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HIV-1 genotype-phenotype discordance

Kara Nyberg, PhD

Assessing resistance to antiretroviral (ARV) drugs has become the clinical standard of care for treating HIV-1-infected patients.^{1,2} Resistance testing is routinely performed by two different means: genotype and phenotype testing. Genotype tests discern the presence of drug-resistance mutations by sequencing the viral genomes of plasma HIV-1. Most genotypic assays employ an algorithm that generates an inferred level of drug resistance based on the mutations found. These tests define resistance based upon known relationships between select mutations and phenotypic and clinical susceptibility. Phenotype tests, on the other hand, gauge the degree of susceptibility of the viruses to drugs within a cell culture system. Phenotypic results are best interpreted based upon an understanding as to what level of phenotypic resistance relative to wild type is associated with poor virologic response (clinical cutoffs), as opposed to simply defining resistance as any significant increase in the 50% inhibitory concentration (IC₅₀) compared to wild-type virus. Both methods of resistance testing are limited by the inability to precisely correlate genotype with phenotype and by the lack of phenotypic clinical cutoffs for all drugs and drug combinations. In addition, minority variants present at frequencies less than 10% to 20% of the total viral population are not reliably detected by either method.^{3,4}


Although the clinical utility of genotype and phenotype testing has been demonstrated in select clinical trials,^{5,6,7,8} there are occasionally discordant results between the two tests when performed

on the same sample.^{3,9,10,11} This discordance may be caused by six different phenomena: (a) HIV-1 mixtures, (b) transitional mutations, (c) antagonistic mutations, (d) thymidine analogue mutations (TAMs), (e) atypical and complex patterns of mutations, and (f) mutations associated with newly approved drugs. This issue of "Navigating HIV Resistance" features more detailed discussion about genotypic and phenotypic discordance resulting from each of these factors, along with examples of clinical situations in which discordance between genotype and phenotype tests occur.

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I. HIV-1 mixtures

Given HIV-1's high rate of mutation, minority variants are present as mixtures with other HIV-1 strains, including wild type, within an individual. As such, ARV selection pressure results in the emergence of drug-resistant variants. Once a mutant virus is present at greater than 20% to 30% of the overall population, these variants can readily be detected by genotype testing, thereby resulting in the assay reporting



decreased susceptibility to the drug affected by this mutation. In contrast, the influence of mixtures of drug-resistant and -susceptible variants on phenotype testing will depend not just on the presence of resistant variants but also by the relative proportion of each virus in the population.³ Mutations present in less than 50% of viruses are likely to have a minimal effect on *in vitro* susceptibility (ie, to raise the IC₅₀). Nonetheless, such mutations are expected to ultimately interfere with the activity of drugs to which these mutations confer resistance, because use of these drugs will cause the enrichment of the minority variants, leading them to become the predominant population within the quasispecies. As an example, if a minority variant harbors the K103N mutation that confers resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs) and this mutation escapes detection by phenotype testing, a patient beginning an NNRTI-containing regimen will rapidly select for this variant species, which will result in a poor virologic response. The genotypic approach is therefore generally considered to be a more sensitive assay for detecting resistant variants when present as a mixture with wild-type virus.

II. Transitional mutations

Certain mutations arising from drug-selection pressure do not confer measurable drug resistance on their own, but rather lower the genetic barrier to resistance. Depending on whether the right combination of accessory mutations is also present, drug resistance as measured by phenotype testing may not be seen. Genotype testing, however, may indicate a degree of drug resistance for a particular transitional mutation depending upon the algorithm or rules used

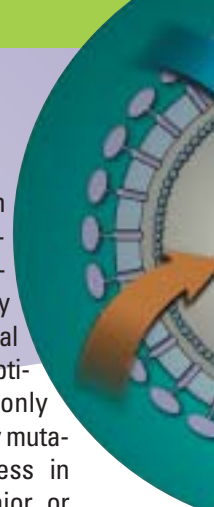
for genotypic interpretation.

