HIV-1 drug-resistance patterns among patients on failing treatment in a large number of European countries

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ABSTRACT

Background: Information about patterns of HIV-1 drug resistance among treatment-exposed patients is crucial for the development of novel effective drugs. Currently no system exists that monitors patterns of resistance in patients failing therapy.

Methods: The study included 1,988 HIV-1 sequences from patients experiencing therapy failure collected between 2000 and 2004 in 15 European countries. Genotypic resistance was interpreted using the ANRS algorithm. Phenotypic resistance was predicted using the Virco geno- to phenotype system.

Results: 80.7% of the sequences included at least one drug-resistance mutation. Mutations were found for NRTIs (73.5%), NNRTIs (48.5%), and protease inhibitors (35.8%). Ninety percent of sequences with genotypic resistance harbored M184V, M41L, K103N, D67N, and/or T215Y. Among NRTIs, resistance was most frequently predicted for lamivudine. About half of all sequences had reduced susceptibility for NNRTIs. Resistance to most boosted protease inhibitors was found in < 25%. No sequence had resistance to all currently available drugs.

Conclusion: Levels of resistance among patients with therapy failure were high. The patterns of resistance reflect resistance to drugs available for a longer time. Fully suppressive regimens can be designed even for the most mutated HIV because boosted protease inhibitors have remained active against most circulating viruses and new drug classes have become available.

KEY WORDS

drug resistance, drug-resistance interpretation systems, treatment failure

Introduction

During the past decade, highly active antiretroviral therapy has reduced mortality among patients infected with HIV (1). However, in a portion of patients complete suppression of virus replication is not achieved, resulting in the appearance of drug-resistant viruses (2).

Insight into the epidemiology of drug resistance among treatment-exposed patients is important for several reasons. One is the benefit it provides for the development of drugs effective against the most fre-

quently found drug-resistant viruses. Second, individuals infected with drug-resistant HIV can transmit these viruses (3–5). Knowledge about the donor population can therefore be helpful in unraveling the dynamics of drug resistance transmission.

Despite its importance, only a few studies have described the epidemiology of drug-resistant HIV among treatment-exposed patients (6–11). Moreover, these studies only reported mutation patterns. Only one study reported the impact of genotypic resistance on drug susceptibility (7).

This study describes the drug-resistance patterns that circulate in a large number of European countries and reports on their impact on predicted genotypic and phenotypic antiretroviral drug susceptibility for currently available drugs.

Methods

HIV-1 protease and partial reverse transcriptase (RT) sequences were collected from routine clinical practice from the plasma of treatment-exposed individuals that virologically failed treatment. The isolates were obtained between 2000 and 2004 from Austria, Belgium, the Czech Republic, Denmark, Greece, Italy, Luxembourg, the Netherlands, Norway, Poland, Portugal, Serbia, Slovenia, Spain, and the United Kingdom.

Nucleotide sequence analysis of the HIV-1 pol gene was performed in the participating centers using their standard local laboratory protocols. Genotypic resistance was defined as the presence of at least one amino acid substitution included in the IAS-USA mutation list of October/November 2006 (12). The classes considered were nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). Other antiretrovirals were not considered because resistance testing for these drugs is not frequently done in clinical practice.

The HIV-1 subtype was assessed using the REGA HIV subtyping tool version 2.0 (13). Genotypic drug resistance was predicted using the ANRS AC rules of 2006. The ANRS algorithm classifies sequences as susceptible, possible resistance, and resistance.

Phenotypic resistance was predicted using the vircoTYPE 4.1.1 system (DB0702), which is developed in a database of more than 45,000 matching genotypes and phenotypes. Using this database, multiple linear regression models have been established to predict the phenotypic fold change for each antiretroviral drug from an individual nucleotide or amino acid sequence (14). Viruses were classified as phenotypically drug resistant (predicted minimal response or reduced response) if the calculated fold change was above the vircoTYPE lower predicted clinical cut-off.

