European surveillance of HIV drug resistance to NRTI, NNRTI and INSTI in newly diagnosed individuals using next-generation sequencing

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• No conflict of interest to declare
The SPREAD surveillance program

SPREAD Database & Interactive map

Database of 20,000 patients from 26 countries: monitoring of HIV transmitted drug resistance (TDR)

Interactive tool with maps of regional surveillance data
Overall weighted prevalence of transmitted drug resistance in patients with newly diagnosed HIV in Europe

Interactive monitoring tool with maps of regional surveillance data from the SPREAD program 2011-2013 dataset (4943 patients, 24 countries):

- Overall prevalence of TDRM in Europe was 8.6% (95% CI 7.6-9.6).
- The prevalence of TDRM was significantly higher in Western Europe (12.2%, 95% CI 10.4-13.9; p<0.01).

Hofstra M et al, CID 2016
Background

- NNRTIs are still frequently used in Central and Eastern Europe

- Minority NNRTI resistant variants were significantly associated with an increased risk of virological failure

- INSTIs is now recommended as a first-line regimen by European and WHO guidelines resulting in a widespread global use

- Analysis of 300 samples of patients from the SPREAD program newly diagnosed in 2006/2007 showed no signature INSTI-resistant variants before INSTIs were introduced in clinical practice.
Objectives

✓ Evaluate prevalence of minority resistant variants in the RT and INT genes in a representative dataset from Europe

✓ Participating countries: Belgium, Bulgaria, Croatia, Cyprus, Finland, France, Greece, Israel, Lithuania, Luxembourg, Poland, Russia and Slovenia

✓ Patients randomly selected from the 2013 dataset of SPREAD (approx. 20 patients per country, Russia).
## Baseline characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Total (N=275)</th>
<th>Reverse transcriptase (N=254)</th>
<th>Integrase (N=214)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis Years, median (IQR)</strong></td>
<td>36 (28 – 44)</td>
<td>37 (29 – 45)</td>
<td>36 (38 – 43)</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>227 (75.9)</td>
<td>194 (76.4)</td>
<td>169 (79.0)</td>
</tr>
<tr>
<td><strong>Route of transmission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>127 (42.5)</td>
<td>115 (45.3)</td>
<td>91 (42.5)</td>
</tr>
<tr>
<td>HSX</td>
<td>98 (32.8)</td>
<td>80 (31.5)</td>
<td>83 (38.8)</td>
</tr>
<tr>
<td>IVDU</td>
<td>23 (7.7)</td>
<td>17 (6.7)</td>
<td>19 (8.9)</td>
</tr>
<tr>
<td><strong>Continent of origin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>247 (82.6)</td>
<td>213 (83.9)</td>
<td>189 (88.3)</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>15 (5.0)</td>
<td>12 (4.7)</td>
<td>11 (5.1)</td>
</tr>
<tr>
<td><strong>HIV RNA load log cps/mL, median (IQR)</strong></td>
<td>4.9 (4.4 – 5.4)</td>
<td>5.0 (4.5 – 5.5)</td>
<td>5.0 (4.5 – 5.5)</td>
</tr>
<tr>
<td><strong>CD4 count cells/μL, median (IQR)</strong></td>
<td>329 (173 – 511)</td>
<td>329 (169 – 489)</td>
<td>302 (165 – 489)</td>
</tr>
<tr>
<td><strong>Subtype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>57 (19.1)</td>
<td>47 (18.5)</td>
<td>45 (21.0)</td>
</tr>
<tr>
<td>B</td>
<td>153 (51.2)</td>
<td>135 (53.1)</td>
<td>118 (55.1)</td>
</tr>
<tr>
<td>C</td>
<td>15 (5.0)</td>
<td>13 (5.1)</td>
<td>11 (5.1)</td>
</tr>
<tr>
<td>D</td>
<td>3 (1.0)</td>
<td>2 (0.8)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>G</td>
<td>5 (1.7)</td>
<td>5 (2.0)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>01_AE</td>
<td>12 (4.0)</td>
<td>11 (4.3)</td>
<td>10 (4.7)</td>
</tr>
<tr>
<td>02_AG</td>
<td>8 (2.7)</td>
<td>7 (2.8)</td>
<td>8 (3.7)</td>
</tr>
</tbody>
</table>

Form of notation is No. (%) unless stated otherwise.
Ultra Deep Sequencing platforms

