

The 118I Reverse Transcriptase Mutation Is the Only Independent Genotypic Predictor of Virologic Failure to a Stavudine-Containing Salvage Therapy in HIV-1-Infected Patients

Nicola Gianotti, MD,* Laura Galli, MSc,* Enzo Boeri, MD,† Anna De Bona, MD,* Monica Guffanti, MD,* Anna Danise, MD,* Stefania Salpietro, MSc,* Adriano Lazzarin, MD,* and Antonella Castagna, MD*

Summary: Patients infected with HIV-1 with more than 1000 HIV-1 RNA copies/mL, who were genotyped within 3 months before starting stavudine and treated for at least 3 months with a stable stavudine-containing highly active antiretroviral therapy, were selected from our database to identify the determinants of response to stavudine. Nonresponse was defined as a failure to achieve HIV-1 RNA level of less than 400 copies/mL or a reduction of more than 2 log₁₀ by week 12. Univariate logistic analysis was used to elicit the failure-associated reverse transcriptase mutations (scored 1 to develop a genotype score). Eighty-one patients were eligible for the analysis, including 75 (93%) who previously received zidovudine. Thirty-five (43%) were nonresponders. Univariate logistic analysis revealed the following failure-associated mutations: 41L ($P = 0.0001$), 44D ($P = 0.02$), 118I ($P = 0.0006$), 184V ($P = 0.04$), 210W ($P = 0.0004$), and 215Y ($P = 0.002$) for a median stavudine score of 2. Failure was observed in 7 (18.9%) of 37 patients with a score less than 2, compared with 28 (63.6%) of 44 patients with a score of 2 or greater ($P < 0.0001$). The multivariable analysis showed that the 118I mutation ($P = 0.04$) was the only independent genotypic predictor of failing on a stavudine-containing highly active antiretroviral therapy.

Key Words: HIV-1 genotype, stavudine, HAART, virologic failure
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One group of reverse transcriptase (RT) mutations (41L, 67N, 70R, 210W, 215Y/F, and 219Q/E) can hamper the response to stavudine. These amino acid substitutions are commonly called *thymidine analogue mutations* (TAMs) because they are selected by thymidine analogues (zidovudine and stavudine), but they can also promote resistance to

almost all nucleoside and nucleotide RT inhibitors (NRTIs)^{1,2} by mediating the adenosine triphosphate and pyrophosphate-dependent hydrolytic removal of NRTIs from a terminated cDNA chain.^{3,4} For this reason, the alternative, more explanatory, use of the term *nucleotide excision mutations* (NEMs) has been proposed⁵ and may be more appropriate.

RT mutations at residues 44 and 118 can contribute to resistance to all NRTIs in the presence of NEMs, but the underlying mechanism is still unknown. For this reason, the definition of *nucleoside/nucleotide-associated mutations* (NAMs) includes NEMs and the 44D and 118I RT mutations.⁶

Three studies have shown that the response to stavudine given as monotherapy or dual therapy is impaired in zidovudine-resistant patients.^{7–9} However, the Novavir study¹⁰ found that neither resistance to stavudine (assessed by means of the algorithm used by the Agence Nationale de Recherche sur le Sida) nor any specific RT mutation correlated with the response to a combination of stavudine, lamivudine, and indinavir in patients failing on zidovudine plus didanosine or zalcitabine.

The aim of this study is to identify the largely unknown determinants of virologic response to stavudine in patients infected with HIV-1 failing on any antiretroviral regimen and starting a stavudine-containing regimen in the context of a highly active antiretroviral therapy (HAART), which now represents the real-world scenario of HIV treatment.

METHODS

Study Design and Patient Selection

Among all of the HIV-1-infected patients attending our clinic on a routine basis, those failing on any antiretroviral regimen were selected from our ongoing database if they (1) had HIV-1 RNA levels of more than 1000 copies/mL, (2) had undergone HIV-1 genotyping (within 3 months before starting stavudine), and (3) had then received a stavudine-containing HAART regimen for at least 3 months without any change in any of the components of the new regimen.

