Antiretroviral Drug Resistance Testing in Adults With HIV Infection

Implications for Clinical Management

Martin S. Hirsch, MD; Brian Conway, MD; Richard T. D'Aquila, MD; Victoria A. Johnson, MD; Françoise Brun-Vézinet, MD; Bonaventura Clotet, MD, PhD; Lisa M. Demeter, MD; Scott M. Hammer, MD; Donna M. Jacobsen; Daniel R. Kuritzkes, MD; Clive Loveday, MD, PhD; John W. Mellors, MD; Stefano Vella, MD; Douglas D. Richman, MD; for the International AIDS Society–USA Panel

Objectives.—To review current knowledge of the biology and clinical implications of human immunodeficiency virus (HIV) resistance to antiretroviral drugs, describe assays for measuring resistance, and assess their use in clinical practice.

Participants.—The International AIDS Society-USA assembled a panel of 13 physicians with expertise in basic science, clinical research, and patient care relevant to HIV resistance to antiretroviral drugs.

Evidence.—We reviewed available data from published reports and presented at national and international research conferences. Basic science research, clinical trial results, and expert opinions were used to form the basis of this report. Data on methods for and characteristics of specific genotypic and phenotypic assays were obtained from manufacturers and service providers.

Consensus Process.—The panel met regularly between October 1997 and April 1998. Panel subgroups developed and discussed different sections of the report before discussing them with the entire panel. Conclusions and suggested approaches to the use of resistance testing were determined by group consensus.

Conclusions.—Plasma HIV RNA level and CD4$^+$ cell count are the primary values that should be used to guide the initiation of antiretroviral therapy and subsequent changes in therapy. Possible causes of treatment failure other than development of drug resistance that should be considered are adherence, drug potency, and pharmacokinetic issues. Genotypic and phenotypic testing for HIV resistance to antiretroviral drugs may prove useful for individual patient management. Assays under development need validation, standardization, and a clearer definition of their clinical roles. Possible current roles of resistance testing for choosing an initial regimen or changing antiretroviral therapy, as well as possible implications of the presence or absence of phenotypic resistance and genotypic changes, are discussed.

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From Harvard Medical School, Boston, Mass (Drs Hirsch, Hammer, and D'Aquila); St. Paul's Hospital, Vancouver, British Columbia (Dr Conway); University of Alabama at Birmingham School of Medicine and Birmingham Veterans Affairs Medical Center, Birmingham (Dr Johnson); Hôpital Bichat-Claude Bernard, Paris, France (Dr Brun-Vézinet); Fundacio irsaCaixa and HIV Unit, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain (Dr Clotet); University of Rochester, Rochester, NY (Dr Demeter); The International AIDS Society–USA, San Francisco, Calif (Ms Jacobsen); University of Colorado Health Sciences Center, Denver (Dr Kuritzkes); The Royal Free Hospital Medical School, London, England (Dr Loveday); University of Pittsburgh Medical Center, Pittsburgh, Pa (Dr Mellors); Istituto Superiore di Sanità, Rome, Italy (Dr Vella); and University of California San Diego and San Diego Veterans Affairs Medical Center, San Diego (Dr Richman).

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Corresponding author: Martin S. Hirsch, MD, Massachusetts General Hospital, Infectious Disease Unit, Grey 5, Boston, MA 02114 (e-mail: hirsch.martin@mgh.harvard.edu).

Reprints: International AIDS Society–USA, 1001-B O'Reilly Ave, Box 29916, San Francisco, CA 94129.

DEVELOPMENT of viral resistance to antiretroviral drugs used for treatment of human immunodeficiency virus (HIV) infection is an important cause of treatment failure[1-7] and limits options for alternative antiretroviral regimens. Prevention, characterization, and clinical management of such resistance is receiving increasing attention. The International AIDS Society–USA assembled a panel to review for clinicians the biologic principles underlying HIV drug resistance, phenotypic and genotypic resistance assays either available or under development, and approaches using viral resistance testing for patient care. The panel consists of persons with expertise in HIV antiretroviral drug resistance and in care of patients with HIV infection. Panel members reviewed relevant clinical and basic science data and evaluated expert opinion. Recommendations were developed by consensus.

See also pp 1977 and 2000.

BACKGROUND

The virus population in a person infected with an RNA virus (eg, HIV-1, hepatitis C virus) has been termed a quasi species,[8] which refers to the existence of genetically distinct viral variants that evolve from the initial virus inoculum. The variants are generated because DNA proofreading mechanisms that preserve the genetic composition of organisms with double-stranded DNA genomes do not exist for RNA viruses.

