RESISTANCE TESTING IN ANTIRETROVIRAL MANAGEMENT

At the Atlanta course, Daniel R. Kuritzkes, MD, discussed characteristics of genotypic and phenotypic resistance testing and the implications of testing for management of antiretroviral therapy.

The rapid turnover of HIV-1 and the high frequency of error of the HIV-1 reverse transcriptase result in great genetic diversity within the viral quasi species. In the absence of selective drug pressure, virus with single mutations associated with antiretroviral drug resistance is constantly produced. Double mutations in an individual genome are of lower probability but are likely to occur with significant frequency, and triple mutations even less likely. Single mutations associated with resistance to antiretroviral drugs thus are likely to be present very early in infection and have been found in

Partial inhibition of virus with anti-retroviral therapy favors the emergence and dominance of resistant variants

patients who are antiretroviral-naive. For some antiretroviral drugs (eg, lamivudine, nevirapine, efavirenz), single point mutations can confer high-level antiretroviral resistance, with drug pressure resulting in rapid selection of resistant mutants and loss of susceptibility within weeks of initiating incompletely suppressive treatment with regimens containing these drugs. For other drugs (eg, zidovudine and such protease inhibitors as indinavir and ritonavir), high-level resistance requires 3 or more

mutations within a single genome. Although single mutations may be present before treatment, accumulation of the multiple mutations conferring high-level resistance is step-wise and requires selective drug pressure.

Clinical experience has provided support for the idea that profound viral suppression with potent antiretroviral combinations delays or prevents emergence of resistance and that partial inhibition favors the emergence and dominance of resistant variants. A number of principles regarding development of resistance under combination therapy have begun to emerge. One is that trough plasma drug concentrations may be important in drug resistance; in the case of ritonavir, the higher the trough concentration of the drug, the more slowly resistance mutations emerge. In addition, the lower the nadir of plasma HIV-1 level, the longer it takes for drug failure to occur. An additional principle is that mutations selected by combination therapy may differ from those expected based on monotherapy experience with each component of the combination.

CHARACTERISTICS OF GENOTYPIC AND PHENOTYPIC ASSAYS

Genotypic analysis and phenotypic analysis, the fundamental approaches to assessing viral resistance, are associated with relative advantages and disadvantages. Current genotypic assays generally require samples with more than 1000 HIV-1 RNA copies, and only HIV-1 variants that constitute 20% or more of the viral population in the sample can be reliably detected. With current assays, all of which require initial amplification through polymerase chain reaction, false-positive results for specific mutations can occur due to carry-over of amplified product from other samples or random polymerase errors in nucleic acid synthesis. Relative advantages of genotypic analysis include its wider availability, briefer time to results (days to 2 to 3 weeks), and fewer technical demands, with much of the testing being automated and not requiring the cell

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culture needed for phenotypic assays. Genotypic analysis may also be more sensitive than phenotypic analysis in that the detection of a resistance mutation in some cases may precede identification of phenotypic resistance; in the case of drugs requiring multiple mutations for high-level resistance, detection of "sentinel" mutations is therefore possible.

Relative disadvantages of genotypic analysis include the fact that the assays provide an indirect measure of susceptibility. Prior investigation is needed to determine which mutations are responsible for phenotypic resistance, and such investigation is required for each new drug being tested, as well as for drug combinations. It also has been found that results on genotypic assessment sometimes do not correlate with phenotypic assay findings, likely due to as yet poorly understood aspects of resistance genotypes. In addition, expert interpretation of genotypic analysis may be required due to the large and growing database on resistance mutations.

With current phenotypic assays, a 4-fold change in 50% or 90% inhibitory concentration (IC_{50} or IC_{90}) is the minimum change that is reliably detectable. Relative advantages of these assays include the fact that they provide a direct measure of drug susceptibility and that results are

expressed in the more familiar terms of IC_{50} and IC_{90} values. Limitations include restricted availability, with only 2 laboratories worldwide currently performing commercial phenotyping, and prolonged time to results (2 to 6 weeks) due to the labor-intensive work involved. Like the genotypic assays, phenotypic assays are insensitive for detecting resistant variants that constitute a minority of the viral population. Finally, clinically significant cut-off values for drug inhibitory concentrations have not been defined, and the clinical relevance of detection of IC_{50} and IC_{90} values for each drug used in combined regimens currently is unknown.