The prototypical examples are the transitional mutations T215S/C/D, also referred to as T215 revertants. The T215Y/F TAMs are two base-pair mutations that develop in response to treatment with nucleoside reverse transcriptase inhibitors (NRTIs), particularly zidovudine (ZDV) and stavudine (d4T), and confer cross-resistance to other NRTIs. When NRTI therapy is discontinued, the T215Y/F mutations are no longer selected for and they transition to

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single base-pair mutations T215S/C/D, which improve viral fitness relative to T215Y/F in the absence of drug therapy.¹² Because these mutations do not cause NRTI drug resistance, their presence will not be detected by phenotype testing. In contrast, it is now known that these revertants are likely to be clinically relevant and will be detected by genotype testing and interpreted as resistant by most algorithms. This information is critical to guide salvage regimen selection, because individuals harboring viruses with T215S/C/D mutations have been shown to rapidly select for viruses with T215Y/F and NRTI resistance once NRTI-containing antiretroviral therapy (ART) is initiated.^{13,14}

The presence of the M46L/I or L90M mutations within the protease represent additional examples of

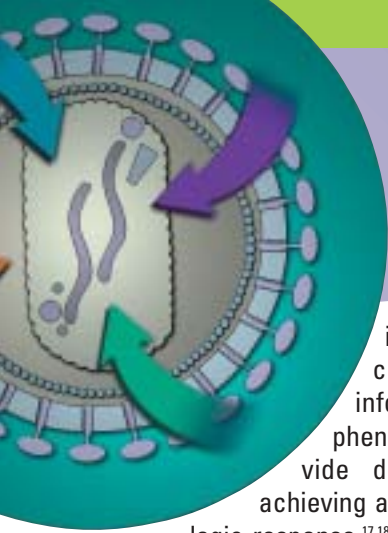


transitional mutations. When these mutations initially develop in individuals taking protease inhibitors (PIs), they may be associated with only minimal reductions in PI drug susceptibility, as they are commonly selected for as compensatory mutations to improve viral fitness in combination with other major or accessory mutations.^{15,16}

III. Antagonistic mutations

Some drug-resistance mutations that develop under selective pressure from one particular ARV drug confer HIV-1 hypersusceptibility to a second drug. For example, the M184V mutation—associated with resistance to lamivudine (3TC) and emtricitabine (FTC)—increases HIV-1 susceptibility to ZDV, d4T, and tenofovir (TDF). L74V and Y181C increase susceptibility to ZDV and TDF, whereas K65R increases susceptibility to ZDV.

When antagonistic mutations exist, genotype testing will detect all mutations that are present but will not distinguish active mutations from those for which the effect is masked by hypersusceptibility-conferring mutations. For example, an individual harboring a strain containing both the K103N mutation conferring resistance to NNRTIs, and also multiple NRTI-resistance mutations, which can result in NNRTI hypersusceptibility, will appear by genotype testing to be NNRTI and NRTI resistant. The fact that NNRTI resistance caused by K103N may be diminished by NNRTI re-sensitization conferred by the NRTI mutations will not be accounted for by this testing method. In contrast, phenotype susceptibility testing will measure only the effect of the active mutations, thus possibly reporting NRTI resistance with NNRTI susceptibility, and failing to detect the presence of latent resistance caused by the



K103N mutation.

In this case, genotypic and phenotypic testing may provide complementary information. The phenotype may provide data useful for achieving a short-term virologic response.^{17,18} In contrast, the genotype results will provide a report of resistance based on the detection of resistance mutations, which may be more predictive of long-term responses. Additional clinical data are needed to better define the clinical relevance of this type of discordance.

IV. TAMs

The TAMs, selected in response to treatment with the thymidine analogues ZDV or d4T, include mutations in reverse transcriptase at positions 41, 67, 70, 210, 215, and 219. These mutations confer biochemical and clinical resistance to each of the NRTIs except 3TC and FTC. However, although the effect of these mutations on all NRTIs can reliably be measured in cell-free enzymatic assays, their effect on didanosine (ddI), zalcitabine (ddC), d4T, and TDF in standard cell-based drug-susceptibility assays (ie, phenotype tests) is subtle and often difficult to detect.