Thus, as single-stranded RNA viruses replicate, each newly copied genome differs from the parental virus on average by a single nucleotide.[9,10]

Viral polymorphisms (genetic variants with apparently equivalent fitness [replication capacity]) are commonly seen in virus populations in infected persons. Nucleotide differences may be "neutral" (no impact on fitness), be deleterious (variants replicating less well or not at all), or confer replicative advantage if selective pressures such as immune responses or drug treatments change. These possibilities illustrate the survival strategy of organisms with high mutation rates that provides a large pool of genetic variants able to adapt rapidly to changing selective pressures.[11,12]

An estimated 10 billion (10^{10}) HIV-1 virions are produced daily in established HIV infection.[13] If each contains on average 1 mutation per 9200-nucleotide genome, replication-competent virus with every possible single drug–resistance mutation is likely to be generated daily. Double mutants are less likely, and the probability of 3 or more drug-resistance mutations in the same genome is very low.[14]

These estimates are supported by observations in infected persons. Virus or HIV-1 RNA with single drug–resistance mutations have been isolated from treatment-naive patients or those infected before antiretroviral drug availability.[15-19] Mathematical modeling of rate of resistance emergence after nevirapine treatment in previously untreated persons permitted estimates of plasma prevalence of HIV-1 variants with nevirapine-resistance mutations before treatment. About 1 in 1000 copies/mL of plasma HIV-1 RNA contains the tyrosine-to-cysteine mutation at amino acid residue 181 (ie, the Y181C mutation) of the reverse transcriptase conferring nevirapine resistance.[2]

When antiviral drug selective pressure is applied to viral quasi species in an infected person, preexisting minor viral species resistant to that drug rapidly become predominant and are selected as the most fit species in the presence of drug. For some antiretroviral drugs such as lamivudine and certain nonnucleoside reverse transcriptase inhibitors (NNRTIs; eg, nevirapine), a single mutation can confer high-level resistance. When these drugs are given in combinations only partially suppressing virus replication, drug-resistant mutants predominate within weeks.[2,20,21]

For some other drugs, such as zidovudine and certain protease inhibitors, high-level resistance requires accumulation of 3 or more resistance mutations in a single viral genome.[22-24] These highly resistant variants emerge more slowly, requiring months to predominate during less than maximum viral suppression,[23-26] supporting the prediction that genetic variants with multiple mutations are present at much lower levels than those with single mutations in untreated patients. Development of high-level resistance to these drugs requires persistent viral replication and selective drug pressure. Persistent viral replication permits further viral evolution leading to high-level drug resistance by cumulative mutation acquisition.

What is known about development of resistance with potent combination therapy? First, the higher the trough plasma concentrations of a protease inhibitor (eg, ritonavir), the more slowly resistance mutations emerge.[24] Second, the lower the nadir of plasma HIV-1 RNA levels, the longer it takes for drug failure to occur.[27] In patients with suppression of plasma HIV-1 RNA to below 50 copies/mL for 1 year, no resistance mutations or other evidence of virus evolution was discerned, even though HIV-1 RNA and DNA and replication-competent virus persisted.[28-30] Conversely, patients with detectable HIV-1 RNA levels had ongoing virus replication and evidence of evolution.

Several practical inferences can be derived from these principles (Table 1). First, drugs for which only a single mutation is required for high-level resistance, eg, lamivudine and nevirapine, should be reserved for use with other drugs in regimens designed to maximally suppress virus replication. Use in less suppressive regimens will select for high-level resistance more quickly.[1-3] Second, combination regimens should be designed to confer potency needed to suppress maximally preexisting genetic variants and prevent replication. Regimens must establish a "genetic barrier" by suppressing minor populations with 1 or 2 mutations that could emerge with individual regimen components, permitting cumulative mutation acquisition. This requirement is more formidable in previously treated patients because prior treatment may have established a genetic archive of drug-resistant virus within peripheral blood mononuclear cells (PBMCs) and other tissue reservoirs.[5]

Table 1.—Practical Implications of the Biology of HIV-1 Drug Resistance*

- Genetic variants of HIV with any single and probably many double mutations (although less likely) preexist in all patients before treatment is started. Thus, partially suppressive regimens containing lamivudine or certain NNRTIs rapidly fail because of breakthrough replication of preexisting resistant variants.
- Genetic variants with 3 or more resistance mutations probably exist rarely, if at all, in untreated patients. Thus, potent combination regimens that require many resistance mutations for viral escape are recommended.
- Preventing cumulative acquisition of resistance mutations requires potent combination regimens that suppress virus replication to below levels of detection of the most sensitive assays available (about 50 copies/mL).
- Complex mixtures of genetic variants exist in all patients. Assays for drug resistance, both genotypic and phenotypic, may provide information only on the predominant circulating variants and may miss minor variants.
- Prior treatment may select for resistant mutants that persist in lymphoid tissues but are no longer predominant or even detectable in the absence of drug pressure. Retreatment with the same drug may not be effective because of rapid selection of these mutants. Thus, genotypic and phenotypic assays must be interpreted in the context of drug treatment history.

*HIV indicates human immunodeficiency virus; NNRTI, nonnucleoside reverse transcriptase inhibitor.

