

# Prevalence of Drug-Resistant HIV-1 Variants in Untreated Individuals in Europe: Implications for Clinical Management

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**Background.** Infection with drug-resistant human immunodeficiency virus type 1 (HIV-1) can impair the response to combination therapy. Widespread transmission of drug-resistant variants has the disturbing potential of limiting future therapy options and affecting the efficacy of postexposure prophylaxis.

**Methods.** We determined the baseline rate of drug resistance in 2208 therapy-naive patients recently and chronically infected with HIV-1 from 19 European countries during 1996–2002.

**Results.** In Europe, 1 of 10 antiretroviral-naive patients carried viruses with  $\geq 1$  drug-resistance mutation. Recently infected patients harbored resistant variants more often than did chronically infected patients (13.5% vs. 8.7%;  $P = .006$ ). Non-B viruses (30%) less frequently carried resistance mutations than did subtype B viruses (4.8% vs. 12.9%;  $P < .01$ ). Baseline resistance increased over time in newly diagnosed cases of non-B infection: from 2.0% (1/49) in 1996–1998 to 8.2% (16/194) in 2000–2001.

**Conclusions.** Drug-resistant variants are frequently present in both recently and chronically infected therapy-naive patients. Drug-resistant variants are most commonly seen in patients infected with subtype B virus, probably because of longer exposure of these viruses to drugs. However, an increase in baseline resistance in non-B viruses is observed. These data argue for testing all drug-naive patients and are of relevance when guidelines for management of postexposure prophylaxis and first-line therapy are updated.

In countries with wide access to antiretroviral therapy, various drugs are currently available to suppress HIV-1 infection. Unfortunately, the frequent development of drug resistance during combination therapy limits the sustained response to antiretroviral drugs in many HIV-1-infected patients. Because cross-resistance is common, failure of therapy can also result in resistance to drugs that were not a part of the failing regimen. Drug-

resistance testing of HIV-1 is therefore an important tool for the selection of subsequent regimens, and it has

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become a standard of care in the clinical management of therapeutic failure [1].

Suggestions have been made that viruses that contain mutations conferring drug resistance may be less transmissible than drug-sensitive virus [2]. However, over the past few years, transmission of all kinds of drug-resistant HIV-1 variants from therapy-experienced patients to newly infected individuals has been observed [3]. Recent publications have shown that the acquisition of an infection with drug-resistant HIV-1 may result in a less-favorable response to therapy [4–6].

Resistance testing before the initiation of therapy may help guide the optimal selection of antiretroviral drugs. Different categories of patients can be identified, depending on the duration of infection. For recently infected patients, current guidelines recommend drug-resistance testing before the initiation of therapy [1, 7]. For chronically infected patients, the guidelines are less concordant, because it is assumed that, in the absence of drug-selection pressure, reversion to or overtake by drug-sensitive virus can occur over time and preclude the detection of drug-resistant variants.

Widespread dissemination of drug-resistant HIV-1 variants has the serious potential to limit therapeutic options in new patients. In addition, the efficacy of postexposure prophylaxis may be affected by the circulation of drug-resistant HIV-1 [8, 9]. To gain insight into the prevalence of transmitted drug resistance in Europe in both categories of therapy-naive patients, a large study of 2208 therapy-naive HIV-1-infected patients from 19 European countries was conducted.

## PATIENTS, MATERIALS, AND METHODS

**Study population.** HIV-1-seropositive individuals ( $\geq 18$  years old) were eligible for the study if they had never been exposed to antiretroviral drugs before the time of sampling. CD4<sup>+</sup> cell counts and HIV RNA loads were determined within 3 months of the moment of drug-resistance analysis. Antiretroviral-naive patients were considered to be chronically infected if they were known to have been infected for  $>1$  year before genotypic analysis was performed. Newly diagnosed cases were considered to be recent infection when HIV-1 antibody was not detectable by EIA with subsequent documented HIV-1 seroconversion within 1 year before the drug-resistance analysis was performed. If no reliable information about the duration of infection was available, newly diagnosed cases were classified as having an unknown duration of infection.

**Data collection.** The Combined Analysis of Resistance Transmission over Time of Chronically and Acute Infected HIV Patients (CATCH) study is a substudy of the European Commission-supported scientific surveillance program Strategy to Control Spread of HIV Drug Resistance (SPREAD), in which clinical centers and public-health institutes from 27 countries across Europe participate. The CATCH study was conducted

as a starting point for assessing the current status of baseline resistance in anticipation of the results of the SPREAD surveillance program. The CATCH study was also open to centers not currently participating in the SPREAD program. More information on the participating centers in the CATCH study and the SPREAD program can be found at <http://www.spread-europe.org>. Data from the period 1996–2002 were collected as part of national surveillance studies designed to investigate the transmission of drug resistance or of the standard clinical practice of baseline sequencing for all newly diagnosed cases in a center. Data were included from the following 19 countries (no. of patients): Austria (84), Belgium (128), Denmark (116), Finland (8), France (249), Germany (62), Greece (40), Israel (104), Italy (365), Luxembourg (161), The Netherlands (25), Norway (23), Poland (35), Portugal (91), Serbia-Montenegro (10), Spain (142), Sweden (153), Switzerland (260), and the United Kingdom (152). Data were retrieved from centers representing different geographical parts of each country, with the exception of Belgium and The Netherlands, where data were collected from only 1 geographical area. Part of the national data sets have been published elsewhere [10–16].

