

## 4'-ETHYNYL-2-FLUORO-2'-DEOXYADENOSINE (EFdA), A TRANSLOCATION DEFECTIVE REVERSE TRANSCRIPTASE INHIBITOR WITH HIGHLY POTENT ACTIVITY AGAINST DIVERSE MULTI-DRUG-RESISTANT HIV-1 VARIANTS

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EFdA is a nucleoside analog that retains a 3'-hydroxyl moiety and is potent against diverse wild-type and drug-resistant HIV-1 strains *in vitro* with IC<sub>50</sub> values of 70 pM~21 nM. This exceptional antiviral activity stems in part from a mechanism of action that is different from approved NRTIs. HIV-1-RT uses EFdA-TP as a substrate more efficiently than dATP. Despite the presence of a 3'-hydroxyl moiety, the incorporated EFdA-MP acts mainly as a *de facto* terminator of further RT-catalyzed DNA synthesis, due to difficulty of RT translocation on the nucleic acid primer possessing 3'-terminal EFdA-MP. EFdA-TP is thus a translocation defective RT inhibitor. When human CEM cells were incubated with <sup>3</sup>H-EFdA and nucleosides/nucleotides within the cells were quantified, amounts of intracellular EFdA-MP, -DP, and -TP increased proportionately with increased EFdA concentrations. Intracellular T<sub>1/2</sub> of EFdATP was 17 hr, which was significantly greater than that of AZT-TP (~3 hr), suggesting that once daily dosing schedule of EFdA could be possible. Moreover, EFdA exerted minimal inhibition to the activity of human DNA polymerases: alpha, beta, and gamma.

When EFdA was administered [20 mg/kg, *ip*, twice a day (BID)] to human peripheral blood mononuclear cell-transplanted, HIV-1-exposed NOD/SCID/Janus kinase 3 knockout mice, EFdA significantly suppressed HIV-1 replication and virtually completely protected CD4+ cells from the cytopathic effect of HIV-1 without any identifiable toxic effect in the mice.

EFdA was also potent against SIV (IC<sub>50</sub>=50 pM). Two macaques, which had reached end-stage SAIDS, were treated with EFdA [2 mg/kg, *sc*, once daily (QD)]. Within 1 week of EFdA treatment, both animals showed a 3-4 log decrease in plasma virus load; this declined to undetectable within 1-2 months. Chronic diarrhea resolved in both animals within one month, and both showed steady gains in body weight for the duration of EFdA treatment. Liver enzyme panels remained normal over the 6-month course of treatment.

The anti-HIV-1 activity and safety profiles of EFdA with the pilot primate study results warrant further development of the compound as a potential therapeutic for individuals harboring wild-type and/or multi-drug-resistant HIV-1.