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DEVELOPMENT OF RESISTANCE TO ANTI-HIV DRUGS IN PRIMARY MACROPHAGES

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Objective of the study: To assess the development of phenotypic resistance to antiviral drugs in macrophages infected by HIV, and to study genotypic variations of HIV reverse transcriptase occurring during treatment with reverse transcriptase inhibitors

Methods: Human primary macrophages were infected by HIV in the presence or absence of reverse transcriptase inhibitors (AZT, 3TC, PMEA; TSAO). Cellular passage was performed every two weeks by coculturing infected macrophages with fresh macrophages, or by transferring supernatants of infected macrophages to fresh macrophages from a different donor.

Results: No evident phenotypic resistance (nor virus breakthrough) to any of the antiviral drugs tested was detected in macrophages cultured for up to 7 passages in vitro (i.e. 105 days). In addition, no mutations conferring resistance to any of the antiviral drugs tested was detected by sequence analysis of the region of reverse transcriptase spanning in the area where the majority of resistance-related mutations occur.

Conclusions: The results cannot be considered conclusive, due to the limited number of in vitro passages assessed in this study. However, virus resistance in CD4-lymphocytes appears within 4 passages (i.e. two weeks of culture in this cellular model) at least for 3TC and TSAO. It is then conceivable that the slower kinetics of reverse transcriptase in macrophages, as well as the lower endogenous 2'-deoxynucleoside-triphosphate pools (that in turn decrease the rate of reverse transcription) may reduce the mutation rate in macrophages, and thus the outgrowth of resistant strains in human organs, like brain, where macrophages represent the majority of cells infected by HIV.

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Kinetic Characterization of HIV-1 Reverse Transcriptases Carrying Mutations Shown to Confer Decreased Susceptibility to PMEA (Adefovir) in Vitro J.M. Cherrington, A.S. Mulato, M.D. Fuller, T. Cihlar and M.S. Chen. Gilead Sciences, Foster City, CA.

Adefovir dipivoxil (bis-POM PMEA), an oral prodrug of adefovir, has shown potent anti-HIV and anti-HBV activity in clinical trials and is currently undergoing further clinical evaluation for the potential treatment of these diseases. Previous in vitro selection experiments showed that either a K65R or K70E mutation in HIV-1 RT could be selected for in the presence of PMEA. Recombinant viruses carrying either of these mutations showed reduced susceptibility to PMEA and 3TC, but full susceptibility to AZT in vitro. Viruses carrying either the M184V or M184I mutation known to confer marked reduced susceptibility to 3TC are fully susceptible to PMEA and AZT in vitro. However, preliminary genotypic analyses of clinical samples obtained after 12 weeks of adefovir dipivoxil monotherapy or 6 months of combination therapy with various antiretroviral agents have not revealed either the K65R or K70E mutation. To further investigate the molecular mechanisms involved in resistance to PMEA, we have cloned, expressed and purified HIV-1 RT enzymes from E. coli carrying the K65R, K70E, or M184V mutations. Representative data are shown in the table below.

(fold increase of Ki above wild type)

Enzyme	PMEApp	3TCTP	AZTTP	ddCTP
K65R	7	13	3	2
K70E	3	3	1	2
M184V	1	>20	2	3

In general, the enzyme kinetic data correlated with the in vitro antiviral susceptibility data. Further experiments are in progress to address additional kinetic parameters (processivity, fidelity, etc.) of these enzymes. Additionally, in vitro competition growth experiments are ongoing to assess the overall fitness of viruses carrying these RT mutations.

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Anti-CD3/2-chloro-5-nitrobenzoic Acid Costimulation Differentially Enhances T-cell Growth Without Inducing HIV-1 Replication. D. Kinchington*, Tony Ng, N. Mathews, M. Tisdale+ D. Devine and W. O. Ayuko+. St. Bartholomew's Medical School, London, England. +Department of Pharmaceutical and Biological Sciences, Aston University, Birmingham, England.

A number of benzoic acid derivatives were investigated for their effect on anti-CD3-induced T-cell growth. In addition, the effect of this costimulation on cells both acutely and chronically infected with HIV was determined. 2, 5 substitution of the benzene ring was important for activity. The 2-chloro, 5-nitrobenzoic acid derivative was chosen for further study and crystallised as the readily soluble sodium salt (CNBA-Na). CNBA-Na augmented anti-CD3-stimulated PBMC proliferation in a dose dependent manner. Supernatants taken from cultures of acutely and chronically infected cells costimulated with anti-CD3/CNBA-Na showed no increase in HIV antigen release compared with cultures not treated with CNBA-Na. This data indicates that these compounds may have differential effects on uninfected and infected cells. The mechanism of action is not entirely clear but may involve the modification of biochemical signals generated from cell surface molecules such as CD28.

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Decreased Thymidine Kinase-1 Activity *in Vitro* and in *ex Vivo* Stimulated PBMC'S of HIV-1 Patients Treated with Different Antiretroviral Agents

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Prolonged antiretroviral monotherapy with AZT showed a decreased thymidine kinase-1 (TK-1) activity in *ex vivo* stimulated PBMC's of HIV-1 infected patients. We investigated whether this phenomenon also occurs in HIV-1 patients treated with nucleoside analogues such as AZT in combination with ddC, 3TC, ddI or D4T and protease inhibitor. Methods were evaluated by the use of a T-lymphoid MOLT-4/8 AZT-resistant cell line. 18 HIV-1 infected patients in different stages of infection in comparison to 10 HIV-1 negative donors were tested in this study. Five of the HIV-1 patients had been treated with combination therapy for less than 6 months, the others were treated for more than half a year up to seven years. EDTA-blood of patients and donors were stimulated three days with PHA. After cell extraction TK-1 activity was determined with a radioactive method. TK-1 activity of HIV-1 patients, treated for more than 6 months with AZT in combination with other nucleoside analogues, is significantly decreased in comparison to HIV-1 negative donors. In contrast HIV-1 patients with antiretroviral combination therapy less than 6 months show a higher TK-1 activity, approximately in the same range as negative donors. We could show for the first time that a prolonged antiretroviral combination therapy with nucleoside analogues induces a significantly decreased activity of cellular kinases, which catalyse the activation of nucleoside analogues in PBMC's. The reduced phosphorylation capacity of AZT in HIV-1 patients could be, in addition to viral resistance, one of the reasons for the decreased efficiency of long-term antiretroviral chemotherapy.