Detection of very low levels of cell-associated HIV-1 RNA in well suppressed children (CARMA)

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Conclusions

Outgrowth assays (VOA, TILDA) will be performed.

Methods

Background and Objective

HIV-1 can persist as latent virus in long-term suppressed patients on antiretroviral treatment (ART). Despite viral suppression HIV-1 still persists in latently infected cells and has the potential to rebound after cessation of ART.

We used two methods (qPCR and dPCR) to quantify cell-associated HIV-1 RNA (CA-RNA) in a well-suppressed, early treated cohort of well-suppressed children (CARMA).

Total and unspliced (US) CA-RNA was measured to complement studies for ongoing HIV replication and/or immune activation in HIV-1 infected individuals.

Detection of very low levels of cell-associated HIV-1 RNA in long-term suppressed children is detectable with either qPCR or dPCR. The Bland-Altman concordance analysis showed 97.4% agreement for both, total and US CA-RNA, suggesting comparability of the methods for detecting low copy numbers of CA-RNA in this cohort. Therefore either method could be used for diagnostic analysis of low-level viremia. However, due to the high sensitivity especially of dPCR, sufficient repeats of negative controls need to be included in each reaction to validate the lower threshold of the method.

Copy numbers of US CA-RNA were generally lower than total CA-RNA, indicating expression of different forms of HIV RNA in general and very low expression of full-length RNA in early treated well-suppressed children. In addition, we measured plasma viral load by an ultrasensitive method. Values of plasma viral load were lower than CA-RNA and there was no correlation observed between them.

Taken together, our data show that expression of HIV-1 CA-RNA in early treated well-suppressed children can be detected by qPCR and dPCR at very low levels. Further work including full-length single genome sequencing is ongoing to investigate whether presence of CA-RNA is correlated with immune activation, and/or if expression of full-length HIV RNA is derived from replication competent proviruses that contribute to the cell-free plasma VL.

To determine whether the expressed full-length HIV RNA is replication competent, whole single-genome sequencing and viral outgrowth assays (VOA, TILDA) will be performed.

Table 1: Detection of CA-RNA in long-suppressed ET (in 40 patients)

<table>
<thead>
<tr>
<th>Method</th>
<th>Total CA-RNA (copies per 106 PBMCs)</th>
<th>US CA-RNA (copies per 106 PBMCs)</th>
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<tbody>
<tr>
<td>qPCR</td>
<td>23 (1-5789)</td>
<td>9 (1-857)</td>
</tr>
<tr>
<td>dPCR</td>
<td>29 (11-857)</td>
<td>21 (11-325)</td>
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</table>

- low-level HIV RNA can be detected with qPCR and dPCR
- different HIV RNA species can be detected with both methods
- detection of very low copy numbers of HIV RNA with qPCR (mean 7.4) and dPCR (1.3) is possible
- assays for quantifying different HIV RNA species were transferable between qPCR and dPCR

Figure 1: Evidence of ongoing RNA transcription in long-suppressed ET (copy numbers per reaction)

- detected copy numbers of US HIV RNA are lower than total HIV RNA copy numbers
- detection of US HIV RNA could indicate ongoing HIV replication

Figure 2: Bland-Altman test (comparison of copy numbers per reaction)

- high agreement (97.4% for LTR and for pol) of both methods
  - 1/38 in Fig. 2A falls out of the 95% confidence interval
  - 1/38 in Fig. 2B falls out of the 95% confidence interval

Figure 3: Cell-associated HIV DNA and RNA in PBMCs and ultra sensitive viral load in plasma (qPCR, paired t-test)

- cell-associated HIV RNA and DNA and plasma HIV RNA can be detected in long-term suppressed children at very low levels
- detection of cell-associated (CA) DNA in PBMCs is higher than detection of CA-RNA or plasma RNA
- no correlation of CA-RNA and plasma RNA amounts (ultra-sensitive method)

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References