BRIEF REPORT

Minor Mutations in HIV Protease at Baseline and Appearance of Primary Mutation 90M in Patients for Whom Their First Protease-Inhibitor Antiretroviral Regimens Failed

Carlo Federico Perno,[1] Alessandro Cozzi-Lepri,[9] Federica Forbici,[1] Ada Bertoli,[2] Michela Violin,[3] Maria Stella Mura,[4] Giampiero Cadeo,[5] Anna Orani,[6] Antonio Chirianni,[7] Carlo De Stefano,[8] Claudia Balotta,[4] and Antonella d'Arminio Monforte,[3] for the Italian Cohort Naive Antiretrovirals Study Group[a]

[1]Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani, and [2]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, [3]Institute of Infectious Diseases, L. Sacco Hospital, Milan, [4]Clinica Malattie Infettive, University of Sassari, Sassari, [5]Divisione Malattie Infettive, Spedali Civili, Brescia, [6]Unità Operativa Malattie Infettive, Ospedale di Lecco, Lecco, [7]II Divisione AIDS, D. Cotugno Hospital, Napoli, and [8]Divisione Malattie Infettive, Ospedale di Potenza, Potenza, Italy; [9]Royal Free and University College Medical School, University College London, London, United Kingdom

The association between minor mutations in human immunodeficiency virus (HIV) protease at baseline and development of common primary mutation 90M at virological failure (conferring some resistance to all protease inhibitors [PIs]) was evaluated in 93 previously drug-naive patients experiencing failure of their first PI-based antiretroviral regimens. In logistic regression analysis, the probability of accumulating a new 90M mutation at virological failure was associated with the presence at baseline of minor mutation 36I (naturally occurring in ~25% of HIV clade B and in >80% of HIV non-clade-B viruses) (adjusted odds ratio, 13.5 [95% confidence interval, 1.89–95.6]; $P = .009$) and, possibly, of 10I/V. This suggests a potential role for the presence of 36I at baseline in predicting the appearance of 90M at virological failure.

Experimental and clinical evidence supports the use of drug-resistance testing in HIV-infected patients receiving therapy and experiencing virological failure [1–4]. In contrast, no similar evidence is yet available for patients with long-term infections who are about to initiate their first antiretroviral therapy regimens (chronically infected, drug-naive patients).

The limited rate of primary mutations present in chronically infected, drug-naive patients may account, in part, for this lack of evidence [5, 6]. With regard to secondary mutations, their correlation with therapeutic outcome is still unclear, but a recent paper from our group has shown that the secondary mutation 36I in the protease (alone or with 10I/V) is associated with an increased risk of virological failure of highly active antiretroviral therapy (HAART) regimens containing a protease inhibitor (PI) [7].

Secondary mutations are often present, as common polymorphisms, in HIV protease of drug-naive patients. They may favor the appearance of primary mutations, such as 90M, which are present in ~40%–50% of patients experiencing multiple virological failures, and are associated with different levels of resistance to all PIs currently in use [8, 9]. This mutation may remain in the viral genome after interruption of a PI-containing regimen, probably because of the relatively high fitness of virus strains containing 90M that is associated with specific secondary mutations [10]. Drug resistance and persistence of the mutation may thus affect future therapeutic attempts and may disrupt strategies based on rotation of drugs belonging to the same class, even after periods of interruption of the PI-containing regimen. For these reasons, the identification of factors favoring the appearance of 90M in the protease is particularly relevant from the clinical standpoint. The aim of the present study was therefore to test whether specific minor mutations present in HIV protease before initiation of a PI-containing HAART regimen correlate with the appearance of 90M at the time of virological failure.

Patients, materials, and methods. One hundred ninety-three patients belonging to the Italian Cohort Naive Antiretrovirals [11] initiated a first antiviral regimen including exactly 1 PI and 2 nucleoside reverse-transcriptase inhibitors (NRTIs) and subsequently experienced virological failure, defined as the time of the first of 2 consecutive determinations of virus levels >500 copies/mL after ⩾24 weeks of therapy. All these patients had a genotypic test performed on a sample stored before initiation of therapy. For 93 of these patients, another plasma sample, stored between 2 weeks before and 6 weeks after the date of defined virological failure, was also available to be tested

Received 8 July 2003; accepted 18 November 2003; electronically published 12 May 2004.

