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Development of Anti-Infective Topical Microbicides 3. Combination Microbicidal Approaches Targeting Multiple Virus Targets or Utilizing Multi-Functional Anti-Infective Agents

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Topical microbicides represent an important potential strategy for preventing the transmission of HIV through sexual intercourse, the predominant mode of HIV transmission worldwide. The number of women with HIV infection and AIDS has been increasing steadily worldwide, accounting for 46% of all adults living with HIV worldwide, and for 57% in sub-Saharan Africa. Thus the dynamics of the epidemic demand the development of safe, effective and acceptable female-controlled chemical and physical barrier methods, including topical microbicides, to reduce HIV transmission. We have been actively developing several novel classes of microbicides which could be used alone or in combination, targeting early steps of HIV replication such as entry, fusion and reverse transcription, as well as agents which result in the direct inactivation of HIV. We have also identified agents which target multiple sexually transmitted organisms. Our strategy requires the development of microbicides which attack HIV at multiple targets through the development of a formulated product which will place the right drug(s) at the right concentration at the right place at the right time to prevent the infection of target cells in the vaginal vault. Agents currently under development include the pyrimidinediones (inhibitors of virus entry and reverse transcription), the phosphorothioate oligonucleotide ISIS 5320 (inhibits CD4-gp120 interactions), the NCp7 zinc finger inhibitors (directl viral inactivation) and a novel natural product derived from a plant (inhibits both HIV and herpes virus). We have evaluated the in vitro microbicidal activity of each of these agents and have shown them to be both safe and effective at preventing virus transmission. We have evaluated potential combination microbicide development approaches with this group of agents to define and prioritize combination therapy strategies. Our formulation strategy exploits the mechanism of action of each compound to deliver each agent to the appropriate site of action upon introduction of the viral inoculum to the vaginal vault. The results of our developmental studies will be presented.

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Peptidomimetic Dimerization Inhibitors of HIV-1 Protease: Further Insights into Structural Variations and Mechanism of Action

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Mutations that occur in response to the HIV-1 protease inhibitors (PIs) are responsible for the development of multi-drug cross-resistance to PIs in AIDS treatment. Virtually all PIs act through the same mechanism: they are transition-state analogs that target the active site of the homodimeric enzyme located at the junction of the two monomers. The emergence of resistance to one PI usually results in cross-resistance to other PIs. One alternative to inhibiting the active site of HIV-1 protease is to target the dimer interface of the enzyme at the antiparallel beta-sheet formed by the interdigitation of the C- and N-ends of each monomer. This region is highly conserved and is responsible for about 75% of the dimer stabilization energy. Here we describe new dimerization inhibitors in which new structural molecular variations have been introduced and the peptidic characteristics have been decreased by introducing peptidomimetic groups that have peptide-like hydrogen bonding properties. This led to an increase of the in vitro efficiency (subnanomolar level) against HIV-1 protease activity. Our dimerization inhibitors proved equally active in vitro against both wild-type and mutated proteases. The mechanism of inhibition was established using a combination of kinetic and biophysical methods. Using analytical ultracentrifugation and NMR, we obtained direct experimental evidence of non-covalent dissociative mode of interaction of the HIV-1 protease dimerization inhibitors.

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A High Throughput HIV-1 Full Replication Assay that Includes HIV-1 Vif as an Antiviral Target

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Background: Although clinically effective when used in combination, current HIV-1 therapies are less than ideal due to drug-related side effects, inconvenient dosing requirements,