

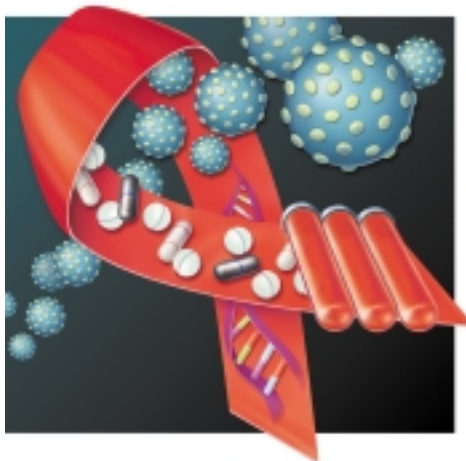
## Testing for HIV Drug Resistance

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**T**oday, many patients with HIV infection are able to live longer and better lives, owing to the use of highly active antiretroviral therapy (HAART). The development of new classes of drugs, the refinement of older drug classes, and an improved understanding of pharmacokinetic and adherence issues have contributed to the improved prognosis of HIV-infected patients. However, much of the progress in patient care that has been made through these pharmacologic advances can easily be nullified by a naturally occurring phenomenon—the development of drug resistance. The transmission of highly resistant strains of HIV is an alarming and perhaps growing component of the AIDS epidemic.<sup>1</sup>

Drug resistance is not a new issue in the management of infectious diseases. Since the onset of the antibiotic era, clinicians have been working to eradicate infections in the face of rapidly developing drug resistance among pathogens. By taking note of the resistance patterns in a given set of circumstances, physicians have often successfully avoided the administration of drugs that would have proved ineffective. However, a more scientific and effective approach, which has been utilized in the treatment of bacterial infections, is to define a pathogen's drug susceptibilities for the individual patient. Unfortunately, for patients infected with HIV, the technology for resistance testing had not been an option until recently. As a result, patients infected with HIV who experience treatment failures attributed to drug resistance have classically been treated empirically, usually by altering an entire drug regimen.<sup>2</sup> This drastic measure has been necessary even if the resistant strain is resistant to only 1 drug of the regimen, because identifying the ineffective component of the multidrug therapy has been impossible.



Fortunately, a new era for managing HIV infection is rapidly approaching. As a result of recent technologic refinements, the dream of individually testing every patient with HIV for his or her optimal pharmacologic profile may soon become a reality. Reliable drug-resistance testing will tremendously facilitate research into the epidemiology of the transmission of highly resistant strains. This article reviews the basic biological aspects of HIV drug resistance,

the techniques used to identify resistance, and the current utility of these techniques.

### BIOLOGICAL ASPECTS OF DRUG RESISTANCE

*Drug resistance* is defined as the natural ability of a microorganism to withstand the effects of a drug that are lethal to most members of its species.<sup>3</sup> Although host and pharmacologic factors play a role in the development of a drug-resistant strain of HIV, the most significant contributors to the process are those factors related to the virus itself.

HIV is a retrovirus with the ability to reproduce its genetic material through a process called *replication*. An active HIV infection may produce as many as 10 billion new virions in a 24-hour period.<sup>4</sup> However, not every replicated virion will have a genetic pattern identical to its precursor, because the reverse transcriptase gene responsible for replication is known to frequently make errors.<sup>5</sup> The results of these errors in transcription are mutations of the original genome. The rapid viral replication rate and the relatively low fidelity of

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reverse transcriptase in making genetically correct copies result in the potential formation of thousands of mutations of the HIV genome in a single individual on a daily basis. If a mutation occurs on a part of a gene that codes for an enzyme that is the target of an anti-retroviral medication (eg, an essential protease) then the enzyme that results from the mutated gene may have a new structure or conformation that makes it resistant to the medication (eg, a protease inhibitor).

HIV infections are polymorphic: every patient's viral profile consists of several different viral strains, reproducing at various rates within the patient. The different strains may be transmitted to the patient at the original time of infection; these are referred to as *primary mutant strains*, because they were acquired at the time of infection. Alternatively, strains may develop as a result of faulty replication after the original infection; these are referred to as *secondary mutant strains*. Either scenario may contribute to treatment failure for any given patient.

