Treatment-Experienced Patients*

Study, y	Study Name or Setting (Patients, No.)	Drug Class Studied	Type of Resistance Testing	Results
Retrospective				
D'Aquila et al, ³ 1995	ACTG Protocol 116B/117 (187)	nRTI	Phenotype	Zidovudine resistance independently associated with greater risk of AIDS or death
Japour et al, ⁴ 1995	ACTG Protocol 116B/117 (188)	nRTI	Genotype	Zidovudine resistance independently associated with greater risk of AIDS or death
Miller et al, ⁵ 1998	University clinic (43)	nRTI	Phenotype and genotype	Zidovudine resistance (phenotype) most strongly associated with lack of response to zidovudine-lamivudine therapy
Harrigan et al, ⁶ 1998	Vancouver cohort (64)	PI	Phenotype and genotype	Protease phenotype and genotype associated with response to ritonavir-saquinavir therapy
Zolopa et al, ⁷ 1999	University clinic (54)	PI	Genotype	Protease genotype most strongly associated with response to ritonavir-saquinavir therapy in multivariate analysis
Deeks et al, ⁸ 1999	Urban hospital clinic (20)	nRTI-NNRTI-PI	Phenotype	The number of drugs to which virus is sensitive associated with virologic response to multidrug therapy
Lorenzi et al, ⁹ 1999	Swiss HIV Cohort Study (62)	nRTI-PI	Genotype	Resistance mutations predictive of virologic response
Prospective				
Durant et al, ¹⁰ 1999	VIRADAPT (108)	nRTI-NNRTI-PI	Genotype	Significantly better virologic response after 6 months with genotype-directed drug selection vs standard of care (no genotype data)
Clevenbergh et al, ¹¹ 1999	VIRADAPT (92)	nRTI-NNRTI-PI	Genotype	Benefits of resistance testing maintained for 1 year
Baxter et al, ¹² 1999	GART (153)	nRTI-NNRTI-PI	Genotype	Significantly better virologic response after 8 weeks with treatment regimen based on genotype and expert advice vs standard of care (no genotype data or expert advice)
Cohen et al, ¹³ 2000	VIRA 3001 (127, interim analysis)	nRTI-first PI	Phenotype	Significantly better response after 16 weeks with phenotype-directed drug selection vs standard of care (no phenotype data)

*HIV indicates human immunodeficiency virus; nRTI, nucleoside reverse transcriptase inhibitor; AIDS, acquired immunodeficiency syndrome; PI, protease inhibitor; and NNRTI, nonnucleoside reverse transcriptase inhibitor. For the ACTG 116B/117 and VIRADAPT studies, the investigations involved the same patient population.

a drug for an isolate. Results also can be presented as *fold-change* in IC_{50} or IC_{90} for each isolate as compared with a drug-susceptible control strain or prior isolate from the same patient.

Measurement of phenotypic HIV drug susceptibility for management of HIV infection is now practical via rapid, high-throughput automated assays based on recombinant DNA technology. These approaches involve amplification of plasma viral RNA, obviating the need for a virus isolate, and are capable of testing more drugs (at least 12) more rapidly than prior assays. Two recombinant virus phenotypic assays are now commercially available. Both amplify HIV protease and reverse transcriptase as a unit from plasma virus and generate a recombinant virus with other HIV genes from a laboratory construct. Using automated methods and reporter genes, drug susceptibility is then assayed with results usually available 2 to several weeks after sample receipt by the laboratory.

These 2 recombinant virus assays differ in technical aspects related to recombinant virus construction and to detection of virus replication. One assay (ViroLogic, South San Francisco, Calif) uses cloning to insert the protease and reverse transcriptase gene sequences into a modified HIV-1 vector carrying a luciferase reporter gene in place of the viral envelope gene. Replication in the presence of protease or reverse transcriptase inhibitors is monitored by quantitating expression of the luciferase gene product, and reflects the level of drug resistance in the patient isolate. The recombinant virus IC_{50} for each drug is expressed relative to a specific reference HIV strain (NL4-3). Based on replicate studies performed by the company, an increase in IC_{50} of more than 2.5-fold that of the reference strain is reported as less susceptible.¹⁸

