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THE NNRTI-RESISTANT Y181C HIV-1 REVERSE TRANSCRIPTASE IS HYPER-SUSCEPTIBLE TO 4'-ETHYNYL-2-FLUORO DEOXYADENOSINE (EFdA)

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4'-Ethynyl-2-fluoro deoxyadenosine (EFdA) is a translocation-defective reverse transcriptase inhibitor (TDRTI). Our laboratory has shown that EFdA blocks viral replication by preventing translocation of RT after its incorporation into the nascent DNA strand. Pre-clinical development of EFdA requires knowledge of how well this inhibitor suppresses viral replication of other drug-resistant mutants. Hence, the present study focuses on the effect of EFdA on viral replication of NNRTI-resistant HIVs.

Using cell-based assays, we initially tested NNRTI-resistant viruses that carried the following RT mutations: Y181C, V106A, V106/Y181C, and K103N/Y181C. Our results demonstrated that all these strains were hyper-susceptible to EFdA to various extents. To determine the mechanism of this enhanced susceptibility to EFdA, we used biochemical approaches to study the susceptibility of the purified RTs of the corresponding viruses in more detail.

Using gel-based DNA extension assays, we found that Y181C RT had more increased susceptibility to EFdA than V106A. In a "rescue" type of assay where primer extension was monitored in the presence of ATP that can unblock EFdA-terminated primers, Y181C was more susceptible to EFdA than V106A and WT RT. ATP- and pyrophosphate-dependent excision assays of EFdA-MP-terminated primers showed a decrease in the efficiency of removal of incorporated EFdA-MP by RTs containing the mutations Y181C and Y181C/V106A. Using steady-state kinetic experiments, we also determined that V106A had an increased efficiency of EFdA incorporation compared to WT RT. Together, these results demonstrate that NNRTI-resistant mutations enhance susceptibility to EFdA mainly by decreasing the excision of EFdA-terminated primers but also by an increase in the relative incorporation efficiency of the inhibitor. The most hyper-susceptible NNRTI-resistant RT is Y181C.

These data suggest that EFdA would be an excellent candidate for therapies in combination with NNRTIs.