**Genotypic resistance analysis.** Population-based nucleotide sequence analysis of HIV *pol* was performed by local laboratories. Sequence alignment was performed with Clustal X (version 1.81; available at: <http://bips.u-strasbg.fr/fr/Documentation/ClustalX/>) [17]. Genotypic resistance was defined as the presence of  $\geq 1$  resistance-related mutation as specified by the consensus mutation figures of the International AIDS Society–USA (IAS; May/June 2002 version) [18]. The substitutions at codon 215, which are listed as footnotes in the IAS figures and are considered to be indicators of transmitted resistance, were included in the analysis as well [6]. The minor mutations in protease and the mutations E44D and V118I in reverse transcriptase (RT) were not taken into account, because it is not possible to determine whether these mutations result from natural variation or from exposure to antiretroviral drugs (for a complete list, see table 2 below).

**Sequence quality verification.** To assure the quality of the CATCH data set, each submitted sequence was checked before inclusion. Sequences that contained stop codons and individual resistance codons with ambiguities consisting of  $>2$  bases per nucleotide position or of  $>2$  ambiguities per codon were excluded from the analysis.

**Prediction of susceptibility.** Assessment of the possible impact of transmitted drug resistance on the therapeutic response was performed by use of resistance interpretation algorithms. For this purpose, the FASTA files of strains carrying drug-resistance mutations were analyzed by use of 2 freely available algorithms, the Stanford drug-resistance algorithm (beta test version 3.6; available at: <http://hivdb.stanford.edu>) and RetroGram (version 1.6; Virology Education; available at: <http://>

**Table 1. Patient characteristics.**

| Characteristic                                                                  | All          | Chronically infected (>1 year) | Newly diagnosed   |              |
|---------------------------------------------------------------------------------|--------------|--------------------------------|-------------------|--------------|
|                                                                                 |              |                                | Infection ≤1 year | Unknown      |
| Patients, no.                                                                   | 2208         | 607                            | 777               | 824          |
| Age, <sup>a</sup> mean (SD), years                                              | 36 (10)      | 36 (10)                        | 34 (9)            | 37 (11)      |
| Sex, <sup>b</sup> %                                                             |              |                                |                   |              |
| Male                                                                            | 73           | 68                             | 81                | 70           |
| Female                                                                          | 27           | 32                             | 19                | 30           |
| Viral subtype B, no. (%)                                                        | 1535 (70)    | 442 (73)                       | 620 (80)          | 473 (57)     |
| Route of transmission, <sup>c</sup> %                                           |              |                                |                   |              |
| Homosexual contact                                                              | 43           | 32                             | 51                | 35           |
| Heterosexual contact                                                            | 41           | 41                             | 30                | 54           |
| Injection drug use                                                              | 15           | 25                             | 13                | 10           |
| Other                                                                           | 1            | 1                              | 6                 | 2            |
| Baseline values                                                                 |              |                                |                   |              |
| CD4 <sup>+</sup> cell count, <sup>d</sup> median (range), cells/mm <sup>3</sup> | 408 (1–1764) | 313 (1–1433)                   | 500 (21–1764)     | 330 (1–1262) |
| HIV-RNA load, <sup>e</sup> mean (SD), log copies/mL                             | 4.82 (0.86)  | 4.65 (0.75)                    | 5.03 (0.94)       | 4.71 (0.79)  |

<sup>a</sup> Data available for 1958 patients.

<sup>b</sup> Data available for 2195 patients.

<sup>c</sup> Data available for 1571 patients.

<sup>d</sup> Data available for 1417 patients.

<sup>e</sup> Data available for 1856 patients.

www.retrogram.com). The Stanford HIV Database algorithm assigns a “drug penalty score” for each drug-resistance mutation. The total score for a drug is derived by adding individual mutation scores and is translated into 5 levels of inferred drug resistance: susceptible (S), potential low-level resistance (PLR), low-level resistance (LR), intermediate resistance (IR), and high-level resistance (HR) [19]. RetroGram is an expert opinion-based algorithm that predicts the potential clinical efficacy of antiretroviral drugs [20]. Genotype interpretation by RetroGram results in a 4-category suitability ranking (A, B, C, or D). The outcome of the algorithms was converted into the following levels of susceptibility: susceptible (S and A), low-level resistance (PLR, LR, and B), intermediate resistance (IR and C), and high-level resistance (HR and D). Reduced susceptibility to a drug was scored when one of the systems indicated the presence of any level of resistance. In cases of discordance, the highest level of resistance was recorded.

