BRIEF REPORT

Declining Prevalence of HIV-1 Drug Resistance in Antiretroviral Treatment-exposed Individuals in Western Europe

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HIV-1 drug resistance represents a major obstacle to infection and disease control. This retrospective study analyzes trends and determinants of resistance in antiretroviral treatment (ART)-exposed individuals across 7 countries in Europe. Of 20 323 cases, 80% carried at least one resistance mutation: these declined from 81% in 1997 to 71% in 2008. Predicted extensive 3-class resistance was rare (3.2% considering the cumulative genotype) and peaked at 4.5% in 2005, decreasing thereafter. The proportion of cases exhausting available drug options dropped from 32% in 2000 to 1% in 2008. Reduced risk of resistance over calendar years was confirmed by multivariable analysis.

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The suppression of human immunodeficiency virus type 1 (HIV-1) replication with antiretroviral therapy (ART) translates in clinical benefits and reduces viral transmission [1, 2]. HIV-1 drug resistance (HIVDR) can be acquired as a consequence of incomplete viral suppression during ART and represents a major obstacle to infection control [3, 4]. HIVDR at failure is influenced by the type of regimen, previously transmitted or selected resistance and testing time [5], factors that may vary over countries.

The aims of this study are to describe the evolution of prevalence, patterns. and determinants of HIVDR in patients exposed to ART in several European countries between 1997 and 2008 and its impact on the activity of available drug options.

METHODS

The SEHERE database merged several projects and cohorts within 7 countries in Western Europe (see details in [5]). All the single data providers had previously obtained patients' informed consent, following the protocols' approval by the relevant Ethics Boards.

From each cohort, HIV-1 *pol* gene sequences of ART-experienced patients (at least 90 days of treatment) sampled between 1997 and 2008 were collected. For a subset of patients, only mutation strings (with respect to consensus B) were available. One genotype per patient per calendar year was collected. For each sequence, the corresponding patients' demographics, HIV RNA load, and $CD4^+$ T-cell counts within 30 days of the date of genotype sample, plus information on ART history, were matched. Viral subtype was determined by the Rega tool [6]. Unresolved classifications were decided by the first BLAST match upon a Los Alamos HIV-1 subtype reference set [7].

We defined "resistance mutations to an antiretroviral class" as the presence of ≥1 drug resistance mutation included in the IAS-USA list [8], considering the nucleoside/tide reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), and major resistance mutations to the protease inhibitors (PIs). In addition, "resistance mutations to 3 classes" was defined as the presence of at least one resistance mutation in each class (major in the case of PI).

Drug resistance interpretation was performed only for cases with viral nucleotide sequence available, using the Rega v.8.0.1 algorithm [9]. This interpretation uses a weighted output accounting for drug susceptibility, genetic barrier, and potency: predicted drug activity was scored 0 for all drugs to whom the virus was interpreted as resistant, 0.25 for intermediate resistant nevirapine and efavirenz, 0.5 for intermediate resistant NRTIs, etravirine, and unboosted PIs, 0.75 for intermediate resistant boosted PIs, 0.75 for susceptible NRTIs, 1 for susceptible NNRTIs and unboosted PIs, 1.5 for susceptible boosted PIs. Susceptibility to enfuvirtide, raltegravir, and maraviroc was assumed to be 0 if used prior to or during resistance testing. If the drugs had never been used before, enfuvirtide and raltegravir were scored 1, whereas for maraviroc, susceptibility was assumed to be 0.5 considering that about 50% of treatment-failing patients never exposed to CCR5 receptor antagonists carry a susceptible virus. Predicted extensive 3-class HIVDR was defined as the absence of any fully active NRTI, NNRTI, or PI. Exhaustion of drug options was defined as an arithmetic sum of the weighted genotypic susceptibility score <2, counting only drugs that were commercially available in Europe at given calendar years (see Supplementary Figure 2 legend). For this calculation, at most one agent per drug class was allowed within a combination, with the exception of NRTIs, which were allowed unlimited combinations, except stavudine + zidovudine and emtricitabine + lamivudine.

We used linear regression and logistic regression to test for changes over calendar years of continuous or categorical variables, respectively. Multivariable analysis was employed to identify predictors of the presence of any major NRTI/NNRTI/PI resistance mutation, any resistance mutation, and 3 antiretroviral class resistance, focusing on calendar year. Separate models were fitted using either HIVDR interpreted using the last available resistance genotype or the cumulative genotype [10]. All analyses were performed using SPSS v.18 (SPSS Inc, Chicago, IL).

