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## Basic Principles & Clinical Implications

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### SUBTYPE-SPECIFIC AMINO ACID POLYMORPHISMS IN THE HIV-1 REVERSE TRANSCRIPTASE CONNECTION SUBDOMAIN OF CRF01\_AE ARE ASSOCIATED WITH HIGHER 3'-AZIDO-3'-DEOXYTHYMIDINE RESISTANCE

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**BACKGROUND:** We previously showed that mutations in the connection subdomain (cn) of HIV-1 subtype B reverse transcriptase (RT) increase 3'-azido-3'-deoxythymidine (AZT) resistance in the context of thymidine analog mutations (TAMs) by altering the balance between nucleotide excision and template RNA degradation, thereby providing more time for RT to carry out NRTI excision. To determine whether the balance between polymerization and RNase H activity affects drug resistance in other HIV-1 subtypes, AZT resistance in CRF01\_AE was analysed.

**METHODS:** Extensive RT subdomain swapping and mutagenesis was used to determine AZT susceptibility of wild-type and treatment-experienced CRF01\_AE. The ability of wild-type and mutant RTs to excise AZT-monophosphate (AZTMP) from a blocked primer was determined in an excision-extension assay using a 19/42mer RNA/DNA and DNA/DNA hybrid. Primary RNase H cleavages were quantified in an RNase H assay using an 18/18-mer RNA/DNA hybrid substrate.

**RESULTS:** Interestingly, CRF01\_AE-containing TAMs exhibited 64-fold higher AZT resistance (relative to wild-type B) in comparison to subtype B containing the same TAMs (13-fold), which in turn correlated with higher levels of AZTMP excision on both RNA and DNA templates. The high AZT resistance exhibited by CRF01\_AE was primarily associated with the T400 residue, which is present in wild-type CRF01\_AE CN. A400T substitution in subtype B increased AZTMP excision on both DNA

and RNA templates and reduced RNase H cleavage. Substituting the T400 in CRF01\_AE with alanine restored AZT sensitivity and reduced AZTMP excision on both DNA and RNA templates, suggesting that the T400 increases AZT resistance in CRF01\_AE at least in part by directly increasing the efficiency of AZTMP excision.

**CONCLUSIONS:** These results demonstrate that the amino acid composition of CRF01\_AE in the background of TAMs exhibits higher AZT resistance than subtype B containing TAMs, and this resistance is associated with the T400 amino acid in the CRF01\_AE CN. Our results show that mixing the RT *pol*, CN and RH domains from different subtypes, specifically the CRF01\_AE *pol* and the subtype B CN and RH, can underestimate AZT resistance levels. These observations indicate the need to develop subtype-specific genotypic/phenotypic assays to provide more accurate estimates of drug resistance.

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29

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