

## Perspective

# Antiretroviral Drug Resistance and Resistance Testing

*Antiretroviral resistance testing should be performed in newly diagnosed patients with acute or recent HIV infection and at the time of treatment failure, and there is growing support for testing in newly diagnosed, treatment-naive patients with chronic infection as well. Genotypic testing is preferred for baseline screening, because it is more sensitive than phenotypic testing for the presence of mixed populations of drug-susceptible and -resistant virus and because it is less expensive. Phenotypic testing provides quantitative information on the degree of resistance and is also able to assess interactions among mutations. As a result, it can be particularly useful in determining treatment options for treatment-experienced patients with multi-drug resistant virus. In many cases, there may be advantages to the use of both tests. This article summarizes a presentation on antiretroviral resistance and resistance testing by Joel E. Gallant, MD, MPH, at the 8th Annual Clinical Conference for Ryan White CARE Act clinicians in New Orleans in June 2005.*

### Resistance Testing in Treatment-naive Patients

The International AIDS Society–USA, the US Department of Health and Human Services, and European guidelines recommend antiretroviral resistance testing for primary HIV infection and in cases of treatment failure. These guidelines also recommend or encourage consideration of testing in chronic infection of less than 2 years' duration.

US surveillance data indicate that transmission of resistant virus is a substantial and growing problem. Data gathered in 2003 and 2004 from 787 newly diagnosed antiretroviral therapy-naive subjects from 89 sites in 6 states indicate the presence of resistance to at least 1 antiretroviral drug class in 14.5% of cases, including resistance to nucleoside analogue reverse transcriptase inhibitors (nRTIs) in 7.1%, nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs) in 8.4%, protease inhibitors (PIs) in 2.8%, and 2 or more

classes in 3.1% (Bennet, CROI, 2005). Although it was once believed that acquired resistant strains would be quickly replaced by wild-type virus, it has become clear that resistant virus persists for prolonged periods. This phenomenon probably reflects the fact that only the resistant strain is transmitted, so that reversion to wild-type virus requires “back mutation,” a more time-consuming process than the selection of pre-existing wild-type strains that occurs in treatment-experienced patients. In one recent study, the average time to reversion to wild-type virus after identification of resistant strains among newly infected patients was 375 days for NNRTI-resistant virus and 362 days for nRTI-resistant virus; no reversion was seen with up to 2 years of follow-up for PI-resistant virus (Little, CROI, 2004). For this reason, there is growing support for resistance testing in chronically infected, treatment-naive patients. However, despite the persistence of mutant virus after infection, the diagnostic yield of resistance testing is highest with earlier testing. It is therefore recommended that resistance testing be performed at the time of HIV diagnosis, regardless of the current need for therapy; it can be assumed that transmitted resistance mutations will still

be present, even if not detected in the circulation, when therapy is eventually initiated. Genotypic analysis is preferred over phenotypic testing in this setting, primarily because it is more sensitive for the detection of mixtures of susceptible and resistant virus, which would be expected if reversion to wild-type had begun to occur at the time of testing. Genotyping is also faster and less expensive.

### Resistance to nRTIs

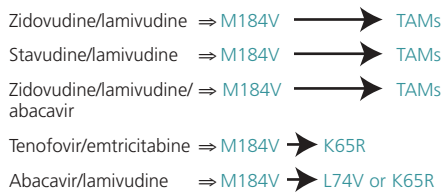
Resistance patterns after initial failure of commonly used nRTI backbones and the resistance map for the individual nRTIs are shown in Figure 1. In patients in whom a combination that includes lamivudine plus either zidovudine or stavudine is failing, the M184V mutation is always the first to appear. It is eventually followed by cumulative acquisition of thymidine analogue-associated mutations (TAMs) if treatment with the nonsuppressive regimen is continued. It should be noted that TAMs are completely preventable mutations; they should become less common in the future, since patients with suppressive options should not be left on a failing thymidine analogue-based regimen. There are 6 TAMs: M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N/R; the presence of these mutations confers cross-resistance to all nRTIs, with the degree of nRTI cross-resistance increasing as the number of TAMs increases (Johnson et al, *Top HIV Med*, 2005). The acquisition of TAMs is slowed by the presence of the M184V mutation, which also serves to partially counter the effects of TAMs on susceptibility to zidovudine, tenofovir, and stavudine. Figure 2 shows the fold change in drug susceptibility as a function of the number of TAMs with or without the presence of the M184V resistance mutation.

