making this tissue a primary site of viral infection. Coinfection with human herpesviruses 6 and 7 or measles virus selectively modulates infection by R5 and X4 HIV by changing chemokine release and the expression of HIV receptors and coreceptors. In conclusion, we describe an HIV-triggered complex cycle of infection-activation-infection that creates new targets for HIV as well as for other viruses via cell activation, involving new cells in productive infection, upregulating cytokines and triggering apoptosis. This cycle is greatly affected by coinfecting pathogens, which by these means can determine the course of HIV disease progression. Intervention in the interactions between HIV and other pathogens may provide a new tool for antiviral therapy.

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Oral Session II: Respiratory and West Nile Viruses

8

Identification and Biochemical Characterization of Small Molecule Inhibitors of West Nile Virus Serine Protease by a High Throughput Screen

Radhakris Padmanabhan [1,*], Niklaus Mueller [1], Nagarajan Pattabiraman [2]

[1] Department of Microbiology and Immunology, Georgetown University; [2] Biomids Inc.

West Nile Virus (WNV) and dengue virus (DV) are mosquito-borne members of *Flaviviridae* that cause widespread human disease for which there is no vaccine or chemotherapy. These viruses, like all flaviviruses, encode a serine protease (NS3-pro) that is essential for polyprotein processing, a required step in viral replication. In this study, we report the development and validation of an in vitro, high throughput screening (HTS) assay for WNV protease. Using this assay, more than 32,000 small molecule compounds were screened, from which three core chemical structures were identified among them that inhibit the protease. A secondary screen of seven compounds selected from the three core structure groups, identified two compounds (A and B) as strong WNV protease inhibitors with K_i values as low as ~3 μM. Based on molecular docking of compound B with the recently reported crystal structure of WNV protease, we propose that compound B binds in the vicinity or within the substrate-binding pocket involved in the interaction with the P1 residue of the substrate. Furthermore, we suggest a plausible mechanism of protease inhibition by this group of compounds. This assay will be useful to identify other potent inhibitors of the flaviviral protease and lead the way for development of antiviral therapeutics against WNV and related flaviviruses.

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Discovery of a New Class of Polycyclic RSV Inhibitors

Silas Bond [1,*], Alistair Draffan [1], John Lambert [1], Chin-Yu Lim [1], Bo Lin [1], Angela Luttick [1], Jeff Mitchell [1], Craig Morton [1], Roland Nearn [1], Vanessa Sanford [1], Simon Tucker [1]

[1] Biota Holdings Limited; [2] MedImmune Inc.

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under 1 year of age and is a leading cause of severe lower respiratory infections in infants and young children. Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

We have discovered a new class of imidazoisoindolone RSV inhibitors with general structure as depicted in Fig. 1. The synthesis of this novel series of compounds will be described with the identification of key features important for antiviral activity. Medicinal chemistry has been applied to develop highly active and specific small molecules species that inhibit RSV.

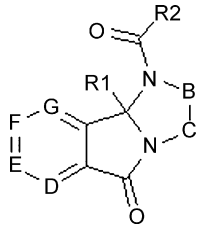

Fig. 1.

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Potent Inhibition of Viral Entry and Replication of SARS-CoV by siRNAs Targeting the Genes Encoding the Cellular ACE2 Receptor or the Viral Nucleocapsid Protein

Xin Yan [1,2,3,*], Hua Shen [2], Yan Feng [1], Jun Wang [2], Shiwen Lou [4], Liping Wang [5], Gillian Wong [1], Zhaoxiong Yang [2], Hongjian Jiang [6], Xinqi Wu [6], Dan Hu [5], Yi Guan [4], Fiona Smaill [1], Chengsheng Zhang [1]

[1] Department of Pathology & Molecular Medicine, McMaster University, Canada; [2] New Medical Science Research Institute, New York, USA; [3] Institute of Chongqing Traditional Chinese Medicine, Chongqing, China; [4] Department of Microbiology, University of Hong Kong, Hong Kong; [5] Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA; [6] Children's Hospital, Harvard Medical School, Boston, USA

Severe acute respiratory syndrome (SARS), which is caused by a newly identified human coronavirus named SARS-associated

coronavirus (SARS-CoV), killed more than 800 people and posed significant challenges for the public health systems and hospital workers during the outbreak in 2002–2003. SARS may still remain a threat to the public health since the natural reservoir of SARS-CoV remains largely unknown and there are no specific treatments and effective vaccines available for SARS-CoV infection. To further identify molecular targets for the development of novel strategies against SARS-CoV infection, we have employed siRNA mediated RNA interference technology to examine the potential effects of a panel of siRNA molecules on the viral entry and/or replication of SARS-CoV by targeting the genes encoding the human angiotensin-converting enzyme 2 (ACE2) receptor or the viral nucleocapsid protein (NP). We first found that some of the siRNA duplexes that were treated by Yan Xin Life Science and Technology (YXLST) could dramatically and specifically down-regulate the cellular ACE2 receptor or viral NP expression in a dose-dependent manner in human 293T cells. We then showed that the siRNA directed against ACE2 receptor could potently suppress the viral entry of the spike protein pseudotyped viruses. We further demonstrated that these siRNA molecules targeting ACE2 or NP genes could also markedly suppress the cytopathic effect (CPE) of the SARS-CoV infected cells, and potently inhibit the viral replication. Therefore, our study has identified two highly conserved molecular targets for the siRNA-mediated RNA interference against SARS-CoV infection.

