

POSTER 13**CHARACTERIZATION OF INHIBITOR INTERACTION WITH THE RNase H DOMAIN OF HIV-1 REVERSE TRANSCRIPTASE**

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HIV-1 Reverse Transcriptase (RT) is a multifunctional enzyme that carries out RNA- and DNA-dependent DNA synthesis as well as RNA degradation in DNA/RNA duplexes. These functionalities are respectively located in the spatially separated polymerase and RNase H (RNH) domains of RT. RNH has been underexplored as a target for antiviral drug discovery and development. Recent work in our laboratory has identified a number of new RNH inhibitors, and NMR studies have suggested that these inhibitors bind in the RNH domain of RT. Residues E428, S499, I526, E529 and V536 showed substantial spectral shifts upon in the presence of inhibitor in 2D HSQC NMR experiments, and the side chains of D498, Q500, Y501, A502 and I505 were identified in 3D HCONH experiments. Our aim was to use site specific mutagenesis to validate these RNH residues implicated in interaction with the inhibitors. Most single mutations conferred only low level resistance to the inhibitors. However, the V536L substitution in RT provided from 2- to 10-fold resistance depending on the inhibitor. Interestingly, the V536L mutant possessed attenuated RNH activity, functioning at only 52% of the wild type enzyme. Introduction of 2 mutations (A502G + V536L) resulted in substantially increased resistance to all inhibitors. However, the double mutant was severely compromised in RNH activity at only 30% of wild type levels. RT RNH may thus represent an interesting target for antiretroviral development as resistance to RNH inhibitors may correlate with substantially reduced virus fitness.

Acknowledgement: This research was supported by NIH grants GM066671 and AI073975.