There are different pathways to

---

Joel E. Gallant, MD, MPH, is Associate Professor of Medicine and Epidemiology at Johns Hopkins University School of Medicine, Associate Director of the Johns Hopkins AIDS Service, Baltimore, Maryland, and a member of the Board of Directors of the International AIDS Society–USA.

<b>Abacavir</b>	K65R; L74V; Y115F; M184V
<b>Didanosine</b>	K65R; L74V
<b>Emtricitabine</b>	K65R; M184V/I
<b>Lamivudine</b>	K65R; M184V/I
<b>Stavudine</b>	M41L; E44D; K65R; D67N; K70R; V118I; L210W; T215Y/F; K219Q/E
<b>Tenofovir</b>	K65R
<b>Zidovudine</b>	M41L; E44D; D67N; K70R; V118I; L210W; T215Y/F; K219Q/E



**Figure 1.** Top: Mutations that affect susceptibility to nucleoside (or nucleotide) analogue reverse transcriptase inhibitors (nRTIs). Adapted with permission from the International AIDS Society–USA (Johnson et al, *Top HIV Med*, 2005). For user notes and updated figures, visit [www.iasusa.org](http://www.iasusa.org). Bottom: Resistance patterns after initial failure of commonly used nRTI backbones. The longer arrow indicates the delay in emergence of thymidine analogue-associated mutations (TAMs) caused by M184V, which does not occur with K65R or L74V, for which the arrow is shorter.

resistance for nRTIs. In the case of zidovudine or stavudine therapy, as yet unidentified factors can result in acquisition of either the M41L, L210W, and T215Y TAMs or the D67N, K70R, and K219Q/E/N/R mutations. The former pathway is associated with higher-level zidovudine resistance, greater nRTI cross-resistance, and a smaller decrease in resistance in the presence of the M184V mutation. The D67N, K70R, and K219Q pathway is associated with a lower level of zidovudine resistance, less nRTI cross-resistance, and a greater decrease in resistance when M184V is present. Unfortunately, it appears to be the less common pathway. When abacavir, tenofovir, or didanosine are used with lamivudine or emtricitabine in the absence of thymidine analogues, the likely mutations are M184V, K65R, and L74V, which result in variable decreases in susceptibility to abacavir, tenofovir,