Reprints or correspondence: Dr. Carlo Federico Perno, Dept. of Experimental Medicine, University of Rome Tor Vergata, Via Montpellier 1, Rome 00133, Italy (cf.perno@uniroma2.it).

The Journal of Infectious Diseases 2004; 189:1983–7

0022-1899/2004/18911-0003$15.00

Presented in part: 1st European Drug Resistance Workshop "From Basic Science to Clinical Applications," Luxembourg, March 2003.

Financial support: Italian Ministry of Health (Project AIDS and Ricerca Finalizzata e Corrente); European Community (contract EC QLK2-CT-2000-00291). The Italian Cohort Naive Antiretrovirals network is supported by an unrestricted educational grant from Glaxo-Wellcome, Italy.

[a] Study group members are listed after the text.

for genotypic resistance. Characteristics of these 93 patients were similar to those of the remaining 100 patients (for whom plasma samples were not available), in terms of proportion of males ($P = .11$), mode of transmission ($P = .40$), Centers for Disease Control and Prevention (CDC) stage ($P = .48$), presence of 10I/V ($P = .50$) or 36I ($P = .34$) before initiation of HAART, median age ($P = .07$), median pre-HAART CD4 cell count ($P = .96$), and median virus load change from baseline ($P = .09$).

Viral RNA was extracted from 500 μL of each plasma sample by use of the QiaAmp Viral RNA kit (Qiagen) and was retrotranscribed by use of reverse-transcriptase (RT) polymerase chain reaction (Viroseq 2; APPLERA). Protease and the first 340 aa of RT were sequenced by use of an ABI PRISM dye terminator cycle in a capillary-automated DNA sequencer (ABI-3100; both Applied Biosystems), in accordance with a well-standardized method [5, 7]. Mutations considered in the present study are those reported in the most recent update of the International AIDS Society–USA table of mutations [10]. A mutation was defined as "new" if detected at the time of virological failure but not at baseline.

The end point was the probability of having accumulated a new 90M mutation at the time of virological failure. The proportions of patients with 90M at the time of virological failure, according to baseline characteristics, were compared, and differences were tested by use of a χ^2 statistic.

A multivariable logistic regression analysis was used to identify factors associated with this end point [12]. The following covariates have been considered: sex, age, modality of HIV transmission, CD4 cell count nadir, pretherapy virus load, preexisting mutations in the protease regions (i.e., 10I/V, 36I, 63P, and 77I), and each drug initiated. For the pretherapy virus load, 2 binary variables were considered by use of the arbitrary, a priori–chosen cut-off values of 1 $\log_{10}$ and 1.5 $\log_{10}$ copies/mL. The analysis was performed in accordance with an intention-to-treat principle, by ignoring treatment switches. A sensitivity analysis, in which patients who changed treatment before resistance testing were instead excluded, was also performed.

Results. The baseline characteristics of the 93 HIV-infected patients included in the present study were the following: median HIV RNA level at baseline was 108,705 copies/mL (interquartile range [IQR], 48,000–280,000 copies/mL); 49 patients (52.7%) had >100,000 HIV RNA copies/mL. Median CD4 cell count was 265 cells/μL (IQR, 83–453 cells/μL); 36 patients (38.7%) had a CD4 cell count <200 cells/μL at baseline; 18 patients (19.4%) were in stage C, according to CDC 1993 classifications [13].

Zidovudine (79.6%) and lamivudine (57.0%) were the NRTIs most represented in the first HAART regimens. Hard gel (HG)–saquinavir and indinavir (always administered 3 times daily in an unboosted regimen) were the most used PIs in the initial regimen (50.5% and 31.2%, respectively). Overall, the most frequent combination regimens were zidovudine plus lamivudine plus indinavir ($n = 21$; 22.6%), zidovudine plus lamivudine plus HG-saquinavir ($n = 19$; 20.4%), and zidovudine plus zalcitabine plus HG-saquinavir ($n = 12$; 12.9%).

At baseline, 18 patients (19.4%) harbored a completely wild-type *(wt)* virus in both the RT and the protease regions. Three patients (3.2%) harbored mutations only in the RT region, 68 (73.1%) only in the protease region, and 4 (4.3%) in both the RT and the protease regions. Of 7 patients with RT mutations, 6 harbored 1 of the following: 70R, 106A, 118I ($n = 2$ patients), 184V, or 210W; 1 harbored both 41L and 215Y.