The other commercially available assay (Virco, Mechelen, Belgium) combines patient and HIV vector sequences using in vitro recombination. Measurement of reporter gene induction by viral replication provides an assessment of the level of recombinant virus replica-

tion at a given drug concentration. The recombinant virus isolate IC_{50} for each drug is expressed relative to a reference HIV strain (HXB-2). Based on replicate studies performed by the company, an increase in IC_{50} of more than 4-fold that of the reference strain is reported as reduced susceptibility.¹⁹

Since only predominant circulating viral populations are sampled to yield IC_{50} or IC_{90} values in the susceptibility assays, minority drug-resistant species (<10%-50% of the viral population), which could contribute to drug failure or transmission of resistant HIV, may not be detected.^{20,21} Phenotypic assay results showing low-level resistance may be caused by a small change in IC_{50} of the whole virus population or a mixture of resistant and susceptible virus. Because of assay complexities and expense, it is likely that assay testing will remain centralized in large commercial laboratories.

Genotype Assays

Certain mutations in HIV-1 genes targeted by antiretroviral drugs can confer drug resistance (FIGURE). Whereas phenotype testing measures virus drug susceptibility, genotype testing detects mutations that confer phenotypic resistance. Polymerase chain reaction (PCR)-based assays for detecting mutations associated with these genes have been summarized.¹ The initial step in these assays is similar to that in the recombinant virus phenotypic assays, ie, amplification of HIV-1 sequences from plasma containing at least 500 to 1000 HIV RNA copies/mL. Depending on mutations assessed and the laboratory performing the test, genotype assays may differentiate a mutant at a level of 10% to 50% in a mixture of viruses.^{20,33}

Several companies are developing kits for HIV resistance mutation analysis, either by genotyping all relevant codons (Visible Genetics, Toronto, Ontario; ABI/Perkin Elmer, Foster City, Calif; and Affymetrix, Santa Clara, Calif) or by hybridization-based detection of selected codons (Innogenetics, Alpharetta, Ga; and Chiron, Emeryville, Calif). These kits should allow results

to be generated in several hours to a few days. The complexity of data generated from sequencing has led to different guidelines for interpretation of results, based on the best scientific evidence available. However, there may be varying interpretations regarding level of phenotypic resistance conferred by a specific mutational pattern. As new data are generated, there is a risk of providing inadequate or even incorrect interpretations.

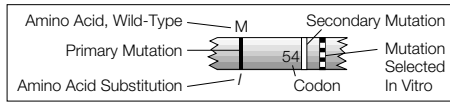
Many commercial or academic laboratories are now performing genotype assays to provide data to assist in clinical management. An evaluation of quality in 35 primarily academic genotyping laboratories worldwide in 1999 using a test panel showed that although there was improvement over 1998, the presence of HIV mutant populations was still underestimated.²¹ However, 2 recent reports showed high concordance for genotype results between 2 experienced laboratories, suggesting that operator experience may correlate with assay performance.^{7,21,34}

Unresolved Technologic Issues

Unresolved technical issues regarding genotypic or phenotypic drug resistance testing remain, including an ongoing need for adequate standardization and clinical validation. In general, patient blood samples should be collected, stored, and shipped according to laboratory recommendations regarding plasma HIV RNA assays. Quality control guidelines are needed at the laboratory level such as ascertaining sample integrity at accessioning, inclusion of quality control indicators to assess assay performance at key steps during resistance test vector assembly, and inclusion of well-characterized drug-susceptible and drug-resistant reference viruses in each batch of samples tested.

