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## IMPAIRED RESCUE OF CHAIN-TERMINATED DNA SYNTHESIS ASSOCIATED WITH THE L74V MUTATION IN HIV-1 REVERSE TRANSCRIPTASE

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**OBJECTIVE:** The M184V, K65R, and L74V mutations in the RT of HIV-1 share a number of characteristics that include: 1. similar discriminatory mechanisms in regard to incorporation of relevant NRTI inhibitors of reverse transcription; 2. all result in diminished RT processivity and viral replicative capacity; 3. they are associated with diminished error rates during reverse transcription as measured in biochemical assays. In addition, both M184V and K65R have been shown to cause a reduction in the efficiency of the excision reaction associated with RT. Our study was performed to assess whether L74V might also have this effect.

**METHODS:** Recombinant wt and L74V-containing RTs were chromatographically purified as previously described. The rescue of chain-terminated DNA synthesis was studied at a single template position using an assay in which a pre-hybridized duplex of PPT-57 template and PPT-18 primer was incubated with either wt or mutated RT in a buffer containing 10  $\mu$ M dCTP and 10  $\mu$ M zidovudine triphosphate (ZDV-TP). The excision of the ZDV-terminated primer was initiated by adding a mix containing 3.5 mM ATP, 100  $\mu$ M dTTP, 10  $\mu$ M dGTP and 100  $\mu$ M ddATP. DNA synthesis was monitored in time course experiments.

**RESULTS:** We found that the presence of the L74V mutation in RT caused an approximate 50% reduction in the efficiency of excision of ZDV-monophosphate from newly synthesized viral DNA. In addition, ATP-dependent unblocking of the ZDV-terminated primer and the continuation of DNA synthesis were compromised when the L74V mutation was present. Furthermore, wt enzyme was able to unblock 50% of the ZDV-terminated primer after  $\approx$ 35 minutes in this reaction, whereas L74V-containing RT required  $\approx$ 85 min to accomplish this task. Studies were also performed with recombinant

RTs containing K65R or M184V, and, in general, it appears that the effect of M184V on excision exceeds that of L74V and K65R, which behave similarly to each other in this regard.

**CONCLUSIONS:** These findings add to the evidence that K65R, L74V and M184V should be regarded as a group with regard to shared mechanisms of resistance to NRTIs and their consequences on RT enzymatic function.

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