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The Microbicide Pipeline: Clinical Development Success and Failure

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Oral Session 7: Microbicides, Drug Design and Late Breaker Presentations

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Design of Intravaginal Ring for Simultaneous Delivery of Antiretroviral Drugs

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Microbicides have recently become an attractive prophylactic method to prevent the male-to-female sexual transmission of HIV. Vaginal rings have shown high patient compliance and efficacy as a contraceptive and are thus being developed to deliver anti-HIV compounds. We developed a monolithic vaginal drug delivery system capable of delivering two drugs, with different mechanisms of action against HIV. The ring consists of two polyurethane segments that are optimized to release two different drugs with differing hydrophobicity—the first segment is composed of a hydrophobic polyurethane and incorporates the non-nucleoside reverse transcriptase inhibitor Dapivirine. The second segment is composed of a hydrophilic polyurethane and incorporates the nucleoside reverse transcriptase inhibitor Tenofovir. We observed an in vitro near-linear release profile of both drugs for a duration of 14 days. The release rates of Dapivirine and Tenofovir were loading-dependent, indicating that loading can be adjusted to reach therapeutic levels to inhibit viral infection. In solution, Dapivirine and Tenofovir were both stable up to 30 days at elevated temperature. In the ring dosage form, both Dapivirine and Tenofovir were stable for 90 days at 40 °C/75% humidity. Lastly, the two-component ring had similar mechanical properties to the contraceptive vaginal ring Nuvaring®, indicating that our ring would likely be well tolerated in vivo.

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Biophysical Mechanisms in Microbicide Pharmacokinetics

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Rational design of vaginal microbicide products should include delivery systems with properties giving rise to target pharmacokinetics. These depend upon the site of antiviral action, viz. the vaginal lumen (e.g. for entry inhibitors) or outer vaginal epithelium (e.g. for RTIs or NNRTIs). Delivery systems for the active pharmaceutical ingredients (APIs) can be semi-solid (e.g. gels) or solid (e.g. intravaginal rings, IVRs). We have created computational compartmental drug delivery models for all these scenarios. The models are being used to compute performance measures for current vaginal microbicide products and in the design of new, improved ones. Model inputs include: (1) diffusion coefficients of APIs and HIV virions in gels and vaginal fluids and tissue; (2) release fluxes from IVRs and gels; (3) vaginal coating thickness distributions by gels; (4) gel rheological properties (whole and during interaction with vaginal fluids); (5) vaginal mechanical properties, e.g. wall elasticity; (6) geometry of IVRs and of the vaginal canal. In vitro assays are measuring (1), (2) and (4), including development of new techniques for (1). We measure (3) in women using an optical vaginal scanning probe. Results to date suggest that vaginal coating layers ~100 μm can be sufficient to deliver doses of luminal APIs that limit infectious concentrations of HIV from contacting epithelial surfaces. Layer effectiveness is enhanced to the extent that HIV mobility within the layer is diminished. However, total protection for luminal APIs depends upon maintenance of such coating over a large fraction (>90%) of the surfaces. API transport via ambient vaginal fluids – which may be menstrual cycle phase dependent – can also play a significant role in increasing API delivery. This is particularly true for the delivery kinetics of intra-epithelial APIs by IVRs: here, model computations have shown that cyclic variations in vaginal fluid production give a range of time intervals after which prophylactic API concentrations are achieved in tissue, from hours after IVR insertion to days.

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Virtual Reality Applications in Antiviral Drug Design

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Structure-based drug design is a creative process that displays several features that make it closer to human reasoning than to machine automation. However, very often the user intervention is limited to the preparation of the input and analysis of the output of a computer simulation. In some cases, allowing human interactive intervention directly in the process could improve the quality of the results by applying the researcher's intuition directly into the simulation. Virtual reality applications can provide an effective way to convey information about a molecular environment in real time, engaging more than one sense at the same time.

We developed an immersive molecular mechanics simulator, where the user can probe a biological target and its interactions with a ligand, feeling the forces and molecular interactions on his/her hand while having a complete three-dimensional visual feedback. The movement of the user's hand are tracked in real time and used to stir the simulation.

We applied this methodology in the design of novel potential inhibitors of HCV helicase and the results obtained will be reported in this presentation.