**Phylogenetic analysis.** Subtypes were assessed by the construction of phylogenetic trees by use of the neighbor-joining method. Pairwise distance matrices were generated by use of the Kimura 2-parameter distance estimation method with a transition:transversion ratio of 2.0. The consistency of the phylogenetic clustering was tested by bootstrap analysis with 100 replicates. Bootstrap values >70 were considered to be sufficient for subtype assignment. Trees were based on *pol* sequences and were constructed for each country, to exclude cross-contamination between the samples.

**Statistical methods.** A weighted analysis was performed to examine whether differences in the number of patients included

per country distorted the prevalence estimates. The weight for each country was the number of patients living with HIV/AIDS in 1999 (available from EuroHIV and the Joint United Nations Programme on HIV/AIDS). For statistical analysis and reporting of resistance, the calendar years were stratified into 3 intervals: 1996–1998, 1999–2000, and 2001–2002.

Differences in the percentage of strains that were resistant for each interval of time were analyzed by means of the  $\chi^2$  test. Logistic regression was used to study the association between transmission of resistance and both subtype and duration of infection. Patients with an unknown duration of infection were excluded from time-trend analysis.

## RESULTS

**Study population.** We enrolled 2208 HIV-1-infected therapy-naive patients from whom a blood sample for HIV *pol* nucleotide sequence analysis was available during the period 1996–2002. Table 1 shows the characteristics of the patients, grouped according to duration of infection. All patients were naive for therapy at the time of testing; 1601 were newly diagnosed cases of HIV infection, and 607 were known to be chronically infected (>1 year). Of the newly diagnosed cases, 777 patients were identified to have a recent infection (≤1 year) on the basis of negative or indeterminate HIV serological results. The remaining newly diagnosed cases (824) presented with an unknown duration of infection. Transmission routes were identified for 1571 patients. The most common transmission route was sexual contact, 678 (43%) through sex between men and 641 (41%)

through heterosexual contact. Other transmission routes were intravenous drug use (239 [15%]) and exposure to HIV-infected blood (13 [1%]). At the time of resistance analysis, the median CD4<sup>+</sup> cell count was higher in recently infected patients (500 cells/mm<sup>3</sup>) than in patients with chronic infection (313 cells/mm<sup>3</sup>) or patients with an unknown duration of infection (330 cells/mm<sup>3</sup>) ( $P < .001$ ). The mean HIV RNA load was slightly higher in recently infected patients (5.03 log HIV RNA copies/mL) than in patients with chronic infection (4.65 log HIV RNA copies/mL) or an unknown duration of infection (4.71 log HIV RNA copies/mL) ( $P < .001$ ). The proportion of recently infected patients varied between countries.

**Resistance analysis.** The percentage of antiretroviral-naïve patients carrying HIV-1 with  $\geq 1$  resistance-related mutation in Europe during 1996–2002 was 10.4% (95% confidence interval [CI], 9.1%–11.7%) (230/2208). The weighted analysis resulted in a comparable prevalence of 11.1% (95% CI, 9.6%–12.2%). The rate of transmission of drug resistance in each country varied from 0% (0/8) in the small data set from Finland to 23% (14/62) in Germany. Recently infected patients (105/777 [13.5%]) harbored resistant HIV variants more frequently than did patients with chronic infection or patients with infection of unknown duration (53/607 [8.7%] and 72/824 [8.7%], respectively) (odds ratio [OR], 1.6 [95% CI, 1.2–2.3];  $P = .006$ ). In the subset of patients for whom information on the route of transmission was available, we did not find statistically significant differences in the frequency of resistance (data not shown).

**Genotypic profiles and predicted susceptibility.** Table 2 shows the frequency of resistance-related mutations. Mutations associated with thymidine analogues (TAMs) (138/229 [60.3%]) were the most predominant. Among the TAMs, substitutions at RT codon 215, including those associated with partial reversion to drug-sensitive virus, were detected most frequently (93/229 [41%]). The most common nonnucleoside RT inhibitor (NNRTI)-related mutation was K103N, and the most common protease inhibitor (PI)-related mutation was L90M. Resistance to  $>1$  class of drugs was observed in 19% (45/231) of patients carrying drug-resistant HIV variants, whereas resistance to all 3 classes was seen in only 3.5% (8/226).

The possible impact of transmitted resistance on the susceptibility to the different antiretroviral drugs was calculated with 2 resistance interpretation algorithms. The predicted reduction in susceptibility was quite extensive for the class of nucleoside RT inhibitors (NRTIs; 18%–65% of all strains harboring drug-resistance mutations), was intermediate for NNRTIs (36% for all), and was less pronounced for the PIs (20%–25%) (figure 1).