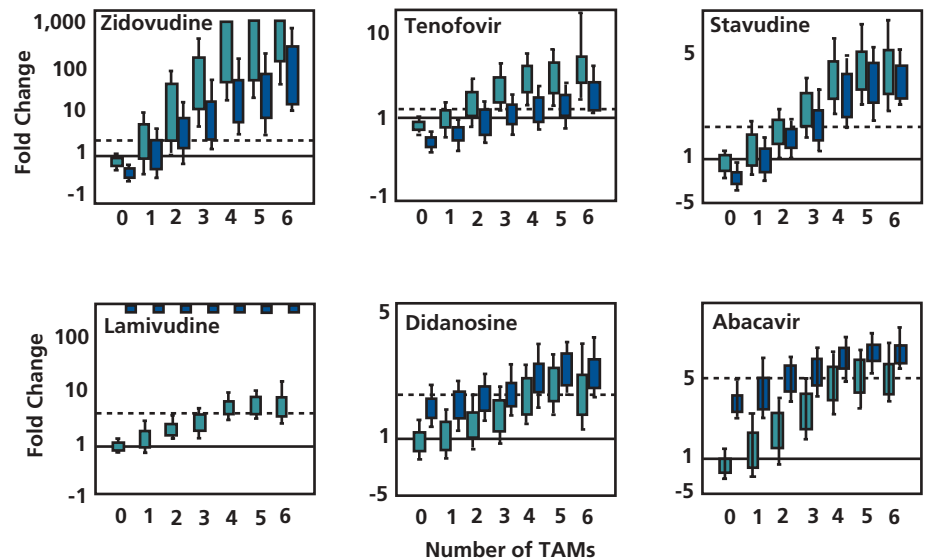
and didanosine but maintained susceptibility to thymidine analogues. K65R is the signature mutation of tenofovir; it can also emerge with abacavir, though L74V is more common. In the presence of zidovudine (and, to a lesser extent, stavudine), the pathway involves the acquisition of the M184V mutation and TAMs, resulting, as already noted, in resistance to both thymidine analogues and other nRTIs.

A brief profile of nRTI resistance mutations and their effects is shown in Table 1, and some sequencing options in cases of specific mutations are shown in Table 2. Options in the presence of TAMs depend on whether M184V is present and on the particular resistance pathway exhibited. Phenotypic testing may be useful when multiple TAMs are present.

### Resistance to NNRTIs

Resistance mutations selected by NNRTIs are shown in Figure 3. NNRTI-associated resistance mutations, the most common of which is K103N, are common at the time of virologic failure and may occur as the first resistance mutations, even preceding the detection of M184V. Most NNRTI resistance is associated with high-level cross-resistance to other drugs in the

class. There are limited prospects for sequential use of currently available NNRTIs following virologic failure with resistance. It had been proposed that efavirenz would be active in cases of nevirapine failure due to the Y181C mutation, but poor results were observed with clinical sequencing, possibly because of the presence of low levels of K103N not detected by commercial assays. Mutations at codons 190 and 225 are associated with delavirdine hypersusceptibility, but there are no clinical data to support delavirdine use in this setting. There are some investigational second-generation NNRTIs that are promising with regard to potential sequential use, such as TMC-125 and TMC-278, and it is hoped that such drugs will remain active in the presence of the K103N and other mutations. However, it is currently advisable to use NNRTIs only in fully virologically suppressed patients. NNRTI resistance mutations do not reduce replicative fitness of the virus as do some resistance mutations to nRTIs or PIs. In addition, continued use of an NNRTI in a non-suppressive regimen allows accumulation of additional NNRTI resistance mutations that may jeopardize the future use of the second-generation NNRTIs now in development.



**Figure 2.** Change in susceptibility as a function of the number of thymidine analogue-associated mutations (TAMs) and the presence of the M184V mutation. Green indicates presence of wild-type M184V; blue indicates presence of mutant M184V. Adapted with permission from Whitcomb et al, *J Infect Dis*, 2003.

**Table 1.** Selected Important Information on Nucleoside (and Nucleotide) Analogue Reverse Transcriptase Inhibitor Resistance