ANTIRETROVIRAL RESISTANCE GENOTYPES

Resistance Mutations Selected During Antiretroviral Treatment

Figure 1 lists common mutations selected by protease inhibitors (A) as well as nucleoside reverse transcriptase inhibitors (nRTIs) and NNRTIs (B), identified largely in studies of monotherapy.[31] Current listings are available at http://hiv-web.lanl.gov/ or at http://www.viral-resistance.com. In general, there is good concordance between mutations seen in laboratory selection experiments and those in clinical isolates from patients with failing treatment. However, some in vitro mutations are not found in patients in whom that particular drug has failed, eg, the stavudine-selected V75T mutation[32] and delavirdine-selected P236L mutation[33] conferred resistance during in vitro virus passage experiments, but were rarely identified in patients in whom the drugs failed.[34,35]

Some mutations selected by antiretroviral drugs directly affect viral enzymes and cause resistance via decreased drug binding, whereas others have indirect effects.[36-42] It is useful to categorize resistance mutations as primary or secondary (Figure 1). Primary mutations are generally selected early in the process of resistance mutation accumulation, are relatively inhibitor specific, and may have a discernible effect on virus drug susceptibility. Secondary mutations accumulate in viral genomes already containing 1 or more primary mutations. Many secondary mutations alone have little or no discernible effect on resistance magnitude but may be selected because they improve viral fitness rather than decrease drug binding to target enzymes.

The distinction between primary and secondary mutations depicted in Figure 1, A, may help explain protease inhibitor cross-resistance. There seems to be little overlap in primary mutations selected by different protease inhibitors (eg, saquinavir-selected L90M and G48V; nelfinavir–selected D30N; and amprenavir-selected I50V). By themselves, these primary mu-