Results

Study population and viral characteristics

Table 1 shows the characteristics of the study population. Most patients were male (71%). Men-having-sex-with-men (MSM) and heterosexual contact (both 36%) were generally reported as the route of transmission. Sequences were predominantly of subtype B (59%), followed by G (15%) and C (7%). The high proportion of subtype G sequences was due to Portugal, where this subtype predominates in the HIV-epidemics.

Drug resistance–associated amino acid substitutions

Among the 1988 sequences that were included, 1,605 (80.7%) had one or more drug resistance–associated amino acid substitutions. Resistance was most frequently found for NRTIs (73.5%), followed by NNRTIs (48.5%) and protease inhibitors (35.8%). Drug resistance was complex, as illustrated by the substantial proportion of 46.0% of the sequences containing "dual resistance" (i.e., resistance-associated mutations relevant for at least two different classes of antiretrovirals). The breakdown of "dual resistance" was as follows: 42.5% of the sequences had resistance-associated mutations to at least one NRTI and one or more NNRTIs, 34.8% to NRTIs and protease inhibitors, and 20.4% to NNRTIs and protease inhibitors. 19.9% contained "triple" resistance (i.e., to at least one NRTI, one NNRTI, and one protease inhibitor).

The five most frequently observed mutations were M184V (47.0%), M41L (29.4%), K103N (29.4%), D67N (27.9%), and T215Y (27.7%). Importantly, these five amino acid substitutions were present in 90.0% of all sequences containing evidence of genotypic resistance.

Genotypic drug susceptibility

The frequently found M184V substitution is associated with resistance to lamivudine and emtricitabine (15). Hence, the ANRS rules predicted reduced susceptibility to lamivudine in the majority of the sequences (51.7%). Among other NRTIs, resistance was also commonly predicted for stavudine (46.3%) and zidovudine (45.7%). Conversely, the prevalence of reduced drug susceptibility was relatively low for abacavir (23.4%), didanosine (24.3%), and tenofovir (29.1%; Fig. 2).

Resistance was frequently predicted for NNRTIs. Due to strong cross-resistance, identical figures were

Table 1 Patient characteristics

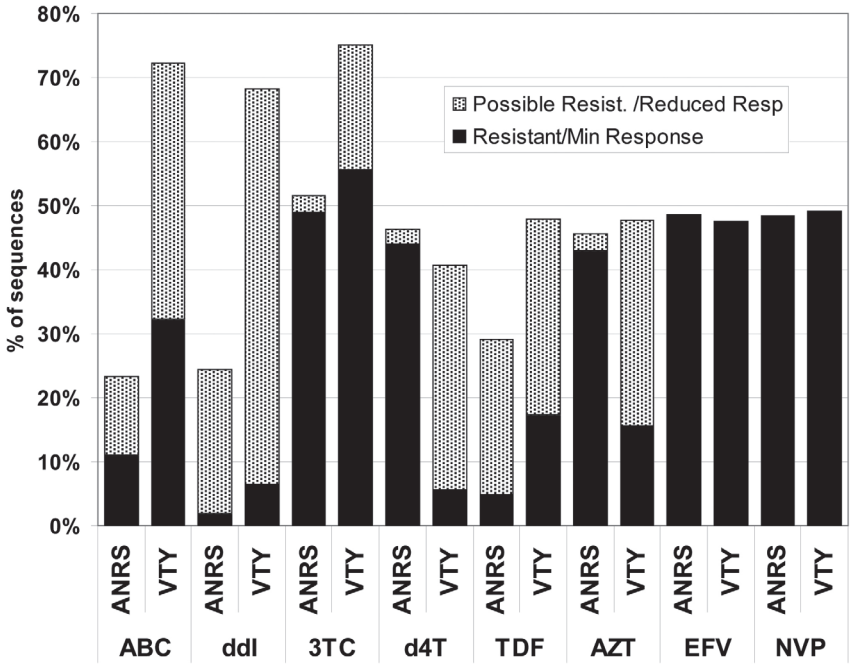
Characteristic	Value
Number of patients	1988
Age[a], median (IQR) [a], years	39 (33-46)
Sex[b], %	
Male	71
Female	29
Route of transmission[c], %	
Men-having-sex-wwith-men	36
Heterosexual contact	36
Injection drug use	16
Other	12
Subtypes, %	
A	6
B	59
C	7
D	2
F	2
G	15
CRF02_AG	5
Other	4
HIV-RNA load[d], median (IQR) log copies/ml	4.12 (3.53-4.82)

