HIV-1 subtype B-infected MSM may have driven the spread of transmitted resistant strains in France in 2007–12: impact on susceptibility to first-line strategies

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Background: Our study describes the prevalence of transmitted drug resistance (TDR) among 1318 French patients diagnosed at the time of primary HIV-1 infection (PHI) in 2007–12.

Methods: HIV-1 resistance-associated mutations (RAMs) were characterized using both the 2009 WHO list of mutations and the French ANRS algorithm. A genotypic susceptibility score was estimated for each first-line recommended ART combination.

Results: Patients were mainly MSM (72.6%). Non-B variants were identified in 33.7% of patients. The proportion of TDR was estimated as 11.7% (95% CI 10.0–13.5). The prevalences of PI-, NRTI-, first-generation NNRTI and etravirine/rilpivirine-associated RAMs were 2.5%, 5.2%, 3.9% and 3.2%, respectively. Single, dual and triple class resistance was found in 9.6%, 1.0% and 1.1% of cases, respectively. Additionally, 5/331 strains isolated in 2010–12 had integrase inhibitor (II)-related RAMs (isolated E157Q mutation in all cases). TDR was more common among MSM than in other groups (12.9% versus 8.6%, \( P = 0.034 \)), and in case of B versus non-B subtype infections (13.6% versus 7.9%, \( P = 0.002 \)). The proportions of fully active combinations were ≥99.2%, ≥97.3% and ≥95.3% in cases of PI-, II- and NNRTI-based regimens, respectively. In 2010–12, the proportion of fully active efavirenz-based ART was lower in cases of subtype B versus non-B infection (\( P = 0.021 \)).

Conclusions: Compared with our previous studies, the proportion of NNRTI- and first-generation NNRTI-related TDR has continued to decline in French seroconverters. However, subtype B-infected MSM could drive the spread of resistant HIV strains. Finally, we suggest preferring PI- or II- to NNRTI-based combinations to treat PHI patients.

Keywords: HIV-1, resistance-associated mutations, primary HIV infection

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Introduction
European studies have reported that the prevalence of HIV-1 transmitted drug resistance (TDR) ranged from 8.3% to 14%, with stable or decreasing rates of NRTI-, NNRTI- and PI-related TDR in recent years.\(^1\)\(^-\)\(^8\) In most of these studies, reverse transcriptase (RT) and protease (PR) mutations were identified from the 2009 WHO consensus list for genotypic resistance surveillance of TDR.\(^9\) This list did not include mutations conferring resistance to second-generation NNRTIs or to integrase inhibitors (IIIs), which have recently taken a major place in recommended first-line combined antiretroviral therapies (cART). Indeed, few data have been published about the prevalence of rilpivirine-\(^10\)\(^-\)\(^12\) and II-associated TDR.\(^1\)\(^,\)\(^13\) The present work aimed to evaluate trends of TDR prevalence in patients diagnosed with primary HIV-1 infection (PHI) in France in 2007–12.

Patients and Methods

Study population
The study population comprised 1318 patients infected for less than 6 months (see enrolment criteria in Supplementary Methods, available as Supplementary data at JAC Online) and recruited in the PRIMO Cohort study (ANRS CO6),\(^6\)\(^,\)\(^8\) or in the ANRS 147 Optiprim trial,\(^1\)\(^,\)\(^9\) or through the ANRS ACT11 Resistance group within the network of French virology laboratories. The study was approved by the Comité consultatif sur le traitement de l’information en matière de recherche dans le domaine de la santé and the Commission nationale de l’informatique et des libertés.

Genotypic resistance analysis
Genotypic resistance tests were performed on plasma samples collected before initiation of cART (virological methods are detailed in Supplementary Methods). HIV-1 resistance-associated mutations (RAMs) were defined using both the 2009 WHO RAMs list\(^9\) and the French ANRS algorithm v23 (updated in September 2013; www.hivfrenchresistance.org). The ANRS algorithm includes all of the rilpivirine-, etravirine- and II-related RAMs reported in the 2013 IAS-USA resistance mutations list,\(^1\)\(^6\) except for the F227C mutation (conferred resistance to rilpivirine) and the T97A mutation (conferred resistance to elvitegravir or raltegravir), which have not been studied here. The studied RAMs are listed in the Supplementary Methods. The genotypic susceptibility score (GSS) was estimated using the list of RAMs. Each drug, except ritonavir, was scored as 1 or 0 in case of ‘susceptibility’ or ‘intermediate/high resistance’, respectively, according to the ANRS interpretation algorithm. For each of the first-line recommended cART combinations in French and IAS guidelines,\(^17\)\(^,\)\(^18\) the arithmetic sum of the individual scores for the specific drugs provided the total treatment-related GSS.

