

HYPERSENSITIVITY OF K65R HIV-1 REVERSE TRANSCRIPTASE TO 4'-ETHYNYL-2-FLUORO DEOXYADENOSINE (EFdA)

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4'-Ethyne-2-fluoro deoxyadenosine (EFdA) is a highly potent Nucleoside Reverse Transcriptase Inhibitor (NRTIs) that suppresses replication of wild-type and drug-resistant HIV viruses. Unlike all approved NRTIs that lack a 3'OH and act as chain terminators, EFdA retains a 3'OH group and has substitutions at the 4' position of the deoxyribose sugar and the 2 position of the base. Using EFdA triphosphate, we recently showed that EFdA exerts its antiviral activity by inhibiting efficiently HIV RT and that EFdA acts as a chain terminator despite the presence of an accessible 3'OH. We elucidated its molecular mechanism of action and showed that this highly potent chain termination activity arises mainly from a difficulty of the primer 3'-terminus to translocate following incorporation of the compound. Therefore, we have termed EFdA to be a Translocation-Deficient Reverse Transcriptase Inhibitor (TDRTI).

We now report that the tenofovir-resistant HIV virus that carries the K65R substitution in HIV RT exhibits a pronounced hypersensitivity to EFdA, as determined in single replication cycle experiments. EFdA showed a 5-fold increase in antiviral potency against K65R HIV-1 (IC_{50} of 0.5 +/- 0.02 nM) compared to that against wild-type HIV-1 (2.6 +/- 0.3 nM).

To determine the mechanism of hypersensitivity to TDRTIs we investigated the effect of the K65R substitution on EFdA incorporation using steady-state and pre-steady-state kinetic experiments. Preliminary results from the kinetic experiments show that K65R RT incorporates EFdA approximately 5 times more efficiently than dATP. Interestingly, this preference is template-sequence-dependent and correlates with the impairment in translocation at the specific sequence of the primer/template substrate. Iron footprinting assays have revealed that K65R RT translocation is more difficult than wild-type RT in sites where the EFdA is incorporated more efficiently than dATP. However, translocation was similar in sites where EFdA and dATP incorporation were comparable. To investigate excision of EFdA-MP terminated primers, we found that excision is less efficient for wild-type than for K65R RT in sites where the primer/template does or does not favor RT translocation. This study confirms cell-based results and provides support to the mechanism of action of EFdA, a member of a new class of antiretroviral drugs.