The patients failing to achieve an HIV-1 RNA level of less than 400 copies/mL or a reduction of more than 2 log₁₀ by week 12 (virologic failure) were defined as nonresponders.

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From the *Clinic of Infectious Diseases, Vita-Salute San Raffaele University, and †Diagnostica and Ricerca San Raffaele, Milan, Italy.

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Reprints: Nicola Gianotti, MD, Divisione di Malattie Infettive, Ospedale San Raffaele, Via Stamira d'Ancona 20, 20127 Milan, Italy (e-mail: nicola.gianotti@hsr.it).

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TABLE 1. Baseline Characteristics and Their Comparison Between Responder and Nonresponder Patients

	All (N = 81)	Responders, n = 46 (56.8%)	Nonresponders, n = 35 (43.2%)	P*
Zidovudine experienced	75 (92.6)	—	—	—
Stavudine experienced	60 (74.1)	30 (65.2)	30 (85.7)	0.02
NNRTI experienced	48 (59.3)	21 (45.7)	27 (77.1)	0.003
PI experienced	65 (80.2)	35 (76.1)	30 (85.7)	NS
No. of previously used drugs	7 [5–9]	6 [4–8]	8 [6–10]	0.03
HIV-1 RNA (log ₁₀ copies/mL)	4.81 [4.18–5.28]	4.81 [4–5.22]	4.9 [4.46–5.34]	NS
CD4 T lymphocytes (cells per microliter)	270 [138–423]	315 [189–435]	206 [106–371]	NS
No. of NEMs	2 [0–4]	1 [0–3]	3 [2–4]	0.001
Off-treatment at genotyping	34 (42)	24 (52.2)	17 (48.6)	NS
Received a new drug class together with stavudine	18 (22.2)	14 (30.4)	4 (11.4)	NS

Values are given as n (%). Continuous variables: median values [interquartile range].

NNRTI indicates nonnucleoside reverse transcriptase inhibitor; NS, not significant; PI, protease inhibitor; NEMs, nucleotide excision mutations.

*Responders vs nonresponders.

Virology

HIV RNA was assessed by means of nucleic acid sequence–based amplification (Nuclisens HIV-1; Bio-Merieux, Boxtel, the Netherlands); the quantification limit was 80 copies/mL.

The sequence of the HIV-1 *pol* gene was performed by extracting HIV RNA using the QIAamp Viral RNA kit (Qiagen GmbH, Hilden, Germany). The RNA was reverse-transcribed to cDNA using Expand Reverse Transcriptase (Roche Diagnostics, Mannheim, Germany), and the cDNA was amplified by means of 2 nested reactions using the Expand High Fidelity polymerase chain reaction system kit (Roche Diagnostics) and oligonucleotide primers (sequence copyright by Virco, Mechelen, Belgium). The amplified fragments were purified using the QIAquick kit (Qiagen GmbH) and sequenced by 7 oligonucleotide primers (sequence copyright, Virco) using MegaBace 1000 (Amersham Biosciences, Piscataway, NJ). The sequence analysis was performed by the software Sequencher (Gene Codes Corporation, Ann Arbor, MI).

Genotypic mixtures were reported if the second highest peak was 30% of the highest. The residues were interpreted as mutated even in the presence of a mixture with wild type.

Statistical Analysis

The Mann-Whitney rank sum test for nonparametric data was used to compare the mean independent values of the continuous variables. The associations between discrete variables were tested by means of the χ^2 test or Fisher exact test, as appropriate.

The RT mutations related to resistance to NRTIs⁶ and detected in more than 10% of the patients¹¹ were considered in the univariate logistic regression analysis, which was applied to identify those associated with failure. Each failure-associated mutation was scored 1 to develop a stavudine genotypic score for each patient.

The receiver operating characteristic curve was used to evaluate the performance of the stavudine genotypic score and identify the optimal clinical cutoff point that minimized classification errors (virologic response or failure).

