

A NOVEL MOLECULAR MECHANISM OF DUAL RESISTANCE TO NRTIS AND NNRTIS

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It was recently shown by us and others that specific mutations in the connection subdomain (cn) and RNase H domain (rh) of HIV-1 reverse transcriptase (RT) exhibit dual resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTIs and NNRTI). In this study we propose a novel mechanism by which mutations in the cn and rh of HIV-1 RT confer resistance to NNRTIs. We hypothesize that during NNRTI exposure, mutations in cn and rh that reduce RNase H cleavage will allow more time for the NNRTI to dissociate from the NNRTI-RT-template/primer complex (NNRTI-RT-T/P), allowing the resumption of DNA synthesis, thereby resulting in enhanced NNRTI resistance. The effect of the reduction in RNase H cleavage on NNRTI resistance is dependent upon the affinity of each NNRTI to the RT and further influenced by the presence of NNRTI-binding pocket (BP) mutants. We observed that mutants D549N, Q475A and Y501A, which reduce RNase H cleavage, enhance resistance to nevirapine (NVP) and delavirdine (DLV), but not to efavirenz (EFV) and etravirine (ETR), consistent with their increase in affinity for RT. Combining D549N with NNRTI BP mutants further increases NNRTI resistance from 3- to 30-fold to all four NNRTIs, supporting the role of affinity of NNRTIs to the RT in our NNRTI resistance model. The *in vivo* RT template switching frequency was also dependent on the affinity of each NNRTI to RT and could be modulated by the presence of NNRTI BP mutations. We also demonstrated that cns from treatment-experienced patients, previously reported to enhance NRTI resistance, also reduce RNase H cleavage and enhance NNRTI resistance in the context of the patient RT pol domain or a wild-type pol domain. Together, these results support key predictions of our NNRTI resistance model and provide support for a unifying mechanism by which cn and rh mutations can exhibit dual NRTI and NNRTI resistance.