

## POSTER 6

### BIOCHEMICAL CHARACTERIZATION OF THE HIV REVERSE TRANSCRIPTASE CONNECTION DOMAIN MULTI-CLASS DRUG RESISTANCE MUTATION N348I

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We previously identified clinical isolates with phenotypic resistance to the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) in the absence of known NNRTI mutations. We showed that this resistance is caused by N348I, a mutation in the connection domain of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) which confers dual-class resistance to NNRTIs (nevirapine) and NRTIs (zidovudine [AZT], and didanosine [ddI]). N348I has been shown to be a common mutation with a prevalence of 12% among patients treated with antiretroviral therapy, and <2% among untreated HIV patients.

In order to understand the effect of the N348I mutation on the biochemical functions of HIV RT, we carried out detailed characterization of enzymes containing the N348I mutation. Using subunit-specific mutagenesis, we constructed enzymes with the N348I mutation in p66 (p66<sub>N348I</sub>/p51<sub>WT</sub>), in p51 (p66<sub>WT</sub>/p51<sub>N348I</sub>), or in both subunits (p66<sub>N348I</sub>/p51<sub>N348I</sub>) of RT. RNase H activity assays with these enzymes indicate that the previously reported changes in RNase H activity appear to be the result of the mutation in the p51 subunit. However, changes in both subunits appear to affect susceptibility to nevirapine. We also find that the N348I enzyme is significantly more processive than WT RT and this processivity is affected by mutation in either subunit. Pre-steady-state kinetics analysis and gel-shift assays demonstrated that N348I does not exhibit a significant decrease in the ability to bind nucleic acid ( $K_{d^{DNA}}$  was unchanged with respect to the WT enzyme). However, transient kinetics experiments revealed that N348I RT has impaired efficiency of incorporation as compared to the WT enzyme, mostly because of a defect in binding of dNTP ( $K_{d^{dNTP}}$ ). The efficiency ratio ( $k_{pol}/K_{d^{dNTP}}$ ) for the WT enzyme was ~7 fold higher than that for N348I RT. We also found that N348I has an increased ability to extend mismatched template/primers through template "T" positions.

These results provide mechanistic information and insights into possible roles of the N348I mutation in resistance to anti-HIV drugs.