454 FLX Genome Sequencer (Roche, Manheim)

Illumina MiSeq platform (UMC Utrecht, Dr M Nijhuis)
Pipeline analysis

✓ Roche 454 and Illumina sequences were filtered according to their quality and signals

✓ Sequencing files were imported into CLC Genomics Workbench (v.10.1.1)

✓ Sequences were checked for quality and trimmed using a minimum score of 0.02 (PHRED score 20)

✓ Reads were aligned to the HIV HXB2 reference sequence. Low frequent variant calling was performed with a required significance of 1% and a minimum coverage of 500 reads

✓ Stringent quality criteria: only sequences with 80% of the full-length sequence with both forward and reverse sequences were kept

✓ No homo-polymer stretches were detected at positions of drug resistance mutations.
Predominance of all substitutions above 20%, between 5% and 20% or below 5% of frequency

✓ Interpretation of drug resistance mutations:
  RT: Stanford and WHO lists for surveillance of drug resistance mutations
  IN: all substitutions, mutation scoring of Stanford HIVdb v8.7
Overall frequency of RT mutations

NRTI: 16.8% (Stanford)  
14.8% (WHO)  

NNRTI: 22.8% (Stanford)  
5% (WHO)
Prevalence of RT mutations by frequency

WHO list
- 5.6% of TDR above 20%
- 4.8% of additional TDR between 5 and 20%
- 10% of additional TDR below 5%
Conclusions RT

✓ The prevalence of TDR above 20% is in line with the SPREAD analysis 2012-2013: no increase of TDR

✓ A significant proportion of low abundance transmitted drug resistance to NRTI (14.8%) and NNRTI (5.6%) was detected in this representative set of newly diagnosed therapy-naïve HIV-patients from Europe

✓ Association of minority variants with virologic failure is dose-dependent (above 1 or 5%) and most prominent in those with NNRTI-resistance mutations

✓ Minority NRTI mutations are more prevalent than NNRTI mutations, in particular between 5 and 20%, which could have an impact on the virological failure of some patients (K65R).
- No INSTI mutations above 20%
- Polymorphic mutations: L74M, T97A, E157Q, S230N with a similar frequency than before the introduction of INSTI in clinical practice
**INSTI substitutions**

- **Below 5%:**
  - S147G in 2 individuals
  - Y143C in combination with L74M, E157Q and S230N (over 20%)
  - E138K in 4 individuals and only one in combination with L74M (over 20%)
  - polymorphic T97A in 1 individual

**Substitutions between 5 and 20%**

**Substitutions below 5% of frequency**

- **Stanford HIV db score**

  - BIC/DTG: 10
  - RAL/EVG 15
  - RAL/EVG 10
  - BIC/DTG: 5
  - EVG: 10
  - RAL: 60
  - EVG: 60
Conclusions INSTI

✓ No evidence of increasing TDR for INSTIs in Europe. The observed low prevalence of TDR to INSTIs 2013 is in line with the low prevalence observed in several national surveys (2016-2018)

✓ Signature INSTI resistance mutation was observed in only three individuals as minority variants below 5%. There is limited data on the clinical relevance of minority INSTI resistant variants

✓ Nguyen T et al, JAC 2018: the prevalence of baseline minority resistant variants did not differ between patients with and without virological failure. The minority variants that were present at baseline were not detected at time of virological failure

✓ There is currently no clinical need to perform integrase genotyping before initiating INSTI therapy, however continued surveillance of INSTI resistance in Europe is warranted.
Acknowledgements

Substudy within the SPREAD program:
Belgium: J. Ruelle, A. Vandamme; Bulgaria: I. Alexiev, D. Beshkov; Croatia: S. Zidovec Lepej; Cyprus: L. Kostrikis; Finland: K. Liitsola; France: D. Descamps; Greece: D. Paraskevis; Israel: O. Mor; Lithuania: A. Griskevicius; Luxembourg: C. Seguin-Devaux, O. Hunewald, C. Lambert, J.Y. Servais; Netherlands: C.A.B. Boucher, L.M. Hofstra, T.C.M. de Jong, M. Nijhuis, A.M.J. Wensing; Poland: M. Parczewski; Russia: M. Bobkova; Serbia: M. Stanojevic; Slovakia: D. Stanekova; Slovenia: M. Poljak

More information about the SPREAD program and how to join is available at www.esar-society.eu or via info@esar-society.eu