Phylogenetic analysis
The HIV-1 subtype was determined by phylogenetic analysis of RT sequences, as previously described.\(^1\)\(^9\)

Statistics
Variables were summarized as proportions for categorical variables, the median and range for continuous variables. The \(\chi^2\) test or Fisher’s exact test was used to compare categorical variables and the Mann–Whitney U-test was used to compare continuous variables between groups. The 95% CIs were computed using a binomial distribution. A logistic regression model was used to identify which population groups had the greatest risk of being infected with resistant viruses. Analyses were performed with IBM SPSS statistics software version 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Study population
Overall, 1318 patients were included in our study (Table 1). Patients were mainly MSM (72.6%); the proportion of MSM increased significantly between 2007–09 and 2010–12. The regions of birth were different between risk groups: MSM originated from France, other European countries and sub-Saharan African countries in 82.4%, 3.1% and 1.5% of cases, respectively, versus 58.5%, 4.0% and 22.4% for heterosexuals.

HIV-1 diversity
Subtype B strains were isolated in 66.3% of patients (Table 1). The most prevalent non-B variant was CRF02_AG (17.1%). The prevalence of non-B strains increased in heterosexuals between 2007–09 and 2010–12 (47.7% versus 69.1%, \(P<0.001\)) and concurrently decreased in MSM (30.7% versus 24.0%, \(P=0.027\)).

TDR over-represented in MSM and subtype B
RAMs were identified in 114/1318 strains (8.6%, 95% CI 7.2–10.3) using the WHO 2009 list, and in 154/1318 viruses (11.7%, 95% CI 10.0–13.5) using both the WHO list and the French ANRS 2013 algorithm (Table 1). The prevalences of PI-, NRTI-, first- and second-generation NNRTI-associated RAMs were stable between 2007–09 and 2010–12. Viruses with single, dual and triple class resistance were isolated in 127 (9.6%), 13 (1.0%) and 14 (1.1%) patients, respectively. Additionally, 5/331 (1.5%) strains, isolated in 2010–12, had II-related RAMs (E157Q mutation in all cases, without any other mutation in RT or PR genes).

Factors associated with the presence of RAMs are shown in Table 2. Compared with other transmission groups, viruses isolated in MSM more frequently harboured RAMs (12.9% versus 8.6%, \(P=0.034\)) and PI-associated RAMs (3.0 versus 1.1%, \(P=0.048\)), and tended to have more frequent NRTI-associated RAMs (6.0 versus 3.3%, \(P=0.07\)). Compared with non-B variants, subtype B viruses more frequently harboured RAMs (13.6% versus 7.9%, \(P=0.002\)) and NRTI-associated RAMs (3.2 versus 1.1%, \(P=0.024\)) and 6.8% versus 2.3%, \(P<0.001\), respectively). Viruses with dual or triple class resistance were mainly isolated in MSM (22/26, 84.6%) and in case of subtype B infection (23/26, 88.5%). Logistic regression analysis showed that MSM infected with B subtype was the group with the highest risk (OR: 2.2 (1.2–2.9)) of harbouring virus with RAMs (Table 2).

A majority of patients with TDR had singleton resistance mutations
Single RAMs were isolated in 64.8% of viruses. Fifty-six strains (35.2%) harboured several RAMs, conferring single, dual and triple class resistance in 29, 13 and 14 cases, respectively (Tables S1 and S2). Overall, the prevalence of TDR related to the most commonly prescribed PIs, NRTIs and IIIs was very low: 0.1% of strains were resistant to atazanavir; 0.2% were resistant...
to lamivudine/emtricitabine; 0.2% were resistant to tenofovir; 0.3% were resistant to abacavir; 0.3% were resistant to lopinavir; 1.5% were resistant to raltegravir; 1.5% were resistant to elvitegravir; and none of them was resistant to darunavir or dolutegravir. These results contrast with the prevalence of NNRTI-related TDR: 4.1%, 4.3% and 4.3% of strains were resistant to efavirenz, nevirapine and ritapivirine, respectively.

Impact of TDR on NNRTI-first line strategies

The proportions of fully active (GSS = 3) regimens were: 99.7% for tenofovir/emtricitabine/dolutegravir; 99.5% for abacavir/lamivudine/atazanavir/ritonavir, tenofovir/emtricitabine/atazanavir/ritonavir, abacavir/lamivudine/darunavir/ritonavir and tenofovir/emtricitabine/darunavir/ritonavir; 99.4% for abacavir/lamivudine/dolutegravir; 99.3% for abacavir/lamivudine/lopinavir/ritonavir; 99.2% for tenofovir/emtricitabine/lopinavir/ritonavir, abacavir/lamivudine/raltegravir and abacavir/lamivudine/raltegravir and tenofovir/emtricitabine/elvitegravir/cobicistat (considering that the E157Q polymorphic mutation may impact on susceptibility to raltegravir and elvitegravir); 95.8% for abacavir/lamivudine/efavirenz and tenofovir/emtricitabine/efavirenz; 95.6% for tenofovir/emtricitabine/nevirapine; and 95.3% for abacavir/lamivudine/efavirenz and tenofovir/emtricitabine/efavirenz.

The proportion of fully active combinations did not differ between transmission groups or between B versus non-B
However, when restricting the analysis to the patients infected in 2010–12, a lower proportion of fully active efavirenz-based ART (with tenofovir/emtricitabine or abacavir/lamivudine) was observed in case of subtype B versus non-B infections (94.2% versus 98.5%, \( P = 0.021 \)).