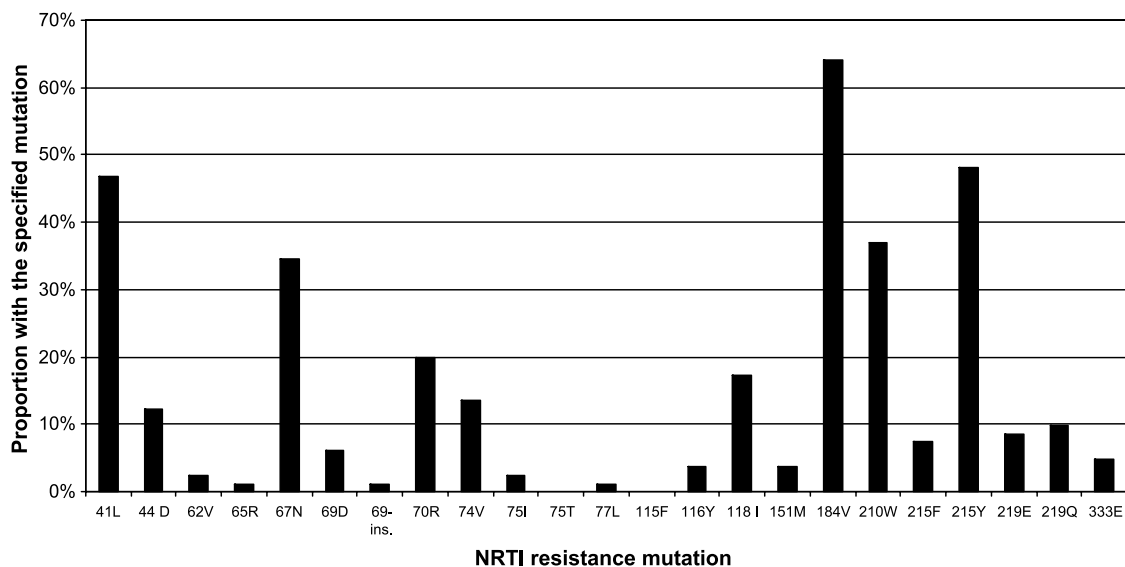


FIGURE 1. Distribution of HIV-1 NRTI-resistance mutations at baseline.

TABLE 2. Antiretroviral Drugs Used in Combination With Stavudine

Drug	No. of Patients (%)
Didanosine	45 (55.6)
Lamivudine	27 (33.3)
Abacavir	8 (9.9)
Tenofovir	13 (16)
Nevirapine	4 (4.9)
Efavirenz	15 (18.5)
Saquinavir soft-gel capsules	3 (3.7)
Saquinavir hard-gel capsules	1 (1.2)
Full-dose ritonavir	4 (4.9)
Boosting-dose ritonavir	40 (49.4)
Indinavir	3 (3.7)
Nelfinavir	5 (6.2)
Amprenavir	8 (9.9)
Lopinavir	34 (42)
Atazanavir	3 (3.7)
Enfuvirtide	1 (1.2)

Subsequently, multiple logistic regression was used to identify the independent predictors of a virologic failure on stavudine-containing HAART using 2 models. The variables included in the first were (1) baseline log₁₀ HIV-1 RNA and CD4 T lymphocyte levels, being off-therapy before starting stavudine; (2) the use of a new drug class, together with stavudine, in the new regimen; (3) previous stavudine treatment(s); (4) the number of previously used drugs; (5) the number of NEMs at baseline; and (6) the stavudine genotypic score (<2 vs. ≥2). In the second model, the last 2 variables were replaced by the individual RT mutations identified as being statistically related to failure using univariate analysis.

The statistical analysis was made using SAS software (release 8.02; SAS, Cary, NC). The continuous variables were given as median values (and interquartile range).