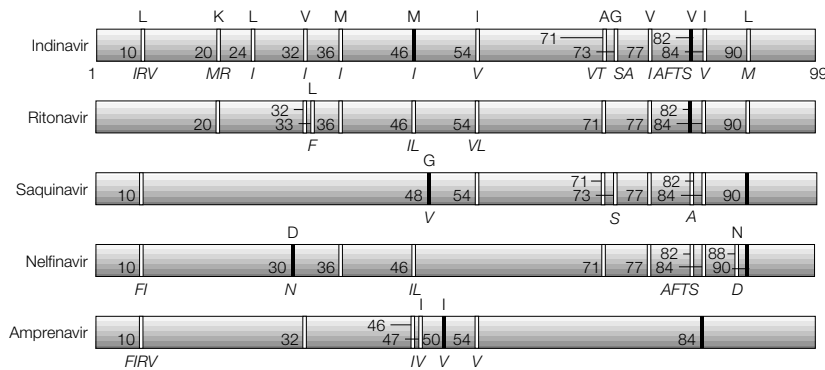
The genetically diverse HIV strains from different clades largely found outside North America may be less well amplified for phenotyping or genotyping vs the more common clade B viruses in Europe and North America. Variable PCR amplification rates have been described in Europe where non-B

Figure. Most Common Mutations in HIV-1 Genes Conferring Drug Resistance



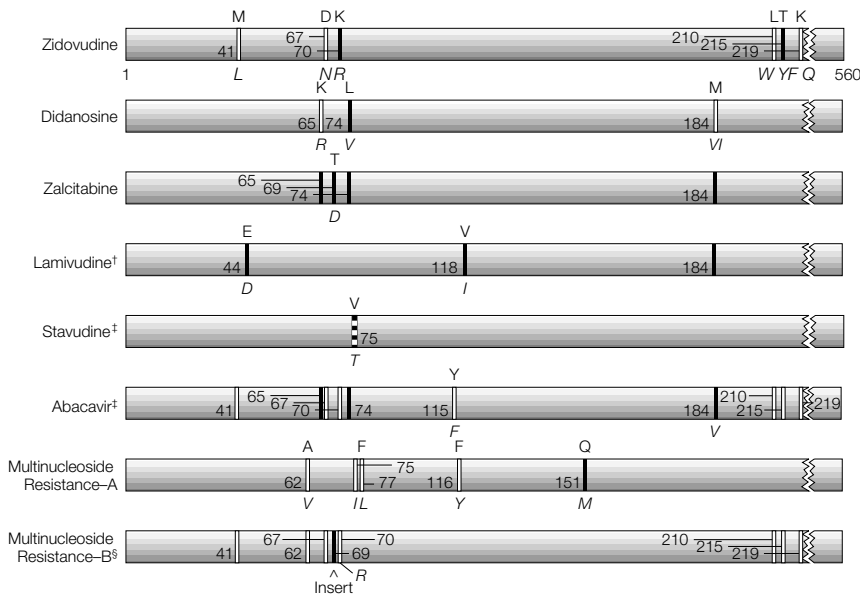
MUTATIONS IN THE PROTEASE GENE SELECTED BY PROTEASE INHIBITORS

Protease Inhibitors*

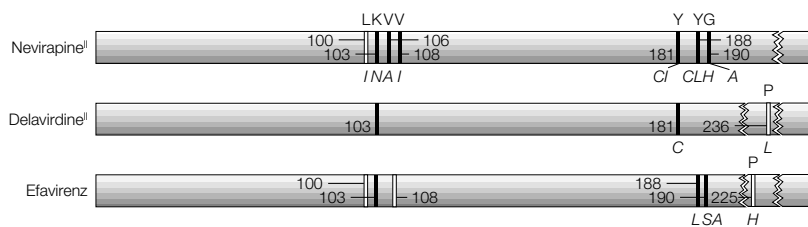


MUTATIONS IN THE REVERSE TRANSCRIPTASE (RT) GENE SELECTED BY RT INHIBITORS

Nucleoside RT Inhibitors



Nonnucleoside RT Inhibitors



For each amino acid residue, the letter above indicates the amino acid associated with wild-type virus; the italicized letters below, substitutions that confer viral resistance. Primary mutations (black bars) generally cause decreased inhibitor binding and are the first mutations selected. Secondary mutations (white bars) also contribute to drug resistance and should be considered as evidence of resistance, although they may have less direct effect on inhibitor binding in vitro than primary mutations. The mutation selected in vitro (black-and-white bar) is rarely seen in patients having treatment failure. For indinavir, the mutations listed as primary may not be the first mutations selected, but they are selected in most patient isolates in combination with other mutations. For zalcitabine, all mutations are listed as primary because of inadequate clinical data to determine the most frequent initial mutation. Amino acid abbreviations are: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Multinucleoside resistance mutational patterns A and B each cause resistance to zidovudine, stavudine, lamivudine, didanosine, zalcitabine, and abacavir. Current listings are also available at <http://www.iasusa.org/>.

