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

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Abstract

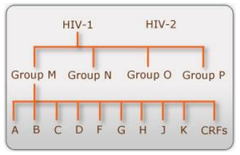
Nevirapine (NVP) is a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). However, with the emergence of resistance mutations due to a low genetic barrier under NVP pressure, new (second generation) NNRTIs have been approved. **Rilpivirine (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.

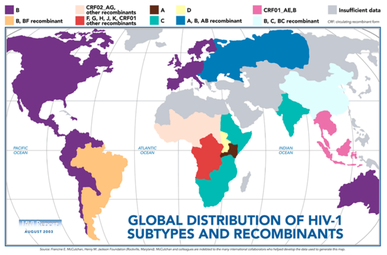
Our results show that overall, NVP binds RTs with lower affinity than RPV, suggesting that NVP has lower effectiveness than RPV. However, NVP binds 02_AG RT with better affinity than RPV. Hence, NVP may still be effective for patients infected with 02_AG. Furthermore, RPV binding affinity with HIV-1C 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.

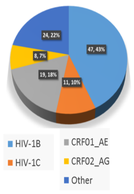


Do HIV-nonB patients fail RPV easier than HIV-1B?

Therapy outcome of 117 patient Swedish InfCare Cohort

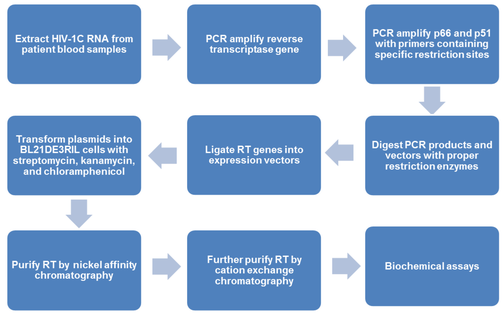
Primary (RPV) Failure*	HIV-nonB N (%)	HIV-1B N (%)	P-value
No	54 (75)	41 (91.11)	0.03*
Yes	18 (25)	4 (8.89)	

*Patients harbored RPV-associated mutations



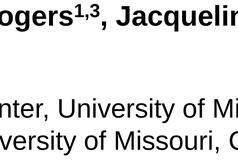
Methods

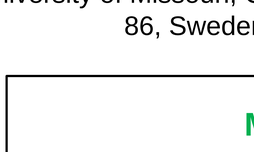
Cloning, expression and purification of RT from patient samples



Kinetics of dNTP binding

Rapid Quench Flow (RQF)



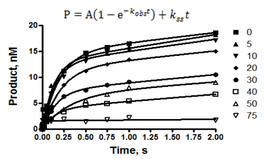


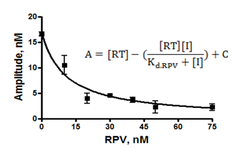
1. Run dNTP incorporation reactions in a rapid quench flow machine under single turnover conditions.
2. Analyze the products on a 20% urea gel. Plot the amount of product at different dNTP concentrations.
3. Determine observed rate constants (k_{obs}) using a burst equation.
4. Plot the observed rates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

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

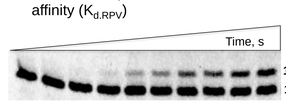
3'- CAG TGA CAA GCT CGT GGT TAC GAT AGA TAC C-5' Template 31
5'- GTC ACT GTT CGA GCA CCA -3' Primer 18

Kinetics of NNRTI (RPV) binding



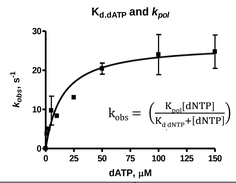


1. Run dNTP incorporation reactions in a rapid quench flow machine under single turnover conditions in presence of increasing concentration of RPV.
2. Analyze the products on a 20% urea gel. Plot the amount of product at different RPV concentrations.
3. Determine amplitude using a burst equation.
4.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



Results

Kinetic parameters of HIV-1C RT on hetero-polymeric (31/18-mer) DNA/DNA template-primer



Enzyme	$K_{d.dATP}$ (μM)	kpol (s-1)	efficiency ($\mu M^{-1}s^{-1}$)
HIV-1B RT	3.4	12.5	3.7
HIV-1C RT	14.54	26.69	1.8
01_AE RT	2.0	10.8	5.4
02_AG RT	2.1	10.38	4.9

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

NVP binding affinity ($K_{d.NVP}$) to HIV-1B and HIV-non B RTs

Enzyme	$K_{d.NVP}$ (nM)
HIV-1B RT	100.7 ± 17
HIV-1C RT	101.1 ± 32
01_AE RT	78.1 ± 7
02_AG RT	21.2 ± 1

Nevirapine binding affinity varies among different subtypes

02_AG appears more susceptible to NVP

RPV binding affinity ($K_{d.RPV}$) to HIV-1B and HIV-non B RTs

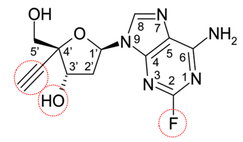
Enzyme	$K_{d.RPV}$ (nM)
HIV-1B RT	21 ± 2
HIV-1C RT	66 ± 7
01_AE RT	31 ± 4
02_AG RT	21 ± 3

Rilpivirine binding affinity varies among different subtypes

HIV-1 Subtype C appears less susceptible to RPV

Alternative approach

Adenosine analog RT inhibitor has been designed by our lab and collaborators



EFdA binding affinity ($K_{d.EFdA}$) to HIV-1B and HIV-non B RTs

Enzyme	$K_{d.EFdA}$(μM)
HIV-1B RT	0.17
HIV-1C RT	0.23
01_AE RT	0.95
02_AG RT	0.20

EFdA binds most of subtypes efficiently

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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