**EFFECTIVENESS OF CURRENT ANTI-HIV REGIMEN IN LOW- AND MIDDLE-INCOME COUNTRIES**

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**Abstract**

Nevirapine (NVP) is a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). However, with the emergence of resistance mutations due to a low genetic barrier under NVP pressure, new (second generation) NNRTIs have been approved. Rilpivirine (RPV), a second generation NNRTI, is not frequently used in low- and middle-income countries (LMICs) that bear the major HIV burden. RPV has been co-formulated with tenofovir (TDF) and emtricitabine (FTC) and has been recommended for patients with viral loads <100,000 copies/mL, inhibiting viruses that are resistant to NVP. It is now being considered in many LMICs.

To understand RPV efficacy in HIV-1 subtypes prevalent in LMICs, we cloned RT genes from patients infected with four different HIV-1 subtypes: subtype B (HIV-1B), subtype C (HIV-1C), and recombinant forms CRF01_AE and CRF02_AG. HIV-1B is most prevalent in western countries and accounts for only ~12% of all infections. However, HIV-1C, which accounts for ~52% of all HIV infections, is most prevalent in LMICs. In vitro inhibition assays were performed with the four patient-derived RTs.

Our results show that overall, NVP binds RTs with lower affinity than RPV, suggesting that NVP has lower effectiveness than RPV. However, NVP binds 02_AG RT with better affinity than RPV. Hence, NVP may still be effective for patients infected with 02_AG. Furthermore, RPV binding affinity with HIV-1C is lower than other subtypes. This result is consistent with clinical results, showing less efficacy of RPV among HIV-1C infected patients.

**Background**

HIV types, groups and subtypes and worldwide distribution

HIV -1C comprises more than 50% of the world’s HIV cases.

**Do HIV-nonB patients fail RPV easier than HIV-1B?**

Therapy outcome of 117 patient Swedish InfCare Cohort

**Methods**

Cloning, expression and purification of RT from patient samples

**Kinetics of dNTP binding**

**Rapid Quench Flow (RFQ)**

1. Run dNTP incorporation reactions in a rapid quench flow machine under single turnover conditions.
2. Analyze the products on a 20% urea gel. Plot the amount of product at different dNTP concentrations.
3. Determine observed rate constants (kobs) using a burst equation.
4. Plot the observed rates against increasing dNTP concentrations.
5. Fit the data points to obtain the optimal polymerization rate (kpol) and dNTP binding affinity (Kd.dNTP).

DNA/DNA Template/Primer used in this study Sequence (31/18mer)

3’ - CAG TGA CAA GCT GTG TAG GAT AGA TAG C-5’ Template 31’

5’ - GTC ACT GTT CGA GCA CCA 3’ Primer 18

**Kinetics of NNRTI (RPV) binding**

1. Run dNTP incorporation reactions in a rapid quench flow machine under single turnover conditions in presence of increasing concentration of RPV.
2. Analyze the products on a 20% urea gel. Plot the amount of product at different dNTP concentrations.
3. Determine amplitude using a burst equation.
4. Plot amplitude with increasing RPV concentrations.
5. Fit the data points to obtain RPV binding affinity (Kd.RPV).

**Results**

**Kinetik parameters of HIV-1C RT on heteropolymeric (31/18-mer) DNA/DNA template-primer**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Kd.dATP (μM)</th>
<th>kpol (s-1)</th>
<th>efficiency (μM·s-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1B RT</td>
<td>3.4</td>
<td>12.5</td>
<td>3.7</td>
</tr>
<tr>
<td>HIV-1C RT</td>
<td>14.54</td>
<td>26.69</td>
<td>1.8</td>
</tr>
<tr>
<td>01_AE RT</td>
<td>2.0</td>
<td>10.8</td>
<td>5.4</td>
</tr>
<tr>
<td>02_AG RT</td>
<td>2.1</td>
<td>10.38</td>
<td>4.9</td>
</tr>
</tbody>
</table>

HIV-1C RT is ~ 2-fold less efficient than other subtype RTs

**NVP binding affinity (Kd.NVP) to HIV-1B and HIV-non B RTs**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Kd.NVP (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1B RT</td>
<td>100.7 ± 17</td>
</tr>
<tr>
<td>HIV-1C RT</td>
<td>101.1 ± 32</td>
</tr>
<tr>
<td>01_AE RT</td>
<td>78.1 ± 7</td>
</tr>
<tr>
<td>02_AG RT</td>
<td>21.2 ± 1</td>
</tr>
</tbody>
</table>

Nevirapine binding affinity varies among different subtypes

02_AG appears more susceptible to NVP

**RPV binding affinity (Kd.RPV) to HIV-1B and HIV-non B RTs**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Kd.RPV (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1B RT</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>HIV-1C RT</td>
<td>66 ± 7</td>
</tr>
<tr>
<td>01_AE RT</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>02_AG RT</td>
<td>21 ± 3</td>
</tr>
</tbody>
</table>

Rilpivirine binding affinity varies among different subtypes

HIV-1 Subtype C appears less susceptible to RPV

**Conclusions**

More HIV-nonB patients failed therapy (25%) than HIV-1B (9%)

NVP & RPV binding affinity varies among subtypes indicating its different efficacy in different HIV subtypes

Both clinical and biochemical experiment results suggest that NNRTIs has different susceptibility for different HIV-1 subtypes

Data suggest that NVP can be used for 02_AG infections efficiently

Data suggest that RPV is not a good anti-HIV drug for subtype C infections

Data suggest that Efda can be used for all subtypes as a potent anti-HIV drug

**Acknowledgments**

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**Figure Legends**

- **Table 1**: Kinetic parameters of HIV-1C RT on heteropolymeric (31/18-mer) DNA/DNA template-primer
- **Table 2**: NVP binding affinity (Kd.NVP) to HIV-1B and HIV-non B RTs
- **Table 3**: RPV binding affinity (Kd.RPV) to HIV-1B and HIV-non B RTs

**Graphs**

- **Graph 1**: Kinetics of dNTP binding
- **Graph 2**: Kinetics of NNRTI (RPV) binding

**References**