The NRTIs are prodrugs that must be triphosphorylated to compete with dNTPs for incorporation into newly synthesized, elongating DNA chains, where they cause chain termination. NRTI triphosphorylation rates *in vivo* are generally not as high as the triphosphorylation rates of cells used in culture assays. The activated lymphocytes used in phenotype assays triphosphorylate some NRTIs at higher rates than others, thus obscuring the potency of certain NRTIs such as ddI, d4T, and TDF.¹⁹ In addition, high dNTP levels *in vitro* adversely affect the primer unblocking mechanism of drug resistance in the presence of the NRTIs ddI, ddC, d4T, and TDF, thereby making

these drugs appear more susceptible than they are in the presence of viruses containing multiple TAMs.²⁰

V. Atypical and complex patterns of mutations

Genotypic interpretation algorithms typically classify a given mutation as drug resistant if reduced drug susceptibility has been documented using site-directed mutants or has been reported in a large number of clinical isolates. Less prevalent resistance mutations among clinical

Genotypic algorithms are limited in their ability to predict the consequences of the interaction of multiple mutations on phenotype, and the extent of cross-resistance among drugs in a class. A typical example includes ABC, where multiple TAMs are associated with increasing phenotypic resistance.

isolates or those mutations not yet assessed by site-directed assays will hence be excluded from genotypic algorithms. For example, whereas the K103N mutation conferring resistance to efavirenz (EFV) is a signature mutation accounted for by genotypic interpretation algorithms, less common EFV-resistance mutations such as K101P and K103S may not be included in the algorithm. Consequently, resistance to EFV conferred by K101P and/or K103S would escape detection by genotype testing. In contrast, phenotype testing would detect the presence of such resistance mutations based on the decreased susceptibility of the viral population to EFV in cell culture assays.

Genotypic algorithms are also limited in their ability to predict both the consequences of the interaction of multiple mutations on phenotype and the extent of cross-resistance among

drugs in a class. A typical example includes abacavir (ABC), where multiple TAMs are associated with increasing phenotypic resistance.²¹ Whereas phenotypic clinical cutoffs are reasonably well characterized to detect decreased ABC susceptibility, the degree of reduced susceptibility caused by multiple TAMs varies greatly between different genotypic algorithms, thus making it difficult to predict those that may have some versus no response to treatment.^{22,23} A similar problem applies to the detection of PI resistance by genotype testing—resistance to lopinavir (LPV) is a classic example²⁴—as the development of multiple mutations confers increasing resistance to any of several different PIs.

VI. Mutations associated with newly approved drugs

Algorithms used for reporting the results of genotype testing are based upon an understanding of the common resistance mutations seen in clinical isolates, or they are assessed by site-directed mutagenesis associated with phenotypic resistance. Since the introduction of new drugs often precedes the characterization of resistance patterns to the agent, algorithms may initially be incomplete. In contrast, phenotype testing can detect the presence of such resistance if the new drug is included in the panel of ARV agents. Nevertheless, even with phenotypic testing, the reporting of clinically relevant cutoffs is usually delayed. This currently applies to atazanavir (ATV) for which precise rules for predicting resistance have not been developed beyond the presence of its signature mutation I50L. Even for this mutation, the clinical relevance in the context of ritonavir (RTV)-boosted ATV is not known.

VII. Conclusion

Although genotype and phenotype testing can provide useful information for clinicians treating patients with drug-resistant HIV-1, both methods

have their limitations, as detailed above. The interpretation of results from each test depends on criteria subject to continuous improvement—updating interpretation algorithms for genotype testing and updating clinical cutoff values for phenotype testing. When used in combination, the tests can provide complementary information that may help the clinician make more informed decisions regarding the selection of salvage regimens.

Understanding the sources of discordance between the two assays can help to guide the

Phenotypic testing may be more useful during the later stages of salvage therapy and may offer advantages in selecting drugs for which there is likely to be some level of susceptibility, despite complex resistance patterns.

selection of the appropriate drug resistance test and can assist in the optimal interpretation of test results when both tests have been performed. In general, genotype testing is probably more sensitive for detecting resistance but can be more difficult to interpret with complex resistance patterns. Consequently, genotype testing is typically suitable in early stages of

salvage therapy when the degree of drug experience and the resulting complexity of resistance mutations are expected to be low. Phenotypic testing may be more useful during the later stages of salvage therapy and may offer advantages in selecting drugs for which there is likely to be some level of susceptibility, despite complex resistance patterns. This is particularly true for PIs (especially RTV-boosted PIs) and drugs such as ABC.