RESULTS

A total of 20 323 records fulfilled the criteria and were used for the final analysis. Country of genotyping was UK (43.4%), Italy (36.6%), Portugal (11.2%), Germany (3.4%), Sweden (2.5%), Spain (2.3%), and Belgium (0.7%). The predominant gender was male (74.4%), the recorded risk factors were men having sex with men (MSM; 36.1%), heterosexual contacts (27.9%), injecting drug users (IDU; 15.8%), transfusion of blood products or vertical transmission (1.7%), and unknown (18.5%); viral subtypes were predominantly B (64.8%), followed by G (3.7%), C (2.7%), CRF 02_AG (1.8%); and other (16.2%) , whereas for 10.8% subtyping was not possible. At genotyping, the median age (interquartile range [IQR]) was 40.1 years (35.3–45.6), median CD4 cell count was 277 cells/μL (155–430), HIV RNA was 3.98 $\log_{10}$ copies/mL (3.30–4.66), year of genotyping was 2003 (2001–2005), and time since ART initiation was 64 months (33–100). A history of mono-dual NRTI therapy was present in 54.0%. All except 7 sequences belonged to patients with a history of exposure to NRTI, 63.4% to NNRTI, 78.8% to any PI, 45.3% to boosted PI, and 2.3% to novel drug classes; 32.1% had been exposed to 3 classes. Over calendar year of genotyping there was an increase of patient age, a decrease of the proportion of males, and of MSM with a corresponding increase of heterosexual patients, an increase of CD4 counts, a decrease of viral load at genotyping, and an increase of non-B HIV-1 subtypes (not shown).

Over calendar years of genotyping, there was a significantly decreased use of NRTI (from 98.3% in 1997 to 94.0% in 2008, $P < .001$), increased use of NNRTI (from 5.9% in 1997, peaking in 2000 at 41.1%, and leveling off thereafter with 32.3% in 2008; $P < .001$), decreased use of unboosted PI (from 55.1% in 1997 to 6.6% in 2008; $P < .001$), and increased use of boosted PI (from 0.7% in 1997 to 48.0% in 2008; $P < .001$). Novel drug classes began to be used in 2002 (0.9%) with a peak in 2006 (3.6%, $P < .001$). Over calendar year there were significant increases in the duration of prior ART exposure, number of drugs, and proportions of genotyped patients having experienced ≥3 drug classes: (from 0.8% in 1997 to a peak in 2006 at 46.0% and a 41.7% in 2008; $P < .001$ for all comparisons).

The viral sequences showing at least one resistance mutation to any of the NRTI or NNRTI or at least one major PI mutation were 16 278 of 20 323 (80.1%). In total, 67.4% carried resistance to NRTI, 51.1% to NNRTI, and 32.5% major mutations to PI. Resistance to 1, 2, and 3 of these drug classes was observed in 25.6%, 38.2%, and 16.3%, respectively. The most prevalent resistance mutations involving NRTI resistance were M184V/I (42.0%) and thymidine analogue mutations (TAM), 47.0%, with 33.3% type 1 TAM (M41L, D67N, L210W, and T215Y), and 31.8% type 2 TAM (K70R, T215F, and K219E/Q). K103N (20.4%), Y181C/I (11.8%), and G190A (8.8%) were the most frequent mutations involving NNRTI resistance. The most prevalent major protease resistance mutations were L90M (17.8%), M46I/L (15.2%), V82A/F/L/S/T (13.6%). and I84V (7.5%). Evolution of the overall and class-specific resistance mutations over calendar year is illustrated in Figure 1. There was a clear evidence of a reduction of overall resistance mutations and of mutations to NRTI and PI over calendar years, in particular after 2001 ($P < .001$ for all comparisons). The probability of detecting NNRTI resistance mutations initially increased, peaking in 2004, and later declined ($P < .001$). Multivariable models confirmed an independent association of more recent calendar years with reduced probability of resistance (Supplementary Table 1). In particular, resistance mutations to any class, to 3 classes, to NRTI, and to

