

ABSTRACT FORM
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**Abstract
Title:**

Q509L in HIV-1 RT INCREASES AZT RESISTANCE BY PROMOTING POLYMERASE-COMPETENT VS. RNASE H-COMPETENT BINDING ON RNA/DNA T/P WITH SHORT DUPLEX LENGTHS

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Text:

OBJECTIVES: A371V and Q509L were selected by AZT in combination with TAMs (D67N/K70R/T215F) and increase AZT resistance 50-fold compared to TAMs alone. Initial biochemical studies show that TAMs/Q509L and TAMs/A371V/Q509L increase AZT-monophosphate (AZT-MP) excision from RNA/DNA template/primers (T/P) by decreasing secondary RNase H cleavage events that reduce the RNA/DNA T/P duplex length to less than 12 nucleotides (nt). However, the precise mechanisms responsible for the decreased RNA cleavage and increased AZT-MP excision have not been defined.

METHODS: RT containing D67N, K70R, T215F, A371V and/or Q509L was over-expressed and purified. The rates for RNase H cleavage of AZT-MP terminated RNA/DNA T/P were determined using transient or steady-state kinetic approaches, both in the absence and presence of a nucleic acid trap. The ability of the wild-type or mutant RTs to bind RNA/DNA T/P with duplex lengths less than 18nt in a DNA polymerase- or RNase H-competent mode was assessed by measuring DNA polymerization or RNase H cleavage at defined times after the addition of trap to a pre-formed RT-T/P complex.

RESULTS: Initial RNase H cleavages for all enzymes were similar, suggesting that A371V and Q509L do not directly affect the catalytic activity of the RNase H active site. However, the rates for the subsequent RNase H cleavages, which occur after T/P dissociation and rebinding, were reduced 2.2- and 2.3-fold for the TAMs/Q509L and TAMs/A371V/Q509L RTs, respectively. RT-T/P binding assays showed that the Q509L mutation promoted RT binding to short T/P duplexes in a polymerase-competent mode favoring AZT-MP excision, rather than an RNase H-competent mode allowing additional cleavages and dissociation of the T/P complex.

CONCLUSIONS: The Q509L mutation does not have a direct effect on RT RNase H catalytic activity, but increases AZT resistance by promoting RT binding to RNA/DNA T/P duplexes <18 nucleotides in a polymerase-competent mode that favors excision rather than an RNase H-competent mode that favors further cleavage and T/P dissociation. These findings provide new insights into the mechanism by which mutations in the C-terminal domain of RT confer NRTI resistance.

CATEGORY OF EMPHASIS

(check all that apply)

Bioscience

Education/Prevention