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In Vivo Selection by Monotherapy of HIV-1 Variants Resistant to Multiple Protease Inhibitors. J.H. CONDRA, W.A. SCHLEIF, O.M. BLAHY, L.J. GABRYELSKI, D.J. GRAHAM, J.C. QUINTERO, A. RHODES, H.L. ROBBINS, E. ROTH, M. SHIVAPRAKASH, D.L. TITUS, T. YANG, H. TEPPLER†, K.E. SQUIRES††, P.J. DEUTSCH* and E.A. EMINI. Departments of Antiviral Research and *Clinical Pharmacology, Merck Research Laboratories, West Point, PA 19486 USA; †Division of Infectious Diseases, Thomas Jefferson University, Philadelphia, PA 19107 USA, and ††Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

The aspartyl protease of HIV-1 catalyzes the cleavage of the viral polyprotein and is essential for viral infectivity. Numerous inhibitors of this enzyme have been described, and several are being evaluated as potential antiviral agents. Among these, L-735,524 has been undergoing clinical trials during the past year. Treatment of HIV-1-infected patients with this inhibitor has led to the emergence of resistant viral variants. We have identified multiple amino acid substitutions in the protease whose appearance in viral isolates from treated patients correlates with resistance *in vitro*. Because the patterns of appearance of these mutations are complex and variable, it has not been possible to discern the minimal requirements for resistance by sequence analyses of clinical isolates. To address this issue, we have introduced many of these substitutions, alone or in combination, into an HIV-1 proviral plasmid and recovered mutant viruses by transfection of cultured cells. These viruses were tested for inhibitor susceptibility. Phenotypic resistance to L-735,524 correlated with substitutions of valine 82 to either alanine, phenylalanine or threonine, and/or of leucine 90 to methionine. These mutations alone, however, were insufficient for resistance and required complex interactions with other amino acid substitutions that occur during therapy. Following extended therapy with L-735,524, several viral isolates exhibited a striking cross-resistance to all members of a panel of six structurally diverse protease inhibitors. These are all compounds or closely related analogs that are either in clinical trials or are being considered for such trials. DNA sequence analysis of these variants revealed that this broad cross-resistance arose through multiple genetic pathways that were unique to each isolate. These data indicate that simultaneous resistance to multiple protease inhibitors occurs readily *in vivo*, even under the pressure of a single selective agent. Further, they suggest that combination therapy with multiple protease inhibitors may readily yield multiply resistant variants, and that initial therapy with any one inhibitor may limit the benefit of future treatment with others.

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Antiviral Agents Reduce Viral Load and Delay the Detection of Infected Cells *In Vivo* in the HIV-Infected Hu-PBMC SCID Mouse Model. Michael A. Ussery[1], Dennis D. Broud[1], Owen L. Wood[1], Steven C. Kunder[1], Paul L. Black[1], Peter J. Dailey[2], Judith C. Wilber[2], Limei Yang[3], Michael Piatek, Jr.[3], and Jeffery D. Lifson[3]. [1]FDA, Rockville, MD 20857; [2]Chiron Corp., Emeryville, CA 94608; and [3]Genelabs Technologies, Inc, Redwood City, CA 94063.

In an effort to validate a murine model of HIV infection, we have studied the HuPBMC-SCID mouse model, which involves the I.P injection of 5-10X10^7 adult human PBMC into 5-7 wk old female SCID mice. Two weeks after reconstitution, mice were infected I.P with 10^3 $TCID_{50}$ HIV-1 9320 (AZT-sensitive isolate A018, D. Richman). The extent of infection in blood cells, splenocytes, lymph nodes (LN) and peritoneal cells (PC) was assayed by quantitative coculture with human PBMC blasts 1 and 3 wk later. At 1 wk after infection, HIV was recovered from all mice by coculture of cells from all the 4 sites examined. Additionally, HIV could be detected in the plasma of infected mice by coculture and by QC-PCR. In contrast, HIV infection could be detected in some, but not all animals treated continuously with AZT at 1 mg/ml in the drinking water beginning 1 day before infection. Furthermore, HIV was recovered from fewer sites in the AZT-treated Hu-PBMC-SCID mice, compared with untreated animals, with PC being most frequently positive and LN least. Additionally, viral load was quantitated by the Chiron branched DNA assay, and the results were consistent with those of cocultures. HIV RNA was detected in splenocytes from untreated mice, but not from any AZT-treated mice (or uninfected mice). When HuPBMC-SCID mice were examined 2½-3 wk after infection, the results were quite different. HIV was recovered from all AZT-treated mice, but only about half of untreated mice. The almost complete disappearance of human lymphocytes (especially $CD4^+$ T-cells) from some untreated, infected mice parallels the inability to recover HIV from them. Further experiments are underway to confirm and expand these results, as are studies with an HIV protease inhibitor.