

Perspective

Incorporating Novel Virologic Tests into Clinical Practice

Virologic assays continue to evolve in order to meet the needs of HIV-infected patients and their health care providers. Genotypic and phenotypic assays for resistance to reverse transcriptase inhibitors, protease inhibitors, and fusion inhibitors have clear roles in disease management, with both types of assay having advantages and disadvantages. The failure of current assays to identify or measure the presence of minority resistant variants has clinical implications, since presence of such variants is associated with increased risk of virologic failure. Viral fitness may be relevant to disease management, but clinical role of available assays has not been determined. HIV coreceptor tropism assays will also be a crucial tool in the use of coreceptor antagonists, and data are emerging that will define pathways to treatment failure when using these new agents and the new integrase inhibitors. One clear finding for all antiretroviral drugs is that they select for resistance and must be used with good optimized background therapy to avoid virologic failure. This article summarizes a presentation on viral assays made by Eric S. Daar, MD, at an International AIDS Society–USA Continuing Medical Education course in Chicago in May 2007. The original presentation is available as a Webcast at www.iasusa.org.

Drug Resistance Testing for Antiretroviral Agents

Genotypic testing currently provides a report of antiretroviral resistance mutations within the HIV reverse transcriptase and protease genes. These reports can be difficult to interpret in terms of deciding on disease management when numerous resistance mutations are present. Drugs that have only recently become available may not be included in the reports. In addition, minority HIV variant populations constituting less than approximately 25% of the total viral pool in the individual patient are not detected in genotypic testing. Mixtures of wild-type and mutant variants present in proportions greater than this threshold, however, are reported.

Phenotype resistance tests are available for reverse transcriptase inhibitors, protease inhibitors, and the fusion inhibitor enfuvirtide. Results of phenotyping are reported as fold

change from wild-type virus with an indication provided as to whether the virus is susceptible, intermediate-susceptible, or resistant to each antiretroviral. Phenotyping provides an average of susceptibility of the viral population in the individual patient and does not account for how mixtures of wild-type and mutant variants separately affect susceptibility. Thus, for example, susceptibility to a particular antiretroviral might be reported on phenotyping when genotyping shows mixtures of resistant and wild-type virus to the agent. In this case, the presence of the mutant variants might well argue against use of this drug. Phenotypic testing has the advantage of providing quantitative information in the setting of complex resistance patterns. Clinical cut-off values, reporting likelihood of virologic response based on phenotype, are increasingly being investigated for individual antiretrovirals and reported in phenotypic testing. As with genotypic testing, information on susceptibility values and clinical cut-offs may not be available for newer drugs.

Discordance between genotype and phenotype results can occur because of the presence of mixtures of resistant and wild-type variants that has not yet sufficiently decreased average sus-

ceptibility of the entire infecting pool of virus. Discordance may also occur because of interactions of mutations, since mutations for some antiretrovirals can increase or decrease overall susceptibility to other antiretrovirals. For example, the M184V mutation may improve susceptibility to zidovudine and tenofovir and reduce susceptibility to abacavir and didanosine.

Virtual phenotyping testing is an approach to analyzing genotypes that assigns an expected fold change in susceptibility by comparing the patient's actual genotype with a database with matching genotypes and phenotypes. In cases in which mixes of resistant and susceptible virus are present on genotyping, virtual phenotyping will report the sensitivity of both the resistant and susceptible variants, unlike routine phenotyping.

Thus far, there are insufficient data comparing different resistance testing techniques to determine relative usefulness in predicting response and guiding treatment. In general, all have advantages and disadvantages that must be kept in mind for optimal interpretation of results, and all provide information that is helpful in making clinical decisions (see also: Johnson et al, this issue). However, the inability of these tests to identify minority variants is likely to be relevant. A recent report showed that allele-specific polymerase chain reaction (PCR), which is capable of detecting minority variants in frequencies as low as 0.4% to 2.0%, detected minority variants in 30 of 205 samples shown as wild-type on standard genotyping. Use of allele-specific PCR to detect K103N, Y181C, and M184V minority mutations in baseline samples from patients in the CNA 30021/30024 trial examining efavirenz/lamivudine plus abacavir or zidovudine (95 with virologic failure and 220 with viral suppression) showed that virologic failure occurred in 7 of 9 patients with minority resistant variants. Logistic regression anal-

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ysis showed that the presence of the minority variants was associated with an 11-fold increased risk of virologic failure ($P = .004$; Johnson, 14th CROI, 2007). Allele-specific PCR is expensive, labor intensive, has limitations, and not available for clinical use. Nevertheless these findings underscore the need to closely monitor patient response to therapy, regardless of findings in routine genotyping or phenotyping.