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Thiazolides: A New Class of Broad-Spectrum Antiviral Drugs Targeting Virus Maturation

M. Gabriella Santoro [1,*], Alessandra Ciucci [1], Patrizia Gianferretti [1], Giuseppe Belardo [1], Simone La Frazia [1], Stefania Carta [1], Jean-Francois Rossignol [2]

[1] Department of Biology, University of Rome Tor Vergata, Rome, Italy; [2] Division of Gastroenterology and Hepatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Nitazoxanide [2-acetolyloxy-*N*-(5-nitro-2-thiazolyl)benzamide, NTZ] is a new anti-infective thiazolide used in the United States for treating Giardia- and Cryptosporidium-originated enteritis. We have recently shown that NTZ is effective in reducing clinical symptoms in hospitalized pediatric patients with severe rotavirus infection (Rossignol, J.F., et al., 2006. Lancet 368, 124–129). We now report that NTZ and its active circulating metabolite tizoxanide [2-hydroxy-*N*-(5-nitro-2-thiazolyl)benzamide, TIZ], have a broad-spectrum antiviral activity, effectively inhibiting the replication of several RNA and DNA viruses in experimental in vitro models. The thiazolides were found to be effective at low micromolar concentrations, which were non toxic to uninfected cells, against viruses belonging to seven different families including: simian SA11 and human Wa G1P1A rotaviruses, influenza A (PR8 and WSN strains) viruses, Sendai virus (SV), respiratory syncytial virus (RSV), coronavirus (CCoV), vesicular stomatitis virus (VSV), adenovirus (Ad5) and herpes simplex virus type 1 (HSV-1). IC_{50} and S.I. varied between 0.5 and 2 μg/ml and, 25 and >100, respectively, in the different experimental models examined. In the case of rotavirus and paramyxovirus infection, both drugs were found to protect the host cell from the cytophatic effect caused by the virus for at least 24 h p.i. Approximately, 20 NTZ derivatives have now been tested for antiviral activity, some of which were found to be more effective than the parent compound. The mechanism of the antiviral activity was studied in cells infected with rotaviruses and influenza viruses. Thiazolides do not inhibit viral RNA transcription and do not cause a general block of virus protein synthesis, but act at post-translational level interfering with the correct processing of selected viral glycoproteins, thus hindering the formation of mature viral particles.

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Oseltamivir-Ribavirin Combination Therapy for Highly Pathogenic H5N1 Influenza Virus Infection in Mice

Natalia A. Ilyushina *, Robert G. Webster, Elena A. Govorkova

St. Jude Children's Research Hospital, Memphis, TN 38105, USA

The unusual severity of disease caused by H5N1 influenza viruses in humans raises concern that contemporary antiviral drugs may be ineffective against systemically replicating highly pathogenic viruses. Combination therapy with drugs that interfere with different stages of the virus replication cycle and/or affect different aspects of virus pathogenicity may provide several advantages over single-drug treatment. To test this hypothesis, we studied the effect of combinations of oseltamivir (neuraminidase inhibitor) and ribavirin (non-specific inhibitor of viral polymerases) against two highly pathogenic H5N1 viruses (A/Vietnam/1203/04 and A/Turkey/15/06) representing two different clades of the H5 phylogenetic tree. BALB/c mice were treated with oseltamivir (10, 50 or 100 mg/kg/day), ribavirin (37.5, 55 or 75 mg/kg/day), or combinations of the two drugs twice daily for 8 days by oral gavage, starting 4 h before inoculation with 5 MLD_{50} of each H5N1 virus. Single-drug oseltamivir produced a dose-dependent antiviral effect against both H5N1 viruses ($P < 0.01$). A higher dose was required for the greatest effect against A/Turkey/15/06 virus (90% survival rate), whereas oseltamivir 10 mg/kg/day resulted in 70% survival of mice infected with A/Vietnam/1203/04 virus. Single-drug ribavirin showed a similar dose-dependent effect against both strains: dosages of 37.5 and 75 mg/kg/day significantly delayed death and provided 10% and 50% survival rates, respectively ($P < 0.01$). The mode of drug interaction in vivo was characterized by the three-dimensional model of Prichard and Shipman. The combination of two drugs produced additive-to-synergistic effects against A/Turkey/15/06 (H5N1) virus, with no enhancement of host toxicity. Combination treatment with 10 mg/kg/day oseltamivir and 37.5 mg/kg/day ribavirin completely inhibited