**Time trends.** For patients with a recent infection, the presence of  $\geq 1$  resistance mutation to any antiretroviral drug varied among time periods (figure 2). The initial relatively high proportion of resistance to NRTIs decreased significantly over time,

**Table 2. Resistance profiles.**

| Mutation <sup>a</sup>          | Frequency in all patients, no. (%) <sup>b</sup> | Frequency in patients with drug-resistant HIV, % |
|--------------------------------|-------------------------------------------------|--------------------------------------------------|
| Any (NRTI, NNRTI, major PI)    | 230 (10.4)                                      | ...                                              |
| NRTI                           |                                                 |                                                  |
| Any                            | 165 (7.6)                                       | 72.1                                             |
| 1                              | 89 (4.1)                                        | 38.9                                             |
| $\geq 2$                       | 76 (3.5)                                        | 33.2                                             |
| M41L                           | 57 (2.6)                                        | 24.9                                             |
| D67N                           | 33 (1.5)                                        | 14.4                                             |
| K70R                           | 22 (1.0)                                        | 9.6                                              |
| M184I/V                        | 31 (1.4)                                        | 13.5                                             |
| L210W                          | 21 (1.0)                                        | 9.2                                              |
| T215F/Y                        | 34 (1.6)                                        | 14.8                                             |
| T215A/C/D/E/N/S/V <sup>c</sup> | 59 (2.7)                                        | 25.8                                             |
| K219Q/E                        | 25 (1.1)                                        | 10.9                                             |
| Any TAM <sup>d</sup>           | 138 (6.3)                                       | 60.3                                             |
| $\geq 2$ TAMs                  | 72 (3.3)                                        | 31.4                                             |
| Any TAM + M184 V               | 16 (0.7)                                        | 7.0                                              |
| NNRTI                          |                                                 |                                                  |
| Any                            | 64 (2.9)                                        | 27.8                                             |
| 1                              | 53 (2.4)                                        | 23.0                                             |
| $\geq 2$                       | 11 (0.5)                                        | 4.7                                              |
| K103N                          | 33 (1.5)                                        | 14.3                                             |
| V108I                          | 12 (0.5)                                        | 5.2                                              |
| Y181C                          | 11 (0.5)                                        | 4.8                                              |
| PI                             |                                                 |                                                  |
| Any                            | 54 (2.5)                                        | 23.7                                             |
| 1                              | 44 (2.0)                                        | 19.3                                             |
| $\geq 2$                       | 10 (0.5)                                        | 4.4                                              |
| M46I/L                         | 22 (1.0)                                        | 9.6                                              |
| V82A/F/S/T                     | 12 (0.6)                                        | 5.3                                              |
| L90M                           | 23 (1.1)                                        | 10.1                                             |

**NOTE.** NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation.

<sup>a</sup> In addition to the mutations listed in the table (frequency,  $>0.5\%$ ), the following mutations were observed: A62V, K65R, T69D, L74V, V75A, F77L, L100I, F116Y, Y188C/H/L, Q151M, G190A/S, P225H, and M230L in RT and D30N, G48V, and I84V in protease. Not observed were V75I/M/S/T, Y115F, V106A/M, V108I, Y181I, and P236L in RT and I50L/V in protease.

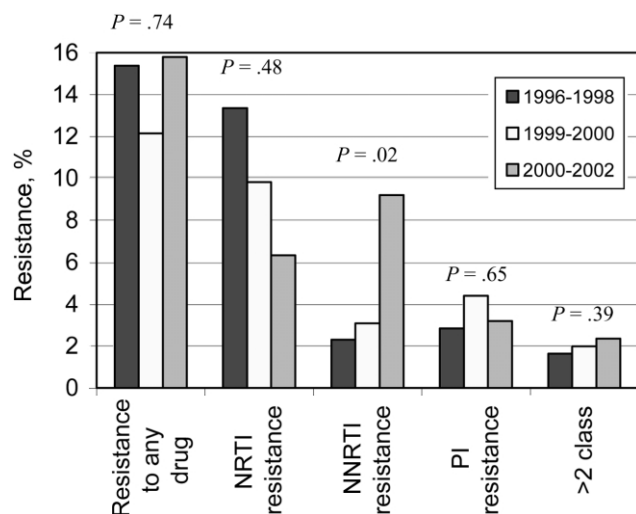
<sup>b</sup> No. of samples analyzed for NRTIs, 2177; for NNRTIs, 2190; and for PIs, 2178.

<sup>c</sup> Frequency of individual mutations at codon 215: A, 2; C, 5; D, 23; E, 1; N, 4; S, 20; N/S, 1; and V, 3.

<sup>d</sup> M41L, D67N, K70R, L210W, T215A/C/D/E/F/N/S/V/Y, and K219Q.

from 13.4% (29/217) in 1996–1998, to 9.8% (44/448) in 1999–2000, and to 6.3% (6/95) in 2001–2002 ( $P = .048$ ). In contrast, NNRTI-related resistance was initially low and displayed a significant increase through time, from 2.3% (5/217) in 1996–1998, to 3.1% (14/454) in 1999–2000, and to 9.2% (8/87) in 2001–2002 ( $P = .02$ ). PI-related resistance remained relatively stable over time (2.8%, 4.4%, and 3.2%, respectively;  $P = .65$ ).