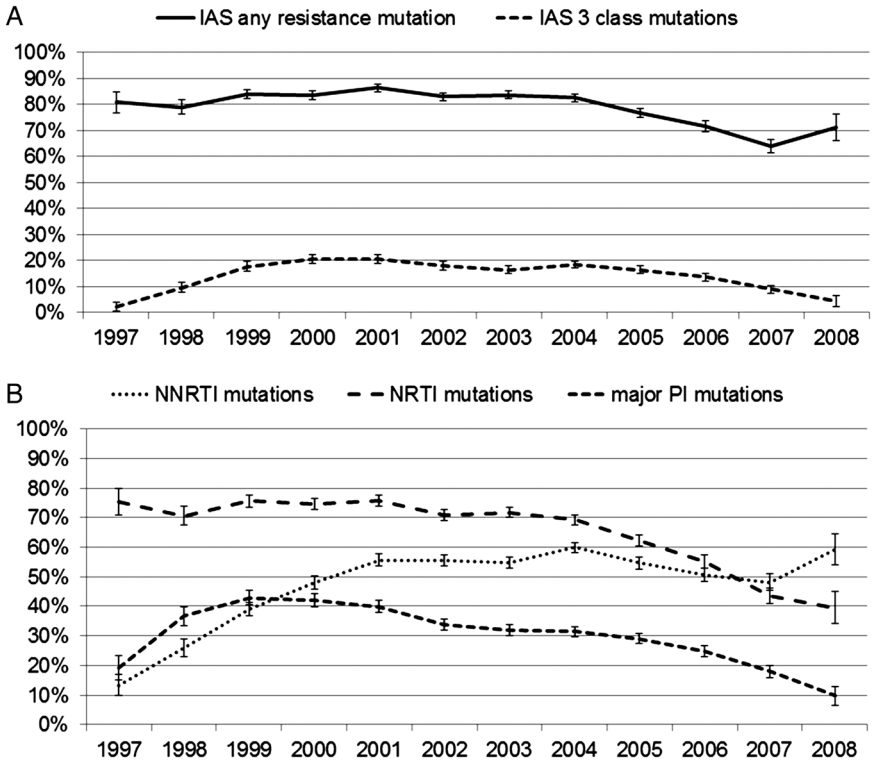

Figure 1. Evolution of the (*A*) overall and (*B*) class-specific resistance mutations over calendar years (vertical bars represent 95% confidence intervals). Abbreviations: NRTI, nucleoside/tide reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

PI showed a progressive decrease after 2001, whereas an independent association of more recent calendar year with a reduction of NNRTI resistance was observed after 2004. Other variables independently associated with the detection of any resistance mutations were MSM as compared to heterosexual contacts, non-B subtypes as compared to subtype B, history of suboptimal therapy, second or higher-line compared to first-line failure, and duration of ART exposure. As compared to failing regimens based on NRTI + NNRTI, those failing with novel drug classes were associated with higher odds (probably an artifact due to confounding by indication, given that these classes were used after previous failure), whereas those failing with NRTI + PI were associated with lower odds of any resistance mutation.

Of note, the presence of NRTI resistance mutations was less probable in patients with a PI in their current therapy and more frequent in carriers of non-B subtypes as compared to B; non-B were also associated with a higher odds of NNRTI and PI resistance mutations as compared to B. Supplementary Figure 1 reports the evolution of specific mutational patterns. Notably, all most frequent resistance mutations and mutational patterns declined over calendar year. Some new mutational patterns tended to emerge over time but either remained of limited frequency, sometimes also declining again during the most recent years. The most frequent NNRTI resistance mutations increased to a peak in 2001–2002 and declined afterward. Viral nucleotide sequences were available for resistance interpretation in 17 718 cases (87.2%).

Extensive drug resistance to the 3 historical classes was present in 2.1% using the last genotype and 3.2% using the cumulative genotype. It increased from 0% in 1997 to a peak of 3.0% (4.5% with the cumulative genotype) in 2005 (Figure 2*A*).

In multivariable models, using the cumulative genotype, the odds ratios of predicted extensive 3-class resistance were lower in the earlier calendar years as compared to 2001–2005, whereas in the following years the resistance tended to decrease slightly (not shown).

The overall proportion of cases with an exhaustion of drug options was 10.6%. It decreased from a peak of 27.4% in 2000 to 0.3% in 2008 (Figure 2*B*), and from 31.8% in 2000 to 1.0% in 2008 using the cumulative genotype. This decrease over calendar time was confirmed by multivariable analysis that showed a drop of the adjusted odds ratios relative to calendar year 2004 from about 14–17 times higher in 1999–2001 to more than 7 times lower in 2007–2008 (Supplementary Figure 2).