Viral Fitness

Numerous studies have provided at least indirect evidence that development of multiple resistance mutations to antiretrovirals can reduce the ability of mutant HIV to replicate. For example, some studies have shown continued immunologic benefit in patients with documented drug resistance and virologic failure, and others have shown precipitous declines in CD4+ cell counts when treatment to which resistance has developed is discontinued. A true measure of viral fitness is one that analyzes the ability of the patient's population of diverse viral variants to grow in the *in vivo* milieu subjected to immunologic and drug pressures. Such measures are not readily available. One assay under study assesses replicative capacity—ie, how the virus replicates in the absence of drug by determining how a virus derived from the polymerase gene of the patient's virus replicates relative to a reference strain. There is some indication that measures of fitness or replication capacity can relate to what happens in clinical practice. For example, in the E-184V study, patients on lamivudine-containing therapy with HIV RNA levels above 1000 copies/mL and CD4+ counts above 500 cells/ μ L who requested a treatment interruption were randomized to interruption or lamivudine monotherapy (Castagna et al, *AIDS*, 2006). Reduction in CD4+ count to below 350 cells/ μ L or development of an opportunistic infection (which occurred in 2 patients on treatment interruption) occurred in 68% of the interruption group and 44% of the lamivudine monotherapy group. Overall, mean CD4+ cell count de-

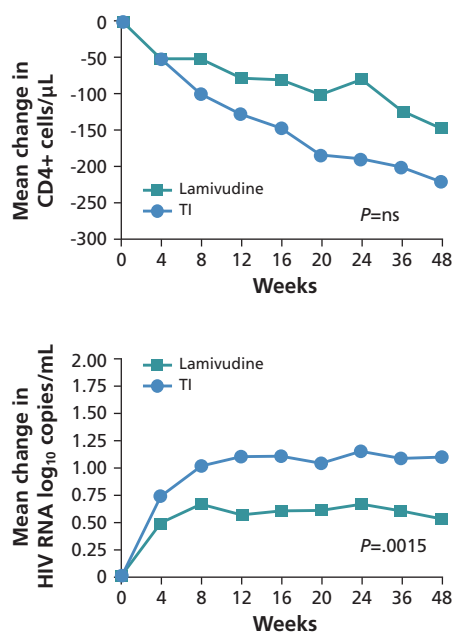


Figure 1. Change in CD4+ cell count (top) and HIV RNA level (bottom) with lamivudine monotherapy or treatment interruption (TI) in the E-184V study. Adapted from Castagna et al, *AIDS*, 2006. ns indicates nonsignificant.

clined in both groups with the reduction being nonsignificantly smaller in the lamivudine group than in the interruption group; viral load increased in both groups, with the increase being substantially smaller in the lamivudine group (Figure 1). Use of a replication capacity assay showed a 2.36-fold increase in the lamivudine group versus a 9.75-fold increase in the treatment interruption group ($P = .013$). The differences in outcome between the 2 groups are likely partially explained by a reduction in viral fitness associated with the persistence of the M184V mutation under lamivudine selection pressure, which appears to be reflected by the difference in the replication capacity findings.

Coreceptor Tropism

An antagonist of the CCR5 coreceptor was recently approved by the US Food and Drug Administration (FDA). The advent of this new class of drugs provides additional motivation for being able to identify and measure levels of CCR5-using (R5) virus with and CXCR4-using

(X4) virus (see next section).