**Phylogenetic analysis.** Phylogenetic analysis revealed that 70% of the patients were infected with subtype B virus (1535/2208). Thirty-six strains (36/2208 [2%]) could not be deter-



**Figure 1.** Predicted susceptibility of all viruses carrying drug-resistance-related mutations. The level of resistance to a specific antiretroviral drug was calculated on the basis of the genotypic profiles by use of 2 Web-based algorithms. Information on the protease inhibitor (PI) atazanavir is only available in the Stanford algorithm, whereas information on the boosted PIs (except for lopinavir/r) is available only in RetroGram.

mined, because they did not cluster with any known subtype and were therefore scored as unclassified non-B subtypes.

Among non-B subtype, subtype C was the most prevalent (221/2208 [10%]), followed by subtype G (96/2208 [4%]) and CRF02\_AG (94/2208 [4%]). The proportion of infections with non-B subtypes varied among countries, from 0% in Poland and Germany to >70% in Israel (82/104) and Portugal (69/91). Patients with an infection of known duration more frequently carried subtype B viruses (915/1431 [64%]) than did patients with an infection of unknown duration (473/824 [57%]) ( $P = .002$ ).

Interestingly, subtype B viruses displayed a higher frequency of baseline drug resistance (198/1535 [12.9%]) than did non-B viruses (32/673 [4.8%]) (OR, 3.0 [95% CI, 2.0–4.4]) ( $P < .001$ ). When the analysis was restricted to the subgroup of recently infected patients, the risk of being infected with a drug-resistant virus was 4 times higher in subtype B infections (99/620 [16%]) than in non-B infections (6/157 [3.8%]) (OR, 4.2 [95% CI, 1.9–9.4];  $P < .001$ ).

A multivariate analysis in the subset of patients with an infection of known duration showed that the difference in resistance between B and non-B subtypes was not confounded by time and duration of infection. The ORs of the univariate and multivariate analysis were 3.8 (95% CI, 2.1–6.7) ( $P < .001$ ) and 3.7 (95% CI, 2.0–6.6) ( $P < .001$ ), respectively. Nevertheless, a time-dependent increase in the rate of resistance was observed in all newly diagnosed cases of non-B infection: from 2.0% (1/49) in 1996–1998, to 3.0% (8/265) in 1999–2000, and to 8.2% (16/194) in 2000–2001.

## DISCUSSION

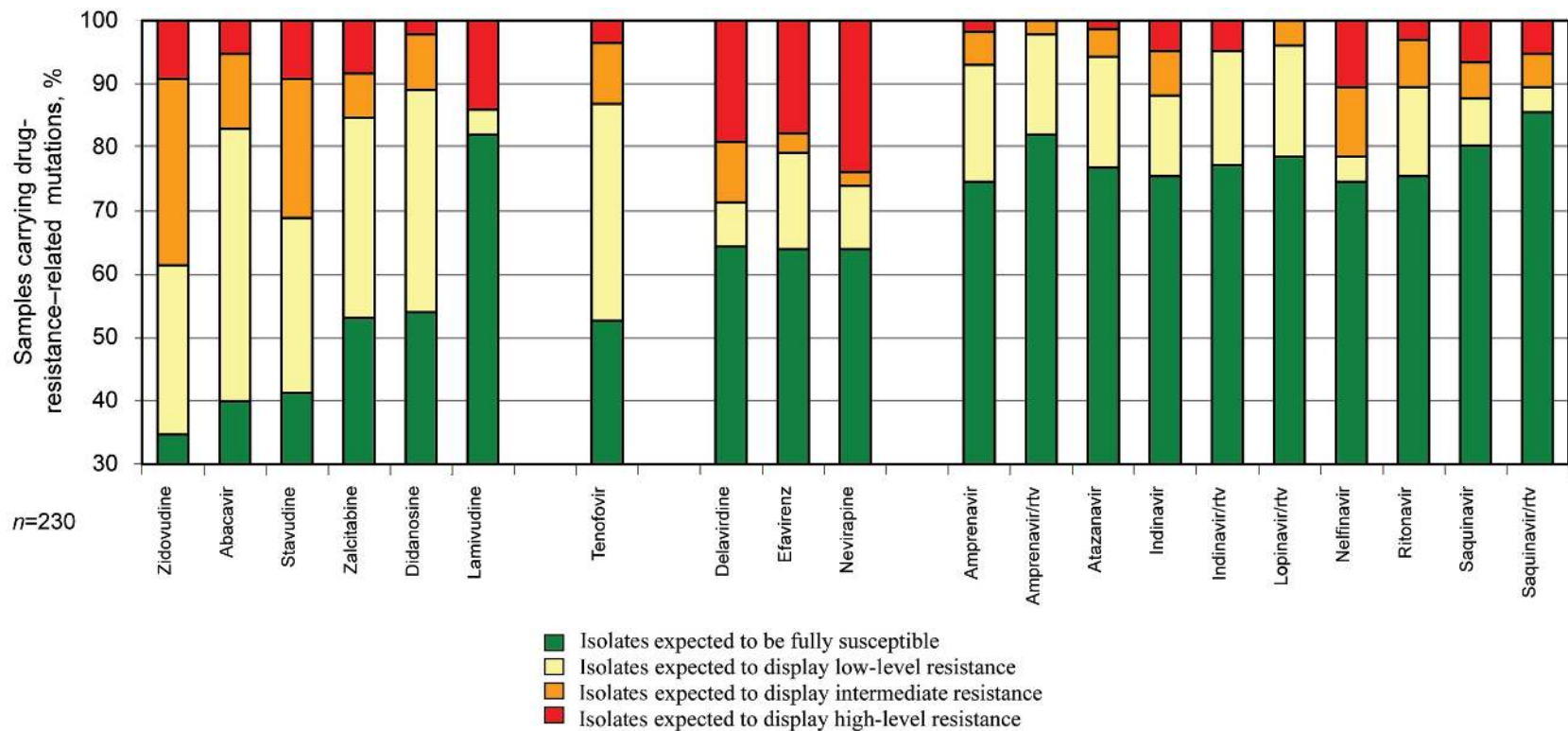
We studied the rate of transmission of drug-resistant HIV-1 over time in Europe. The results of this international study showed that 10.4% of patients who had never been exposed to antiretroviral therapy carried HIV with  $\geq 1$  drug-resistance mutation. This rate differs from that in several earlier reports. The previous studies included relatively small numbers of participants, were often limited to single countries, and varied widely in the type of mutations considered or the method used to determine resistance. As a consequence, the rate of new infections by drug-resistant HIV has been difficult to estimate on a continentwide scale [3]. To our knowledge, our study is the first to have analyzed a large resistance data set with uniform definitions that has taken into account duration of infection and variations in subtype.