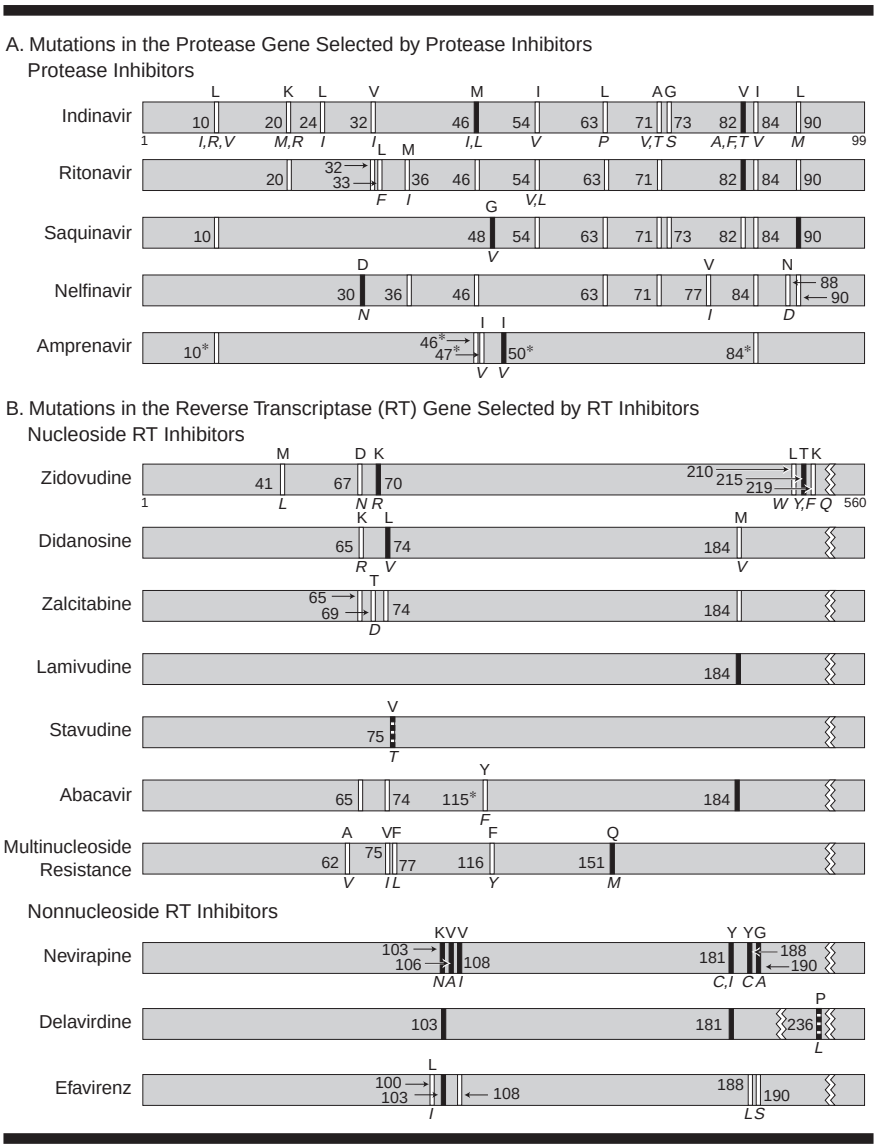

Figure 1.—The most common human immunodeficiency virus 1 mutations selected by protease inhibitors (A), and nucleoside and nonnucleoside reverse transcriptase inhibitors (B).[31] For each amino acid residue listed, the letter above the listing indicates the amino acid associated with the wild-type virus. The italicized letter below the residue indicates the substitution that confers drug resistance. The drug-selected mutations are categorized as "primary" (black bars) or "secondary" (white bars). (The black-and-white bar indicates a mutation selected in vitro, but rarely seen in specimens from patients in whom therapy fails.) Primary mutations generally decrease inhibitor binding and are the first mutations selected. For indinavir, the mutations listed as primary may not be the first mutations selected, but they are selected in most patients' isolates in combination with other mutations. For zalcitabine, all mutations are listed as secondary because of inadequate clinical data to determine a common initial mutation. For nevirapine and delavirdine, each mutation can occur as either an initial or subsequent mutation and affect inhibitor binding. The asterisk indicates that the mutation has been reported in vitro, but relevance for clinical drug failure is uncertain. Amino acid abbreviations are as follows: 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 viruses have phenotypic resistance to most nucleoside reverse transcriptase inhibitors. Current listings are also available at http://hiv-web.lanl.gov/ or at http://www.viral-resistance.com.

tations may not cause cross-resistance to other protease inhibitors. However, there is an overlapping spectrum of secondary mutations in the protease gene selected by all protease inhibitors (Figure 1, A). Many of the secondary changes are compensatory, improving fitness of virus containing primary mutations without actually increasing inhibitor resistance.[36,37] The mutations may improve enzymatic function by altering protease catalytic activity or by affecting protease substrates (eg, making sites in gag or other viral precursor polypeptides more easily cleavable).

The nRTIs can select for a single primary mutation (eg, lamivudine), any one of a few primary mutations (eg, didanosine and zalcitabine), or an accumulation of primary and secondary mutations (eg, zidovudine) (Figure 1, B). Secondary mutations that compensate for replication impairment caused by primary resistance mutations are also selected by reverse transcriptase inhibitors.[38-42]

Cross-resistance among nRTIs can be mediated by inhibitor-specific mutations and less specific secondary mutations, especially among drugs that bind to similar or adjacent viral target residues (evident for didanosine and zalcitabine, which select for similar mutations [Figure 1, B]). Similarly, the primary mutation commonly selected by lamivudine confers high-level phenotypic resistance to this drug as well as low-level phenotypic resistance to didanosine, zalcitabine, and abacavir in vitro. The clinical significance of cross-resistance among these drugs has not been determined.

Mutations selected by drug combinations may differ from those expected based on monotherapy experience.[1] A unique mutation pattern in the reverse transcriptase gene that confers broad cross-resistance to all nRTIs includes the Q151M mutation associated with 3 or 4 additional mutations (Figure 1, B), occasionally seen in patients with long-term exposure to nRTIs,[43] and first described in association with exposure to zidovudine-didanosine combination therapy[44,45] or weekly alternating zidovudine-zalcitabine monotherapy supplemented briefly with didanosine.[45]

The NNRTIs (nevirapine, delavirdine, and efavirenz) select for mutations in 2 different reverse transcriptase regions (codons 98 to 108 and 179 to 190). None of the mutations overlaps with mutations conferring resistance to nRTIs (Figure 1, B). However, some of the mutations cause broad cross-resistance among all members of the NNRTI drug class (eg, K103N).

Interactive Effects of Mutations on Drug Susceptibility

Some mutations selected by one drug suppress phenotypic effects of another mutation, eg, suppression of zidovudine resistance by didanosine-selected L74V, NNRTI-selected Y181C, and lamivudine-selected M184V.[46-49] Molecular mechanisms for these interactions are not well understood.

Lamivudine primarily selects for reverse transcriptase codon 184 mutations whether it is given as monotherapy or in combination. Suppression of the zidovudine resistance phenotype or delay in its emergence due to M184V is common during zidovudine-lamivudine combination therapy.[50] Nevertheless, additional reverse transcriptase mutations emerge with combination zidovudine-lamivudine therapy and eventually overcome the suppressive effect, resulting in high-

level resistance to both drugs.[51-53] The M184V mutation effect is thus likely to be transient and its induction less useful than maximizing HIV suppression. Presence of this mutation should prompt consideration of change in therapy, unless no satisfactory therapeutic options remain.

Techniques for Genotyping

Assays for detecting HIV-1 genome mutations are based on polymerase chain reaction (PCR) as the first methodological step. The PCR amplifies an RNA fragment (after a reverse transcription step) or DNA to quantities large enough for genotyping (the second step). Most laboratories now analyze protease and reverse transcriptase gene DNA sequences but may not investigate other genome regions relevant for drug resistance (eg, gag cleavage sites). It is still a technical challenge to amplify and genotype all regions implicated in protease and reverse transcriptase inhibitor resistance.