RESULTS

Eighty-one patients seen between January 1998 and December 2003 fulfilled the inclusion criteria. Seventy-three percent were men, 89% were white, 6% were American Hispanics, 5% were Africans, and the median interquartile range (IQR) age was 42 years (37–47 years). Baseline characteristics are illustrated in Table 1. Figure 1 shows

TABLE 3. Univariate Logistic Regression Analysis of the HIV-1 RT Mutations (Present in >10% of Patients) Associated With Failure to a Stavudine-Containing Salvage HAART

RT Mutation	Relative Risk	95% CI	P
41L	6.35	2.4–16.82	0.0001
44D	6.52	1.29–33	0.02
118I	11.45	2.37–55.71	0.0006
184V	2.84	1.06–7.55	0.04
210W	6.17	2.28–16.67	0.0004
215Y	4.51	1.76–11.58	0.002

TABLE 4. Distribution of the Stavudine Genotype Score Calculated on the Presence of the Following HIV-1 Reverse Transcriptase Mutations: 41L, 44D, 118I, 184V, 210W, and 215Y (Each Mutation Was Scored 1)

Stavudine Genotypic Score	No. of Patients
0	18
1	19
2	10
3	8
4	12
5	12
6	2
25th Percentile	1
Median	2
75th Percentile	4

the baseline distribution of NRTI resistance mutations. In 30 patients (37%), a mixture of wild-type and mutated residues was found in at least 1 RT position. The mutations present in more than 10% of the patients were 41L, 44D, 67N, 70R, 74V, 118I, 184V, 210W, and 215Y, which were therefore used to develop the stavudine genotype score.

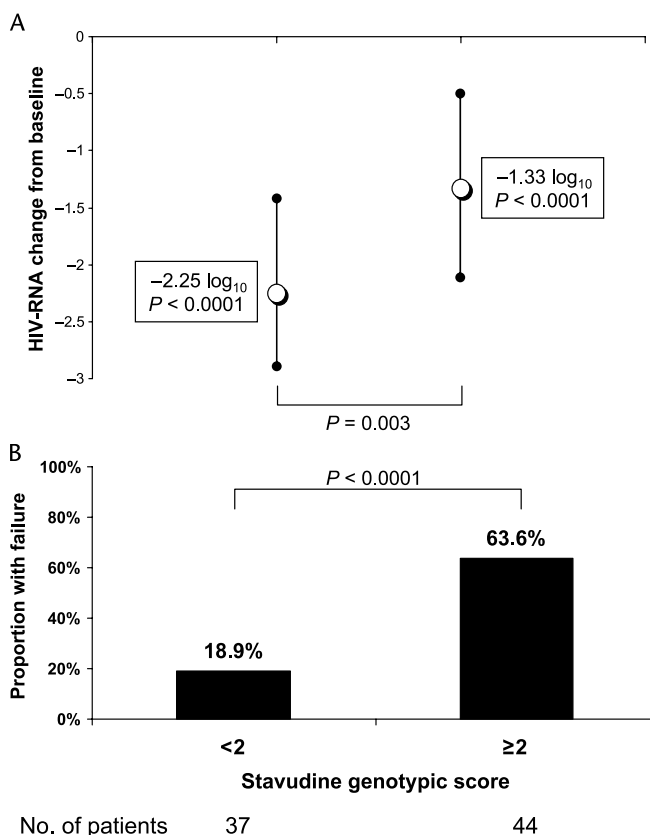


FIGURE 2. Virologic outcome of stavudine-containing HAART by stavudine genotypic score (<2 or ≥2). A, Week 12 changes in log₁₀ HIV-1-RNA vs baseline. Open circles indicate median values; bars bordered by filled circles, interquartile ranges. B, Week 12 proportion of patients with virologic failure.

Twelve (92.3%) of 13 patients harboring an HIV variant bearing the 118I mutation (HIV-1_{118I}) and 48 (78.7%) of 61 not harboring an HIV-1_{118I} previously received stavudine ($P =$ not significant).

The drugs combined with stavudine in the salvage HAART are listed in Table 2. Eighteen patients (21%) received a new drug class together with stavudine.

