

Inhibition Mechanism of EFdA, a Highly Potent Inhibitor of HIV Reverse Transcriptase

Eleftherios Michailidis¹, Bruno Marchand¹, Eiichi N. Kodama², Kamleendra Singh¹,
Karen A. Kirby¹, Yee T. Ong¹, Emily M. Ryan¹, Eva Nagy³,
Noriyuki Ashida⁴, Mickey Murphey-Corb³, Hiroaki Mitsuya⁵, and Michael A.
Parniak³, and Stefan G. Sarafianos¹

¹Christopher Bond Life Sciences Center, Department of Molecular Microbiology & Immunology, University of Missouri, Columbia, Missouri, ²Division of Emerging Infectious Diseases, School of Medicine, Tohoku University, Sendai, Japan, ³Department of Molecular Genetics & Biochemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, ⁴Yamasa Corporation, Chiba, Japan, ⁵Department of Internal Medicine, Kumamoto University, Kumamoto, Japan & Experimental Retrovirology Section, HIV/AIDS Malignancy Branch, NIH, Bethesda, Maryland

The nucleoside 4'-ethynyl-2-fluoro-deoxyadenosine (EFdA) is one of the most potent antiretroviral nucleosides yet described, inhibiting replication of wild-type and multidrug-resistant HIV-1 strains *in vitro* (PBMC cells) with an EC₅₀ as low as 50 pM. Our laboratory works in collaboration with academic, government and pharmaceutical industry laboratories, to characterize the mechanism of action of EFdA, and help develop it as a therapeutic for the treatment of HIV-infected patients, and as a topical microbicide to minimize sexual transmission of HIV. We have recently shown that the potency of antiviral activity stems in part from a mechanism of action not shown by any of the clinically used nucleoside antiretrovirals. Unlike other Reverse Transcriptase (RT) inhibitors, EFdA has a 3'-OH group which is necessary for nucleotide incorporation, yet it acts as a chain-terminator of retroviral DNA synthesis. Using biochemical techniques, we have determined that EFdA is incorporated very efficiently into the nascent viral DNA chain and blocks the incorporation of incoming nucleotides by stopping the translocation/movement of RT. Therefore, we have dubbed EFdA as a Translocation-Defective RT Inhibitor (TDRTI).

A pilot collaborative study spearheaded by collaborators Parniak and Corb demonstrated that EFdA treatment of Rhesus Macaques resulted in a 2-3 log decrease in simian immunodeficiency virus (SIV) within seven days; these levels

declined to undetectable levels (5-log reduction) within 2 months and essentially remained so for the duration of therapy.

Hence, EFdA is a highly potent HIV RT inhibitor with *in vitro* and *in vivo* antiviral activities that warrant further development of the compound as a potential therapeutic for individuals harboring wild-type and/or multi-drug resistant HIV-1.