As in any study of the transmission of resistance, it cannot be ruled out that patients who are considered to be antiretroviral naive might not have been truthful about their lack of exposure to therapy, which would result in an overestimation of baseline resistance. However, the prescription procedures in Europe (which are mainly done by HIV-specialized centers), reimbursement for therapy by national healthcare programs, and the high level of insurance coverage do not give reasons for hiding previous exposure to therapy.

Theoretically, differences in sampling strategy and size of the data sets could have influenced the results. To minimize sampling biases, no isolated clinical samples were included. All samples in the study were collected either as part of surveillance, as part of a standard practice of baseline resistance testing, or, less frequently, as baseline samples for transmission studies. We consider the influence of sample sizes of the data sets to be small, given that a weighted analysis showed that differences in sample size among countries barely influenced the overall rate of transmission.

Differences in the duration of infections may be more relevant. We found a significant difference ( $P = .006$ ) in the level of baseline resistance between recently infected patients (13.5%) and patients infected for >1 year (8.7%). The lower prevalence of resistance in chronic infection is most likely due to a lower exposure to drug-resistant virus in the past. In addition, the lower prevalence can be explained by reversion from resistant variants to sensitive wild-type viruses over time [21]. Interestingly, drug-resistance mutations could still be detected in the plasma of quite a few chronically infected patients, which indicates that complete reversion does not always occur. Indeed, recent studies have indicated that drug-resistant viruses can persist for a couple of years in the plasma of treatment-naive HIV-infected patients [22, 23].

Differences in data sets among countries may also have resulted from specific biological and cultural characteristics as they relate to national epidemics, such as transmission routes,



**Figure 2.** Baseline resistance in recently infected individuals (*n* = 777) over time. The calendar years were stratified into 3 intervals of time. Differences in the percentage of resistant isolates were analyzed by the  $\chi^2$  test for trend. Nucleoside reverse-transcriptase inhibitor (NRTI)-related resistance decreased significantly over time, whereas nonnucleoside reverse-transcriptase inhibitor (NNRTI)-related resistance increased significantly over time. No differences were seen in protease inhibitor (PI)-related resistance.

the proportion of non-B viruses, and prescription guidelines. For instance, the proportion of non-B subtypes seems to have a substantial influence on the frequency of baseline resistance. Resistance was higher in antiretroviral-naïve patients who were infected with subtype B (12.9%) than in those infected with non-B viruses (4.8%). Moreover, in recently infected patients, the risk of being infected with drug-resistant HIV was 4 times higher in patients with subtype B infections (16%) than in patients with non-B infections (4%).

Subtype B was originally the predominant subtype in Europe and in North America, whereas non-B subtypes have spread widely in Africa and Asia. Differences in the prevalence of baseline resistance may thus likely reflect the longer period during which subtype B viruses have been exposed to antiretroviral drugs. Interestingly, we noticed a consistent increase in baseline resistance in non-B viruses. This trend is consistent with the increasing number of patients identified with non-B viruses in Europe who are currently being exposed to therapy [14, 24].

The most commonly observed mutations in our study were those associated with resistance to the thymidine analogues zidovudine and stavudine. Zidovudine and, to a lesser degree, stavudine have been extensively used as monotherapy or as part of dual therapy in the past and are frequently present in current regimens. Also, viruses with TAM resistance profiles may have better transmissibility and/or preservation in a drug-free environment, compared with viruses that contain other resistance mutations that more extensively compromise viral fitness.