[a] *Data available for 1544 patients. IQR=Inter-Quartile Range*

[b] *Data available for 1629 patients*

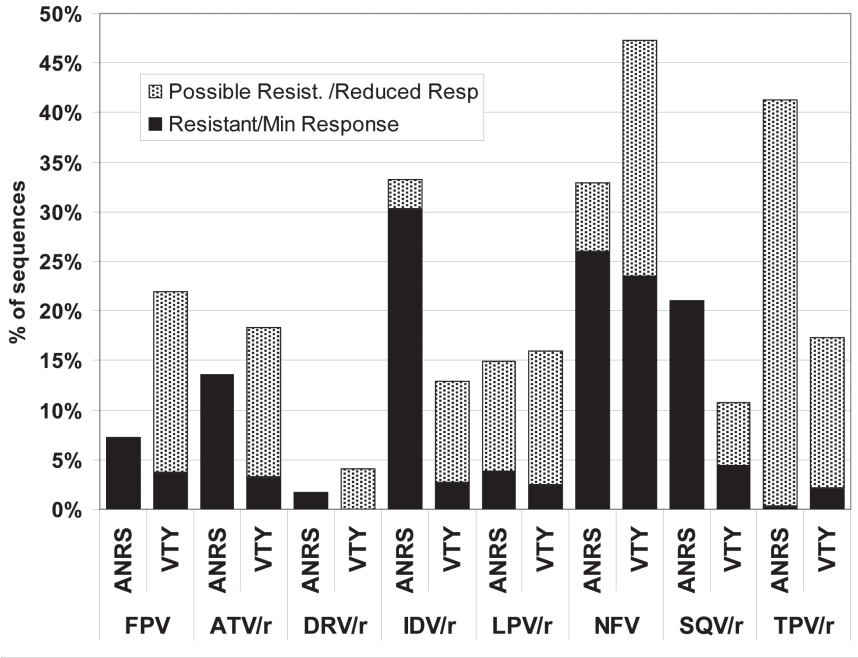
[c] *Data available for 766 patients*

[d] *Data available for 1879 patients*



ABC=Abacavir,
ddi=Didanosine,
3TC=Lamivudine,
d4T=stavudine,
TDF=tenofovir,
AZT=zidovudine,
EFV=efavirenz,
NVP=nevirapine

Figure 1 – Comparison genotypic(ANRS) and phenotypic (VTY=VircoType) resistance to RT inhibitors.



FPV=Fosamprenavir, ATV/r=atazanavir, DRV/r=darunavir, IDV/r=Indinavir, LPV/r=lopinavir, NFV=nelfinavir, SQV/r=saquinavir, TPV/r=tipranavir

Figure 2 – Comparison genotypic(ANRS) and phenotypic (VTY=VircoType) resistance to protease inhibitors.

found for efavirenz (48.8%) and nevirapine (48.8%; Fig. 2).

Resistance was generally less common to particular protease inhibitors. Surprisingly, among individual protease inhibitors, reduced susceptibility was most frequently found for the recently approved drug tipranavir (42.1%). The levels of reduced susceptibility were considerably lower than to the other novel protease inhibitors atazanavir (14.0%) and, especially, darunavir (1.7%). Similarly, lower levels of reduced susceptibility were found for fosamprenavir (18.5%) and lopinavir (14.6%; Fig. 3). No sequence had genotypic resistance to all available antiretrovirals.