Mutation(s)	Selected by	Effects on other Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)
M184V	Lamivudine, emtricitabine	<ul style="list-style-type: none"> <li>Loss of susceptibility to lamivudine, emtricitabine</li> <li>↓ Susceptibility to abacavir, didanosine (clinically insignificant)</li> <li>Delayed TAMs and ↑ susceptibility to zidovudine, stavudine, tenofovir</li> </ul>
TAMs	Zidovudine, stavudine	<ul style="list-style-type: none"> <li>↓ Susceptibility to all nRTIs based on number of TAMs</li> <li>Greatest loss of susceptibility with the M41L/L210W/T215Y pathway</li> </ul>
Q151M complex, T69 insertion	Zidovudine/didanosine, didanosine/stavudine	<ul style="list-style-type: none"> <li>Q151M complex: resistance to all nRTIs except tenofovir</li> <li>T69 insertion: resistance to all nRTIs</li> </ul>
K65R	Tenofovir, abacavir, didanosine	<ul style="list-style-type: none"> <li>Variable ↓ susceptibility to tenofovir, abacavir, didanosine (and lamivudine, emtricitabine)</li> <li>↑ Susceptibility to zidovudine</li> </ul>
L74V	Abacavir, didanosine	<ul style="list-style-type: none"> <li>↓ Susceptibility to abacavir, didanosine</li> <li>↑ Susceptibility to zidovudine, tenofovir</li> </ul>
E44D, V118I	Zidovudine, stavudine	<ul style="list-style-type: none"> <li>Increased nRTI resistance (with M41L/L210W/T215Y pathway)</li> </ul>

TAMs indicates thymidine analogue-associated mutations; nRTIs, nucleoside (and nucleotide) analogue reverse transcriptase inhibitors.

## Resistance to PIs

PI resistance mutations and resistance patterns after initial failure of commonly used PIs are shown in Figure 4, and the major mutations for each agent are highlighted in bold. Some major mutations are not associated with PI cross-resistance, notably the D30N nelfinavir resistance mutation and the I50L atazanavir resistance mutation. However, many other major PI mutations, such as mutations at codons 82, 84, and 90, are associated with significant cross-resistance. Accumulation of minor mutations, which by themselves do not cause resistance, can increase cross-resistance in the presence of major mutations. It is notable with regard to PIs that HIV does not always seem to “know the rules” regarding resistance, making sequencing after failure problematic. For example, although it has been argued that nelfinavir failure is

not associated with cross-resistance to other PIs because of the emergence of the unique D30N mutation, it is now known that it is not uncommon for L90M to emerge instead of D30N. Finally, PI resistance mutations are emerging less frequently today because of the widespread use of ritonavir-boosted regimens. Data from previously PI-naïve patients show that failure with several of the ritonavir-boosted PIs is not associated with PI resistance. Furthermore, since the accumulation of PI mutations is gradual and sequential, like that of TAMs, it can be prevented by responding promptly to early virologic failure rather than allowing continued therapy with a nonsuppressive PI-based regimen.

## Resistance to Enfuvirtide

As with NNRTIs, there appears to be a low genetic barrier to resistance to the

fusion inhibitor enfuvirtide. Resistance to this agent has been observed at early virologic failure, and failure often is associated with rapid viral rebound to baseline HIV RNA level. In one study, enfuvirtide was stopped in 22 patients with detectable viremia while on enfuvirtide, and only a small viral rebound was observed when the drug was discontinued. Phenotypic susceptibility to the drug reemerged by week 16 in most of these patients, and although there was increased replicative fitness of the virus as resistance faded, there was very little change in plasma viral load. Such findings suggest that there is no benefit to continuing enfuvirtide once resistance has emerged and underscore the importance of using enfuvirtide in combination with other active agents.

## Phenotypic Testing

Advantages of phenotypic testing for resistance include relatively easy interpretation, the ability to provide quantitative information on the degree of resistance, the ability to assess interactions among resistance mutations on overall resistance and susceptibility, and the fact that it does not require an understanding of genotypic corre-

**Table 2.** Some Nucleoside (or Nucleotide) Analogue Reverse Transcriptase Inhibitor Sequencing Options for Specific Genotypic Resistance Patterns

Mutation(s)	Sequencing Options
K65R	Zidovudine > stavudine, with or without abacavir
K65R with M184V	Zidovudine > stavudine, with or without tenofovir
L74V with or without M184V	Zidovudine, tenofovir > stavudine
M184V	Zidovudine, stavudine, tenofovir > abacavir, didanosine
TAMs with or without M184V	Depends on the number of TAMs and the TAM pathway

TAMs indicates thymidine analogue-associated mutations.