Only 1 of 93 patients harbored a major mutation (46L) in the protease region at baseline; among minor mutations (or polymorphisms), 63P was found in 46 patients (49.5%), 36I and 77I in 23 patients each (24.7%), 10I and 71T in 5 patients each (5.4%), 10V in 4 patients (4.3%), and 33F in 2 patients (2.2%); 20R, 53L, and 671V were found in 1 patient each (1.1%). Thirty-five patients showed >1 mutation in the protease region.

Virological failure occurred a median of 40 weeks (range, 24–154 week) after the initiation of HAART. The median HIV RNA level at the time of virological failure was 3.65 $\log_{10}$ copies/mL (IQR, 3.18–4.32 $\log_{10}$ copies/mL), and the median CD4 cell count was 390 cells/μL (IQR, 178–593 cells/μL). Median time from baseline to the date of the second genotypic test was 48 weeks (range, 21–152 weeks); 34 patients (36.6%) had modified their regimen before the second genotypic test, but, at the time of the test, they were all still receiving a PI-containing HAART regimen.

Plasma samples sequenced at the time of virological failure showed 76 new mutations in the RT region (occurring in 56 patients), and 72 new mutations in the protease region (occurring in 37 patients) (table 1). Overall, at the time of virological failure, 25 patients (26.9%) had both new RT and protease mutations, 31 (33.3%) had only new RT mutations, 12 (12.9%) had only new protease mutations, and 25 (26.9%) had no new RT or protease mutations. The most frequent new RT mutation was 184V, which occurred in 42 patients (all treated with lamivudine-containing regimens). With regard to the protease region, the most frequent new protease mutation was 90M, which occurred in 13 patients and was associated with other new mutations in 11 of 13 patients. Mutation 90M appeared in 3 (33.3%) of 9 patients carrying 10I/V at baseline and in 10 (11.9%) of 84 patients not carrying the mutation ($P = .08$, χ^2 test); similarly, 90M appeared in 6 (26.1%) of 23 patients carrying 36I at baseline and in 7 (10%) of 70 patients not carrying the mutation ($P = .05$, χ^2 test). Of interest, the frequency of 90M was greater than that for all new minor mutations occurring at the time of virological failure (table 1).

Table 2 shows the crude and adjusted odds ratios (ORs) of having the 90M mutation at the time of virological failure, by logistic regression analysis. In this model, the presence of either 10I/V or 36I before initiation of HAART was significantly associated with the risk of developing 90M, after adjusting for

Table 1. New mutations appearing in the reverse transcriptase (RT) and protease regions of HIV-1, harbored by 93 patients at the time of virological failure.

New mutation	Mutation(s), no. (%)
RT	
41L	6 (7.9)
62V	1 (1.3)
67N	9 (11.8)
70R	7 (9.2)
184V	42 (55.3)
210W	1 (1.3)
215Y	7 (9.2)
219E	1 (1.3)
219Q	2 (2.6)
Total	76 (100.0)
Protease	
10I	3 (4.2)
10R	1 (1.4)
10V	1 (1.4)
20M	1 (1.4)
20R	3 (4.2)
30N	2 (2.8)
32I	1 (1.4)
33F	2 (2.8)
36I	8 (11.9)
46I	2 (2.8)
46L	1 (1.4)
48V	2 (2.8)
54V	2 (2.8)
63P	5 (6.9)
71T	6 (8.3)
71V	6 (8.3)
73S	3 (4.2)
82A	5 (6.9)
82S	1 (1.4)
82T	1 (1.4)
84V	3 (4.2)
90M	13 (18.1)
Total	72 (100.0)