*Mutations selected by protease inhibitors in *gag* cleavage sites are not listed because their contribution to resistance is not fully defined.

† A preliminary report identifies mutations E44D and V118I as conferring moderately reduced (about 10-fold) susceptibility to lamivudine with uncertain clinical significance.²² This contrasts with the greater than 100-fold reduced susceptibility to lamivudine conferred by M184V or M184I, which is associated with virologic rebound.¹⁴⁻¹⁶

‡ The mutations listed for zidovudine above contribute to reduced susceptibility to abacavir in vitro and in vivo and are listed as secondary, even though they may be present before abacavir is introduced.^{23,24} They have also been reported to be uncommonly selected by stavudine plus didanosine even in the absence of prior zidovudine exposure.²⁵⁻²⁷ Phenotypic resistance of these mutations to stavudine or didanosine in vitro was not identified. The clinical significance of these mutations and of V75T on in vivo response to stavudine is not known.

§ Several insertions of 2 amino acids have been reported following T69S (or rarely T69A), including Ser-Ser; Ser-Gly; Ser-Ala; Glu-Ala; and Thr-Ser.^{28,29}

|| For nevirapine or delavirdine, each mutation can occur as an initial or subsequent mutation and affect inhibitor binding.³⁰⁻³²

subtype prevalence is higher than in North America.³⁵ However, studies have reported amplification and successful resistance analysis for all group M (A-H) HIV-1 subtypes.^{18,36}

CLINICAL TRIAL DATA ON RESISTANCE TESTING

Prospective Studies

Two prospective randomized trials examining the benefits of genotype testing in patient management have been reported. The VIRADAPT study, which was conducted in Europe,^{10,11} prospectively studied 108 patients with plasma viral loads above 10 000 copies/mL and protease inhibitor (PI) experience for at least 3 months. Patients were randomly assigned to have their therapy changed according to the usual standard of care or with access to genotype testing results. During the 6-month controlled portion of the study, changes in viral load (-1.15 vs -0.67 log₁₀ copies/mL) and proportion of patients with HIV RNA values below 200 copies/mL (32% vs 14%) were better in the group managed using genotype data. After 6 months, resistance test information was provided for all patients with detectable viral loads. After another 6 months, with 92 of 108 patients still available for follow-up, the 2 groups had similar virologic responses (-1.15 vs -0.98 log₁₀ copies/mL change in viral load from baseline and 30% of patients with values <200 copies/mL in both groups).¹¹ Thus, the benefits of resistance testing were maintained for 1 year and were still present even if strategy implementation was delayed for 6 months.

In the GART (Genotypic Antiviral Resistance Testing) study, conducted in the United States,¹² changes in therapy for the genotype-assigned group were recommended by an expert panel; no such recommendations were made for the control group. Study design and size were similar to the VIRADAPT protocol: there were 153 patients with a median viral load of 26 000 to 29 000 copies/mL (depending on the group), half of whom had experienced virologic breakthrough with their first antiretroviral treatment. Resistance testing results influenced choice

of subsequent therapy, leading to a more favorable virologic response (-1.17 vs -0.62 log₁₀ copies/mL plasma for 4-8 weeks) than with the usual standard of care. This difference was likely due to the broader use of drugs expected to be active against virus in the group that had resistance test results and expert advice. However, sustained virologic responses were not universally achieved, with viral loads below detection in only one half of the resistance group at 8 weeks, falling to one third at 12 weeks. Of note, half of the patients with genotyping did not receive the therapy recommended by the expert panel.