The week 12 change from baseline in HIV-1 RNA levels was -1.61 (IQR, -2.56 to -0.98) \log_{10} copies/mL. Thirty-five patients (43%) were nonresponders.

RT mutations 67N, 70R, and 74V were not associated with either a virologic failure or a virologic response. The relative risks of a virologic failure in the presence of RT mutations 41L, 44D, 118I, 184V, 210W, and 215Y at baseline are shown in Table 3.

The median stavudine genotype score of the 81 subjects was 2 (IQR, 1–4); the distribution of the scores is shown in Table 4. The receiver operating characteristic curve indicated that a score of 2 was the best cutoff point for virologic failure ($C = 0.79$; 95% confidence interval [CI], 0.69–0.89; $P < 0.0001$).

Virologic failure occurred in 7 (18.9%) of 37 patients with a score of <2 and in 28 (63.6%) of 44 of those with a score of ≥ 2 ($P < 0.0001$). The week 12 changes from baseline in HIV-1 RNA levels were significant both in patients with a score <2 (-2.25 \log_{10} copies/mL IQR, -2.87 to -1.45 ; $P < 0.0001$) and in those with a score of ≥ 2 (-1.33 \log_{10} copies/mL; IQR, -2.17 to -0.50 ; $P < 0.0001$); the difference between these 2 subgroups was also significant ($P = 0.003$; Fig. 2).

The results of the multivariable analysis are shown in Table 5. In the first model, a previous treatment with stavudine ($P = 0.04$) independently predicted virologic failure, whereas the use of a new drug class independently predicted a virologic response ($P = 0.049$). When the stavudine genotypic score and the number of NEMs were

substituted by the individual mutations associated with virologic failure at univariate analysis, only the 118I mutation ($P = 0.04$) remained independently predictive of this outcome.

DISCUSSION

The present study emphasizes that the role of 118I HIV-1 variants in impairing virologic response to stavudine-containing regimens might be more relevant than that hypothesized based upon in vitro phenotypic data and than that attributed to NEMs. It is possible that this is the first study to find a correlation between HIV-1_{118I} and an impaired response to stavudine, simply because such a correlation has never been investigated before. A relationship between the detection of the 44D/A and/or 118I mutation and previous treatments with didanosine or stavudine has been found in 2 independent retrospective analyses.^{12,13} When we included this mutation in the multivariable model, the previous use of stavudine (and the introduction of a new drug class in the new regimen) was no longer predictive of the virologic outcome.

Phenotypic resistance to stavudine increases proportionally with the number of NEMs.¹ Our univariate analysis showed that 6 RT mutations were related to the virologic outcome of stavudine-containing HAART, but not all of the RT mutations were associated with a lack of response to stavudine. This finding confirms the fact that phenotypic data cannot be directly translated into clinical practice, and that RT mutations and NEMs cannot be considered as a whole, but each mutation may have different impacts on the virologic response to different drugs.¹⁴

Consistent with phenotypic data, our patients with higher stavudine genotype scores showed a worse virologic response, but the score itself was not an independent predictor of failure. This was possibly because (1) about

TABLE 5. Multiple Logistic Regression Analysis of the Independent Predictors of Virological Failure to a Stavudine-containing Salvage HAART

