be developed as a component of a combination microbicide product to prevent the sexual transmission of viral, bacterial and fungal organisms.

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dsRNA binding characterization of full length recombinant wild type and mutants *Zaire ebolavirus* VP35

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The multifunctional VP35 protein is a major virulence factor by which the highly pathogenic Ebolaviruses (EBOVs) evade host innate immune response. VP35 is essential for EBOVs replication as a component of the viral RNA polymerase complex, it is a key participant to the nucleocapsid assembly, and it also binds to dsRNA antagonizing RIG-I like receptors antiviral signaling and, ultimately, inhibiting the host interferon (IFN) production. Insights in the VP35 dsRNA binding, ascribed to its C-terminal IFN inhibitory domain (IID), have been recently revealed through structural and functional analysis of the C-terminal truncated version of the protein. Here, we report the first biochemical characterization of the dsRNA binding of the full length, His-tagged recombinant VP35 (rVP35) of the *Zaire ebolavirus* species. For this purpose we established a new *in vitro* magnetic pull down assay, validating it for compound screening also by assessing the inhibitory ability of the auryntricarboxylic acid which showed an IC_{50} value of 50 μg/mL. Optimal biochemical parameters for dsRNA binding and K_d values for dsRNA with different length were obtained through competition binding studies. Furthermore, the dsRNA binding properties of the R305A, K309A and R312A rVP35 mutants, known for their defective dsRNA binding-mediated inhibition of the host IFN response in cell culture were assessed. Interestingly, results showed that, as compared to wild type rVP35, all three rVP35 mutants displayed a modified migration pattern in gel mobility shift assays and, when tested in the magnetic pull down assay, they displayed a significantly increase of the K_d values for dsRNA binding.

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Role of Cathepsin A and Lysosomes in the Intracellular Activation of Novel Anti-papillomavirus Agent GS-9191

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GS-9191, a bis-amidate prodrug of nucleotide analogue 9-(2-phosphonylmethoxyethyl)-N^6-cyclopropyl-2,6-diaminopurine (cPrPMEDAP), was designed as a topical agent for the treatment of papillomavirus-associated proliferative disorders such as genital warts. It has been previously shown that cPrPMEDAP is deaminated to guanine nucleotide that is further metabolized to active nucleoside triphosphate analog. In this study, we investigated the mechanism of conversion of GS-9191 to cPrPMEDAP. We observed that GS-9191 is hydrolyzed in the presence of lysosomal carboxypeptidase cathepsin A (CatA) in vitro and is less efficiently metabolized in CatA-deficient fibroblasts compared to control cells. In addition, knock-down of CatA by siRNA reduced the intracellular accumulation of GS-9191 metabolites. However, intracellular CatA levels did not correlate with the susceptibility of tested cell lines to GS-9191, indicating that the CatA step is unlikely to be rate-limiting for the activation of GS-9191. Further analysis showed that upon the hydrolysis of the carboxylester bond in one of the GS-9191 amidate moieties, the unmasked carboxyl group displaces L-phenylalanine 2-methylpropyl ester from the other amidate moiety. The formed cPrPMEDAP-L-phenylalanine conjugate (cPrPMEDAP-Phe) is not metabolized by Hint1 (histidine triad nucleotide binding protein 1) phosphoramidase, but undergoes a spontaneous degradation to cPrPMEDAP in acidic pH that can be significantly enhanced by the addition of SiHa cell extract. Pre-treatment of SiHa cells with bafilomycin A or chloroquine resulted in 9-fold increase in the intracellular concentration of cPrPMEDAP-Phe metabolite and the accumulation of GS-9191 metabolites in lysosomal/endosomal fraction. Together, these observations indicate that the conversion of GS-9191 to cPrPMEDAP occurs in lysosomes via CatA-mediated ester cleavage followed by the release of cPrPMEDAP, most likely through the combination of enzyme-driven and spontaneous pH-driven hydrolysis of cPrPMEDAP-Phe intermediate.

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Dioxolane L-Nucleoside Analogs Prevent Varicella-Zoster Virus Replication in Fibroblasts and Skin Organ Culture

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The α-herpesvirus varicella-zoster virus (VZV) causes chickenpox (varicella) and shingles (zoster). Current treatments are acyclovir and its derivatives, phosphonoformate, and brivudin (Europe only). Live, attenuated vaccines (Varivax, Zostavax) lower the incidence of primary and recurrent infections. Additional antiviral drugs with increased potency are needed, especially for resistant VZV strains and to treat post-herpetic neuralgia. We found that the bromovinyl uracil derivative (L-BHDU) was effective against VZV in culture and in a mouse model, so 3 related prodrugs were evaluated for their effects on VZV-BAC-Luc replication in HFFs and skin organ culture (SOC). Virus spread was measured by bioluminescence imaging. The ethyl- and methylphosphoamidate derivatives were similar to L-BHDU, with EC_{50} 0.1–0.3 μM in HFF at 48 hpi. In SOC, the EC_{50} of L-BHDU and the ethyl derivatives were similar (methyl not tested). The valyl derivative was most potent, with an EC_{50} of 0.038 μM in HFFs and 0.05 μM in SOC at 6 dpi. At 2 μM, these compounds did not affect HFF proliferation, and they were nontoxic up to 200 μM over 3 days. HFF cells treated with these compounds (2 μM) appeared normal and VZV plaque size was reduced. Additional tests will be conducted to evaluate these compounds against VZV strains resistant to acyclovir, their effects on viral DNA synthesis, and their effectiveness in the SCID-Hu skin implant mouse model. Overall, the results indicate that L-BHDU prodrugs, especially the valyl derivative, show promise as novel antiviral agents for treating VZV infections.