In a prospective, randomized trial, treatment-experienced patients fared better when antiretroviral therapy was chosen in conjunction with the availability of phenotypic test results than when treatment decisions were made without having this information.¹³

Retrospective Trials

Data regarding clinical utility of drug resistance assays are also available from retrospective studies (Table 1). Decreased zidovudine susceptibility in nucleoside reverse transcriptase inhibitor (nRTI)-treated individuals was significantly correlated with a poor clinical outcome.³⁻⁵ Other studies also found strong correlation between genotypic or phenotypic evidence of resistance and viral load in treatment-experienced patients.^{6-8,24,37-42}

Collectively, the prospective and retrospective studies show that the presence of drug susceptibility is predictive of treatment response and adds to knowledge obtained from either drug history or viral load measurements alone.^{7,42} However, a good response is not uniformly seen when virus is sensitive at baseline. This may reflect the presence of minority drug-resistant viral subpopulations or factors such as suboptimal drug levels and poor adherence.¹

CLINICAL MANAGEMENT

Although available data support a role for HIV drug resistance testing in selecting drugs in many clinical situa-

tions, these test results should not be used as the principal criterion for decisions on initiating or changing antiretroviral therapy. Such decisions should be based primarily on plasma viral load.

Several factors in addition to resistance testing also must be considered in choosing drugs for a new regimen after the prior regimen has failed. Drug treatment history, viral load, medication tolerance, future adherence likelihood, and concomitant medical conditions or medications are important considerations. Blood and cellular levels of drug are also crucial and may vary among persons based on drug-drug interactions, adherence, or genetically determined differences in absorption or metabolism. In the VIRADAPT study, maximal virologic benefit was observed in patients receiving resistance genotyping in whom optimal PI trough levels in the blood were maintained at several time points.⁴³ However, therapeutic drug monitoring is not yet clinically validated in large studies or widely available.

Drug selection pressure at the time of resistance testing may also affect the result. False-negative results may occur if blood is drawn for resistance testing after therapy is changed or stopped because susceptible variants outgrow the resistant mutants, eg, reverse transcriptase M184V predominance is rapidly lost after lamivudine withdrawal.^{44,45} Thus, blood should be drawn for resistance testing before the failing drug regimen is stopped. Whether susceptible virus emergence after drug interruption improves treatment options is an important area for further investigation, but studies have indicated that an inducible archive of virus persists in resting memory CD4⁺ cells harboring latent proviral genomes.^{46,47}

Interactions among resistance mutations may also complicate interpretation of genotype results, eg, the M184V mutant may partially "reverse" phenotypic zidovudine resistance conferred by mutations in codons 41, 67, 70, 210, 215, and 219.⁴⁸ However, this effect is limited by development of other mutations that restore zidovudine resis-

tance, thus leading to zidovudine and lamivudine coresistance.⁴⁹⁻⁵¹ This complexity can lead to varying interpretations from different laboratories.

Different interpretations regarding PI resistance and cross-resistance now occur because not all of the many possible effects of combinations of mutations and polymorphisms have been elucidated. However, the existence of 2 or more key mutations (eg, D30N; G48V; I50V; V82A, F, T, or S; I84V; L90M) is likely to confer broad cross-resistance to most currently available PIs.^{9,52}

Rules for judging the degree of resistance of specific sets of mutations have been clinically validated to a limited extent.¹² However, the many possible permutations of multiple mutations may limit such a rules-based approach to interpretation of genotyping results. Frequent updating is needed given the continually accumulating data on resistance mutations and the availability of new drugs. Another approach is to report only the phenotype that is expected based on the identified genotype(s) (*virtual* phenotype). This approach is based on a proprietary database of mutational patterns and their phenotypes⁵³ but may lack usefulness regarding genotypes not previously identified (eg, selected by a new drug or drug combination). Databases directly linking mutational patterns or phenotypes to clinical responses are beginning to be developed.

