7

A NEW ANTISENSE CONTRUCT FOR THE GENETIC TREATMENT OF ANTIGEN-SPECIFIC HUMAN LYMPHOCYTES: AN APPROACH TO ADOPTIVE IMMUNOTHERAPY OF AIDS

G. Palu[1], L. Del Pup[2], D. Fenoglio[2], G. La Pira[2], D. Saverino[2], M. Gimelli[3], C. De Giuli-Morghen[3], F. Manca[3]

[1]Institute of Microbiology of the University of Padova, Italy, [2]Dep. Immunol. S. Martino Hospital, Adv. Biotechn. Center Genova, Italy, [3]Department of Pharmacology of the University of Milano, Italy.

We have just proposed the retroviral delivery of a hybrid tRNA carrying an antisense molecule targeted at *tat*-mRNA, as a strategy to impair HIV-1 replication that might provide the basis for a new therapy of AIDS (1).

We evaluated, in vitro, the comparative anti-HIV efficiency of constructs expressing single and multiple tRNA units.

We were also able to show that CD4 cells specific for antigens of opportunistic pathogens can be expanded in vitro and rendered resistant to HIV-1 replication. The genetic treatment did not affect the functional properties of specific CD4 lymphocytes, namely proliferative response to antigen, production of lymphokines upon stimulation and expression accessory molecules for interaction with antigen presenting cells and for homing to inflammatory sites. When challenged with HIV-1, the transduced cells required a 20-10000fold higher virus doses to become infected and to produce infectious virus, as compared to untreated controls.

To our knowledge, this is the first report to describe HIV-1 targeted gene therapy of antigen specific human CD4 lymphocytes and demonstrated preservation of essential functions. This strategy may represent a new form of gene therapy of AIDS aimed at restoring the immune repertoire specific for opportunistic pathogens.

(1) Biasolo et al. 1996 J. Virol. 70:2154-2161

8

Design of Novel, Conformationally Restricted HIV Protease Inhibitors

R.D.Tung, F.G.Salituro, G.B.Rao, C.T.Baker, D.D. Deininger, B.Li, P.M.Novak, W. C. Schairer, J. J. Court, M.Porter and S.Pazhanisamy

Vertex Pharmaceuticals, Inc.
Cambridge, MA USA

HIV aspartyl protease inhibitors have proven to be the most potent class of agents directed against this virus to date. Certain combination regimens including these inhibitors have proven capable of reducing plasma viral RNA loads to below the limits of the currently most sensitive assays. However, poor patient compliance, inconvenient dosing protocols and dose limiting toxicities associated with the currently approved agents have been shown drive the formation of resistant viral species. Therefore, there is a continuing need for the development of additional classes of compounds which address protease-resistant viral strains.

Our initial design efforts in the HIV protease area resulted in the development of VX-478, a novel hydroxyethylamine sulfonamide currently undergoing clinical development by Glaxo-Wellcome as 141W94 and by Kissei Pharmaceuticals in the Far East as KVX-478. We have continued to apply the design precepts utilized in this program to the generation of new classes of inhibitors. In this presentation we will discuss the application of structurally-driven molecular design to a new class of potent, low molecular weight, conformationally restricted HIV protease inhibitors.

9

Nb-containing Polyoxometalate HIV-1 Protease Inhibitors.

Craig L. Hill[†], Deborah A. Judd,[†] Jordan Tang,[§] Jim Nettles,[†] and Raymond F. Schinazi[‡] [†]*Department of Chemistry, Emory University, Atlanta, Georgia 30322,* [‡]*Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Decatur, Georgia 30033, and* [§] *Oklahoma Medical Research Foundation, 825 Northeast 13th, Oklahoma City, Oklahoma 73104*