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Characterization of determinants of genotypic and phenotypic resistance to enfuvirtide in baseline and on-treatment HIV-1 isolates

Sista PR, Melby T, Davison D, *et al.*
AIDS 2004;18:1787-1794.

BACKGROUND

Enfuvirtide (ENF) is the first of a novel class of drugs that blocks HIV gp41-mediated viral fusion to host cells. Viruses with mutations at positions 36 through 38 in HIV-1 gp41 and/or reduced susceptibility to ENF have been selected both *in vitro* and *in vivo*.

METHODS

An analysis of baseline and on-treatment ENF susceptibility in virus samples from Phase II clinical trial patients treated with ENF as functional monotherapy for 28 days (TRI-003) or in combination with oral antiretrovirals for ≥ 48 weeks (T20-205, T20-206 and T20-208). Population sequencing identified amino acid (aa) substitutions at positions 36-45 of gp41 in plasma HIV-1. ENF susceptibility of virus isolates was tested in the cMAGI assay and viral DNA was sequenced for selected isolates.

RESULTS

HIV-1 gp41 aa 36-45 were highly conserved in virus from ENF-naïve patients, except for a 15% incidence of N42S which did not reduce sensitivity to ENF. Virus from patients experiencing viral load rebound exhibited reduced susceptibility to ENF and substitutions in gp41 aa 36-45. The most common substitutions observed on treatment were at positions 36, 38, 40, 42, and 43. On-treatment changes in the phenotypic susceptibility of virus isolates to ENF were generally associated with genotypic changes in aa 36-45. There was a relatively lower incidence of ENF resistance in patients with baseline sensitivity to more oral antiretrovirals in comparison to patients sensitive to fewer antiretrovirals.

CONCLUSIONS

These data identify the importance of HIV-1 gp41 aa 36-45 in the emergence of resistance to ENF.

ANALYSIS

Several large randomized controlled trials have shown that ENF can be an important part of combination therapy for patients with multidrug-resistant HIV-1. *In vitro* selection studies, confirmed by site-directed mutagenesis experiments, have previously demonstrated that substitutions in amino acids 36-38 of the HR-1 domain of gp41 confers reduced susceptibility to ENF. These mutations have also been shown to emerge within 14 days of monotherapy in a small number of patients studied in a Phase I/II clinical trial.


The study by Sista *et al* expands our understanding of ENF resistance by assessing baseline genotypic patterns and phenotypic susceptibility in subjects enrolled in clinical trials of ENF monotherapy, as well as in those given combinations of antiretrovirals. The investigators characterized changes in the HR-1 domain at amino acids 36-45 of gp41 and used *in vitro* peripheral blood mononuclear cell (PBMC) assays to measure phenotypic susceptibility of viral isolates cultured from the subjects' own PBMCs.

Consistent with other data, this group demonstrated that HIV-1 from ENF-naïve subjects had only modest variation in gp41 at these amino acids. Nevertheless, despite this region remaining relatively conserved in those never treated with ENF, there was greater than 400-fold difference in phenotypic susceptibility between isolates with 50% inhibitory concentrations (IC_{50} s) varying from <0.001 to 0.480 $\mu\text{g/ml}$ (median = approximately 0.02 $\mu\text{g/ml}$). Additional analyses were performed on plasma HIV-1 and viral isolates from numerous subjects treated with ENF alone or as part of combination therapy in clinical trials.

Study TRI-003 provided 28 days of ENF monotherapy with 26 of 67 (39%) subjects found to have developed changes in amino acids 36-45 of gp41, most frequently identified at positions 36, 38, 42, and 43. In a subset of patients with baseline and end-of-treatment viral isolates, it was seen that 24% had a greater than 10-fold reduction in viral susceptibility to ENF emerge during the 28-day period of treatment. Similar studies were performed in those receiving more prolonged treatment with ENF-containing combination therapy. These studies were limited to analyzing virus from either early specimens from subjects who ultimately experienced good viral suppression, or from a small number that actually were observed to have virologic rebound on treatment. Not surprisingly, those who ultimately had good viral suppression on therapy rarely developed mutations at amino acids 36-45 of gp41. In contrast, mutations in this region were observed in those experiencing viral rebound. Many of these isolates had in excess of a 10-fold increase in viral IC_{50} to ENF compared to their baseline isolates.