RETROSPECTIVE ANALYSES OF PREDICTIVE ABILITY OF DRUG RESISTANCE ASSAYS

A number of retrospective analyses have shown the ability of genotypic and phenotypic analyses to predict response to treatment. Lanier and colleagues demonstrated that the virological response to the nucleoside reverse transcriptase inhibitor (nRTI) abacavir was reduced in patients whose baseline virus carried zidovudine and lamivudine resistance mutations, mutations that had not been predicted to confer abacavir resistance in initial in vitro studies. Phenotypic analysis of virus isolates from these patients showed that decreased abacavir susceptibility correlated with a smaller decrease from baseline in plasma HIV-1 RNA level; it also showed that although the majority of patients responding to abacavir had abacavir-susceptible virus, many patients with susceptible virus did not have a good virologic response. Such a scenario, in which resistance is predictive of lack of response but susceptibility does not assure response, is common in the setting of antimicrobial treatment for bacterial infections (eg, mycobacterial infections.

Deeks and colleagues analyzed the significance of phenotypic drug resistance as measured by a recombinant virus assay (ViroLogic, Inc.) in patients in whom combination therapy that included indinavir was failing. Patients with plasma HIV-1 RNA levels greater than 2500 copies/mL who had received indinavir-containing therapy for more than 6 months were given a salvage regimen of saquinavir, nelfinavir, abacavir, and a

nonnucleoside reverse transcriptase inhibitor (NNRTI). At the time of the switch in treatment the median CD4+ count was 290 cells/μL and the median HIV-1 RNA level was 4.4 $\log_{10}$ copies/mL. Results of the phenotype testing showed that patients with isolates sensitive to 1 or none of the drugs in the regimen had a 0.14-log decrease in plasma HIV-1 RNA level, whereas those with isolates sensitive to 2 or 3 drugs had a 2.25-log decrease (P=0.007).

Patick and colleagues conducted an analysis in a group of patients in the nelfinavir expanded access program who had failed other protease inhibitor treatment (n=65; mean CD4+ count of 110 cells/μL, mean plasma HIV-1 RNA of 4.58 log). Nelfinavir treatment resulted in a decrease in HIV-1 RNA of at least 0.5 log for 24 weeks in 14 of 32 patients for whom the analysis was performed. Genotypic analysis for the protease inhibitor resistance mutations G48V, V82A/T/F, I84V, and L90M in 28 patients showed that 16 of 19 with 1 or no mutations had a response to nelfinavir whereas only 3 of 9 with 2 or more mutations had a response (P=0.013). In a group of 84 protease inhibitor-experienced patients studied by Harrigan and colleagues, treatment with ritonavir and saquinavir led to a response

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in half. Genotypic and phenotypic analyses, each performed in approximately three quarters of patients, showed that 80% of patients with resistant isolates were nonresponders. Genotypic evidence of resistance conferred a more than 5-fold risk of treatment failure (odds ratio [OR]

5.6; P<0.05), and phenotypic resistance conferred a more than 3-fold risk of failure (OR, 3.4; P<0.05) compared with wild type. Breakthrough viruses had multiple mutations, including mutations at codons

> *In the GART study, HIV-1 RNA level decreased in proportion to the number of active drugs in the salvage regimen*

10, 36, 48, 54, 71, 82, 84, and 90. Finally, Zolopa and colleagues reported on response to ritonavir/saquinavir in 51 patients in whom at least one prior protease inhibitor had failed; complete response (reduction of HIV-1 RNA to levels below detection [<500 copies/mL]) occurred in 37%, partial response (>0.5-log reduction) in 27%, and no response in 35%. Predictors of response included disease stage, CD4+ cell count, and HIV-1 RNA at baseline (P<0.03), as well as number of prior protease inhibitors and RTIs used (P<0.05). Regression analysis showed that after accounting for prior therapy, the number of protease inhibitor resistance mutations was strongly associated with the likelihood of treatment response (P<0.0001), with the presence of 3 or more mutations at codons 30, 46, 48, 54, 82, 84, and 90 being highly predictive of a poor response.

