Whole genome deep sequencing of HIV reveals extensive multi-class drug resistance in Nigerian patients failing first-line antiretroviral therapy

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International Workshop on HIV Drug Resistance & Treatment Strategies
8th November 2017
Background

- Nigeria has a “low” HIV prevalence ~ 3%...
  
  … but 2\textsuperscript{nd} largest epidemic

- Diverse HIV-1 subtypes and recombinants

- No routine viral load monitoring/genotypic testing

Aim

- To describe the nature and frequency of drug resistance mutations associated with first-line ART failure
Methods

• Adults with first-line failure from a second-line ART cohort, University of Abuja Teaching Hospital, 2006-2016

• First-line virological failure = HIV RNA >1000 copies/mL (viral load performed at clinician’s request)

• Deep sequencing of participants’ stored first-line failure samples

• IAS-USA drug resistance mutations: frequency 2-20%, 20-90%, >90%

• Stanford HIVdb interpretation of ART susceptibility (consensus sequence)
Participant selection and characteristics

700 people received second-line ART at UATH

111 with plasma sample from first-line failure

44 excluded:
- 32 sample missing
- 9 sequencing failed
- 1 had received a PI
- 2 ART-naïve

60 people with HIV sequence from first-line failure

Participants (n=60)

<table>
<thead>
<tr>
<th>Age, median years (IQR)</th>
<th>30 (28 - 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: female, n (%)</td>
<td>44 (73%)</td>
</tr>
<tr>
<td>HIV-1 subtype, n (%)</td>
<td></td>
</tr>
<tr>
<td>CRF02_AG</td>
<td>34 (57%)</td>
</tr>
<tr>
<td>G</td>
<td>22 (37%)</td>
</tr>
<tr>
<td>CRF06_cpx</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>C</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>CD4+ count, median cells/mm³ (IQR)</td>
<td>110 (63 - 191)</td>
</tr>
<tr>
<td>ART exposure during first-line, n (%)</td>
<td></td>
</tr>
<tr>
<td>NRTI: 3TC</td>
<td>55 (92%)</td>
</tr>
<tr>
<td>AZT</td>
<td>40 (67%)</td>
</tr>
<tr>
<td>TDF</td>
<td>33 (55%)</td>
</tr>
<tr>
<td>FTC</td>
<td>29 (48%)</td>
</tr>
<tr>
<td>d4T</td>
<td>20 (33%)</td>
</tr>
<tr>
<td>ABC</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>NNRTI: NVP</td>
<td>51 (85%)</td>
</tr>
<tr>
<td>EFV</td>
<td>11 (18%)</td>
</tr>
<tr>
<td>HIV RNA at failure, median log₁₀ copies/mL (IQR)</td>
<td>4.9 (4.4 - 5.4)</td>
</tr>
<tr>
<td>Duration of first-line ART, median months (IQR)</td>
<td>28 (18 - 41)</td>
</tr>
</tbody>
</table>
- Total of 367 drug resistance mutations in 60 participants (all in RT)
- 17% of all mutations were minority variants (in 58% of participants)
- 59% were high-frequency substitutions (in 93% of participants)
Type and prevalence of RT mutations

*mutations present in >2 participants

- 57% of participants had TAMs (30% had ≥3 TAMs)
- 95% had other NRTI mutations
- 100% had NNRTI mutations
- 48% had TDF intermediate/high + M184V/I + NNRTI
- None had K65R + ≥3 TAMs (13% had K65R and 1-2 TAMs)
### Time on first-line ART

<table>
<thead>
<tr>
<th>Mutations (frequency within sample)</th>
<th>Median number of mutations (IQR) by ART duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 year (n=8)</td>
</tr>
<tr>
<td>NRTI</td>
<td>3 (2.5 - 3.5)</td>
</tr>
<tr>
<td>&gt;20% frequency</td>
<td>2.5 (1.5 - 3)</td>
</tr>
<tr>
<td>NNRTI</td>
<td></td>
</tr>
<tr>
<td>&gt;20% frequency</td>
<td></td>
</tr>
</tbody>
</table>

Highly resistant viruses emerged during failure, regardless of ART duration
Predicted second-line options - Stanford interpretation
(consensus sequence - no minority variants included)

NRTI

- TDF: 75% predicted resistance (52% intermediate/high-level)
- AZT: 40% predicted resistance (37% intermediate/high-level)
- ABC: 95% predicted resistance (75% intermediate/high-level)
- FTC/3TC: 92% predicted resistance (90% intermediate/high-level)

In practice: were they susceptible to the prescribed second-line regimen?
- 53% had no fully active NRTIs
- 40% had one fully active NRTI
- 7% had two fully active NRTIs

Protease/integrase inhibitors

Predicted to be susceptible
Conclusions

• Diverse Nigerian HIV clades had extensive RTI resistance at first-line ART failure

• High-frequency mutations (>90%) were common, indicating fixation in the viral population, even in the first year of ART

• Routine viral load monitoring and adherence support are crucial from the outset of therapy

• Second/third-line agents were predicted to be efficacious, but clinical effectiveness is unknown in the context of NRTI resistance

• Limitations:
  – pre-ART samples not available (wild-type at baseline? PDR?)
  – potential bias from clinician-driven viral load testing
  – selection of participants from second-line cohort
Acknowledgements

All study participants and members of the UATH community support group