<b>Delavirdine</b>	K103N; V106M; Y181C; Y188L; P236L
<b>Efavirenz</b>	L100I; K103N; V106M; V108I; Y181C/I; Y188L; G190S/A; P225H
<b>Nevirapine</b>	L100I; K103N; V106A/M; V108I; Y181C/I; Y188C/L/H; G190A
<b>Multi-NNRTI Resistance</b>	K103N; V106M; Y188L
<b>Multi-NNRTI Resistance: Accumulation of Mutations</b>	L100I; V106A; V181C/I; G190S/A; M230L

Figure 3. Mutations selected by nonnucleoside reverse transcriptase inhibitors. Adapted with permission from the International AIDS Society–USA (Johnson et al, *Top HIV Med*, 2005). For user notes and updated figures, visit [www.iasusa.org](http://www.iasusa.org).

lates of resistance. Phenotypic analysis determines the degree to which a drug inhibits replication of the patient’s virus, with the results showing the fold change in 50% inhibitory concentration (IC<sub>50</sub>) compared with a wild-type reference HIV. Interpreting the results is not as straightforward as it may appear, however, since it may be unclear what fold-change cutoff should be used to indicate decreased susceptibility. The likelihood of response to a drug decreases gradually with increasing fold change, as in the example shown in Figure 5, so that the cutoff used on a phenotypic report turns quantitative data into a qualitative determination of resistance versus susceptibility. Studies with tenofovir showed that the best virologic response was achieved in patients whose fold change was less than 1.4, partially reduced when at 1.4 to 4, and that the drug was virtually inactive in the setting of greater than 4-fold resistance (Figure 5; Miller et al, *J Infect Dis*, 2004). Knowledge of such gradations in response are helpful for treatment-experienced patients with limited options, for whom it may be necessary to use drugs with partial susceptibility. Phenotypic reports also indicate whether cutoffs are derived from clinical data or simply from in vitro testing; more confidence can be placed in

clinical cutoffs than biologic ones.

Phenotyping should be considered after multiple regimen failures when mutation patterns and their interpretation are likely to be complex, or when there may be limited treatment options making even partial activity desirable. The presence of multiple TAMs or PI mutations can be especially difficult to interpret genotypically. Phenotyping can also be used to evaluate viral susceptibility to newer drugs, or for patients infected with nonsubtype-B HIV, since in both cases genotypic correlates of resistance may not be well-established. Finally, phenotypic testing may prove useful in correlating resistance with blood drug levels (ie, in determining inhibitory quotient) or viral fitness (replication capacity).

### Other Approaches to Genotypic Testing

One form of genotypic testing uses a database to predict phenotype, as opposed to the standard algorithmic approach to interpretation. In this

case, the genotype is used to provide an estimated, or “virtual,” phenotype based on comparison with a large database of paired genotypic and phenotypic test results. The cost of the test is slightly more than that of genotyping alone.

### Combined Resistance Testing

Another popular, though expensive, approach is combination genotypic-and-phenotypic testing, which offers the advantages of both tests and is particularly useful when mixtures of virus are present (eg, when there is low-level emerging or reverting resistance). In addition to indicating the results of both tests, the report provides a net assessment of viral susceptibility to each drug, which is useful in cases of discordance between genotypic and phenotypic results. In general, if phenotypic test results indicate resistance and genotypic test results indicate susceptibility, it is assumed that the phenotype is a more accurate measure of susceptibility. The same is true when phenotyping suggests sus-