The compounds, α_1-$K_7[P_2W_{17}(NbO_2)O_{61}]$ (α_1**1**) and α_2-$K_7[P_2W_{17}(NbO_2)O_{61}]$ (α_2**1**) have been prepared in 63 and 86% yields, respectively, from reaction of α_1-$K_9Li[P_2W_{17}O_{61}]$ or α_2-$K_{10}[P_2W_{17}O_{61}]$ and $K_7H[Nb_6O_{19}]$ in aqueous H_2O_2. Thermolysis of α_1**1** and α_2**1** (72-96 h in refluxing H_2O) converts them to α_1-$K_7[P_2W_{17}NbO_{62}]$ (α_1**2**) and α_2-$K_7[P_2W_{17}NbO_{62}]$ (α_2**2**) in 66 and 52% yields, respectively. The identity and high purity of all four compounds were confirmed by ^{31}P NMR and ^{183}W NMR. All four are quite active in cell culture against HIV-1 (EC_{50} values: 0.17-0.83 μM) and minimally toxic (IC_{50} values: 50 to >100 μM). The IC_{50} values for inhibition of HIV-1 protease by α_1**1**, α_2**1**, α_1**2** and α_2**2** are 2.0, 1.2, 1.5, and 1.8 μM, respectively. The compounds show moderate inhibitory activity in cells chronically infected with HIV consistent with protease inhibition activity. Molecular mechanics and dynamics calculations using HIV protease and polyoxometalate X-ray crystal structures indicate that these compounds may bind at the active site in the flaps-open conformation or at a cationic region on the HIV-1P surface near the base of the flap.

In summary, we have identified a mechanism by which POM interacts with HIV in addition to fusion and reverse transcriptase. The research was supported by NIH.

10

Synthesis and In Vitro Antiviral Activity of Long-Chain 5'-(O-Alkoxycarbonylphosphinyl)-3'-azido-3'-deoxythymidines Against Drug-Sensitive and AZT- or PFA-Resistant Human Immunodeficiency Virus Type-1 (HIV-1).

A. Rosowsky[1], H. Fu[1], N. Pai[1], J.W.Mellors[2], D.D. Richman[3,4] and K.Y. Hostetler[3,4]. [1]*Dana Farber Cancer Inst. and* [1]*Dept. Biol. Chem. & Mol. Pharmacol., Harvard Univ., Boston, MA 02115;* [2]*Dept. Med., Univ. Pittsburgh and* [2]*VA Med. Center, Pittsburgh, PA 15213; and* [3]*Dept. Of Med. & Pathol., Univ. Cal. San Diego and* [4]*VA Med. Center, San Diego, CA 92161.*

Development of effective drug regimens for AIDS can be aided by knowledge of the resistance patterns evoked by the agents. Recently foscarnet(PFA)-resistant strains of HIV have been identified that exhibit increased susceptibility to AZT. Conversely, AZT resistance mutations can reverse PFA resistance. HIV strains that are coresistant to PFA and AZT have not yet been identified by in vitro selection or in treated patients. We therefore synthesized double conjugates of AZT-PFA which consist of a carboxyester with a long chain alcohol of 18 to 22 carbons and tested their antiviral activity against wild type and PFA- or AZT-resistant HIV-1. Plaque reduction assays were done in HT4-6C cells. Against the highly AZT-resistant strain A018-post, 3'-azido-3'-deoxy-5'-O(1-docosanyl-oxycarbonylphosphinyl)thymidine inhibited viral replication by 50% (EC_{50}) at 0.77 μM; by comparison the EC_{50} values of PFA and AZT were 65 μM and 3.2 μM, respectively. Against the PFA-resistant HIV-1strain E89K, the double prodrug had an EC_{50} of 0.17 μM versus >1000 μM for PFA and 0.01 μM for AZT. In HT4-6C cells, 3'-azido-3'-deoxy-5'-O(1-docosanyloxycarbonylphosphinyl)thymidine was cytotoxic (TC_{50}) at 320 μM. Antiviral activity and cytotoxicity will also be reported for similar prodrugs having either 18 or 20 carbon alkanols esterified at the carboxyl of AZT-PFA. We conclude that AZT-PFA lipid prodrugs may be useful for treatment of strains of HIV which have become resistant to AZT and PFA.