It has long been recognized that the phenotypes of nonsyncytium-inducing (NSI) virus, typically present in early infection, and syncytium-inducing (SI) virus, typically emerging in later infection, are associated with different rates of progression in HIV infection, with the presence of SI virus being associated with more rapid immunologic deterioration. It remains unclear, however, whether there is in fact emergence of a more virulent strain of HIV or whether disease progression itself allows for emergence of the SI virus. It is now recognized that for the most part these different phenotypes correspond to coreceptor use in viral binding: the R5 viruses are the NSI viruses, which account for most transmitted variants and are prevalent in early disease; the X4 viruses are the SI viruses found more frequently in later disease and associated with rapid immunologic decline. There are also dual-tropic viruses that can use both coreceptors and some people have mixtures of X4 and R5 viruses. Phenotypic assays for receptor tropism have been developed, with one currently being widely used in clinical trials. This assay, which amplifies the entire viral envelope, identifies virus as R5 only, X4 only, or dual or mixtures of these variants. The assay, which detects X4 virus and thus nonresponsiveness to an R5 inhibitor, has a turnaround time of 16 days, a screen failure rate of 4% to 6% (based on 15,000 clinical trial samples), is 100% accurate at detecting minority variants at a 10% mixture and 83% accurate at a 5% mixture, and has 94% sensitivity at HIV RNA levels of 500 copies/mL to 1000 copies/mL. A more sensitive assay for detection of CXCR4-utilizing minority variants is in development. Investigators are also attempting to develop and validate other phenotypic assays as well as genotypic algorithms based on mutations associated with coreceptor-tropism in the gp120 V3 loop; however, the sensitivity of the latter approach may be limited.

Three studies using the phenotypic assay in treatment-naïve patients (evaluating from 325 to 979 patients) have shown R5-only virus in 81% to 88%

of samples, dual and mixed virus in 12% to 19%, and X4-only virus in 0% to 0.1%. In treatment-experienced patients, 5 studies (evaluating from 117 to 1076 subjects) have found R5-only in 49% to 67%, dual and mixed in 22% to 48%, and X4-only in 2% to 5% of samples. A study by Dr Daar's group in a hemophilia cohort using a single assessment of coreceptor tropism at baseline showed that the presence of dual and mixed-tropic virus was associated with a greater than 4-fold increased risk of clinical progression compared to those with only R5 virus.

Resistance to Novel Targets: CCR5 Coreceptor Antagonists and Integrase Inhibitors

CCR5 Coreceptor Antagonists

The rationale for developing CCR5 coreceptor antagonists is that homozygosity and heterozygosity for the CCR5 $\Delta 32$ gene deletion, resulting in absence and reduction, respectively, of CCR5 receptors on the cell surface, are associated with protective benefits. Homozygotes (~1% of the white population) appear to be protected from infection, with some cases of acquisition due to X4 virus being observed. These individuals appear to be otherwise healthy (although there is some evidence of greater viremia in hepatitis C virus

infection and greater severity of West Nile virus encephalitis in this setting). Heterozygotes (~15% of whites) have delayed progression of HIV disease compared with HIV-infected individuals with wild-type CCR5.

A concern with treatment with CCR5 antagonists is the potential selection or enrichment for X4 or dual and mixed virus. In a recent study, treatment-experienced patients who had X4-only or dual and mixed or nonphenotypable virus were randomized to optimized background therapy alone or with once- or twice-daily maraviroc for 24 weeks (Mayer et al, 16th IAC, 2006). Overall, maraviroc was well tolerated and appeared to be safe. Virologic outcomes were similar in all groups, although there appeared to be a trend to better response at the higher maraviroc dose; CD4+ cell count increases were somewhat greater with maraviroc, and included increases with maraviroc versus decreases with placebo in a small number of patients with X4 virus who had virologic failure. Although these findings suggest that patients with dual and mixed virus are not likely to derive the greatest benefit from CCR5 antagonist treatment, they also suggest that the potential enrichment of dual and mixed or X4 virus was not associated with immunologic decline in the relatively short term.