Not every mutant strain, whether primary or secondary, will survive, however. Many strains will lack the necessary biologic characteristics to subsist; conversely, other strains will have growth and survival capabilities exceeding that of the standard, or "wild-type" strain.<sup>6</sup> However, even if 1 HIV strain becomes predominant, other strains (possibly drug resistant) may still circulate in very low and virtually unmeasurable concentrations. Furthermore, resistant strains may "hibernate" in lymphoid tissue or other reservoirs within the body. Should the predominant moiety be successfully attacked by antiretroviral agents, a previously obscure drug-resistant strain may quickly multiply to become the predominant strain. This phenomenon is known as emergence under selective drug pressure.

#### DRUG-RESISTANCE TESTING TECHNIQUES

The goal of drug-resistance testing is simply to identify which drugs will be helpful in controlling an HIV infection. The 2 types of tests for HIV drug resistance are termed *genotypic*, in which the HIV genome existing within the patient is identified, and *phenotypic*, in which a viral sample from the patient is subjected to various medications and growth characteristics are studied. A schema for integrating resistance testing into clinical practice appears in **Figure 1**.

##### Genotypic Testing

Genotypic testing involves taking an HIV sample from the patient (via a blood sample), and with the use of polymerase chain reaction or other technologies, examining the viral sample for the presence of mutations on

various parts of the genome. The analysis is generally confined to the specific areas of the genes (codons) serving as codes for proteins, such as various proteases, on which the antiretroviral medications are known to exert their action. The viral genetic material from the patient is compared with known mutation patterns that have been recognized as correlating with specific patterns of resistance. Thus, if the HIV genome existing within a patient has a pattern associated with resistance to a specific anti-retroviral agent, that drug may be purposely avoided in his or her therapy.