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Identification of a Small-molecule Antiviral with Broad-spectrum Application to Multiple, Lethal Virus Types

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We report herein the identification of a small molecule therapeutic, FGI-103, which displays potent and broad spectrum in vitro inhibition of lethal viral hemorrhagic fevers to include Ebola, Rift Valley Fever and Dengue. Using lethal in vivo mouse model of Ebola

virus, we further demonstrate that FGI-103 can protect animals from an otherwise lethal infection when used either in a prophylactic or therapeutic setting and that a 'single treatment', administered after infection, is sufficient to confer protection from lethal Ebola or Marburg virus challenge. 100% efficacy from a single, low dose treatment provides a proof-of-concept direct in vivo validation that FGI-103 in its current composition is bioavailable, efficacious and a viable lead for further therapeutic development. Exploratory in vitro antiviral testing further identified inhibitory activity against members of many different, otherwise unrelated virus families to include RSV, PIV and HBV, suggesting that perhaps FGI-103 potentially interferes with a common 'host' – target or – pathway utilized to varying extent by these different viruses. Altogether, these findings suggest FGI-103 may provide a much-needed 'broad-spectrum' opportunity to target multiple and otherwise intractable viral diseases.

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In vitro transport, activation and antiviral evaluation of new HPMPA prodrugs synthesized on a solid support

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9-[(2*S*)-3-Hydroxy-2-phosphonomethoxypropyl]adenine (HPMPA) is a broad spectrum antiviral agent that is highly potent against orthopox viruses, including cowpox, vaccinia, and variola (smallpox) virus. Unfortunately, it exhibits low oral bioavailability due to the presence of a phosphonic acid group, which is ionized at physiological pH. We report here extension of our phosphonate ester amino acid prodrug approach (*Mol. Pharm*, **2008** 5, 598) to HPMPA. One negative charge in the drug is masked by conversion to its cyclic form (cHPMPA) and the other by installation of the promoiety via esterification of the remaining POH with the side chain hydroxyl group of an appropriate single amino acid or dipeptide, containing a free N-terminal amine function and a C-terminal carboxylate alkyl ester group. A small library for SAR studies consisting of derivatives with varying C-terminal ester alkyl functions, amino acid stereochemistry, and P-O-C linkages was constructed using solid phase synthetic chemistry, including (L)-Ser(OMe)-cHPMPA, (D)-Ser(OMe)-cHPMPA, (L)-Ser(O*i*-Pr)-cHPMPA, (L)-Val-(L)-Ser(OMe) and (L)-Val-(L)-Ser(O*i*-Pr). The relative advantages of the new solid phase *vs*. conventional solution approaches to preparation of these compounds will be discussed. The stability and hydrolysis products of the compounds in tissue homogenates were evaluated by LC-MS. In intestinal homogenate, the amino acid prodrugs released cHPMPA in yields up to 90%. (L)-Ser(O*i*-Pr)-cHPMPA showed increased stability in both homogenate and phosphate buffer media compared to (L)-Ser(OMe)-cHPMPA. The dipeptide prodrugs had longer half-lives and different mechanisms of activation. The anti-viral (cowpox, vaccinia, CMV) potential of the new prodrugs will be assessed, based on data from *in vitro* assays.

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New peptidomimetic prodrugs of acyclic and cyclic cidofovir: sar studies of chemical and enzymatic activation mechanisms

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Cidofovir (HPMPC) and its similarly potent cyclic form (cHPMPC) are possible therapies for orthopox virus infections, but are limited in this role by low oral bioavailability. We have previously reported the synthesis of several dipeptide prodrugs of cHPMPC (aa-Ser-CO_2R cHPMPC) in which the amino acid stereochemistry or the peptide carboxyl R group were modified. These modifications resulted in significant differences in stability and transport properties. When the dipeptide prodrugs were investigated in phosphate buffer and tissue homogenates, both chemical and enzymatic activation pathways were observed. In the current study, a series of novel single amino acid (Ser, Thr, and Tyr) and dipeptide prodrugs of cHPMPC and HPMPC were synthesized and evaluated for their mechanism of activation and intestinal transport potential. The presence of a free N-terminal amino group in the Ser and Thr conjugates of cHPMPC catalyzes cleavage of the promoiety and releases cHPMPC in 80-90% yield, while the Tyr conjugate requires enzymatic activation. Dipeptide HPMPC conjugates, prepared from their corresponding cHPMPC analogues, show enhanced stability and require phosphatase activation. The effects of these and other structural modifications, including methylation of the dipeptide amido nitrogen [C(O)-N(CH_3)] and reversing the dipeptide sequence (Ser-Ala-CO_2R), on the activation pathways and transport potential of the compounds will be discussed, together with *in vitro* antiviral (cowpox, vaccinia, CMV) data. The results illustrate the versatile tunability of these side chain-linked peptidomimetic acyclic and cyclic cidofovir derivatives with respect to optimizing their transport and activation properties.

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