The total number of recently infected patients with drug resistance displayed a fluctuating pattern over time (figure 2). This pattern results from changes in resistance to RT inhibitors and can be explained by several mechanisms. The initial high level of NRTI resistance most likely reflects less-effective viral suppression and the easy selection of drug resistance at the time of prescription of suboptimal monotherapy and dual NRTI therapy. The second phase reflects a decline in NRTI resistance after the introduction of highly active antiretroviral therapy (HAART), which results in a more-effective suppression of HIV replication. Because a low viral load has been associated with a reduced risk of sexual transmission [25], the introduction of HAART may account for the observed decrease in transmitted NRTI drug resistance.

The third phase, which is characterized by a sharp increase in NNRTI resistance, may reflect the enhanced use of NNRTI-based regimens, compared with PI-based regimens. An additional explanation may be a higher level of NNRTI resistance in the general population, given that NNRTIs have a lower genetic barrier to resistance and that just one point mutation can be sufficient to confer high-level resistance to this class of drugs [26]. In addition, viruses with NNRTI resistance may be

more fit and therefore more able to establish infection in the new host, compared with PI resistance-related variants.

Our results may have important consequences for clinical management. The continuous transmission of drug-resistant viruses to newly infected patients demonstrates that a portion of HIV-infected individuals receiving antiretroviral medication is still engaging in risk-related behavior, despite awareness of their infection status [27]. These patients are receiving medical attention and should therefore be accessible targets for prevention programs.

Likewise, major concerns exist about the clinical impact of transmitted drug resistance. In the present data set, the loss in predicted susceptibility was most extensive for NRTIs, whereas predicted high-level resistance was more pronounced for drugs with a low genetic barrier, such as lamivudine and NNRTIs. The precise impact of transmitted resistance might be difficult to assess, because the correlation between baseline resistance and therapeutic response is influenced by the knowledge of baseline resistance patterns at time of the initiation of therapy and by the availability of alternative treatment options. Nevertheless, preliminary data show that baseline resistance may compromise the response to antiretroviral therapy. In 3 studies, the time to viral suppression was significantly prolonged in individuals infected with drug-resistant HIV [4, 5, 28]. Also, the time to virological failure was shorter if baseline resistance was present [5]. Additionally, individuals from the ICONA cohort harboring revertants or atypical mutants at position 215 of RT had an increased risk for selecting drug-resistance mutations and experiencing virological failure [6].

Recently updated guidelines recommend drug-susceptibility testing for patients presenting with recent infection (<1 year) and for all newly diagnosed cases when the regional prevalence in an area increases to >5% and >10%, respectively [1, 7]. Moreover, the results of modeling studies have suggested that offering genotypic resistance testing before the initiation of therapy was cost-effective in a US healthcare setting at a 4% prevalence of baseline resistance [29]. At present, most clinical centers in Europe do not perform baseline resistance testing as standard procedure. However, we have shown that considerable baseline drug resistance can be found in antiretroviral-naïve patients even after they have been infected for >1 year. Therefore, our data support genotypic resistance testing for all antiretroviral-naïve individuals before combination therapy is selected.

Additionally, when making decisions about initial therapy, one should consider the possibility that more-complex patterns are transmitted but that certain mutations are not frequently identified in antiretroviral-naïve patients because of reversion and the resistance test's inability to detect minority variants. In the case of an inadequate virological response to initial ther-

apy, resistance testing should be repeated early, to identify the rapid selection of former minority variants.

Finally, the resistance patterns observed in the present study represent the viruses that are currently circulating in Europe. Because the failure of postexposure prophylaxis has been described after exposure to drug-resistant HIV-1, the reduced susceptibility of these viruses should be taken into account when selecting prophylaxis for those patients for whom no source-related information is available [8, 9, 30].

In conclusion, in 1 of 10 antiretroviral-naïve patients in Europe, viruses with resistance to at least 1 drug are found. This high prevalence should be taken into account when decisions are made about initial regimens for therapy-naïve individuals and about the selection of drugs for prophylaxis. Continuous surveillance of the spread of drug-resistant HIV-1, as well as the distribution of non-B viruses, is of utmost importance.