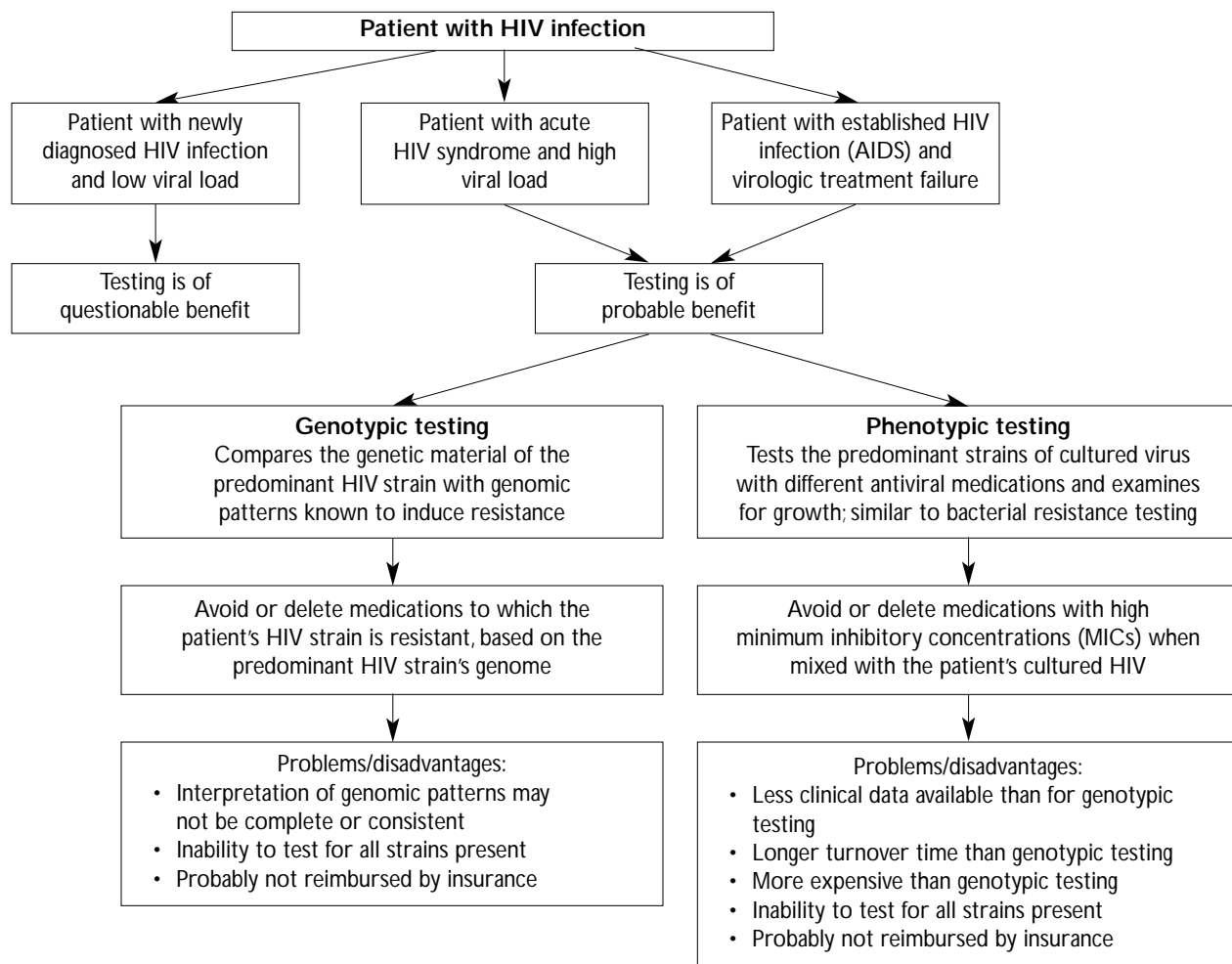
##### Phenotypic Testing

Phenotypic testing, which is routinely performed for bacteria and other human pathogens, addresses the ability of the patient's pathogen to grow when subjected to various medications. The virus is grown in culture by using peripheral blood mononuclear cells or similar media. After this, it is mixed with different concentrations of various antiretroviral agents. Viral growth is then examined, leading in turn to the calculation of the minimum inhibitory concentration (MIC), the concentration of a particular drug necessary to inhibit in vitro growth of the pathogen, in this case HIV. Instead of being subject to the interpretation dilemmas associated with genotypic testing, the results of phenotypic testing are drawn directly from observation of viral growth after exposure to the drug.

##### Drawbacks Associated With Genotypic and Phenotypic Techniques

The most prominent deficiency associated with both modes of testing is the lack of comprehensiveness: not all strains within a given patient are examined. Assay techniques test only the most concentrated strains found in serum. Any one strain constituting less than 20% of the total number of different circulating strains will essentially be missed. In many acute and subacute infections, drug-susceptible wild-type strains vastly predominate, and this domination means that resistant strains found in small concentrations will not contribute to the results of either genotypic or phenotypic testing. As a result, resistant strains may emerge late in the infection under selective pressure once the non-resistant strains have been treated with a given regimen, even though resistance testing had not originally indicated that some viral strains may have been resistant to that regimen.

The most serious problem associated with genotypic testing is interpretation. The genomes of measurable HIV strains from the patient are first defined and then compared with known genomic templates that



**Figure 1.** A schema for integrating resistance testing into clinical practice.

are related to drug resistance. Unfortunately, the resistance patterns associated with all possible single mutations have not been identified, which is not surprising considering that the HIV genome contains 9,200 nucleotides. Additionally, multiple mutations may appear on a single genome, complicating interpretation of known resistance patterns, considering that a mutation in one part of the genome may suppress or even obviate the effect of a mutation in another part of the genome.<sup>7,8</sup> Finally, a recent study examining the interpretation of standardized HIV genomes in different laboratories revealed significant interlaboratory differences, indicating that even for known mutation-induced resistance patterns, not all experts are in agreement.<sup>9</sup> As stated in a *Lancet* editorial, “the Achilles heel of the [genotypic] technique is interpretability.”<sup>10</sup>

Additional issues include the costs of the procedures (\$400 to \$900) and the time required to attain results (days to weeks); these obstacles are considerably more significant for the phenotypic technique. Notably, neither genotypic nor phenotypic assays have received approval for general use from the United States Food and Drug Administration. This lack of approval more often than not translates into lack of insurance coverage. Interestingly, issues of safety, especially regarding the culturing of HIV as part of phenotypic testing, have not been described as prohibitive thus far.