This study contributes to our emerging understanding of how ENF resistance is likely to emerge in clinical practice. Like other studies, this group shows that despite relatively conserved sequences at amino acids 36-45 in HIV-1 in ENF-naïve subjects, there is substantial variability in viral susceptibility that is mediated by unclear mechanisms. This observation alone illustrates how complex it will be to develop phenotypic assays that will define clinically relevant resistance based on a fold change from wild-type criteria. This study also showed that select mutations associated with virologic rebound and shifts in phenotypic susceptibility do emerge in a specific region of gp41 of those treated with ENF, particularly in those on monotherapy and on less than fully suppressive combination therapy. Nevertheless, a better understanding of which specific mutation or combination of mutations are most important still needs to be defined. Moreover, it is very likely that there may be important parts of the viral genome outside of this region of gp41, and possibly even distant from gp41 that may influence susceptibility to ENF, perhaps accounting for the highly variable susceptibility of wild-type strains to this drug.

Eric S. Daar, MD



Sequencing-based detection of low-frequency human immunodeficiency virus type 1 drug-resistant mutants by an RNA/DNA heteroduplex generator-tracking assay

Kapoor A, Jones M, Shafer RW, *et al.*
J Virol 2004;78(13):7112-23.

Drug-resistant viruses may be present as minority variants during early treatment failures or following discontinuation of failed antiretroviral regimens. A limitation of the traditional direct PCR population sequencing method is its inability to detect human immunodeficiency virus type 1 (HIV-1) variants present at frequencies lower than 20%. A drug resistance genotyping assay based on the isolation and DNA sequencing of minority HIV protease variants is presented here. A multiple-codon-specific heteroduplex generator probe was constructed to improve the separation of HIV protease genes varying in sequence at 12 codons associated with resistance to protease inhibitors (PIs). Using an RNA molecule as a probe allowed the simple sequencing of protease variants isolated as RNA/DNA heteroduplexes with different electrophoretic mobilities. The protease gene RNA heteroduplex generator-tracking assay (RNA-HTA) was tested on plasma quasispecies from 21 HIV-1-infected patients in whom one or more protease resistance mutations emerged during therapy or following initiation of salvage regimens. In 11 of 21 cases, RNA-HTA testing of virus from the first episode of virologic failure identified protease resistance mutations not seen by population-based PCR sequencing. In eight of these 11 cases, all of the low-frequency drug resistance mutations detected exclusively by RNA-HTA during the first episode became detectable by population-based PCR sequencing at the later time point. Distinct sets of protease mutations could be linked on different genomes in patients with high-frequency protease gene lineages. The enhanced detection of minority drug resistance variants using a sequencing-based assay may improve the efficacy of genotype-assisted salvage therapies.

ANALYSIS

This study by Kapoor *et al* is important for two reasons. First, the authors describe a new method—RNA-HTA—for detecting minor drug-resistant HIV-1 variants. Second, the application of RNA-HTA to heavily treated patients undergoing genotypic testing shows that standard direct PCR (“population-based”) sequencing often underestimates the extent of drug-resistant virus present within heavily treated patients.

Most methods designed for detecting minor HIV-1 variants belong to one of two types: (1) point mutation assays designed to be highly sensitive at detecting specific mutations present in small proportions of viruses, and (2) sequencing of large numbers of individual clones. Point mutation assays are limited because each assay can detect the presence of only a single mutation. Clonal sequencing is limited because large numbers of clones must be sequenced to reliably detect most minor variants (ie, common variants will be sequenced repeatedly whereas rare variants will be sequenced rarely).

RNA-HTA was designed to physically separate variant HIV-1 protease genes according to their patterns of drug-resistance mutations. The first three steps in RNA-HTA are similar to those of standard direct PCR sequencing: plasma viral RNA extraction, reverse transcription to cDNA, and PCR amplification of cDNA. At this stage, the amplified DNA is usually run on an agarose gel to confirm the presence of an appropriately sized band prior to performing the sequencing reactions.

