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High-throughput *In Vitro* HIV Rev-multimerization Assay

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The HIV Rev protein is essential for efficient viral replication. Rev-monomers multimerize on viral RNAs to export these from the nucleus to the cytoplasm of the infected cell through interaction with the cellular CRM1-mediated pathway for nuclear export. Efforts to develop inhibitors of the Rev-function were focussed mainly on the Rev-RNA and the Rev-CRM1 interaction. However an important aspect to the function of Rev is its necessity to multimerize. So far, no inhibitors targeting the Rev-oligomerization process have been discovered. Therefore, we have developed a solid high-throughput *in vitro* Rev-multimerization assay based on FRET (Fluorescence Resonance Energy Transfer). This technique requires two fluorophores to be in close proximity of each other. Upon excitation of the donor fluorophore, energy is transferred to the acceptor fluorophore from which emission is measured. In our multimerization assay fusion proteins of Rev to ECFP (FRET-donor) and Rev to EYFP (FRET-acceptor) are mixed allowing interaction of Rev-monomers, which results in high FRET-efficiencies. Samples containing combinations of ECFP and EYFP-Rev, ECFP-Rev and EYFP or ECFP and EYFP are used as negative controls. To validate this FRET-assay for the screening of Rev-multimerization inhibitors, increasing amounts of unlabeled Rev were added to the ECFP-Rev/EYFP-Rev sample leading to a dose-dependent inhibition of the FRET-signal. This fast and solid Rev-multimerization assay is adaptable to 96-well plate, 386-well plate and 1536-well plate formats, making the assay suitable for high-throughput screening of Rev-multimerization inhibitors. The discovery of a Rev-oligomerization inhibitor will allow the validation of HIV Rev-multimerization as target for antiviral chemotherapy. The concept of this assay is also widely applicable to the discovery of inhibitors of other protein–protein interactions.

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Pre-clinical Development of IQP-0410, a Highly Potent Dual-acting Agent for the Therapy of HIV-1 Infection

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The primary problems associated with anti-HIV therapy continue to be drug toxicity, drug–drug interactions, patient compliance with prescribed treatment regimens, and the appearance of drug resistant viruses. ImQuest Pharmaceuticals is developing IQP-0410, a highly potent non-nucleoside pyrimidinedione inhibitor of both HIV-1 and HIV-2. Oral dose of IQP-0410 administered via gavage, up to a maximum feasible dose level of 1000 mg/kg, was well tolerated in Beagle dogs. There were no test article-related findings noted during the evaluation of in-life data, clinical pathology or necropsy data. Histopathology examination of selected tissues revealed no drug-related microscopic findings. Preliminary pharmacokinetics studies of IQP-0410 in dogs showed that a significant amount of the compound remained in plasma at 24 h on Day 6, with an average of 37 ng/mL remaining. C_{max} values for Day 1 ranged from 52 to 113 ng/mL and from 71 to 165 ng/mL on Day 7 and the calculated EC95 value for IQP-0410 was 1 ng/mL. Metabolic stability and reaction phenotyping in human liver microsomes indicated that CYP3A4 is a major enzyme responsible for the metabolism of IQP-0410. Safety pharmacology studies showed no signs of pharmacological and toxicological activity. All genotoxicology tests of IQP-0410 were negative except for the mouse lymphoma test that was positive but below the range normally seen with a known positive control compound. This favorable pre-clinical profile suggests that IQP-0410 will be an important addition to the currently available therapeutic regimens used to treat HIV.

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Poster Session 2: Herpesviruses, Poxviruses, Other Antiviral Agents, Medicinal Chemistry and Topical Microbicides

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Antiviral Activity of Monoterpene Components of Essential Oils Against Herpes Simplex Virus

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Herpes simplex virus (HSV) is an important pathogen for humans causing labial herpetic infections and is a serious disease in immunosuppressed patients. The development of resistant strains of HSV to the available drugs, especially acyclovir, has further attempted to identify and develop new alternative agents for management of HSV infections. Essential oils and their components are potential antiviral agents. Eleven monoterpenenes including alpha-terpinene, gamma-terpinene, alpha-pinene, beta-pinene, alpha-terpineol, terpinene-4-ol, limonene, thymol, p-cymene, citral and 1,8-cineol, with hydrocarbon, alcohol, aldehyde and ether structure, which are major components of essential oils, were evaluated for anti-HSV activity. All monoterpenes were examined *in vitro* on RC-37 cells, cytotoxicity of components were evaluated by a standard neutral red assay. The 50% inhibitory concentrations (IC_{50}) of the monoterpenes for HSV plaque formation were determined in dose–response studies. Ten monoterpenes revealed high antiviral activity against free HSV. At maximum noncytotoxic concentration, all monoterpenenes reduced plaque formation by 80–100 %, except for monoterpene ether. The experimental data exhibited a significant higher susceptibility of HSV against the monoterpene hydrocarbons in comparison to oxidized components. In order to identify the mode of antiviral action, the monoterpenes were added to host cells or viruses at different stages of infection. In time addition experiments, the viral infection was reduced when monoterpenes interacted with free virus. Our studies suggest that monoterpenes might be suitable for topical treatment of herpetic infections.

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Preclinical Pharmacokinetic, Toxicokinetic and Toxicology Results for Cyclopropavir, a Promising New Agent for the Treatment of Beta- and Gamma-herpesviruses

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Cyclopropavir (CPV, ZSM-I-62, MBX 400) has been shown to be potent against beta- and gamma-herpesviruses (Kern et al.,