HIV RESISTANCE TO ANTIRETROVIRAL DRUGS

Eleven antiretroviral drugs, representing 3 drug classes, are presently commercially available in the US for the treatment of HIV infection. Viral resistance to the drugs was quickly recognized as these compounds became widely used. HIV resistance now constitutes a major challenge to any drug's or regimen's ability to produce durable suppression of viral replication. Knowledge of the issues regarding the prevention and recognition of viral resistance is therefore central to the clinician's decisions about antiretroviral therapy. At the Atlanta Meeting in February, Victoria A. Johnson, MD, summarized the current understanding of viral resistance to antiretroviral drugs.

Cause of Antiretroviral Failures

Dr Johnson began with a simple clinical question: Why do drugs used to treat HIV fail? Many of the available antiretroviral drugs have potent activity in vitro and are capable of suppressing HIV replication in simple systems, but treatment failure can occur in vivo for a number of reasons (Table 1). First, drug treatment may not completely suppress viral replication in all tissues and cells. Even with optimal drug administration and pharmacokinetics, protected cellular microenvironments exist in certain sites such as macrophages, follicular dendritic cells, and cells in the central nervous system. Antiretroviral drugs do not have an optimal effect in all tissues, and although the total body viral burden may decrease with treatment by 3 or 4 log (eg, from 10^{12} to 10^8 copies/mL). HIV replication may continue in selected microenvironments. Secondly, drug failure can be associated with poor adherence to a regimen. Less than strict adherence can allow ongoing viral replication, which can result in the evolution of drug-resistant virus. The complicated, multi-drug regimens now in use make strict adherence difficult. Two other reasons for drug treatment failure include emergence of more virulent, rapidly replicating forms of viruses, and perturbations or defects in host cell metabolism (and activation) of antiretroviral drugs. Lastly, mutant viruses can emerge that have a lower susceptibility to the antiretroviral effect of a particular drug or drug class. Dr Johnson focused her presentation on this latter issue.

Mechanisms of Viral Resistance

Landmark studies of the kinetics of HIV replication during the chronic, steady-state phase of HIV infection showed that although plasma HIV RNA levels and CD4+ cell numbers appear to be quite stable and constant, there is in fact a very high rate of viral replication and lymphocyte turnover. This high rate of replication results in a higher frequency of mutations, some of which can affect the susceptibility to antiretroviral drugs. Because of the

Table 1. Reasons for Drug Failure

- Incomplete suppression of HIV replication
- Patient nonadherence to antiretroviral therapy
- Emergence of virulent HIV sub-types
- Altered host cell drug metabolism
- Viral resistance to antiretroviral drugs

high replication rate of HIV and the relatively high error (mutation) rate associated with the HIV reverse transcriptase (RT), single and sometimes double mutations that encode for viral resistance frequently preexist in a large population of virions (eg, exist prior to exposure to that drug). The use of an antiretroviral drug that only partially suppresses viral replication results in inhibition of only the HIV that is susceptible to the drug; drug-resistant variants continue to emerge and
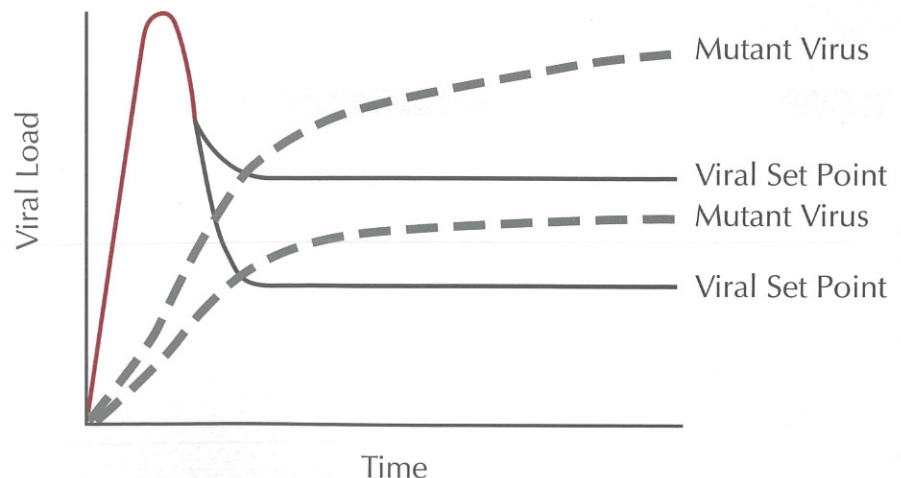

Figure 1. *Relationship between rate of viral replication and prevalence of resistance mutations. Those patients with a higher set point have a higher rate of replication and more mutant virus. Adapted in part from Ho DD. Science. 1996;272:1124; and Coffin JM. Science. 1995;267:483.*

replicate and may eventually dominate the population of infecting virus.

