Improvement of HIV-1 resistance testing by proviral DNA analysis and Next Generation Sequencing

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Problems

- Majority of samples from therapy-experienced patients with VL < 500 copies/ml
  - Low level (LLV) or undetectable viremia
  - In most cases no results with plasma samples
- Restricted detection limit of drug resistance mutations (DRMs)
  - Sanger sequencing: 15-20% sensitivity cutoff
Possible Solutions

- proviral DNA (PBMCs)
  - Unsuccessful testing with plasma RNA
  - LLV or suppressed VL
  - Problem
    - VL often not known at timepoint of sample processing
    - Cost and time intensive unnecessary repeats with plasma samples

- Total nucleic acid (tNA= plasma RNA + proviral DNA)
  - Effective resistance testing with unknown VL
  - Saving cost and time
Comparison of mutation patterns

1. **Viral RNA vs. proviral DNA** of identical blood samples

2. **Viral RNA vs. total NA** of identical blood samples
   - Sanger sequencing vs. NGS
69 samples of TE (n=46) and TN patients (n=23) of the RESINA cohort

- Paired viral RNA and proviral DNA were isolated
- PR and RT genes were amplified
- Sanger sequencing
- 47/69 samples (68%) presented DRMs in RNA and/or proviral DNA genotypes
High concordance of the DRMs in viral RNA and proviral DNA (41.6%), especially the PI mutations (53.2%)

Significant higher frequency of RTI mutations in RNA only (40.5%) compared to PI mutations (23.4%) (p=0.049)
36 different resistance-associated positions in PR and RT

6/36 DRMs were more frequent in proviral DNA (NNRTI and PI mutations)

8/36 DRMs were more frequent in viral RNA (NRTI mutations)

Overall high concordance of DRMs in RNA and DNA
28 samples of TN patients of the RESINA cohort

- median VL=54,433 copies/ml (range 281-10,000,000)
- Plasma viral RNA and total NA were isolated of each sample
- PR und RT genes were amplified

Sequencing

- Sanger Sequencing (Sanger)
- Next Generation Sequencing (NGS)
  - Illumina MiSeq (selected sensitivity cutoff 10%)
### Prevalence of DRMs in viral RNA and tNA

<table>
<thead>
<tr>
<th></th>
<th>viral RNA</th>
<th>total NA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanger (n=27)</strong></td>
<td>DRM</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.19 ± 0.96</td>
<td>1.56 ± 0.70</td>
</tr>
<tr>
<td><strong>NGS (n=28)</strong></td>
<td>DRM</td>
<td>73</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.61 ± 1.50</td>
<td>3.07 ± 1.68</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

- 1.3-fold higher detection rate of DRMs in tNA samples
- 2-fold higher detection rate of DRMs by NGS (p=0.0001)

- tNA analyses provided a slightly increased DRM detection rate
- Significant higher DRM detection rate by NGS
### DRM detected in RNA only, tNA only or both

**Sanger:**
- 6-fold higher detection rate of DRMs in tNA vs. RNA only
- High concordance of DRMs in RNA and tNA (68%)

**NGS:**
- 1.5-fold higher detection rate of DRMs in tNA vs. RNA only
- Increased sensitivity of DRM detection independent of used nucleic acids
Sanger:
- 95% of DRMs are detected by tNA

NGS:
- 74% of DRMs are detected by tNA
- twice as many as detected by Sanger
- superior to Sanger
Summary and Conclusion

- **RNA vs. DNA**
  High concordance of DRMs in viral RNA and proviral DNA
  - Proviral DNA resistance testing could help in cases of unsuccessful RNA genotyping (LLV, suppressed VL)

- **RNA vs. tNA**
  Total NA provided an increased DRM detection rate
  - Total NA could be an alternative to plasma RNA analyses

- **Sanger vs. NGS**
  NGS significantly increased the resistance information
  - NGS could be an alternative in routine diagnostic
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