In the MOTIVATE 1 and 2 studies treatment-experienced patients

with R5-only virus received once- or twice-daily maraviroc or placebo plus optimized background therapy consisting of 3 to 6 antiretrovirals. Rates of virologic response to less than 400 copies/mL and less than 50 copies/mL, were approximately twice as high in the maraviroc groups than in the placebo groups in both studies, with MOTIVATE 1 data shown in Figure 2 (Lalezari et al, 14th CROI, 2007). Analysis of changes in tropism showed that approximately 8% of patients in the combined study populations had a shift between screening and baseline, reflecting patients who had mixed populations that initially were below limits of assay detection. Among all treatment failures, 64% (63 of 98) of those receiving maraviroc had a shift from CCR5 tropism to dual and mixed tropism, compared with only 5% (4 of 84) of those receiving placebo (Nelson et al, 14th CROI, 2007). Although CD4+ cell counts increased in subgroups of patients with virologic failure, increases were smaller in the maraviroc patients who had tropism change (increases of 37/ μ L and 56/ μ L in the 2 dosage groups) than those with failure who maintained the CCR5 tropism (increases of 61/ μ L and 138/ μ L in the 2 dosage groups). The significance of these findings remains somewhat unclear. Results of in vivo resistance testing for the agent suggest that a small number of individuals do have failure with R5 virus with phenotypic resistance to the CCR5 antagonist.

MOTIVATE 1-Week 24

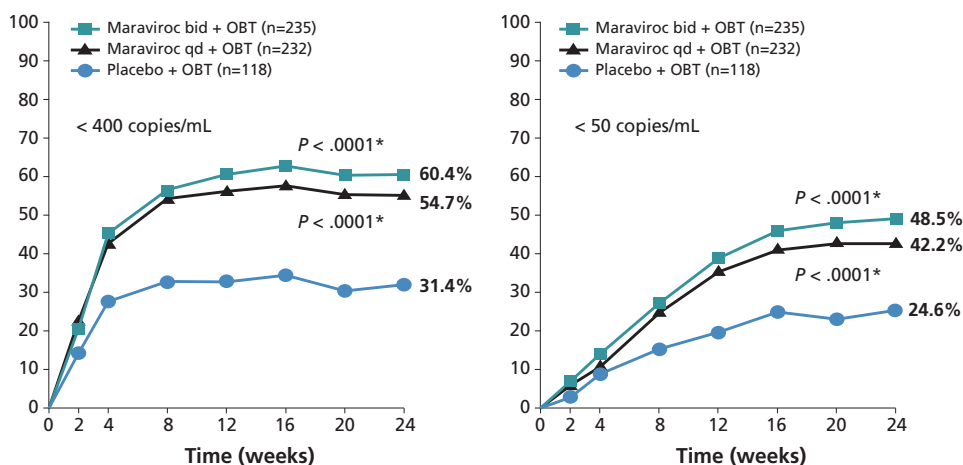


Figure 2. Virologic response in MOTIVATE 1 study. Adapted with permission from Lalezari et al, 14th CROI, 2007. OBT indicates optimized background therapy; bid, twice daily; qd, daily *HIV-1 RNA value imputed as baseline if missing or if patient discontinued before 24 weeks versus placebo + OBT.

Integrase Inhibitors

In the BENCHMRK 1 and 2 trials, the addition of the investigational integrase inhibitor raltegravir to optimized background therapy in treatment-experienced patients was associated with markedly increased rates of virologic response (Cooper et al, 14th CROI, 2007; Steigbigel et al, 14th CROI, 2007). Overall, virologic failure occurred in 16% of raltegravir patients and 51% of placebo patients. Partial analysis based on genotyping in 41 patients in whom raltegravir was failing showed integrase changes in 32 cases and no consistent changes in 9. Two potential primary genetic pathways to resistance were identified: N155H (with the additional

mutations E92Q, V151I, T97A, G163R, and L74M) and Q148K/R/H (with additional mutations G140S/A and E138K). Another potential pathway was Y143R/C (with additional mutations L74A/I, E92Q, T97A, I203M, and S230R). The observed mutations were proximal to the catalytic center and similar to those selected for in in vitro testing.

Summary

Novel virologic assays continue to evolve in order to optimally meet the needs of HIV-infected patients and their health care providers. Genotypic and phenotypic assays for resistance to reverse transcriptase inhibitors, protease inhibitors, and fusion inhibitors have clear roles in disease management, and both types of assays have advantages and disadvantages. Viral fitness appears to be relevant to disease management, but how to use available assays in clinical practice is still being explored. Tropism assays will be crucial tools in the use of coreceptor antagonists, and data are emerging that will define pathways to treatment failure when using these agents and integrase inhibitors. One clear finding for all antiretrovirals is that resistance develops for all of them, and all must be used with good optimized background therapy to avoid virologic failure.

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Suggested Reading

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