<b>Atazanavir</b>	L10I/F/V; G16E; K20R/M/I; L24I; V32I; L33I/F/V; M36I/L/V; M46I/L; G48V; <b>I50L</b> ; I54L/V/M/T; D60E; I62V; A71V/I/T/L; G73C/S/T/A; V82A/T; <b>I84V</b> ; I85V; <b>N88S</b> ; L90M; I93L
<b>Fosamprenavir</b>	L10F/I/R/V; V32I; M46I/L; I47V; <b>I50V</b> ; I54L/V/M; G73S; V82A/F/T/S; <b>I84V</b> ; L90M
<b>Indinavir</b>	L10I/R/V; K20M/R; L24I; V32I; M36I; <b>M46I/L</b> ; I54V; A71V/T; G73S/A; V77I; <b>V82A/F/T</b> ; <b>I84V</b> ; L90M
<b>Lopinavir/ritonavir</b>	L10F/I/R/V; K20M/R; L24I; <b>V32I</b> ; L33F; M46I/L; <b>I47V/A</b> ; I50V; F53L; I54V/L/A/M/T/S; L63P; A71V/T; G73S; <b>V82A/F/T/S</b> ; I84V; L90M
<b>Nelfinavir</b>	L10F/I; <b>D30N</b> ; M36I; M46I/L; A71V/T; V77I; V82A/F/T/S; I84V; N88D/S; <b>L90M</b>
<b>Ritonavir</b>	L10F/I/R/V; K20M/R; V32I; L33F; M36I; M46I/L; I50V; I54V/L; A71V/T; V77I; <b>V82A/F/T/S</b> ; <b>I84V</b> ; L90M
<b>Saquinavir</b>	L10I/R/V; <b>G48V</b> ; I54V/L; A71V/T; G73S; V77I; V82A; I84V; <b>L90M</b>
<b>Tipranavir/ritonavir</b>	L10V; I13V; K20M/R; <b>L33F</b> ; E35G; M36I; K43T; M46L; I47V; I54A/M/V; Q58E; H69K; T74P; <b>V82L/T</b> ; N83D; <b>I84V</b> ; L90M

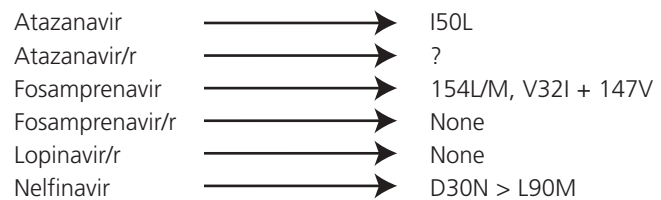


Figure 4. Top: Mutations selected by protease inhibitors. Major mutations are highlighted in bold. Adapted with permission from the International AIDS Society–USA (Johnson et al, *Top HIV Med*, 2005). For user notes and updated figures, visit [www.iasusa.org](http://www.iasusa.org). Bottom: Resistance patterns after initial failure of commonly used protease inhibitors. /r indicates ritonavir-boosted.

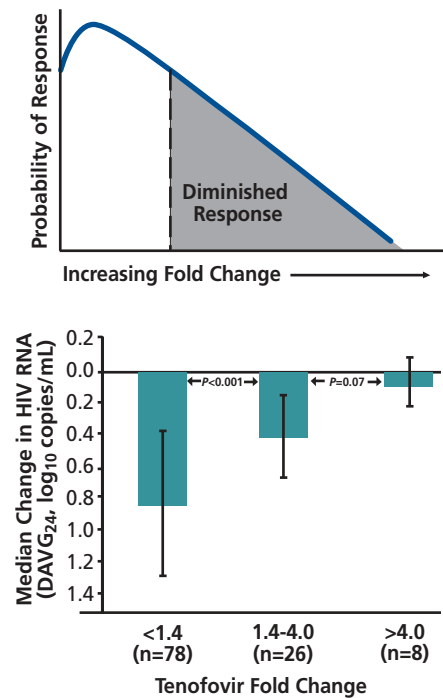


Figure 5. Top: Probability of virologic response to a drug according to fold increase in resistance; the dotted line marks the clinical cutoff (the fold change at which the virologic response begins to decline below wild-type susceptibility). Bottom: Reduction in viral load with tenofovir treatment according to baseline susceptibility of virus (tenofovir fold change). DAVG<sub>24</sub> indicates difference in average HIV RNA level between baseline and week 24. Adapted from data in Miller et al, *J Infect Dis*, 2004.