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## References

1. 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*:113-28.
2. Leigh Brown AJ, Frost SDW, Mathews WC, et al. Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. *J Infect Dis* **2003**; *187*:683-6.
3. Wensing AM, Boucher CA. Worldwide transmission of drug-resistant HIV. *AIDS Rev* **2003**; *5*:140-55.
4. Grant RM, Hecht FM, Warmerdam M, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. *JAMA* **2002**; *288*:181-8.
5. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* **2002**; *347*:385-94.
6. Violin M, Cozzi-Lepri A, Velleca R, et al. Risk of failure in patients with 215 HIV-1 revertants starting their first thymidine analog-containing highly active antiretroviral therapy. *AIDS* **2004**; *18*:227-35.
7. Vandamme AM, Sonnerborg A, Ait-Khaled M, et al. Updated European recommendations for the clinical use of HIV drug resistance testing. *Antivir Ther* **2004**; *9*:829-48.
8. Anonymous. HIV seroconversion after occupational exposure despite early prophylactic zidovudine therapy. *Lancet* **1993**; *341*:1077-8.
9. Beltrami EM, Luo CC, de la Torre N, Cardo DM. Transmission of drug-resistant HIV after an occupational exposure despite postexposure prophylaxis with a combination drug regimen. *Infect Control Hosp Epidemiol* **2002**; *23*:345-8.
10. Balotta C, Berlusconi A, Pan A, et al. Prevalence of transmitted nucleoside analogue resistant HIV-1 strains and pre-existing mutations in *pol* reverse transcriptase and protease region: outcome after treatment in recently infected individuals. *Antivir Ther* **2000**; *5*:7-14.
11. Chaix ML, Descamps D, Harzic M, et al. Stable prevalence of genotypic drug resistance mutations but increase in non-B virus among patients with primary HIV-1 infection in France. *AIDS* **2003**; *17*:2635-43.
12. de Mendoza C, del Romero J, Rodriguez C, Corral A, Soriano V. Decline in the rate of genotypic resistance to antiretroviral drugs in recent HIV seroconverters in Madrid. *AIDS* **2002**; *16*:1830-2.
13. Magiorkinis E, Paraskevis D, Magiorkinis G, et al. Mutations associated with genotypic resistance to antiretroviral therapy in treatment naïve HIV-1 infected patients in Greece. *Virus Res* **2002**; *85*:109-15.
14. Romano L, Venturi G, Ferruzzi R, et al. Detection of genotypically drug-resistant HIV-1 variants and non-B subtypes in recently infected antiretroviral-naïve adults in Italy. *AIDS* **2000**; *14*:2204-6.
15. Van Vaerenbergh K, Debaisieux L, De Cabooter N, et al. Prevalence of genotypic resistance among antiretroviral drug-naïve HIV-1-infected patients in Belgium. *Antivir Ther* **2001**; *6*:63-70.
16. Yerly S, Vora S, Rizzardì P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. *AIDS* **2001**; *15*:2287-92.



17. Chenna R, Sugawara H, Koike T, et al. Multiple sequence alignment with the Clustal series of programs. *2003*; 31:3497–500.
18. D'Aquila RT, Schapiro JM, Brun-Vezinet F, et al. Drug resistance mutations in HIV-1. *Top HIV Med* **2003**; 11:92–6.
19. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res* **2003**; 31:298–303.
20. Torti C, Quiros-Roldan E, Keulen W, et al. Comparison between rules-based human immunodeficiency virus type 1 genotype interpretations and real or virtual phenotype: concordance analysis and correlation with clinical outcome in heavily treated patients. *J Infect Dis* **2003**; 188:194–201.
21. Yerly S, Rakik A, De Loes SK, et al. Switch to unusual amino acids at codon 215 of the human immunodeficiency virus type 1 reverse transcriptase gene in seroconverters infected with zidovudine-resistant variants. *J Virol* **1998**; 72:3520–3.
22. Brenner BG, Routy JP, Petrella M, et al. Persistence and fitness of multidrug-resistant human immunodeficiency virus type 1 acquired in primary infection. *J Virol* **2002**; 76:1753–61.
23. Little SJ, Dawson K, Hellmann NS, Richman DD, Frost SDW. Persistence of transmitted drug-resistant virus among subjects with primary HIV infection deferring antiretroviral therapy. *Antiviral Ther* **2003**; 8: S115.
24. Deroo S, Robert I, Fontaine E, et al. HIV-1 subtypes in Luxembourg, 1983–2000. *AIDS* **2002**; 16:2461–7.
25. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med* **2000**; 342:921–9.
26. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol* **1994**; 68:1660–6.
27. Kozal MJ, Amico KR, Chiarella J, et al. Antiretroviral resistance and high-risk transmission behavior among HIV-positive patients in clinical care. *AIDS* **2004**; 18:2185–9.
28. Harzic M, Pellegrin I, Deveau C, et al. Genotypic drug resistance during HIV-1 primary infection in France (1996–1999): frequency and response to treatment. *AIDS* **2002**; 16:793–6.
29. Weinstein MC, Goldie SJ, Losina E, et al. Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. *Ann Intern Med* **2001**; 134:440–50.
30. Hawkins DA, Asboe D, Barlow K, Evans B. Seroconversion to HIV-1 following a needlestick injury despite combination post-exposure prophylaxis. *J Infect* **2001**; 43:12–5.

In the 15 September issue of the *Journal*, in the article by Wensing et al. (Wensing AMJ, van de Vijver DA, Angarano G, et al. Prevalence of Drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. *J Infect Dis* 2005;192:958–66), there are the following 2 errors:

The legend, or caption, currently below figure 1 should be below figure 2, and the legend currently below figure 2 should be below figure 1; the publisher regrets this error. Also, the second *P* value in figure 1 should be .048, as it is in the text (not .48); the author regrets this error.