

**POSTER 5****INCREASED PROCESSIVITY OF HIV-1 REVERSE TRANSCRIPTASE CONTAINING A LEU74→ILE CHANGE IN THE BACKGROUND OF LYS65ARG LEADS TO A REPLICATION-COMPETENT VIRUS**

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We have previously demonstrated that nucleoside analog-selected mutations K65R and L74V are incompatible, as 65R→K reversion occurs during replication of doubly mutant virus in PBM cells in the absence of drug(s). Recent reports have highlighted the significance of the emergence of L74I in addition to L74V in HIV-infected individuals. We have recently shown (Cold Spring Harbor, Retroviruses, May 18-23, 2009) that a Leu74→Ile change leads to a replication-competent virus in the backbone of reverse transcriptase (RT) containing a K65R mutation. In order to assess the biochemical mechanism of mutant RTs, we created site-directed mutants containing K65R, L74V, L74I, M184V, K65R+L74V, and K65R+L74I in the backbone of provirus NL4-3 and performed *in vitro* processivity assays. Viruses were produced upon transfection of 293T cells and RT activity was determined using template-primer, poly (rA)-oligo (dT), and  $\alpha$ -<sup>32</sup>P-TTP. To determine a single-cycle processivity, RT lysates containing  $5 \times 10^6$  cpm (<sup>32</sup>P-TTP) RT activity were used in the presence of 50-fold excess of poly (rC)-oligo (dG). The percentage reductions of mutant enzymes with respect to WT RT were calculated. We observed a significant difference ( $p = 0.005$ ) among RT activities of 65R+74V ( $45 \pm 5$ ) and 65R+74I ( $65 \pm 15$ ). It was interesting to note that the single-cycle RT activity of double-mutant 65R+74I was consistently higher than the single-mutant K65R and L74V. L74I mutant RT activity was found equivalent to WT RT. Comparing cDNA densities on the autoradiograph revealed that the intensities of bands and the length ( $60 \pm 10$  nt) of the products for WT and L74I were similar. Comparing K65R+L74V and K65R+L74I, the latter RT synthesized larger ( $47 \pm 5$ nt) cDNA product as compared to the former, where the largest product was only 20 nt in length. Also, there was a significant difference ( $p = 0.001$ ) among the intensity of bands between two RTs as 65R+74I RT was much more robust in comparison to 65R+74V. We conclude that the improved replication kinetics of K65R+L74I virus was due to an increase in the processivity of RT containing 65R+74I mutations. The robust polymerase activity of L74I enzyme is likely to have implications in the selection and prevalence of mutant viruses with L74I mutation presumably with thymidine analog mutations. This study was supported by a VA Merit grant to PLS and Veterans Affairs Medical Center. We are thankful to the AIDS Research and Reference Reagent Program, NIAID, NIH for providing 293T cells and proviral clone pNL4-3.