Generally, plasma samples with more than 1000 copies/mL of HIV RNA are needed to generate results. Resistance testing is not as likely to be useful when values are below this level. With current methods, species constituting 20% or more of amplified product can usually be detected. False positivity for mutations is possible from carryover from other HIV-1 samples in the laboratory or from random polymerase errors in vitro during in vitro nucleic acid synthesis. Also, unless molecular clones of PCR products are studied (not routinely done), it is impossible to be certain whether multiple positions in the sequence are physically linked together on the same genome; ie, it is impossible to differentiate a mixture of singly mutant genomes from a mixture having some genomes with accumulated different mutations.

Crucial issues for analysis of genotypic results include laboratory quality assurance, use of appropriate controls, and laboratory report clarity and comprehensiveness. Importantly, expert clinical interpretation is needed to assess likelihood that a given mutation pattern confers cross-resistance to related antiretroviral drugs and to define expected impact of mutation combinations on resistance phenotype. Other factors, eg, treatment history and plasma HIV-1 RNA levels, must also be considered when interpreting resistance data.

Two methods of sequencing the amplified HIV-1 DNA fragment are used: one is based on in vitro copying of amplified DNA templates (dideoxynucleotide terminator cycle sequencing), and the other is based on hybridization of the amplified nucleic acid (sequencing by hybridization). Other methods do not involve sequencing all PCR product positions but interrogate only certain codons.

Dideoxynucleotide terminator cycle sequencing using automated fluorescent dye–based sequencers is the most common approach. Human immunodeficiency virus 1 protease and reverse transcriptase can also be sequenced by hybridization using high-density oligonucleotide arrays;[54] chips with thousands of immobilized oligonucleotides are used to interrogate labeled, fragmented nucleic acid molecules derived from circulating HIV. The hybridization and computerized data analyses are highly automated, minimizing human input after template RNA preparation. Compared with cycle sequencing using automated sequencers, the chip hybridization-based method has yielded virtually identical results.[55] However, it is not currently designed to identify genetic mixtures of mutant and wild-type viruses.

A more targeted genotyping method now commercially available is the line probe assay that interrogates only certain codons.[56] This method involves detecting a nonradioactive colorimetric signal from hybridization of HIV-1 PCR product to oligonucleotide probes immobilized in lines on a paper strip. Data analysis is simple and fast with this method; however, it is now available only for genotyping selected reverse transcriptase codons associated with zidovudine, didanosine, zalcitabine, and lamivudine resistance (reverse transcriptase codons 41, 69, 70, 74, 184, 214, and 215). This assay may have greater sensitivity for detecting minority species in a genetic mixture in some samples but may sometimes give no results because nearby polymorphisms impair hybridization.[56]

Antiretroviral Resistance Phenotypes

Drug-resistant virus phenotypes are detected by measuring the 50% or 90% inhibitory concentration (IC_{50} or IC_{90}) of a drug in vitro. In standardized drug susceptibility assays, cells are infected with a fixed amount of viral inoculum, and various drug concentrations are tested to quantitate drug concentration required to inhibit viral replication (ie, determine dose-response curve) compared with untreated infected control cells. The precise IC_{50} or IC_{90} values obtained depend on the assay used, cell type used, antiretroviral drug tested, input viral inoculum, marker of viral replication selected (eg, measurement of HIV p24 antigen or reverse transcriptase activity), and time in culture.[57] Therefore, IC_{50} or IC_{90} values from one type of assay should not be compared with those obtained by another method.

Drug susceptibility testing measures HIV ability to grow at different drug concentrations vs a drug-susceptible laboratory strain of virus or previous isolate from the same patient. In general, a 4-fold increase is the minimum change reliably detectable in the laboratory. Changes in IC_{50} or IC_{90} values that are clinically important regarding drug activity have not been defined. High-level HIV-1 resistance to zidovudine (ie, isolates for which IC_{50} values are $\geq$1.0 µmol/L) predicted more rapid clinical progression and death in analyses adjusting for other risk factors in patients with advanced HIV disease receiving zidovudine monotherapy.[5,58,59] The clinical relevance of IC_{50} or IC_{90} values for each multidrug regimen component has not been defined. Also, sustained virus suppression may be seen in patients in whom drug-resistant virus has been detected.[3,60] This may result from achieving plasma drug levels in vivo that exceed IC_{50} or IC_{90} values for resistant virus in vitro.

Phenotypic assays may fail to detect evolving resistance that has not yet led to measurable increases in IC_{50} values, eg, the K70R zidovudine resistance–conferring mutation emerges within 12 weeks in nearly half of patients receiving zidovudine monotherapy, yet its presence alone is not associated with measurable increases in zidovudine IC_{50}. Thus, detection of a mutant genotype may be expected to precede detection of an increased IC_{50} value. Moreover, a limitation of all drug susceptibility assays described to date is that only predominant circulating viral populations are sampled to yield IC_{50} or IC_{90} values. Thus, minority drug-resistant species contributing to drug failure or transmission of resistant virus may not be detected.

