

POSTER 15**RNase H CLEAVAGE-MEDIATED DISSOCIATION OF PRIMER-TEMPLATE BY HIV-1 REVERSE TRANSCRIPTASE (RT) CONTAINING M41L, T215Y AND M184V MUTATIONS**

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Replication of HIV-1 is inhibited by azidothymidine (AZT), which lead to chain termination and inhibition of DNA synthesis. Resistance to AZT is frequently the result of mutations that increase the ability of RT to remove the chain-terminating nucleotides after they have been incorporated. It has been proposed that RNase H cleavage of the RNA template can occur when RT is stalled near the site of chain termination and contributes to the inhibition by causing the dissociation of the primer-template before the chain terminator can be excised¹. Mutations in the connection and RNase H domains of RT have been shown to increase excision. It has long been known that resistance to thymidine analogs is conferred by the mutations M41L and T215Y in RT and that this resistance is suppressed by the additional presence of the M184V mutation. Changes in excision activity on DNA templates have been observed with these mutant RTs, but effects on RNase H cleavage resulting in indirect effects on excision activity is also possible with RNA templates. We used 5'-labeled RNA template to evaluate RNA cleavage activity and biotin-tagged chain-terminated oligodeoxynucleotide to monitor primer-template dissociation. RNase H cleavage at secondary cleavage sites (-7, -8) was substantially reduced with M41L-T215Y RT in comparison with wild type RT, and primer-template dissociation was decreased. In contrast, when M184V was present, RNase H cleavage at the secondary cleavage sites and dissociation of the primer-template occurred at the wild type level. These results suggest that RNase H-mediated RNA-DNA template-primer dissociation is influenced by mutations associated with thymidine analog resistance, and that suppression of resistance to nucleoside RT inhibitors by M184V may be partly explained by effects on RNase H cleavage that decrease the time available for excision to occur.

¹Nikolenko GN, Palmer S, Maldarelli F, *et al.* 2005. Mechanism for nucleoside analog-mediated abrogation of HIV-1 replication: Balance between RNase H activity and nucleotide excision. *Proc. Natl. Acad. Sci. USA* 102: 2093-2098.