ceptibility and genotyping indicates resistance, unless mixtures of wild-type and mutant virus are present on the genotype, in which case the phenotype may underestimate the degree of resistance. The detection of mixtures by the genotype indicates the presence at low levels of mutants that may be emerging or that could become predominant with selective pressure. Some practitioners rarely order phenotyping without genotyping because of the concern about missing mixtures. Other reasons for discordance between the phenotypic and genotypic test results include interactions among mutations, as in the case of “resensitizing” mutations, or incomplete genotypic algorithms.

For example, there may be novel resistance mutations whose effects on viral susceptibility are not yet defined, and specific mutations may be under-weighted or over-weighted because their effect on susceptibility is not fully understood. Also, since most of the data on genotypic resistance comes from patients infected with subtype-B virus, interpretation of genotypes in patients infected with other subtypes may be difficult.

### Limitations of Resistance Tests

All currently available forms of resistance testing have limitations. They cannot detect minority populations of virus in a mixture (eg, those accounting for less than about 20% of the sample) and they cannot detect resistant virus archived in viral reservoirs. Because of the ability of wild-type virus to replace mutant virus when selective drug pressure is withdrawn, resistance tests are most reliable at predicting activity of drugs or drug classes that the patient is currently taking. For example, a patient with a distant history of NNRTI failure may no longer demonstrate the presence of the K103N mutation on a genotype, but its presence can and should be inferred, as it would emerge rapidly if NNRTI treatment were to be reinstated. It is crucial to remember that the patient’s viral resistance should be assessed based on the results of the current resistance test plus any and all prior resistance tests, or, when prior tests are not available, based on assumptions made about resistance based on a review of the treatment history. Finally, resistance testing requires a minimum viral load (around 500 to 1000 plasma HIV RNA copies/mL). This can be a problem in patients with early virologic failure, since waiting until the viral load is high enough for resistance testing may sometimes guarantee the emergence of additional resistance mutations.

*Presented in June 2005. First draft prepared from transcripts by Matthew Stenger. Reviewed and updated by Dr Gallant in November 2005.*

*Financial Disclosure: Dr Gallant has received grant and research support or honoraria from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Monogram Biosciences, Pfizer, Roche, Tanox, and Tibotec-Virco. He has also served as a consultant to Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Monogram Biosciences, Tanox, Tibotec-Virco, and Vertex.*

### Suggested Reading

Bennet D, McCormick L, Kline R, et al. US surveillance of HIV drug resistance at diagnosis using HIV diagnostic sera. [Abstract 674.] 12th Conference on Retroviruses and Opportunistic Infections. February 22-25, 2005; Boston, MA.

Hirsch MS, Brun-Vézinet F, Clotet B, et al. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA panel. *Clin Infect Dis*. 2003;37:113-128.

Johnson VA, Brun-Vézinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: Fall 2005. *Top HIV Med*. 2005;13:125-131.

Little SJ, Koelsch KK, Ignacio CC, et al. Persistence of transmitted drug-resistant virus among subjects with primary HIV infection deferring antiretroviral therapy. [Abstract 36LB.] 11th Conference on Retroviruses and Opportunistic Infections. February 8-11, 2004; San Francisco, CA.

Miller MD, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. *J Infect Dis*. 2004;189:837-846.

Whitcomb JM, Parkin NT, Chappey C, Hellmann NS, Petropoulos CJ. Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates. *J Infect Dis*. 2003;188:992-1000.

Yeni PG, Hammer SM, Hirsch MS, et al. Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society–USA panel. *JAMA*. 2004;292:251-265.

*Top HIV Med* 2005;13(5):138-142

Copyright 2005, International AIDS Society–USA