The same mechanism operates during acute HIV infection (Figure 1). At this point, the HIV viral load will typically decline to a particular "set point" in an individual patient. If the set point is relatively high, there is greater opportunity for emergence of viral resistance because there is a higher rate of viral replication. If the set point is low, there is less replication and, subsequently, a lower rate of development of resistance mutations. This is the rationale for the current focus on selecting an initial antiretroviral regimen that produces maximal suppression of HIV replication.

Mathematical Basis for Combination Drug Therapy

Given that the development of viral resistance is related to the rate of viral replication, one means of slowing the development of resistance is the simultaneous use of drugs of different classes early in HIV infection. The goal is to maximally suppress early viral replication and prevent expansion of resistant subpopulations. The use of a combination of drugs may also provide a mathematical advantage. For example, the frequency at which a specific, single base mutation occurs in HIV is typically about 10^{-5}, (ie, one virus with a specific mutation will occur per 100,000 virions). Therefore, in a patient with a viral load of 10^8 virions, there may be approximately 1000 viral mutants of that type ($10^8 \times 10^{-5} = 10^3$), and the odds of resistance developing during monotherapy are very high. However, the frequency at which 2 specific mutations occur in the same virion is greatly reduced. From our knowledge of genetics, if they are independent mutations, the frequency can be calculated by multiplying the individual mutation frequencies, as in the example above: $10^{-5} \times 10^{-5}$, or 10^{-10}. If the viral load is 10^8, the odds of carrying a specific mutation to both drugs are $10^{-10} \times 10^8$, or 10^{-2} (1 in 100). Adding an effective third drug to the combination further increases the mathematical advantage and theoretically, at least, should make drug resistance rare.

Continued Presence of Mutant Viruses

According to Dr Johnson, if the antiretroviral drug pressure is removed by stopping therapy, the predominant HIV population will revert to wild-type virus (susceptible to the drug) over a period of months. However, a subpopulation of mutant variants with resistance to that particular drug will remain at a frequency that is much higher than before treatment was introduced. This mutant population may replicate slowly, but it will persist in lymph nodes or other sequestered sites. When treatment with the same drug or a drug of the same class that is associated with cross-resistance to that drug is initiated, these mutants quickly reemerge and again predominate in the viral population. This resistant virus will reemerge more rapidly than it did initially. The clinical result is that the drug will be less effective when used a second time. For this reason, the patient's first treatment with antiretroviral drugs is currently considered to be the one with the best chance to succeed. For previously treated patients, knowing the complete drug treatment history can be essential to planning changes in the therapy.

Viral Susceptibility and Resistance Testing

Laboratory testing of the susceptibility of an HIV isolate to different drugs in vitro, and for detecting and quantifying resistance mutations, are beginning to become available. These assays can be broadly subdivided into 2 types: (1) phenotyping, and (2) genotyping (See sidebar definitions).

Phenotyping: Direct Measurement of Viral Susceptibility

HIV phenotyping can be considered analogous to determining the minimum inhibitory concentration of a streptococcal isolate to penicillin in vitro. The viral isolate to be tested is exposed to varying concentrations of the antiretroviral drug in cell culture, and the drug concentration that has a specific inhibitory effect is determined. Phenotyping requires the use of infectious virus, requires days to perform, is expensive, and lacks standardization. Furthermore, it is not yet clear what the measurements precisely mean, nor where the cut-off should be made between "susceptible" and "resistant" virus. In addition, the viral population being tested may consist of a mixture of subtypes with varying responses to the drug being evaluated. Nonetheless, this assay method has a relatively long history, and research into making this test faster and simpler is progressing.