Variable	Model 1			Model 2		
	OR	95% CI	P	OR	95% CI	P
BL CD4 + T lymphocytes (per unit increase)	1	0.99–1	NS	1	0.99–1	NS
BL \log_{10} HIV-1 RNA (per unit increase)	0.69	0.29–1.64	NS	0.66	0.27–1.62	NS
Off-therapy at BL	0.46	0.12–1.71	NS	0.7	0.17–2.82	NS
New drug class with stavudine	0.22	0.05–0.99	0.049	0.26	0.05–1.29	NS
Previous treatment with stavudine	10.73	1.17–98.68	0.04	6.71	0.68–65.99	NS
No. previously used drugs (per unit increase)	0.88	0.67–1.14	NS	0.93	0.71–1.23	NS
No. NEMs (per unit increase)	1.34	0.92–1.95	NS	NC	NC	–
Stavudine genotypic score (<2 vs ≥ 2)	0.65	0.13–3.32	NS	NC	NC	–
41L RT mutation	NC	NC	–	2.72	0.39–19.23	NS
44D RT mutation	NC	NC	–	2.09	0.22–20.07	NS
118I RT mutation	NC	NC	–	7.18	1.12–46.19	0.04
184V RT mutation	NC	NC	–	1.95	0.47–8.06	NS
210W RT mutation	NC	NC	–	1.21	0.21–6.87	NS
215Y RT mutation	NC	NC	–	0.96	0.17–5.42	NS

BL indicates baseline; NEMs, nucleotide excision mutations; NC, not considered; NS, not significant; RT, reverse transcriptase.

one third of the responders had a score greater than 2 and (2) nonresponders also showed a significant reduction in HIV-1 viral load.

Our results are consistent with those of the Novavir study,¹⁰ and it has been recently reported that stavudine may retain antiviral activity even in the presence of up to 3 NEMs.¹⁵ However, it must be pointed out that our sample size may have precluded the identification of some correlations that actually exist; for example, the low prevalence of the 75T mutation and of some amino acid substitutions known to confer high-level resistance to stavudine (eg, the 151M mutation or insertions at codon 69) prevented us from evaluating their impact on drug response. As 42% of our patients were off-treatment at the time of genotypic analysis, it is possible that this was also true for other NAMs because the significance of missing mutations in the absence and in the presence of drug selective pressure is very different.

Ideally, the most appropriate way to assess the genotypic determinants of drug response in patients who are failing therapy is to add the studied drug to the ongoing regimen (functional monotherapy). However, this is both unrealistic and unethical as it is inconsistent with shared guidelines for the management of HIV-1-infected patients who are failing therapy, whereas our approach is realistic (it considers the patient as a whole and not only his or her virus genotype), ethical, and reflects the international guidelines. Furthermore, adding a single drug to a failing regimen may lead to its efficacy being underestimated in the presence of a specific genotype because, according to guidelines, a new drug should usually be administered with at least 1 other drug not included in the failing regimens and preferably with a new drug of a new class.

Finally, our study design reflects a consolidated practice for identifying the genotypic determinants of response: the genotypic scores for other drugs^{16–20} have indeed been defined by administering them along with an optimized background regimen and not by the mere addition of 1 drug to the failing therapy.

Overall, our results suggest that, when given as part of HAART regimen, stavudine may be effective in zidovudine-experienced patients, at least in the short term. However, the fact that a significant proportion of patients was off-therapy at genotyping might, in part, affect the applicability of these results in patients who do not interrupt treatment before introducing stavudine. As it has been found that treatment interruption before salvage therapy can favor a better virological response in highly pretreated patients,²¹ it is possible that the efficacy of stavudine in our study was better than expected because of the drug withdrawal preceding the stavudine-containing regimen. By contrast, because most of the patients were also being treated with other NRTIs, the virological failure might also be caused by a higher level of resistance conferred against the combination of NRTIs the patients received and not just by stavudine.

In conclusions, the 118I RT mutation was the only independent genotypic predictor of failure on a stavudine-containing HAART in HIV-1-infected patients failing on other antiretroviral treatments. The results of our study provide a key insight into the determinants of the response to

stavudine in drug-experienced patients and could be included in the clinical decision-making process when evaluating salvage regimens for patients harboring HIV-1 variants bearing NAMs.