CD4 cell count nadir, pretherapy virus load, and number of other mutations in the RT and protease regions (for the presence of 10I/V: adjusted OR, 9.11 [95% confidence interval [CI], 1.09–76.10]; $P = .04$ and for the presence of 36I: adjusted OR, 13.5 [95% CI, 1.89–95.6]; $P = .009$). This effect does not appear to be shared with other minor protease mutations, since natural polymorphisms 63P and 77I, with prevalences similar to or even greater than that of 36I at baseline, failed to be associated with the appearance of 90M (table 2). Results of this analysis remained similar (1) after excluding 1 patient carrying a non-clade-B strain of HIV (adjusted OR between 10I/V and 90M, 10.04 [95% CI, 1.55–139.07]; $P = .03$, and adjusted OR between 36I and 90M, 12.96 [95% CI, 1.89–89.08]; $P = .009$), (2) after excluding 7 patients who carried RT mutations at baseline (adjusted OR between 10I/V and 90M, 13.28 [95% CI, 1.55–114.06]; $P = .02$, and adjusted OR between 36I and 90M, 11.58 [95% CI, 1.71–78.61]; $P = .01$), and (3) after adjusting for the use of HG-saquinavir over the course of the follow-up (adjusted OR between 10I/V and 90M, 13.74 [95% CI, 1.44–131.52]; $P = .02$, and adjusted OR between 36I and 90M, 14.01 [95% CI, 1.76–111.22]; $P = .01$, significant at the Bonferroni-corrected level).

Discussion. The results of the present report show a significant correlation between the presence of minor mutations 36I and 10I/V in HIV protease before initiation of HAART and the appearance of 90M at the time of virological failure. The strength of the association was greater for the presence of 36I (significant also, at a conservative Bonferroni-corrected value of $P = .0125$, on the basis of the 4 mutations tested: 10I/V, 36I, 63P, and 77I). These results are in line with those reported in our previous report, showing a greater risk of virological failure in patients carrying mutation 36I (alone or associated with mutations at codon 10) at baseline, but not other natural protease polymorphisms [7].

Of interest, the multivariable analysis showed that the appearance of 90M was significantly more frequent in patients whose virus load at the time of virological failure was $1-1.4$ $\log_{10}$ copies/mL lower than pretherapy levels, compared with patients whose virus load either had decreased >1.4 $\log_{10}$ copies/mL or had a small or no decrease (table 2). This indicates that partial virus suppression, rather than either major or no suppression, may have increased the risk of appearance of 90M. This also indirectly suggests that drug pressure on a highly replicating virus is necessary to select for resistant mutations; in contrast, low drug pressure (because of poor adherence to the treatment regimen or limited drug absorption) decreases the chances of developing resistance, as the *wt* virus will be the best replicating virus. Whether this also applies to the data described in this report requires a direct measure of adherence, which is not available for these 93 patients.

The interpretation of our results potentially may be limited by some factors. First, statistical analysis was performed by an intention-to-treat approach that ignores therapy switches. However, when the same analysis was performed after excluding patients who changed the initial HAART regimen, the association between 36I and the appearance of 90M at the time of virological failure remained similar (data not shown). Second, these results cannot be extended, in principle, to all PIs currently in use or to ritonavir-boosted PIs (widely used nowadays). Indeed, a large proportion of patients in our cohort were treated with HG-saquinavir (the most frequently used PI at the

Table 2. Crude and adjusted odds ratios (ORs) of acquiring 90M at the time of virological failure, by logistic regression model (intention-to-treat analysis; switches in therapy were ignored).

	Crude analysis		Adjusted analysis[a]	
Covariate	OR (95% CI)	*P*	OR (95% CI)	*P*
Baseline CD4 cell count, per 100 cells/μL higher	0.93 (0.71–1.22)	.60	0.95 (0.69–.130)	.75
Virus load change, $\log_{10}$ copies/mL				
<1 log decrease or an increase			1.00	
1–1.4 log decrease	4.13 (0.91–18.7)	.07	15.5 (1.64–146.8)	**.02**
⩾1.5 log decrease	1.42 (0.29–6.86)	.66	1.54 (0.23–10.5)	.65
10I/V				
No	1.00		1.00	
Yes	3.70 (0.80–17.2)	.09	9.11 (1.09–76.1)	**.04**
36I				
No	1.00		1.00	
Yes	3.18 (0.94–10.7)	.06	13.5 (1.89–95.6)	**.009**
63P				
No	1.00		1.00	
Yes	1.23 (0.38–3.97)	.73	1.21 (0.29–5.07)	.80
77I				
No	1.00		1.00	
Yes	0.51 (0.11–2.50)	.41	0.87 (0.11–6.72)	.89
Other protease mutations				
1 additional	0.48 (0.06–3.63)	.48	0.99 (0.13–7.42)	.99

NOTE. Bold type indicates significant values. CI, confidence interval.