#### **CURRENT UTILITY OF DRUG-RESISTANCE TESTING** **Research Evaluating Drug-Resistance Testing**

In spite of its limitations, AIDS practitioners and researchers are successfully applying the technique of

resistance testing in patient management. Resistance testing is recommended as a useful tool in selecting active drugs when managing antiretroviral regimens in the setting of virologic failure and for suboptimal suppression of viral load after initiation of antiretroviral therapy.<sup>11</sup> Critical support for this recommendation was gained through the VIRADAPT study,<sup>12</sup> which took place in 3 French hospitals. The study evaluated approximately 100 patients whose viral loads could not be reduced despite 3 months of HAART. These patients were randomly assigned to 2 groups: a standard therapy group (control arm) and a group whose physicians would have access to the results of genotypic analysis (experimental arm). The results indicated that at 3 and 6 months, the group that had genotypic analysis had statistically significant lower viral loads. Additionally, at 3 and 6 months, the genotypic analysis group had a greater percentage of patients with unmeasurably low viral loads. The differences between the groups were considered so profound that the research team halted the study so that all the patients whose treatment regimens failed virologically could benefit from genotypic testing. An investigation with comparable methodology, undertaken in the United States, is known as the GART (genotypic antiretroviral resistance testing) study.<sup>13</sup> This study has had similar findings, and an article on the study is in press (AIDS Research Alliance [Chicago], personal communication, July 1999).

These studies tend to support the use of genotypic testing for patients in whom a particular drug regimen failed, assuming adherence-related and pharmacokinetic issues have been ruled out. Resistance testing also has a role in newly infected patients with acute HIV syndrome and its accompanying high viral loads; these patients should be tested to determine whether they have acquired HIV with primary resistance.<sup>11</sup> However, in other newly infected patients with lower viral loads, the role of resistance testing remains unclear, primarily because of the inability of available assays (phenotypic as well as genotypic) to test for strains in low concentrations. As refinement of assay techniques continues, assays may become capable of determining the resistance profiles of viral strains that are present in even the smallest concentrations.

#### **Current Utility of Genotypic Testing**

Genotypic testing appears to be closer to being established in standard clinical practice than does phenotypic testing. Many of the clinical studies of resistance testing have used genotypic testing. For the immediate future, this mode of analysis is the most promising.

#### **Current Utility of Phenotypic Testing**

Phenotypic testing, which more closely links test results with the pathogen's *in vivo* characteristics, may soon improve to the point of being clinically useful, as well. One recent study indicated the potential of phenotypic testing by successfully correlating the results of such assays with the efficacy of salvage regimens in patients whose previous regimens had failed virologically.<sup>14</sup> A second-generation phenotypic technique that provides results in days instead of weeks is in development and will further enhance the clinical utility of this type of test.<sup>15</sup>

Instead of being subjected to the interpretation dilemmas associated with genotypic testing, the results of phenotypic testing are drawn directly from the observation of viral growth after exposure to the drug. Accordingly, phenotypic analysis is considered by many to be the gold standard of resistance testing. Unfortunately, the technological complexities of the technique have thus far limited its entry into mainstream testing.

#### **Epidemiologic Applications of Resistance Testing**

The usefulness of resistance testing is not limited to the care of individual patients; resistance testing is also a powerful tool for public health surveillance. Information from treatment-naive patients, combined with data from clinical trials on development of resistance during treatment, can provide a complete picture of the epidemiology of resistant strains of HIV. The Centers for Disease Control and Prevention have been researching this issue for several years, by interviewing and performing resistance testing on individuals with newly diagnosed HIV infection. The availability of the "detuned" HIV assay, which indicates the vintage of HIV infection, is another technological advance that has facilitated the study of primary resistance in HIV.

#### **SUMMARY**

Drug-resistance testing shows tremendous potential for aiding in the management of the care of patients with HIV infections, by identifying which drugs will be successful in suppressing viral growth in a given patient. Furthermore, resistance testing is becoming an important surveillance tool for determining the transmission characteristics of various HIV strains, and in the investigation of localized epidemics of HIV.<sup>16</sup>

Existing research suggests that testing may have a role in the management of patients in whom standard regimens have failed virologically, although further investigation may be needed to definitively support this position. The usefulness of resistance testing for newly

infected patients, or patients with subacute infections, remains unclear. However, as assays are improved to test for smaller concentrations of strains in the polymorphic HIV infection, effective testing for patients with new infections may become a reality.

Physicians should keep abreast of advances in resistance testing, keeping in mind the following guidelines:

1. There is no substitute for a thorough treatment history when considering the components of an antiretroviral treatment regimen.
2. No therapeutic decision should be made based on the results of resistance testing alone. Virologic failure and patient intolerance to medications are the primary reasons for changing regimens, and the entire clinical picture must be considered.
3. The care of patients with HIV is complex and should be supervised by a physician with expertise and extensive experience in this area. HP

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