Phenotypic data may appear easier to interpret, but there are currently no well-defined, clinically-validated cut-off points that correlate virologic response with IC₅₀ values. The trough blood level of drug needed to suppress replication *in vivo* is not known for any drug, and it likely differs for each drug. Trough levels for some drugs, including PIs, have been correlated with virologic responses.^{43,54} Interpretation of drug levels for nRTIs is more complex because intracellular concentrations rather than serum or plasma levels are important. The degree of resistance (fold-increase in IC₅₀ relative to wild-type) varies for mutants selected by different drugs. In general, it is rea-

sonable to expect that the higher the magnitude of resistance measured *in vitro*, the less activity a drug will have against that virus *in vivo*. However, even low-level resistance may predict poor response to a drug. Neither genotypic nor phenotypic tests assess possible cellular mechanisms of drug resistance⁵⁵⁻⁵⁸ or the relative replicative capacity of resistant mutant virus.

Given these complexities, expert interpretation of both genotypic and phenotypic resistance testing results is recommended, even if a test report lists drugs likely to be active. Standardization of interpretation of mutational patterns would be helpful for the nonexpert clinician. However, assessment of the many clinical and biological factors that affect interpretation of results may still require input from a clinician or virologist with antiretroviral therapy experience and knowledge of drug resistance patterns.

RECOMMENDATIONS

Primary Infection

In 1998, reports appeared from San Francisco, Calif, and Geneva, Switzerland, of sexual transmission of PI-resistant HIV-1 mutants and mutants resistant to drugs from 2 or 3 classes.^{59,60} The possibility that multidrug-resistant virus was geographically limited was excluded by subsequent surveys in other cities in North America and Europe.⁶¹⁻⁶⁵ In the reported cases of transmitted drug-resistant mutant HIV, history of sexual or blood exposure to HIV-infected patients in whom therapy was failing was often not obtained. A slower than expected decline in plasma HIV RNA with a potent combination regimen was common, suggesting the possibility of primary drug resistance during initial treatment of a drug-adherent patient and serving as an indication for resistance testing.

A surprisingly high proportion of recently infected subjects has circulating virus with some degree of reduced susceptibility (<10-fold) to nonnucleoside reverse transcriptase inhibitors (NNRTIs), ritonavir, and nelfinavir.^{61,62,66} This is probably because virus never exposed to these drugs has previously unrecognized polymor-

phisms with some effect on drug susceptibility. Virus selected during NNRTI failure generally has a larger reduction in susceptibility (>50-fold). Thus, it is possible that virus with low-level resistance was not transmitted from patients in whom therapy was failing. One preliminary report found that 20% of polymorphisms are associated with minimal resistance to any NNRTI, but only about 2% involved a diminished initial response to efavirenz-containing regimens and no significant difference in response vs the absence of polymorphisms.⁶⁷ Such baseline-reduced susceptibility was also found to be of little significance in the response to a potent nevirapine-containing regimen.³⁰ Further study is needed, including more extensive characterization of NNRTI-resistant genotypes and detailed clinical evaluation of virologic responses to regimens containing drugs to which there are low levels of reduced susceptibility.

There is evidence that zidovudine- and lamivudine-resistant mutants are being sexually transmitted more commonly.⁶⁸ In studies from North America,⁶⁹ Great Britain,⁷⁰ Italy,⁷¹ Spain,^{72,73} Luxembourg,⁷⁴ and Brazil,^{75,76} up to a 20% prevalence of zidovudine resistance was reported; lamivudine resistance was also noted. Preexisting zidovudine resistance does not preclude prolonged viral load suppression during initial triple combination regimens including zidovudine, lamivudine, and a PI.^{77,78}

Drug resistance testing should thus be considered for all patients presenting with symptomatic acute HIV infection or other evidence documenting infection within several months of presentation, particularly if the source patient was taking antiretroviral drugs. The goal of therapy in this setting is to suppress virus replication quickly to preserve HIV-specific CD4⁺ cell helper responses and alter long-term outcome. The ability of therapy to achieve such a goal is under investigation, although preliminary data appear promising.^{79,80} When therapy is initiated in the setting of recent infection it should

be done before resistance test results are available. Regimens can then be adjusted within a few weeks if resistance to any drug is detected. Suboptimal viral load response to an initial regimen (eg, failure to attain viral load below detectable levels by 16 to 24 weeks of therapy) should also prompt consideration of resistance testing. Adherence should be evaluated in such patients. Ongoing epidemiologic surveillance for PI-resistant and multidrug-resistant virus in patients presenting with acute or early HIV infection is essential.

