

## COMPLEMENTARY MECHANISMS OF NUCLEOSIDE ANALOG RESISTANCE FROM STRUCTURAL STUDIES OF HIV-1 REVERSE TRANSCRIPTASE

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Nucleoside inhibitors (NRTIs) of HIV-1 reverse transcriptase (RT) are among the most important anti-AIDS drugs and are prescribed in nearly all treatment regimens. We have used X-ray crystallography to study the structural basis for the mechanisms of resistance to AZT (zidovudine) and PMPA (tenofovir). A detailed understanding of the distinct mechanisms of resistance to the two drugs and their antagonistic relationship is critical for understanding the complexity of NRTI resistance and for designing new and broadly effective NRTIs.

Resistance of HIV-1 RT to AZT has been shown to involve an ATP-mediated excision reaction in which AZTMP is removed from the terminated primer, forming a dinucleoside tetraphosphate product, AZTppppA. We have solved and analyzed a series of AZT-resistant HIV-1 RT structures, including ternary complexes with a template-primer and the excision product AZTppppA. The structures define the roles played by the major AZT-resistance mutations in the mechanism of AZT resistance. Primary mutations K70R and Y215Y help in binding the ATP molecule to the mutant RT and therefore can be classified as excision-enhancing mutations (EEMs).

The mutation K65R causes resistance to tenofovir and has complex relationships with other NRTI-resistance mutations. We have analyzed structures of K65R HIV-1 RT in ternary complexes with tenofovir diphosphate and dATP. We propose that differential stacking of the guanidinium groups of K65R and R72 creates a “checkpoint” that reduces dNTP incorporation and allows the mutant RT to discriminate between tenofovir diphosphate and the normal substrate, dATP. The structural results also suggest potential mechanisms of complex relationships of K65R mutation with other NRTI-resistance mutations including the antagonistic relationship with EEMs.