Genotyping: Measurement of the Genetic Potential for Resistance

Genotyping assays are used to detect the presence of viral nucleic acid sequences

Table 2. Clinical Application of Viral Resistance Testing

- Viral resistance is only one possible cause of treatment failure; possible other causes should be evaluated, as well.
- The susceptibility of virus found in the plasma may not reflect the susceptibility of virus in sequestered sites.
- Serial quantification of plasma HIV RNA level and CD4+ cell count (the clinical response) should remain the primary guide for evaluating the response to antiretroviral therapy.
- Knowledge of a patient's complete drug history is essential for the clinical management of drug failure.
- Viral susceptibility and resistance assays are not yet standardized or validated, and their clinical utility has not yet been established.
- Interpretation of viral resistance data must consider the patient's complete drug history, plasma HIV RNA level, response to therapy, and patient adherence to dosing regimens.

known to be associated with phenotypic or clinical resistance. The assay does not require live, infectious virus and can use a small amount of plasma, tissue, or other body fluid. However, the specimen must contain a sufficient number of copies of the viral genome (about 1000) to allow adequate amplification of the target HIV nucleic acid. Genotyping can be completed in hours and may be less expensive than phenotyping, but the interpretation of the results is much more complex than are the results from phenotyping methods. Genotyping can detect resistance subtypes of virus in the tested population. However, genotyping detects single mutations that may not be relevant, and may miss important resistance mutations that are rarer. This latter problem can be overcome by complete sequencing of portions of the viral genome; but sequencing is time-consuming and impractical for large numbers of isolates.

Dr Johnson stressed that neither phenotyping nor genotyping alone is optimal at present, and probably both are needed, along with plasma HIV RNA, CD4+ count, and complete drug history data, for the proper clinical use of resistance information.

Specific Viral Resistance Mutations

HIV Reverse Transcriptase Mutations

Figure 2 shows the common viral mutations in the HIV-1 RT gene associated with phenotypic resistance to RT inhibitor drugs. Didanosine, zalcitabine, and stavudine commonly select for resistance mutations between positions 69 and 74, along with lamivudine in the 184 position. There is expectedly some cross-resistance among these compounds, although Dr Johnson indicated that this was sometimes a low-level resistance, as occurs between didanosine and zalcitabine. This moderate degree of resistance may be overcome by using higher doses of a drug to raise tissue concentrations to near the increased 50% inhibitory concentration (IC_{50}) value. For the lamivudine 184 position mutation, and for the nonnucleoside RT inhibitor (NNRTI) nevirapine, only 1 mutation is usually required to cause high-level resistance (eg, a 100- to 1000-fold increase in the IC_{50}). For zidovudine, a step-wise accumulation of 2 mutations must occur for high-level resistance to develop (ie, mutations at positions 41 and 215). Because 2 rather than 1 mutations are required, resistance will appear more slowly than that associated with a single point mutation.

Theoretically, knowledge of the existing genotypes prevalent in a heavily pretreated patient in whom therapy has failed may be useful in determining which new drug regimen is most likely to be virologically effective. For example, a patient with the 184 position mutation may not achieve a satisfactory clinical response by retreatment with lamivudine

DEFINITIONS

Genotype The genetic make-up of a virus, determined by the sequence of nucleotide bases in the viral genome. Changes (or mutations) in the sequence change the genotype and may encode viral proteins that respond differently to an antiretroviral drug. If the mutation causes decreased susceptibility, the change is termed a mutant genotype. Based on prior laboratory analysis, specific genotypes are known to be associated with resistance to specific drugs. Genotyping requires only detectable viral genome, not live virus.

Phenotype The ability of a virus to grow in cell culture in the presence of various concentrations of an antiviral drug. Usually a virus is tested in comparison with a control, wild-type virus known to be drug-susceptible. Infectious (live) virus is required to measure phenotype.

or didanosine. Dr Johnson noted that although this type of application of genotypic information is being studied, there are not yet enough data to use it in routine practice.

Mutations to HIV Protease Inhibitors

There are a significant number of common or overlapping resistance mutations among saquinavir, ritonavir, and indinavir (Figure 3). Cross-resistance

Amino Acid	41M	65K	67D	69T	70K	74L	75V	103K	106V	108V	181Y	184M	188Y	190G	210L	215T	219K	236P
Zidovudine	L		N		R										W	Y,F	Q	
Didanosine		R				V						V						
Zalcitabine		R		D		V						V						
Lamivudine												V						
Stavudine							T*											
Nevirapine								N	A	I	C, I		C	A				
Delavirdine								N			C, I							L*