Phenotypic drug susceptibility

Figure 2 shows that phenotypic resistance to particular RT-inhibitors was common with estimates ranging between 40.7% (stavudine) and 75.2% (lamivudine). Notably, substantial differences between genotypic and phenotypic resistance were found for abacavir and didanosine.

Phenotypic resistance to protease inhibitors was in general found in < 25% of all sequences. Only reduced susceptibility to nelfinavir was found in a higher proportion of sequences. Notably, reduced susceptibility to the new protease inhibitors atazanavir and tipranavir was frequently found (estimates were respectively 18.3% and 17.3%).

Discussion

This study analyzed mutation patterns and implications for drug susceptibility for 2000 HIV-1 samples obtained from a large number of European countries from treatment-exposed patients. Only five amino acid substitutions were found in the vast majority of HIV strains analyzed. The mutation patterns did show substantial complexity with a large proportion of sequences harboring resistance to drugs for different classes of antiretrovirals. No sequence had resistance to all available drugs.

The sequences in this study came from routine clinical practice. For the treatment of most patients, a resistance test is not needed because they have successful suppression of viral replication during treatment (2). The estimates reported in this study are therefore overestimations because they were derived from patients that virologically failed on antiretroviral therapy. However, the results can be used to determine the distribution of resistance-associated mutation and its impact on resistance to particular antiretroviral drugs.

Few resistance-associated amino acid substitutions (M184V, M41L, K103N, D67N, and T215Y) were found in virtually all sequences with evidence of resistance. We did not have access to drug utilization, but the frequent occurrence of these mutations seems to be related to prescribing practices. For instance, M184V strongly reduces the susceptibility of HIV to the widely used drug lamivudine (15). Similarly, M41L,

D67N, and T215Y are part of a complex named thymidine-analogue resistance mutations (TAMs) (16), which can be selected by stavudine and zidovudine. Finally, K103N is selected by the NNRTIs efavirenz and nevirapine.

Our estimates are consistent with values reported in literature (6–11). Other studies, which were also limited to patients failing treatment, found that 71 to 83% of the sequences contained at least one resistance-associated mutation that agrees with our estimate of 80.7%. Similarly, "dual resistance" (ranging in literature between 48% and 58%) and "triple resistance" (13–20%) were consistent with our findings of 46.0% and 19.9%, respectively. A recent study among treatment-exposed patients across France and Switzerland found comparable estimates for the most frequently observed resistance-associated mutations (7). Hence, it seems that mutation patterns of drug resistance are equally distributed in various geographical regions.

Resistance-associated amino acid substitutions generally disappear from the plasma once drug treatment is interrupted. However, drug-resistant HIV can persist for decades in patients by establishing a latent infection in resting memory CD-4 positive cells (2). The genotypic assays used in this study were performed on plasma and thus potentially underestimate resistance. In an individual patient this problem can be partly overcome by adding all resistance mutations detected in previous samples to the most recent measurement, thus estimating a worst-case scenario (6, 17). Unfortunately, we did not have access to past samples and therefore cannot estimate to what extent we underestimate the prevalence of resistance as a function of persistence in the cellular compartment in this study.

In the present study, the genotypic and phenotypic interpretation system showed some differences in the levels of resistance to abacavir, didanosine, tenofovir, and the boosted protease inhibitors amprenavir, indinavir, saquinavir, and tipranavir. Previous studies have also reported discordances between different genotypic drug resistance interpretation systems for drugs such as abacavir (18), didanosine (18, 19), tenofovir (19), and amprenavir (18, 19). The differences reported in this study reflect the difficulty in predicting drug susceptibility for some of these drugs. Genotypic resistance levels to tipranavir could be overestimated because the ANRS rules consider several mutations to be of importance that are polymorphic in several non-B subtypes (20).

In conclusion, the levels of resistance among patients with therapy failure are high. The patterns of resistance predominantly reflect resistance to drugs that have been available for a longer time. Because novel drug classes have recently been approved and the newest boosted protease inhibitors have remained active against most circulating viruses, it remains possible to design a fully suppressive regimen for even the most mutated circulating viruses.

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