pounds would directly inactivate both cell-free and cell-associated virions. Based on data obtained on a series of small molecule thioester inhibitors of NCp7, three lead compounds (designated 19, 89 and 247) were chosen for further elucidation of their microbicide potential. We have utilized standard in vitro assays for the development of vaginal microbicides as a means to define the most potent lead thioester microbicide candidate to be used in combination with other topical microbicides in preclinical and clinical development. These data indicate that the thioesters result in inactivation of all clinical strains of virus tested in fresh human PBMCs and monocyte-macrophages, including subtype C and E strains which predominate in sub-Saharan Africa and South East Asia. These data would indicate that the biological activity of the NCp7 inhibitors was not dramatically affected by the presence of semen or vaginal fluids. Additionally, the NCp7's appear to have a memory effect that reduces virus production substantially for 21 days after initial exposure and resistant virus is unable to be selected for due to the barrier in infectivity only after 4 or 5 passages of selection. These characteristics make the NCp7 inhibitors attractive candidates as part of a combination microbicide product with other molecules that possess a different mechanism of action.

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Development of a Long Lasting Combination Microbicide Product Consisting of Highly Potent Compounds Exhibiting Multiple Mechanisms of Action

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In the absence of an effective HIV vaccine, topical microbicides represent an important strategy for preventing the sexual transmission of HIV, the predominant mode of HIV transmission worldwide. Women now account for 46% of all adults living with HIV worldwide. 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. An approved vaginal microbicide does not yet exist despite extensive development efforts. Thus far, three microbicide candidates have failed in human clinical trials, raising the hurdle for other microbicides in development. Although the microbicide products in clinical trials are tested as single agents, current thinking suggests that a combination product will be the required ideal microbicide. Our laboratories have been actively pursuing the development of combination microbicides that include different classes of molecules targeting multiple steps in the HIV replication cycle. Our strategy focuses on the development of a long lasting microbicide which prevents HIV infection and replication at multiple steps through the development of a combination product which will be formulated and delivered in an optimal fashion to place the right drug(s) at the right concentration at the right place at the right time. Agents under development include the pyrimidinediones (inhibition of both virus entry and reverse transcription), the phosphorothioate oligonucleotide ISIS 5320 (inhibition of virus attachment and fusion via binding to the V3 loop of gp120), and the thioester NCp7 zinc finger inhibitors (direct inactivation of cell-free and cell-associated HIV through removal of the coordinated zinc in NCp7). We have evaluated the in vitro activity of combinations of these agents in a variety of microbicide specific virus transmission assays in order to define and prioritize appropriate combination therapy strategies. Evaluations include the ability of the combination products to inhibit virus replication in PBMCs, activity in a microbicidal transmission sterilization assay, and other virus entry inhibition assays.

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Oral Session 4: Herpesviruses and Poxviruses

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Inhibition of Herpesvirus Replication With 5-Iodo-4′-Thio-2′-Deoxyuridine

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A series of 4′-thionucleosides was synthesized and their antiviral activity was evaluated against orthopoxviruses and herpesviruses. We have reported previously that one analog, 5-iodo-4′-thio-2′-deoxyuridine (4′-thioIDU), exhibited good antiviral activity both in vitro and in vivo against two orthopoxviruses. This compound also has good activity against many of the herpesviruses. It inhibited the replication of herpes simplex virus type 1 and type 2 (HSV-1, HSV-2), and varicella-zoster virus with EC_{50} values of 0.4 μM, 0.5 μM, and 2 μM, respectively. It also inhibited the replication of human cytomegalovirus (HCMV) with an EC_{50} of 5.9 μM, but did not selectively inhibit Epstein-Barr virus, either variant of human herpes virus-6, or human herpesvirus-8. While some acyclovir-resistant strains of HSV-1, and -2 were comparatively resistant to 4′-thioIDU, it retained some activity against these strains (4–12 μM). Some ganciclovir resistant strains of HCMV also exhibited reduced susceptibility to the compound, and appeared to be related to the specific mutations in the DNA polymerase since it was fully active in an HCMV strain that lacked UL97 kinase activity. The activity of this molecule was also evaluated in mice infected intranasally with the MS strain of HSV-2. Twice daily oral administration of 4′-thioIDU at 5 mg/kg, 10 mg/kg or 30 mg/kg was initiated 24 h, 48 h, or 72 h after infection. Although there was no decrease in final mortality rates, the mean day of death was increased significantly ($P < 0.05$) in all animals receiving 4′-thioIDU even when therapy was delayed 72 h post infection. The highest dose of the compound was the most effective and increased the mean day of death irrespective of treatment delay ($P < 0.001$). Studies presented here suggest that 4′-thioIDU is a good inhibitor of some herpesviruses as well as orthopoxviruses and warrants further study as a therapy for these infections.

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Selection and Characterization of (S)-1-[3-Hydroxy-2-(Phosphonomethoxypropyl)-2,6-Diaminopurine [HPMPDAP] Resistant Camelpox Viruses

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The acyclic nucleoside phosphonate (ANP) family of drugs shows promise as therapeutics for treating poxvirus infections by interfer-