One method for detecting viral drug resistance involves drug susceptibility testing in PBMCs using clinical isolates derived from HIV-1–infected PBMCs or plasma.[57,58,61-63] A high-titer viral stock is grown, followed by end point dilution to yield an infectivity titer. An appropriate drug concentration is then used in a subsequent susceptibility assay in PBMCs. The multistage procedure is time-consuming and expensive, and requires expertise beyond the capability of most clinical virology laboratories. The requirement to grow virus stocks from infected PBMCs in long-term culture and need to perform the assays over at least 7 days, may result in selection of viral subpopulations in vitro that do not reflect the majority species in vivo.

More rapid viral phenotypic assays based on recombinant DNA technology (Figure 2) are under development and may soon be available commercially.[64-66] An advantage of recombinant virus susceptibility assays is use of un-

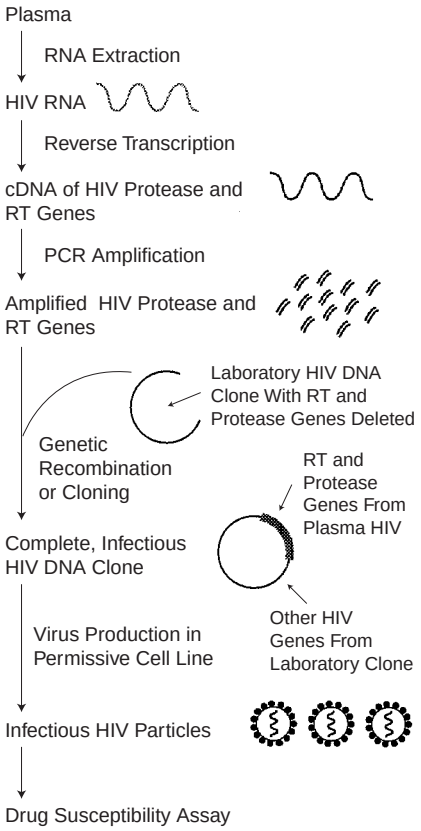

Figure 2.—Schematic representation of a recombinant phenotypic assay. Total RNA is extracted from a small volume of plasma that generally contains at least 1000 copies/mL of human immunodeficiency virus (HIV) RNA. After complementary DNA (cDNA) synthesis using reverse transcriptase (RT) in vitro, the viral protease and reverse transcriptase genes are amplified by polymerase chain reaction (PCR). The resulting amplicons are either cloned or recombined into an HIV vector plasmid, from which the protease and/or RT gene have been deleted. Stocks of recombinant virus are assayed for drug susceptibility.

cultured plasma as starting material, obviating the need to grow virus from infected PBMCs. This approach allows sampling of mutant HIV strains appearing earlier in plasma virus than in PBMC-derived isolates.[20] Although the assay may ultimately lead to faster turnaround time vs the PBMC-based approach, it can still take 2 to 3 weeks to generate results. The ability to test many drugs and isolates via an automated approach based on recombinant technology may provide more rapid monitoring of sequential patient isolates for susceptibility changes from baseline during treatment with combination therapy. Table 2 provides a summary of relative advantages and disadvantages of assays for detecting genotypic changes or phenotypic resistance. A number of these assays show considerable promise; however, they still need adequate standardization and clinical validation before routine use can be recommended.

Table 2.—Comparison of Genotypic and Phenotypic HIV Resistance Assays*

Relative Advantages	Relative Limitations
Genotypic Assays	
Availability	Indirect measure of susceptibility
Shorter time to results (days)	May not correlate with phenotype
Less technically demanding	Expert interpretation required
Mutations may likely precede phenotypic resistance	Most methods insensitive for detecting minor species
Phenotypic Assays	
Direct measure of susceptibility	Restricted availability
More familiar results (eg, IC_{50} or IC_{90})†	Longer time to results (weeks)
	Technically demanding
	Insensitive for detecting minor species
	Clinically significant cutoff values undefined

*HIV indicates human immunodeficiency virus.
†IC_{50} or IC_{90} indicate concentrations of drug required for inhibition of the replication of microorganisms at the level represented by the subscripted number (eg, IC_{50} inhibits replication by 50%, and IC_{90} inhibits replication by 90%).

IMPLICATIONS FOR CLINICAL MANAGEMENT

Role of Resistance Testing in Selecting an Initial Regimen

Transmission of HIV-1 mutants resistant to zidovudine was initially described in 1992.[67] Since then, several cross-sectional surveys to detect primary infection involving drug-resistant virus have been done (Table 3). In Europe and North America, prevalence of primary zidovudine resistance is variable, from 0% to 10% of isolates.[68] Transmission of lamivudine-[69] or nevirapine-resistant[70] virus has also been reported. Primary infection with virus resistant to protease inhibitors has not yet been reported but is expected.