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REFERENCES

- Whitcomb JM, Parkin NT, Chappey C, et al. Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates. *J Infect Dis.* 2003;188:992–1000.
- Clavel F, Hance AJ. HIV drug resistance. *N Engl J Med.* 2004;350:1023–1035.
- Arion D, Kaushik N, McCormick S, et al. Phenotypic mechanism of HIV-1 resistance to 3'-azido-2'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry.* 1998;37:15908–15917.
- Meyer PR, Matsuura SE, So AG, et al. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. *Proc Natl Acad Sci U S A.* 1998;95:13471–13476.
- Shafer RW. Genotypic testing for human immunodeficiency virus type 1 drug resistance. *Clin Microbiol Rev.* 2002;15:247–277.
- Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: 2005. *Top HIV Med.* 2005;13:51–57.
- Shulman NS, Machezano RA, Shafer RW, et al. Genotypic correlates of a virologic response to stavudine after zidovudine monotherapy. *J Acquir Immune Defic Syndr.* 2001;27:377–380.
- Izopet J, Bicart-See A, Pasquier C, et al. Mutations conferring resistance to zidovudine diminish the antiviral effect of stavudine plus didanosine. *J Med Virol.* 1999;59:507–511.
- Calvez V, Costagliola D, Descamps D, et al. Impact of stavudine phenotype and thymidine analogue mutations on viral response to stavudine plus lamivudine in ALTIS 2 ANRS trial. *Antivir Ther.* 2002;7:211–218.
- Descamps D, Flandre P, Joly V, et al. Effect of zidovudine resistance mutations on virologic response to treatment with zidovudine or stavudine, each in combination with lamivudine and didanosine. *J Acquir Immune Defic Syndr.* 2002;31:464–471. [published correction appears in *J Acquir Immune Defic Syndr.* 2003;32:116].
- Brun-Vezinet F, Costagliola D, Khaled MA, et al. Clinically validated genotype analysis: guiding principles and statistical concerns. *Antivir Ther.* 2004;9:465–478.
- Romano L, Venturi G, Bloor S, et al. Broad nucleoside-analogue resistance implications for human immunodeficiency virus type 1 reverse-transcriptase mutations at codons 44 and 118. *J Infect Dis.* 2002;185:898–904.
- Montes B, Segondy M. Prevalence of the mutational pattern E44D/A and/or V118I in the reverse transcriptase (RT) gene of HIV-1 in relation to treatment with nucleoside analogue RT inhibitors. *J Med Virol.* 2002;66:299–303.
- 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.
- Maggiolo F, Callegaro A, Ripamonti D, et al. In vivo determination of stavudine activity in the presence of TAM. Presented at: 12th Conference on Retroviruses and Opportunistic Infections; February 22–25, 2005; Boston, MA. Abstract 706.
- Kempf DJ, Isaacson JD, King MS, et al. Analysis of the virological response with respect to baseline viral phenotype and genotype in protease inhibitor-experienced HIV-1-infected patients receiving lopinavir/ritonavir therapy. *Antivir Ther.* 2002;7:165–174.
- Marcelin AG, Cohen-Codar I, King MS, et al. Virological and

- pharmacological parameters predicting the response to lopinavir-ritonavir in heavily protease inhibitor-experienced patients. *Antimicrob Agents Chemother.* 2005;49:1720–1726.
18. Marcelin AG, Lamotte C, Delaugerre C, et al. Genotypic inhibitory quotient as predictor of virological response to ritonavir-amprenavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients. *Antimicrob Agents Chemother.* 2003;47:594–600.
 19. Marcelin AG, Dalban C, Peytavin G, et al. Clinically relevant interpretation of genotype and relationship to plasma drug concentrations for resistance to saquinavir-ritonavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients. *Antimicrob Agents Chemother.* 2004;48:4687–4692.
 20. Masquelier B, Tamalet C, Montes B, et al. Genotypic determinants of the virological response to tenofovir disoproxil fumarate in nucleoside reverse transcriptase inhibitor-experienced patients. *Antivir Ther.* 2004;9:315–323.
 21. Katlama C, Dominguez S, Gourlain K, et al. Benefit of treatment interruption in HIV-infected patients with multiple therapeutic failures: a randomized controlled trial (ANRS 097). *AIDS.* 2004;18:217–226.