Established Infection

The prevalence of drug-resistant virus in patients with established HIV infection before starting an initial regimen has been assessed.^{66,81-83} These studies had relatively small sample sizes and used different definitions for cut-off values in phenotypic assays or for key mutations associated with resistance in genotypic assays. One study has reported a lower prevalence of drug-resistant variants in established infection than in recent infection in patients in the same geographic regions,⁸² but another did not confirm this observation.⁸¹ Even if resistant mutants are initially present, in the absence of drug selection, wild-type viruses are expected to eventually emerge in an infected person because of better replicative capacity and may become predominant.⁸⁴ Drug-resistant variants, persisting as minority species, might not be detected by current assays, but could emerge rapidly when antiretroviral therapy is initiated.

Prior to starting treatment in patients with established infection, the use of resistance testing should be considered, particularly in areas where the local prevalence of primary drug resistance is appreciable.

Use of Resistance Testing When Changing Therapy

There is evidence that resistance testing in the setting of virologic failure may be useful in selecting an alternative antiretroviral regimen. Retrospective studies have shown that baseline genotypic profiles or phenotypic susceptibilities can

be predictive of virologic response.³⁻⁹ Prospective studies of genotyping (combined with expert interpretation in 1 study) resulted in improved short-term virologic responses.¹⁰⁻¹³ Also, the finding that genotyping combined with optimal PI drug levels resulted in a greater decline in plasma HIV RNA levels illustrates the importance of pharmacokinetic considerations in successful antiretroviral therapy.⁴³

Resistance testing may provide additional insights into drug-host interactions. For example, the determination of the presence of wild-type virus in a patient in whom a current regimen is failing may indicate problems with drug adherence, drug absorption, or drug-drug interactions, leading to lack of selective pressure on the virus. Of note is the finding that virologic failure of an initial PI/dual-nRTI regimen is commonly associated with mutation(s) associated only with the nRTI component of the regimen. Early virologic failure of indinavir-zidovudine-lamivudine or amprenavir-zidovudine-lamivudine is associated initially with the lamivudine-associated M184V mutation in most patients.¹⁴⁻¹⁶ This suggests that differential "genetic barriers" to resistance may determine the temporal pattern of HIV-1 drug resistance and that it may not always be necessary to change all the drugs in a failing regimen. Continuation of 1 or more components, combined with other new drugs, may prove to be a successful strategy in certain settings. However, this approach has not yet been clinically validated, and at present, single-drug substitutions should be avoided and current therapy guidelines followed.^{85,86} Clinicians must be cautious about the potential existence of undetected minority resistant subspecies that could emerge quickly on a non-suppressive regimen. Even if the entire regimen is changed, the knowledge gained from resistance testing may prove useful when a subsequent regimen fails, fewer options are available, and the issue of recycling of drugs arises.

First Regimen Failure. Drug resistance testing is recommended in the setting of virologic failure on an initial regi-

men once poor regimen adherence and pharmacokinetic reasons for failure have been reasonably excluded. The specimens for resistance testing should be obtained before discontinuing the failing regimen to maintain selective pressure on the viral populations. Resistance testing may not be necessary for all first-regimen failures, particularly if the clinician is confident about the patient's drug history, and an alternative regimen is introduced early after failure and at a lower, rather than a higher, viral load. Assuming a high degree of adherence and adequate drug absorption, the settings in which resistance testing is likely to prove helpful are (1) early after therapy initiation if only a minimal plasma HIV RNA decline occurs over the first 4 to 12 weeks, suggesting suboptimal treatment response; (2) during early virologic breakthrough (ie, any confirmed detectable plasma HIV RNA after levels below detection have been attained); (3) during more prolonged virologic replication in which more extensive resistance might be suspected; and (4) prior to therapy institution to provide a baseline for longitudinal testing likely to become a more common component of patient monitoring. In the first setting, resistance testing might show that a drug-resistant strain was acquired and is now reemerging following therapy initiation. In the second setting, it should be noted that resistant variants may emerge at viral load levels as low as 20 to 50 copies/mL.⁸⁷