Epidemiologic surveys of HIV isolates from newly infected patients in representative populations are needed to assess whether prevalence of primary infection with resistant virus is increasing, particularly in adults with primary HIV infection and pregnant women and their newborn children. In our opinion, drug-resistance testing should be considered for use in the design of initial antiretroviral regimens if there is an increased prevalence of resistance in a particular population.

Genotypic or phenotypic testing for drug resistance before antiretroviral therapy initiation in treatment-naive persons cannot be recommended for routine use at this time. Decisions concerning therapy initiation should be made on the basis of plasma HIV RNA level, $CD4^+$ cell count, and clinical status.[71-73] However, transmission of drug-resistant variants is likely to increase with widespread use of antiretroviral drugs. In absence of therapy, isolates with primary drug resistance may only be detectable early in infection, as wild-type strains may have a replication advantage that dominates over time in absence of drug selection. Drug-resistant variants that persist as minority species may be difficult to detect yet would quickly reemerge under drug-selective pressure.

For high-risk occupational, and possibly nonoccupational, HIV exposures, treatment with postexposure prophylactic antiretrovirals should be started as soon as possible and should not be delayed for results of resistance testing of virus from the source patient, whose antiretroviral treatment history should be carefully considered when choosing the prophylactic regimen for the exposed person.[74]

Use of Resistance Testing When Changing Therapy

Resistance is only one possible cause of therapy failure (Figure 3). Increasing evidence, however, indicates that viral resistance and treatment failure are closely linked.[1-7,75] In recent reports, a minority of those taking complex antiretroviral therapy regimens in whom virologic drug failure was observed appeared to have predominantly wild-type HIV isolates from peripheral blood.[76-79] Although these findings could be attributed in part to lack of assay sensitivity, other factors may be operative. First, adherence to increasingly complex drug regimens is often difficult, and some patients discontinue therapy intermittently.[80] The removal of drug pressure leads to replication of wild-type virus and apparent loss of drug efficacy. In this setting, drug resistance may not have developed, but an alternative antiretroviral treatment regimen to which these persons would more likely adhere is advised. Other causes of drug failure may include widely divergent plasma trough drug levels among patients,[81-83] limited drug potency, inadequate intracellular phosphorylation to active drug in the case of nucleosides,[84,85] or ongoing viral replication in sanctuary sites relatively inaccessible to inhibitory drug concentrations.[18,86] Suboptimal drug levels, whatever the cause, will permit ongoing viral replication and favor emergence of resistant virus over time (Figure 3).

A confirmed increase in plasma HIV-1 RNA level should be the main trigger for

Table 3.—Resistance Testing in Clinical Management*

- Drug resistance is only 1 of several possible causes of treatment failure. Other possible causes that should be considered include limited drug potency, poor adherence to medication, individual variation in pharmacokinetics, and inadequate drug activation (eg, inadequate phosphorylation of nucleosides).
- Serial measurement of plasma HIV RNA level and $CD4^+$ cell count should guide initiation of therapy, changes in therapy, and interpretation of HIV resistance test results.
- Current resistance-testing assays and services have not been standardized or validated. The utility of each assay needs to be established independently.
- HIV resistance testing may prove useful in guiding choice of initial or subsequent regimens, but its value in these settings remains to be established.
- Interpretation of resistance testing results must include consideration of treatment history, longitudinal changes in plasma HIV RNA levels, and likelihood of adherence to medication.
- Presence of phenotypic or genotypic resistance indicates that the drug in question may not be sufficiently active; the use of drugs to which the virus is likely not cross-resistant should be considered.
- Absence of resistance to a drug in the setting of previous therapy with the drug does not rule out reservoirs of resistant virus that may emerge rapidly after the drug is reinstituted.
- Transmission of virus resistant to current antiretroviral drugs appears to be uncommon currently. However, greater use of antiretrovirals may lead to increasing prevalence of resistant virus in newly infected persons.
- Epidemiologic studies are needed to monitor prevalence of resistant HIV isolates in specific populations. In our opinion, pretreatment screening for resistance may be useful in certain patient populations (eg, antiretroviral drug-naive pregnant women or patients with primary HIV infection syndromes) if prevalence of drug resistance in these populations is increased.

*HIV indicates human immunodeficiency virus.