Figure 2. Known HIV-1 reverse transcriptase (RT) mutations associated with resistance to RT inhibitor drugs.
*Mutations selected in vitro, but not often found in patients in whom the drug has failed.
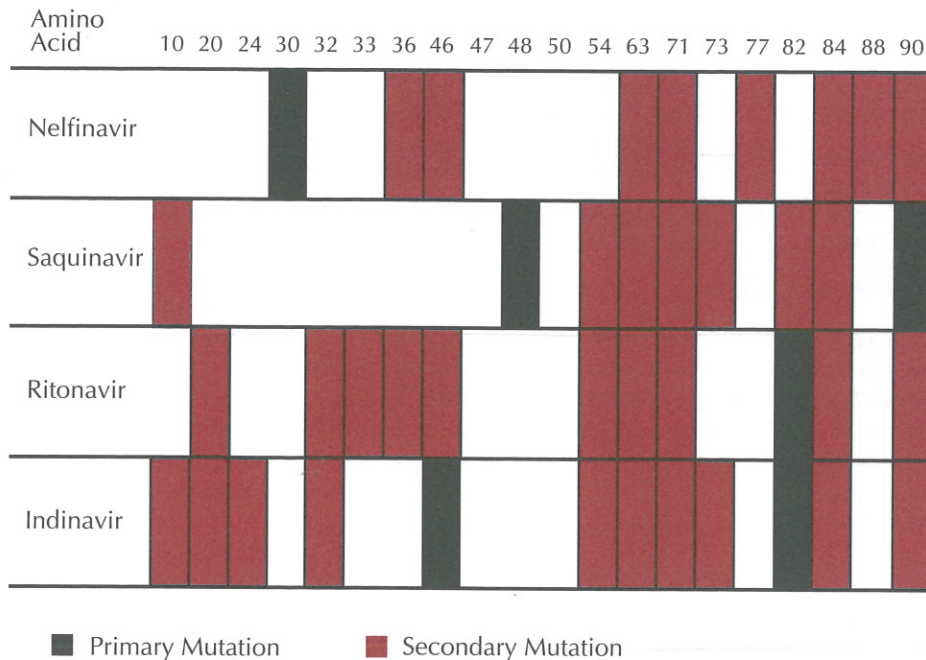
Figure 3. Known HIV-1 protease mutations that are associated with resistance to protease inhibitor drugs. Primary mutations are generally selected early in mutation accumulation and are relatively inhibitor specific. Secondary mutations accumulate in viral genomes already containing one or more primary mutations and may be selected because they improve viral fitness.

Experience in the Clinic

Dr Johnson discussed the application of HIV drug susceptibility and resistance testing in the clinical setting, as summarized in Table 2. The development of HIV viral resistance testing as a clinical tool is very new; there are no standardized kits or accepted and validated test conventions on which to base clinical decisions.

Available resistance data suggest that HIV carrying resistance mutations for RT inhibitors or protease inhibitors can be transmitted from person to person. Furthermore, in certain regions of the country, 5% to 10% of the HIV-infected patients have broad, multiple drug resistance. In this regard, viral resistance testing may become useful in those regions to assist in the design of initial treatment regimens for HIV-infected pregnant women, occupationally exposed health care workers, and in patients with primary HIV infection.

Over the next few years we can expect that the methodology for HIV resistance testing will become simpler, less expensive, and faster. This, along with clinical studies, will facilitate advances in the understanding of the clinical relevance of resistance data and may lead to more rational, less empirical, antiretroviral therapy.

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among the protease inhibitors was first noted when patients who had participated in early studies of saquinavir were later found to have reduced susceptibility to indinavir, even though they had never taken indinavir. As a drug class, protease inhibitors have the greatest degree of cross-resistance, and clinicians usually do not switch from indinavir to ritonavir, or vice versa, in the setting of treatment failures (except when the ritonavir/saquinavir combination is used to increase serum levels of saquinavir). For this reason, it is important that the protease inhibitor in an initial regimen be used at the optimum dose, consistently with strict adherence to the dosing schedule, and in a regimen as one in a combination of drugs.

There is now crystal structure information on the HIV protease molecule revealing the positions in the protein where the various resistance mutations occur. Protease inhibitor molecules fit into the active site pocket of the enzyme. Three common mutations sites for indinavir and ritonavir resistance are at positions 82, 84, and 90 of the protease gene. Saquinavir has a different mutation site, which explains the lower level of cross-resistance between saquinavir and indinavir or ritonavir than between the latter two drugs. An important resistance mutation for nelfinavir is at position 30 on the protease gene. As this drug becomes more widely used, additional resistance mutation sites will most likely be identified.

Multiple mutations are required for HIV to develop high-level phenotypic resistance to indinavir or ritonavir. For example, the IC_{50} of HIV to indinavir may not change with only 1 or 2 active site mutations. The IC_{50} will be increased with the third, fourth, and fifth mutation, by approximately 2.5-fold, 4-fold, and 8-fold, respectively. At the point where there are 4 or 5 mutations, it will be difficult to achieve a high enough drug concentration in vivo to inhibit replication.

Suggested Reading

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Electronic Media

http://hiv-web.lanl.gov/

http://www.viral-resistance.com