PROSPECTIVE STUDY OF TREATMENT MODIFICATION

In addition to these retrospective analyses, there are now data from studies assessing the effects of changes in therapy based on genotypic analysis. In the Viradapt study, decreases in plasma HIV-1 RNA level over 6 months of treatment were greater in patients in whom treatment was guided by genotyping than in patients in whom regimens were selected without this knowledge. In the GART trial

(CPCRA 046), 153 patients with virologic failure (defined as a 3-fold increase in plasma HIV-1 RNA from baseline) after more than 12 weeks of treatment with 2 nRTIs and a protease inhibitor were randomized to genotyping combined with expert advice on choice of regimen or standard-of-care management without genotyping and advice; 73% of patients had major reverse transcriptase inhibitor and protease inhibitor resistance mutations, with 20% having reverse transcriptase inhibitor mutations with no protease inhibitor mutations and 4.6% having no resistance mutations. As reported by Baxter and colleagues, follow up at 12 weeks showed that decreases in HIV-1 RNA were 1.17 log in the genotyping group versus 0.62 log in the control group ($P=0.0001$). Plasma HIV-1 RNA levels were reduced to levels below the limits of detection (<500 copies/mL) in 29% of GART patients and 17% of controls ($P=0.15$). These early findings show that the magnitude of decrease in HIV-1 RNA level increased in association with number of active drugs in the regimen. Plasma HIV-1 RNA levels decreased by 0.25 $\log_{10}$ copies/mL for each additional drug in the new regimen to which virus was susceptible. A total of 86% of patients in the genotyping arm received at least 3 active drugs, compared with 30% in the control arm. It was also found that although treatment changes were recommended in 86% of patients in the genotyping group, treating physicians implemented this advice in only 54% of cases.

DRUG FAILURE WITHOUT RESISTANCE

Despite the findings indicating the ability of genotypic and phenotypic analyses to predict response to treatment, it remains the case, as noted above, that not all drug failure is attributable to resistance. Recent analyses by Holder and colleagues of isolates from time of onset of indinavir failure in patients receiving combined treatment with either lamivudine or efavirenz, have shown that indinavir resistance mutations are detected in a minority of cases. In 1 analysis of 23 patients in whom indinavir failed, only 5 exhibited indinavir resistance mutations, with 17 exhibiting lamivudine resistance mutations. In a similar analysis of 14 patients, indinavir resistance mutations were present in 3 cases, with 11 cases exhibiting efavirenz resistance mutations.

RESISTANCE IN PRIMARY INFECTION

Data from surveys of resistance in primary infection have recently been reported. Surveys of seroconverters in San Diego, Los Angeles, Dallas, Boston, and Denver reported by Little and colleagues indicated that 'major' resistance was present in 4%, including nRTI resistance in 3%, NNRTI resistance in 1%, and protease inhibitor resistance in 3%. Any level of reduced susceptibility was present in 14%, including 3% to nRTI, 14% to NNRTI, and 13% to protease inhibitors. However, the threshold for the reduced susceptibility in this survey (called moderate resistance and defined as increases in IC_{50} of ≥2.5 but less than 10-fold) requires careful assessment as to whether this represents a predictor of therapeutic response (eg, isolates with 4- or 5-fold elevated IC_{50} values for NNRTIs, compared with the 500- or 1000-fold increases indicative of clinically significant resistance). Most of the cases of reduced susceptibility of moderate levels were associated with genetic polymorphisms (variants seen in untreated patients) rather than with primary resistance mutations. Another study, conducted by Wegner and colleagues at the Walter Reed Army Institute for Research (WRAIR), assessed genotype and phenotype in 114 patients with documented seroconversion within 3 years. In the WRAIR study intermediate resistance and high-level resistance were defined as IC_{50} increases of ≥5 fold and >10 fold, respectively. Resistant genotypes and phenotypes were found in 4% and 7%, respectively, for nRTIs; 15% and 20%, respectively, for NNRTIs, and 10% and 1%, respectively, for protease inhibitors. Most isolates were resistant to only 1 class of drug; 2% to 3% exhibited multidrug resistance.

USE OF DRUG RESISTANCE TESTING WHEN CHANGING THERAPY

A confirmed significant increase in plasma HIV-1 RNA level should remain the main trigger for considering a change in antiretroviral therapy due to drug failure. Resistance testing might be used as a complement to a thorough treatment history in selecting a new regimen. If resistance to a drug has ever been detected in a patient, those resistant mutants may persist as a minority viral pool; thus, regardless of a subsequent finding of susceptibility, that drug should probably not be used again unless no other options are available. Currently, resistance testing is likely to have greatest utility in cases of treatment initiation in acute HIV-1 infection (to assess for transmission of resistant virus) and in patients experiencing their first or second failure of treatment. Owing to the possibility of persistence of virus resistant to previously used drugs, resistance tests are likely to be much less helpful in the highly antiretroviral experienced patient in whom there are few or no new drug options available.

Daniel R. Kuritzkes, MD, is Associate Professor of Medicine and Microbiology at the University of Colorado Health Sciences Center, Denver, Colorado.

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