### Discussion

Compared with our previous reports in French seroconverters, the rate of TDR was stable (10.9% in 1996–2006 versus 11.7% in 2007–2012)\(^\text{19}\). However, because the present study was based on the 2013 ANRS algorithm, we described several RAMs that were not listed in the 2009 WHO RAMs list or in our previous reports. Indeed, the high frequency of several second-generation NNRTI-related RAMs, especially the E138A polymorphic substitution, could impact the prevalence of TDRs in the present study. Restricting our analysis to the 2009 WHO RAMs list, we found a similar prevalence of TDR (8.6%) to that recently reported in European cART-naive patients in studies using the same list of RAMs.\(^\text{3,4}\) However, when restricting the comparison to the data about recently infected patients, we herein describe a lower prevalence of TDR than in the UK or in Spain (11.7% in 2007–09 and 13.4% in 2010–12, respectively).\(^\text{3,4}\)

The proportion of NRTI- and NNRTI-related TDR has been continuously decreasing in patients diagnosed with PHI in France over a decade.\(^\text{19}\) Three factors could explain these results. First, the proportion of patients being virologically controlled on cART continues to increase over time in France \([88\% in 2011 (www.ccde.fr)]\). Second, thymidine analogues are no longer recommended. As NRTI-related RAMs mainly consisted of thymidine analogue mutations, viruses with NRTI-related TDR are nowadays rarely transmitted.\(^\text{1–4,6,7}\) Third, the low rate of first-generation NNRTI resistance is probably due to the widespread use of new-generation boosted PIs, which receive preferential use in France. TDR was much higher in HIV-1 subtype B-infected MSM compared with other patients, in line with previous European reports,\(^\text{1,20}\) because most patients who acquired HIV through heterosexual contact were more likely to come from regions with limited access to cART (or to become infected through individuals originating from these regions).\(^\text{20}\) Because subtype B-infected MSM may drive the spread of resistant HIV strains, we confirm the urgent need to increase not only the ART coverage of individuals diagnosed with HIV, which is already high in France (81% of diagnosed individuals, 76% of MSM), but also HIV testing opportunities.

### Table 2. Univariate and multivariable logistic regression analysis of factors associated with the risk of harbouring virus with drug resistance mutations

<table>
<thead>
<tr>
<th>Presence of at least one RAM (%)</th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>no ((n = 1164))</td>
<td>yes ((n = 154))</td>
<td></td>
</tr>
<tr>
<td>Study period</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>2007–09</td>
<td>0.160</td>
<td>1</td>
</tr>
<tr>
<td>2010–12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral subtype</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>non-B variant ((n = 444))</td>
<td>0.002</td>
<td>1</td>
</tr>
<tr>
<td>B subtype ((n = 874))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>female ((n = 147))</td>
<td>0.588</td>
<td>1</td>
</tr>
<tr>
<td>male ((n = 1166))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>missing value ((n = 5))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission group</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>others ((n = 361))</td>
<td>0.034</td>
<td>1</td>
</tr>
<tr>
<td>MSM ((n = 957))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country of birth</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>sub-Saharan Africa ((n = 79))</td>
<td>0.283</td>
<td>1</td>
</tr>
<tr>
<td>other ((n = 1239))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission group and viral subtype</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>non-MSM: non-B variant ((n = 191))</td>
<td>0.007</td>
<td>1</td>
</tr>
<tr>
<td>MSM: non-B variant ((n = 253))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-MSM: B subtype ((n = 170))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM: B subtype ((n = 704))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count (cells/mm(^3)), median (range)</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>502 (3–2872)</td>
<td>0.719</td>
<td>0.9 (0.7–13)</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log(_{10}) copies/mL), median (range)</td>
<td>( P \text{ value} )</td>
<td>( OR (95% CI) )</td>
</tr>
<tr>
<td>5.5 (2.0–8.7)</td>
<td>0.579</td>
<td>0.9 (0.8–1.1)</td>
</tr>
</tbody>
</table>

\( P \text{ values} <0.05 \) are shown in bold.

Subtypes. However, when restricting the analysis to the patients infected in 2010–12, a lower proportion of fully active efavirenz-based ART (with tenofovir/emtricitabine or abacavir/lamivudine) was observed in case of subtype B versus non-B infections (94.2% versus 98.5%, \( P = 0.021 \)).
To sum up, the proportion of NRTI- and first-generation NNRTI-related TDR continues to decline in persons infected with HIV in France. However, TDR seems to be more concentrated in the MSM epidemic. We suggest that preferential choice of PI- or II- over NNRTI-based combinations to treat individuals with PHI be implemented, as the risk of early failure and possible forward transmission of TDR will be reduced. Continuous monitoring of TDR should continue given the potential impact on the recommended first-line therapies. Importantly, monitoring of the prevalence of II-related RAMs will also be key, given the expanding role of these medications.

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Members of the ANRS PRIMO Cohort Study Group


Members of the OPTIPRIM ANRS 147 Study Group


Members of the ANRS AC11 Resistance Study Group


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Supplementary data

Supplementary Methods, Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References