[a] Adjusted for the factors shown in this table.

time of enrolment in the present study [1997–1999]), a drug with poor gut absorption that is therefore more prone to induce a limited pressure on the virus [14]. Indeed, our data show that the effect of having preexisting 36I (but not 10I/V) on the chance of developing 90M was somewhat lower in patients receiving HG-saquinavir (OR, 1.08 [95% CI, 0.23–5.08]) than in patients receiving other PIs (OR, 22.20 [95% CI, 1.92–256.82]; $P = .05$, test for interaction).

Mutation 36I is a natural polymorphism present in >80% of patients infected with non-clade-B strains of HIV [15]. Of interest, recent evidence shows that, in patients treated with nelfinavir-containing regimens, clade-G strains tend to evolve toward mutation 90M, rather than 30N (the latter is typically found in patients infected with clade B strains of HIV at the time of the first virological failure) [16]. Studies of large cohorts of patients that are able to discriminate among different non-clade-B strains of HIV and that use different PIs are now mandatory to clarify whether 36I and other common polymorphisms affect PI-containing first-line HIV therapies in poor-resource countries, where circulating strains belong mainly to clades other than B.

In conclusion, these findings strongly suggest, for the first time, an association between specific polymorphisms and the appearance of a primary mutation in vivo (which is, in turn, strongly related to virological failure). This has obvious practical implications. Resistance tests performed before the initiation of the first PI-containing regimens may help to identify those patients who may be more prone to virological failure of such regimens because they are carrying a virus already harboring certain mutations. In other words, the genetic barrier for PI is lowered by the presence of such mutations, as the virus would need a smaller number of "mutational steps" to overcome the inhibition of the drugs. Further clinical studies are warranted, to confirm this hypothesis and to verify whether it holds true for some PIs more than for others. If so, the use of resistance testing in chronically infected, drug-naive patients before initiation of therapy should become the rule.

ITALIAN COHORT NAIVE ANTIRETROVIRALS (I.CO.N.A.) STUDY GROUP MEMBERS

I.CO.N.A. members are listed by location: Italy—M. Montroni, G. Scalise, A. Zoli, and M. S. Del Prete (Ancona); U. Tirelli and G. Di Gennaro (Aviano [PN]); G. Pastore, N. Ladisa, and G. Minafra (Bari); F. Suter and C. Arici (Bergamo); F. Chiodo, V. Colangeli, C. Fiorini, and O. Coronado (Bologna); G. Carosi, G. P. Cadeo, F. Castelli, C. Minardi, and D. Vangi (Brescia); G. Rizzardini and G. Migliorino (Busto Arsizio); P. E. Manconi and P. Piano (Cagliari); T. Ferraro and A. Scerbo (Catanzaro);

E. Pizzigallo and M. D'Alessandro (Chieti); D. Santoro and L. Pusterla (Como); G. Carnevale and D. Galloni (Cremona); P. Viganò and M. Mena (Cuggiono); F. Ghinelli and L. Sighinolfi (Ferrara); F. Leoncini, F. Mazzotta, M. Pozzi, and S. Lo Caputo (Firenze); G. Angarano, B. Grisorio, and S. Ferrara (Foggia); P. Grima and P. Tundo (Galatina [LE]); G. Pagano, N. Piersantelli, A. Alessandrini, and R. Piscopo (Genova); M. Toti and S. Chigiotti (Grosseto); F. Soscia and L. Tacconi (Latina); A. Orani and P. Perini (Lecco); A. Scasso and A. Vincenti (Lucca); F. Chiodera and P. Castelli (Macerata); A. Scalzini and G. Fibbia (Mantova); M. Moroni, A. Lazzarin, A. Cargnel, G. M. Vigevani, L. Caggese, A. d'Arminio Monforte, D. Repetto, R. Novati, A. Galli, S. Merli, C. Pastecchia, and M. C. Moioli (Milano); R. Esposito and C. Mussini (Modena); N. Abrescia, A. Chirianni, C. Izzo, M. Piazza, M. De Marco, V. Montesarchio, E. Manzillo, and M. Graf (Napoli); A. Colomba, V. Abbadessa, T. Prestileo, and S. Mancuso (Palermo); C. Ferrari and P. Pizzaferri (Parma); G. Filice, L. Minoli, R. Bruno, and S. Novati (Pavia); F. Balzelli and K. Loso (Perugia); E. Petrelli and A. Cioppi (Pesaro); F. Alberici and A. Ruggieri (Piacenza); F. Menichetti and C. Martinelli (Pisa); C. De Stefano and A. La Gala (Potenza); G. Ballardini and E. Briganti (Ravenna); G. Magnani and M. A. Ursitti (Reggio Emilia); M. Arlotti and P. Ortolani (Rimini); L. Ortona, F. Dianzani, G. Ippolito, A. Antinori, G. Antonucci, S. D'Elia, P. Narciso, N. Petrosillo, V. Vullo, A. De Luca, L. Del Forno, M. Zaccarelli, R. Acinapura, P. De Longis, M. Ciardi, G. D'Offizi, M. P. Trotta, P. Noto, M. Lichtner, M. R. Capobianchi, E. Girardi, P. Pezzotti, and G. Rezza (Roma); M. S. Mura and M. Mannazzu (Sassari); P. Caramello, A. Sinicco, M. L. Soranzo, L. Gennero, M. Sciandra, and M. Bonasso (Torino); P. A. Grossi and C. Basilico (Varese); A. Poggio and G. Bottari (Verbania); E. Raise and S. Pasquinucci (Venezia); F. De Lalla and G. Tositti (Vicenza); and F. Resta and A. Chimienti (Taranto); United Kingdom—A. Cozzi-Lepri (London).

