61

RETROVIRAL VECTORS EXPRESSING ANTISENSE AND RIBOZYME RNA DIRECTED AGAINST HIV-1 TAT CAN INHIBIT HIV-1 REPLICATION. K.M. Steve Lo, Maria Angela Biasolo, Ghassan Dehni, Giorgio Palu', and William A. Haseltine, Dana Farber Cancer Institute, Boston, MA., U.S.A.

A hammerhead ribozyme directed against tat is constructed and is shown in vitro to cleave specifically RNA that contains the first exon of tat. The tat ribozyme and a tat antisense sequence are separately cloned into the 3' untranslated region of the neo gene in a Moloney retroviral vector. The vectors are amphotropically packaged and are transduced into the Jurkat human T cell line. Neomycin-resistant clones are selected and are shown by Northern blot hybridization to produce RNA containing the ribozyme and antisense sequences. These clones and controls are challenged with HIV-1 (IIIB). On day 11 of infection, total viral protein production assayed by immunoprecipitation using AIDS patient serum was inhibited 100% in the antisense-producing clones and up to 98% in one of the ribozyme-producing clones. There is also an inhibition of syncytia formation and p24 Gag protein production. Significant cell killing is seen only for the ribozyme-producing clones and control cells, but not the antisense-producing clones. These results demonstrate that a retroviral vector producing tat antisense RNA can inhibit HIV-1 replication better than one producing tat ribozyme RNA. These vectors can potentially be used as a basis for gene therapy against HIV-1.

62

Preclinical Development of Biological Response Modifiers Intended for Use in the Treatment of HIV Infection and Associated Disorders. S. L. Lard, M. A. Ussery, & J. C. Ramsey , Division of Antiviral Drug Products (HFD-530), Center For Drug Evaluation and Research, U.S. Food & Drug Administration, 5600 Fishers Lane Rockville, MD, U.S.A. 20857

The HIV epidemic has brought renewed interest in the development of immune-based therapies to assist in the clinical management of viral diseases. For HIV, immune intervention may take a number of different forms including post-exposure immunization, passive transfer of immunity, use of site-directed immunotoxins and administration of immunomodulatory substances (e.g., cytokines and growth factors or immuno-active drugs). Of the various approaches to immune intervention in HIV infection, immunomodulatory drugs may be the most challenging form of therapy to evaluate successfully in a nonclinical setting. To support clinical studies, sufficient preclinical information about drug activity is needed to establish a rationale for human use, to facilitate the generation of a realistic risk/benefit profile and ultimately, to facilitate the design of appropriate human studies. To this end, a potential immunomodulator should be thoroughly evaluated for activity in a minimum of 2 independent systems. In general, a comprehensive drug development program will utilize at least one animal model of retrovirus infection and appropriate *in vitro* systems to maximize the predictive value of the preclinical data. These studies should include a careful evaluation of clinically relevant parameters, including the effect of dose, schedule and route of administration on drug activity. Direct and indirect effects of the immunomodulator on virus replication should also be considered. Finally, when an immunomodulator is to be administered in combination with conventional antiretroviral therapy, combination drug studies should be performed to characterize potential drug interactions. In most cases, Phase I human studies can begin when sufficient preclinical data are available to demonstrate a positive risk/benefit profile for the drug in the selected patient population. Additional preclinical information pertaining to relevant therapeutic variables and the mechanisms of drug action are needed prior to the initiation of Phase II (efficacy) trials. A "Points to Consider" document has been prepared by representatives of the Division of Antiviral Drug Products at the U.S. FDA to assist drug sponsors in the design of appropriate preclinical activity studies for immunomodulators intended for use in the treatment of HIV infection and associated disorders.