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-

ing with their viral DNA polymerase. In this context, analysis of the mechanisms through which poxviruses acquire resistance to ANPs is an important concern. The molecule HPMPDAP has been shown to be one of the most effective ANPs for the inhibition of poxvirus replication in vitro. The mutations within the viral DNA polymerase gene (*E9L*) involved in the resistance phenotype to HPMPDAP have only been described for vaccinia virus. In this study, camelpox viruses (strains Iran and Dubai) were passaged 30 times in medium containing an escalating dose of HPMPDAP, which selected for mutant viruses exhibiting an approximately 28- to 45-fold-increase in resistance to the drug. HPMPDAP-resistant clones were isolated following plaque purification. The antiviral activities of several ANPs, as well as of phosphonoacetic acid [PAA] and of ST-246 were determined by plaque reduction assays against the different clones. As a general conclusion, it appears that these HPMPDAP-resistant clones exhibit cross-resistance to other ANPs, including cidofovir, and that they also show hypersensitivity to two molecules: 6-[2-(phosphonomethoxy)ethoxy]-2,4-diaminopyrimidine] [PMEO-DAPy] and PAA, a direct viral DNA polymerase inhibitor. Interestingly, (S)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]-3-deazaadenine [3-deaza-HPMPA] retained marked activity against most of these resistant clones. Also, all the resistant clones were as susceptible as the wild type clones to ST-246, a poxviral egress inhibitor. The sequencing of the viral DNA polymerase genes of both wild type and resistant camelpox viruses is currently ongoing, and our results will be compared to those published for HPMPDAP-resistant vaccinia viruses. In conclusion, our studies provide additional insights in the mechanism of action of ANPs at the level of the viral DNA polymerase. Further in vivo experiments are still needed to evaluate the pathogenicity of such resistant viruses.

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## Effects of Anti-human Papillomavirus (HPV) Disease Agents on HPV Episome Levels In Vitro: Cidofovir, Podophyllotoxin, and Pyrrole–Imidazole Polyamides

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Many in vitro studies of anti-HPV agents have misguidedly focused upon cells maintaining integrated rather than episomal copies of human papillomaviruses (HPV). HPV displays genotype-specific tissue tropism and causes hyperproliferative diseases of both cutaneous and mucosal epithelia. Persistent infection with "high risk" HPVs may lead to malignancy. We have taken a novel approach to design a series of pyrrole–imidazole polyamides against the sequences located in the ori of high-risk HPV genotypes. The compounds specifically reduce HPV episome levels in cells maintaining high-risk HPV genomes. In this study, we compared the effects of our targeted polyamides against Cidofovir, which is currently being used off label for treatment of HPV-related disease including recurrent respiratory papillomatosis (RRP), and podophylotoxin which is commonly used to treat cutaneous warts. Monolayer cultures of human foreskin keratinocytes maintaining HPV31 were treated for 48 h with a range of doses of each compound. The effect of this treatment on HPV31 episome levels was measured via Q-PCR normalized to DNA input. Cell viability was also assessed in parallel using an MTT assay. A 50% reduction in HPV31 genome copy number was achieved at a concentration of 1 μM of polyamide NV1020 with no observable cytotoxicity up to the highest dose tested (10 μM). Cidofovir caused a dose-dependent decrease in HPV31 DNA at high doses of compound, although a 50% reduction in viral genomes was never reached for this compound up to 500 μM. The observable loss of HPV31 episomes due to Cidofovir correlated with losses in cell viability. Podophyllotoxin had no effect on HPV31 episome levels, however there was a significant dose-dependent reduction in cell viability (TD50 = 80 μM). These studies demonstrate the following: that podophylotoxin does not effect HPV episome levels but acts primarily via a cytotoxic mechanism; that Cidofovir appears to have an anti-HPV effect that is associated with cytotoxic activity; and that NV1020 effects on HPV episome levels occur in the absence of measurable cytotoxicity.

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## Identification of the Type of Cells Responsible for Transfer of Herpes Simplex Virus (HSV) and Vaccinia Virus (VACV) Infection to Epithelial Cells Grown in 3D

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We have previously shown that organotypic raft cultures of human keratinocytes isolated from neonatal foreskins can be infected with different dermotropic viruses and these cultures can be used as a model to evaluate the activity of antiviral compounds. We have also demonstrated the feasibility of using mononuclear cells (MCs) as viral carriers to transfer infection to organotypic epithelial raft cultures. We have now determined the population of cells responsible for carrying herpes simplex virus (HSV) and vaccinia virus (VACV) infection to the epithelial cells. For this purpose, MCs were isolated from human umbilical cord blood by Ficoll-Hypaque density gradient centrifugation and they were infected with HSV-1, HSV-2 or different VACV strains at a multiplicity of infection of approximately 0.01 and incubated overnight. MCs were washed three times to remove viral inoculum and were used to infect organotypic epithelial raft cultures. A part of the MCs was processed for confocal microscopy. Double-staining with anti-HSV or anti-poxvirus antibodies, and different cell differentiation marker-specific antibodies was performed to identify infected cell types. HSV and poxvirus infection was detected in CD45+ (leukocytes) and CD14+ (monocytes). Purified CD14+ cells either differentiated into macrophages by treatment with phorbol myristate acetate and hydrocortisone or not differentiated were able to support viral replication and transfer the infection to the epithelial cells suggesting that monocytes/macrophages may be considered as a vehicle to transfer infection to epithelial cells.

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## Drug Resistance Mutations in HSV-1 UL5 Selected using a Helicase–Primase Inhibitor: Frequency and Effects on Virus Growth and Pathogenicity

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Helicase–primase inhibitors (HPIs), e.g. BAY 57-1293, are extremely active against HSV in cell culture and animal infection models. They target the helicase–primase (HP) complex which is involved in virus DNA replication. Using BAY 57-1293 at inhibitory concentrations (e.g. 10–100 times the IC50) it was possible to detect HPI-resistant viruses in two different laboratory working stocks of