Multiple Regimen Failures. Drug resistance testing is recommended to help guide management^{85,86} after numerous regimens have failed. Retrospective studies have shown that resistance is strongly predictive of lack of response to therapy. Given the limited drug options available to persons with multiple regimen failures, incorporating resistance testing into patient management should provide physicians and patients with data that will permit the most effective use of approved or investigational drugs and may help to avoid the inconvenience, cost, and toxicity of drugs in a regimen with little likelihood of conferring benefit.

Table 2. Summary of Recommendations for Resistance Testing, Based on Available Data and Expert Opinion*

Clinical Characteristics	Recommendation	Rationale
Primary HIV infection	Consider testing†	Detect transmission of drug-resistant virus; modify therapy to optimize response and maintain HIV-specific immune responses
Established HIV infection‡	Consider testing	Detect prior transmission of drug-resistant HIV although this may not always be possible with current tests
First regimen failure§	Recommend testing	Document drug(s) to which there is resistance
Multiple regimen failures§	Recommend testing	Optimize the number of active drugs in the next regimen; exclude drugs to which response is unlikely
Pregnancy	Recommend testing	Optimize maternal treatment and prophylaxis for the neonate

*HIV indicates human immunodeficiency virus.

†Therapy should not be delayed while waiting for resistance testing results.

‡In untreated established infection, wild-type virus may replace drug-resistant quasispecies over time. Drug resistance results should thus be interpreted with caution.

§The results are most reliable for drugs that are being taken by the patient at the time of testing.

Pregnancy

Drug-resistant virus may be transmitted heterosexually either before or during pregnancy.⁸⁸ In current guidelines it is recommended that zidovudine be included as a component of all regimens designed to prevent mother-to-child transmission.^{89,90} These recommendations were based on studies of zidovudine use in treatment-naïve women; current treatment guidelines discourage withholding combination antiretroviral therapy from pregnant women if otherwise indicated.^{85,89,91} Nevirapine alone may also be useful in this setting, although a study of nevirapine prophylaxis showed that the K103M mutation could be selected in Ugandan women following a single dose of this drug.⁹² Vertical transmission of multi-drug-resistant HIV-1 has been reported with incomplete suppression of maternal plasma viremia and extensive prior antiretroviral exposure.⁹³ In this case, the mother had zidovudine-resistant virus prior to delivery, suggesting that zidovudine was unlikely to prevent transmission. If viremia is present in the mother, it is recommended that resistance testing be performed on maternal virus, particularly when there has been prior antiretroviral exposure or when prevalence of resistant virus in the community is high. Optimally active drugs thus can be identified for the preg-

nant woman and regimen adjustments made to maximize chances of preventing vertical transmission.

SUMMARY AND FUTURE GOALS

Despite uncertainties, available evidence suggests that resistance testing can aid in patient management by improving at least short-term virologic outcome while avoiding use of costly and potentially toxic drugs with little promise of benefit. Specific recommendations are given in TABLE 2. Important issues that remain include long-term benefit in terms of virologic outcome, situations in which genotypic or phenotypic testing is preferred, sensitivity of newer testing methods for detecting minority viral subspecies, how to assess resistance testing results of drugs as components of multidrug combinations, applicability of resistance testing to the drug interruption approach to managing antiretroviral failure, and cost-effectiveness of resistance testing as a routine part of patient monitoring. Resistance testing is currently costly (charges ranging from \$400-\$550 for sequencing of protease and reverse transcriptase to \$700-\$900 for phenotypic testing in the United States). However, proper implementation may actually reduce costs by prompting more appropriate choices in

therapy.⁹⁴ Until studies to address these and other issues are performed, decisions regarding the appropriate role of resistance testing in clinical management will depend on judgment and consensus, as incorporated in these guidelines.

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