In the RNA-HTA assay, amplified viral cDNA is first incubated with an excess of an RNA probe containing deletions at multiple PI resistance positions. The mixture of amplified viral cDNA and RNA probe are then run on an acrylamide rather than agarose gel. Heteroduplexes formed between the RNA probe and amplified cDNA migrate at a slower rate than cDNA-cDNA heteroduplexes because of kinks formed when the viral DNA binds to the multiply deleted RNA probe. The key to the RNA-HTA method is that different protease mutations cause kinks of different shapes and cause variants with different mutations to migrate at different speeds, thus physically separating these variants. Following ethidium bromide staining, RNA/DNA heteroduplexes are isolated from several different locations in the gel and submitted to reamplification and separate sequencing reactions. By sequencing viral cDNA with different rates of migration, this process is more likely to sample the diversity within a virus population than blindly sequencing an equivalent number of randomly selected subclones.

The potential clinical usefulness of the protease RNA-HTA assay was tested in a group of patients found to “develop” at least one new PI-resistance mutation with virologic failure during the second of two consecutive PI-containing regimens. In 11 of 21 patients meeting these criteria, RNA-HTA detected mutations in samples obtained at the time of first virologic failure that population-based sequencing detected only at the time of the second virologic failure. Whether such a high rate of additional mutations would be detected in less heavily treated patients or in patients that were not selected on the basis of having two consecutive virologic failures requires additional study.

Assays for detecting minor drug-resistant HIV variants are unlikely to be used in routine clinical settings for at least several years. However, these methods are useful research tools that shed light on HIV population dynamics *in vivo* and provide insights into the causes of virologic failure. If one lesson can be drawn from this and other studies of minor variants, it is that standard resistance testing will often underestimate the extent of drug resistance, particularly in heavily treated patients. Therefore, genotypic resistance tests must be interpreted in the context of a patient’s past treatments and with the realization that the virus population within an individual is often somewhat more resistant than apparent on any single test.

Robert Shafer, MD

Survey

INSTRUCTIONS

The International Association of Physicians in AIDS Care (IAPAC) is gauging reader perspectives on the clinical management of antiretroviral drug resistance through this case study-based survey mechanism. Answers to each month's survey will be published in the following month's "Navigating HIV Resistance" newsletter.

Note your answers to the following two questions in the answer grid below and fax back to (312) 795-4938. Thank you!

QUESTIONS

1) In which of the following situation(s) would HIV resistance testing NOT generally be recommended?

- a) A 28-year-old man who has just been diagnosed with primary (acute) HIV infection.
- b) A 38-year-old woman who is on a regimen of stavudine (d4T)/lamivudine (3TC)/indinavir (IDV) and has had an HIV RNA level less than 50 copies/ml for 18 months, but the last two HIV RNA levels have been 72 and 110 copies/ml.
- c) A 41-year-old man on zidovudine (ZDV)/3TC/nelfinavir (NFV) whose last four HIV RNA measurements were 256, 865, 1,838, and 3,298 copies/ml.
- d) A 35-year-old woman who previously had an HIV RNA level of 16,700 copies/ml while on a regimen of d4T/3TC/efavirenz (EFV). She admits to spotty adherence while taking that regimen, and she discontinued all of her antiretroviral medications 24 weeks ago. She now wants to restart antiretroviral therapy and states she is highly motivated to have excellent adherence.

2) A 38-year-old HIV-infected woman has extensive antiretroviral therapy experience with several previous virologic failures. She is now seen for evaluation of a possible salvage regimen. Her CD4 count is 128 cells/mm³ and HIV RNA 35,350 copies/ml. An HIV resistance test is ordered. Which one of the following statements is TRUE regarding HIV resistance testing?

- a) Compared with the phenotypic assay, the genotypic assay is more expensive and has a longer turnaround time.
- b) A potential drawback of the phenotypic assay is that clinical relevant resistance thresholds have not yet been determined for some antiretroviral agents.
- c) The genotypic assay detects resistance in greater than 98% of minority populations of HIV that exist within an individual.
- d) When a patient is undergoing a change in antiretroviral therapy because of virologic failure, genotypic resistance testing does not provide any benefit in improving virologic responses.

Editors' Note: Case study questions reprinted here with permission from the Northwest AIDS Education and Training Center (NAETC) and the University of Washington.