References

1. Hirsch MS, Brun-Vezinet 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**; 37:113–28.
2. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet **1999**; 353:2195–9.
3. Tural C, Ruiz L, Holtzer C, et al. Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. AIDS **2002**; 16:209–18.
4. Cingolani A, Antinori A, Rizzo MG, et al. Usefulness of monitoring HIV drug resistance and adherence in individuals failing highly active antiretroviral therapy: a randomized study (ARGENTA). AIDS **2002**; 16:369–79.
5. Perno CF, Cozzi-Lepri A, Balotta C, et al. Low prevalence of primary mutations associated with drug resistance in antiviral-naive patients at therapy initiation. AIDS **2002**; 16:619–24.
6. Ammaranond P, Cunningham P, Oelrichs R, et al. Rates of transmission of antiretroviral drug resistant strains of HIV-1. J Clin Virol **2003**; 26: 153–61.
7. Perno CF, Cozzi-Lepri A, Balotta C, et al. Secondary mutations in the protease region of human immunodeficiency virus and virologic failure in drug-naive patients treated with protease inhibitor–based therapy. J Infect Dis **2001**; 184:983–91.
8. Piana S, Carloni P, Rothlisberger U. Drug resistance in HIV-1 protease: Flexibility-assisted mechanism of compensatory mutations. Protein Sci **2002**; 11:2393–402.
9. D'Aquila RT, Schapiro JM, Brun-Vézinet F, et al. Drug resistance mutations in HIV-1. Top HIV Med **2002**; 10:21–25.
10. Martinez-Picado J, Savara AV, Sutton L, D'Aquila RT. Replicative fitness of protease inhibitor–resistant mutants of human immunodeficiency virus type 1. J Virol **1999**; 73:3744–52.
11. d'Arminio Monforte A, Cozzi-Lepri A, Rezza G, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naive patients. AIDS **2000**; 14:499–507.
12. McCullogh P, Nelder A. Generalized linear models. 2nd ed. London: Chapman & Hall.
13. CDC 1993 revised classification system for HIV infection and expanded surveillance case definition of AIDS among adolescents and adults. MMWR Morb Mortal Wkly Rep **1992**; 41(RR-17):1–19.
14. Cardiello PG, Monhaphol T, Mahanontharit A, et al. Pharmacokinetics of once-daily saquinavir hard-gelatin capsules and saquinavir soft-gelatin capsules boosted with ritonavir in HIV-1–infected subjects. J Acquir Immune Defic Syndr **2003**; 32:375–9.
15. Los Alamos HIV database Web site. Available at: http://www.hiv.lanl.gov/content/index. Accessed October 2003.
16. Gomes P, Diogo I, Gonçalves MF, et al. Different pathways to nelfinavir genotypic resistance in HIV-1 subtypes B and G [abstract 46]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**.