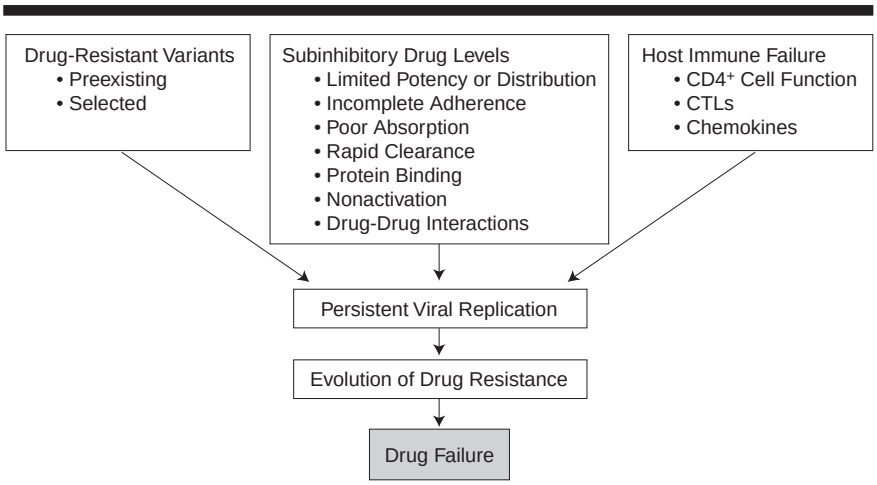

Figure 3.—Factors that contribute to antiretroviral drug failure due to resistance. Ongoing viral replication leads to the emergence of resistant virus, and ultimately to drug failure. The existence or emergence of resistant virus, the lack of drug levels adequate to inhibit viral replication, and host immune function each play a role. CTLs indicates cytotoxic T lymphocytes.

considering change in therapy.[71-73] Resistance testing, therefore, should not be the primary assay used to decide when to change therapy. Once issues of adherence (or related factors) are excluded, it may be reasonable to conduct resistance testing to help guide the choice of alternate antiretroviral regimens. When patients have received complex regimens, however, a search for known mutations conferring resistance to an individual drug may yield results that are difficult to interpret. There is no substitute for a thorough treatment history in guiding choice of appropriate regimens in such patients.

Mutants selected by a drug from a previous regimen may not be currently detected by available resistance assays, and may rapidly reemerge within days to weeks of "recycling" the drug. Similar considerations would apply to initiating a drug known to share cross-resistance with the first. A longitudinal record of resistance test results from time of initial presentation (including formal evaluation prior to therapy) may ultimately prove useful, but would be costly and require validation in controlled clinical studies.

If a person in whom therapy is failing never received a given antiretroviral drug or one inducing cross-resistant mutants, it can be assumed that absence of mutations known to confer resistance will lead to acceptable drug activity when used as part of a potent regimen. The presence of resistance-conferring mutations in a patient in whom therapy is failing, however, indicates that the drug in question may not be sufficiently active and that other antiretroviral drugs should be considered. This has been shown for zidovudine or didanosine, for which resistance is associated with lack of clinical efficacy.[7,75,87-89] These drugs should be replaced when resistance is seen in the setting of confirmed detectable plasma HIV RNA levels. Similar predictive data are emerging for other antiretroviral drugs, eg, phenotypic resistance to abacavir in vitro (ie, >8-fold increase in IC_{50}) appears to be associated with poor virologic response to abacavir therapy in vivo.[65,90,91] Protease inhibitor failure has also been associated with demonstrable resistance in vitro, and identification of mutations associated with decreased susceptibility to these drugs should prompt a change in therapy.[36] Although cross-resistance to all other protease inhibitors may not be present, development of broad cross-resistance under drug selective pressure may be rapid.[36] Further research is needed to determine the best strategies for serial use of protease inhibitors when resistance emerges to one member. If resistance to any given drug has ever been detected, that drug should probably not be used again, even if current test results suggest viral susceptibility, unless no other options are available. The safest approach is to change all members of a failing regimen, regardless of resistance-testing results. Prevention of perinatal transmission is a special situation in which many, although not all, experts believe that zidovudine should be included in the antiretroviral regimen, regardless of history of zidovudine use, because it is the only drug shown to date to reduce HIV transmission to neonates.[92]

SUMMARY

Drug-resistant HIV strains emerge readily in the setting of ongoing viral replication during antiretroviral therapy. In patients receiving sequential multidrug regimens, complex interactions involving multiple mutations can occur. In such settings, results of susceptibility testing and mutational analyses require clinical interpretation that also considers drug history and plasma viral load information. However, emerging evidence suggests that in drug-experienced patients, genotypic or phenotypic evidence of resistance to a drug in vitro is associated with poor virologic response to the drug in vivo. Thus, resistance testing will likely be useful for identifying drugs that will not be optimally active in a treatment regimen. The absence of phenotypic or genotypic evidence of resistance in the setting of previous therapy, however, does not necessarily predict a good response, since minor variants may not be detected by current assays. Thus, a confirmed increase in plasma HIV RNA level should remain the main trigger for considering a change in therapy.

Epidemiologic research is needed to track drug resistance prevalence in populations. In our opinion, routine testing for certain patients, eg, antiretroviral drug–naive pregnant women or persons with primary HIV infection, should be considered when prevalence of drug

resistance in that population is increased. As sequential data are generated about patterns of resistance in drug-naive patients starting therapy, such information may also guide selection of initial antiretroviral regimens.

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