YOUR ANSWERS

- | | | | | |
|----|---|---|---|---|
| 1) | A | B | C | D |
| 2) | A | B | C | D |

Case study

A 31-year-old man is diagnosed with HIV in 2003 when presenting with mild thrush and seborrheic dermatitis. He was probably exposed to HIV as a result of sex with men, and he reported that his last negative HIV antibody test was in 2000. At the time of presentation his CD4 count was 97 cells/mm³ and plasma HIV RNA was 115,000 copies/ml. The patient was convinced that he was ready to start therapy, and after repeat lab tests demonstrated similar results, he was started on trimethoprim/sulfamethoxazole with zidovudine (ZDV)/lamivudine (3TC)/abacavir (ABC)/efavirenz (EFV) at standard doses. After four weeks of therapy he experienced a truncal rash, fever to 100.2°F, diarrhea, and myalgia. Therapy was stopped because of concerns of hypersensitivity reaction (HSR) to ABC. Plasma HIV RNA at that time was found to be 1,200 copies/ml. His therapy was restarted with ZDV/3TC/atazanavir (ATV) 400 mg once daily.

Over the ensuing months, plasma HIV RNA decreased to less than 1,000 copies/ml, and then between months five and 10 remained at 400 to 800 copies/ml. The patient stated that he was adherent with the regimen, was not experiencing any adverse events, and denied taking any antacids, H2 blockers, or proton pump inhibitors that might impair ATV absorption. Multiple attempts to perform a genotype test were unsuccessful because the patient's viral load was reported as insufficient for testing. After approximately 10 months on this regimen, his HIV RNA was approximately 1,100 copies/ml and a genotype test showed M41L, K70R, M184V, and I50L resistance mutations. Therapy was modified to tenofovir (TDF)/didanosine (ddI) plus lopinavir/ritonavir (LPV/RTV), and plasma HIV RNA has subsequently been

less than 50 copies/ml for approximately four months with CD4 counts of 300 to 400 cells/mm³.

This patient's clinical course was complicated by a probable drug-related adverse event, as well as the limitations of resistance testing to assist in guiding therapy in the setting of low-level viremia. The initial adverse reaction was consistent with an abacavir HSR, although it is conceivable that it could have been an EFV reaction. Nevertheless, stopping all drugs to manage this potential complication allowed for the possible selection of nonnucleoside analogue reverse transcriptase inhibitor (NNRTI) resistance due to the long half-life of EFV. Because of this concern, it was decided that the next regimen would not include an NNRTI, but instead would use the protease inhibitor (PI) ATV. The initial response was not sufficient to achieve undetectable virus levels; however, viral loads were maintained at levels that limited the ability to perform resistance testing. Ultimately, the low-level viremia was associated with the selection of nucleoside analogue reverse transcriptase inhibitor (NRTI) and PI resistance mutations. Therapy was changed to TDF/ddI plus LPV/RTV with at least an initially good viral response.

The resistance pattern seen in this patient was consistent with the rapid selection of 3TC resistance in the setting of a less than fully suppressive regimen. The delay in modifying therapy, related at least in part to the inability to perform resistance testing, resulted in the emergence of the signature mutation associated with ATV failure, and the

accumulation of thymidine analogue mutations (TAMs). In fact, the number of TAMs, with associated NRTI cross-resistance, would likely have gotten worse had therapy been continued. The response to an LPV/RTV-based regimen is consistent with the known lack of cross-resistance to other PIs associated with ATV resistance, and certainly demonstrates the advantage of using PIs that do not initially select for cross-resistance. It is intriguing to consider whether this patient may have responded to intensification of ATV with RTV. However, currently there are no data demonstrating whether the increased levels seen with RTV-boosted ATV are sufficient to overcome the resistance associated with the I50L mutation. Similarly, consideration could be given to what the outcome might have been if ATV had been RTV-boosted initially. Would the enhanced potency have resulted in improved initial suppression? And, what mutation pattern might have been selected in this setting? To date, no data exist regarding efficacy or the pattern of resistance selected for when using RTV-boosted ATV in PI-naive subjects.

For now, the most important lessons learned from this case are that the best strategy remains to identify early virologic failure or sub-optimal response, and to assure both adequate adherence with the regimen and that there are no important drug-drug or drug-food interactions that might impair the utility of drugs in the regimen.

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