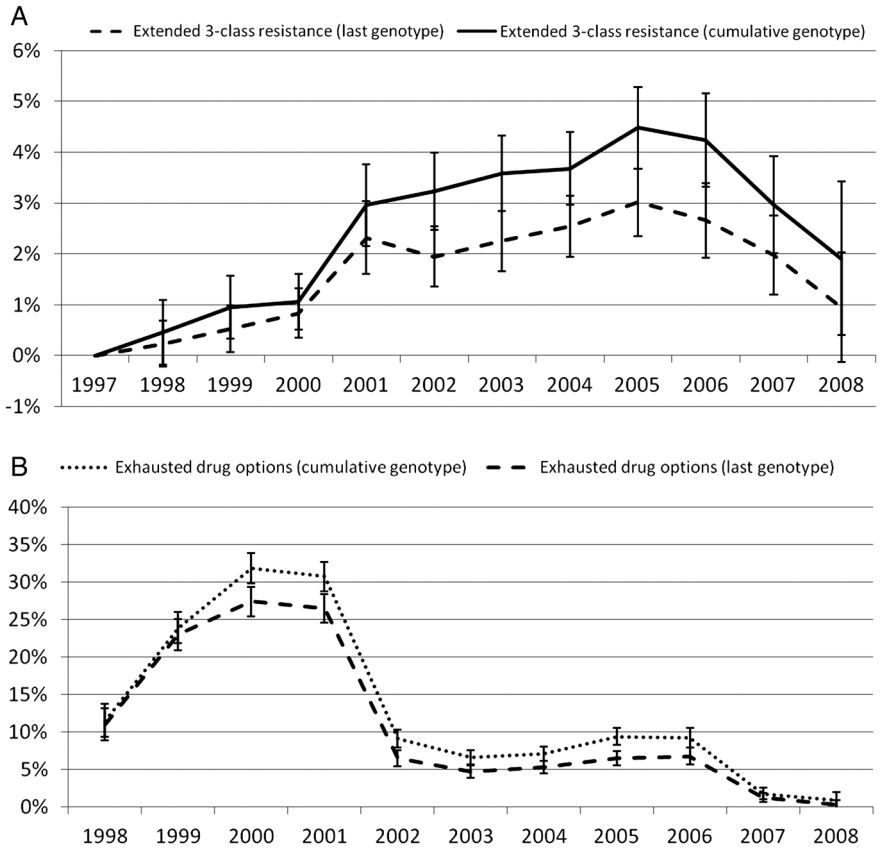

Figure 2. Proportions with extensive 3-class resistance over calendar years by the last and the cumulative genotype (*A*) and proportion of cases with an exhaustion of drug options by the last and the cumulative genotype (*B*) (vertical bars represent 95% confidence intervals). The following drugs were considered available during calendar years: from 1997 zidovudine, stavudine, didanosine, zalcitabine, nevirapine, lamivudine, indinavir, ritonavir, and saquinavir; from 1998, nelfinavir; 1999 efavirenz; 2000 abacavir, amprenavir/r, indinavir/r, and saquinavir/r; 2001 lopinavir/r; 2003 enfuvirtide, tenofovir, tipranavir/r, and atazanavir/r; 2005 emtricitabine; 2007 darunavir/r; 2008 etravirine, raltegravir, and maraviroc.

DISCUSSION

The main finding from this multicohort, European study is that in ART-exposed genotyped patients, HIVDR mutations to all historical drug classes declined during the more recent calendar years. This effect emerged despite accumulating drug exposure over time, involved all classes and almost all different mutation patterns, and persisted after adjusting for potential confounders.

Results from previous smaller national studies are herewith extended to a European multinational scale [11–13]. A North American cohort study showed similar declining trends of resistance mutation patterns involving NRTI/NNRTI/PIs [14]. The reduction of HIVDR over calendar time was independent from the type of third drug in the failing regimen, suggesting that the improvement involved the whole ART sequencing strategies (eg, switching faster at lower viral load failure, with less use of suboptimal regimens in the previous treatment history) as well as the genetic barrier of whole regimens [5, 15]. It is reassuring to observe how the effect of increased drug exposure is more than counterbalanced by the reduced impact determined by the more recent regimens and the newer switching strategies on HIVDR.

An exception is represented by the small but initially increasing proportion of individuals with extensive 3-class resistance. However, its prevalence decreased slightly after 2005. Moreover, the approval of new agents allowed overcoming the exhaustion of drug options for almost every patient.

The significant reduction of HIVDR at treatment failure may be a benefit also for the community, reducing the sources of HIVDR transmission.

A potential limitation of this study is its retrospective design, based on a convenience sampling. However, at least for Italy, UK, Portugal, and Sweden, the analyzed viral sequences represent relevant proportions of those performed. Moreover, multivariable analyses, adjusting for several potentially confounding factors, confirmed a declining prevalence over the years, although the presence of selection bias in initial

years, before resistance testing became clinical practice, cannot be excluded.

In conclusion, this large observational Western European meta-cohort shows how, despite increasing drug exposure, HIVDR in ART-experienced individuals decreased during the more recent calendar years. Continued monitoring of acquired HIVDR will be necessary to follow its evolution.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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