v3xUL97, which had similar levels of sensitivity to GCV as wild type GPCMV. These studies indicate the feasibility of using a UL97 humanized GPCMV for antiviral pathogenicity studies in the guinea pig model.

doi:10.1016/j.antiviral.2010.02.344

Oral Session 5: Respiratory Viruses, Emerging Viruses and Biodefense

Chairs: Graciela Andrei, Ph.D. and Peter Silvera, Ph.D., 1:00–5:30 pm, Grand A

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ST-246, a Therapeutic for Smallpox

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Background: ST-246, a small-molecule inhibitor of poxviruses has demonstrated safety and efficacy profiles in various animal model systems. ST-246 is being developed as a promising antiviral for smallpox and is currently in Phase I clinical trials. Here, we evaluated the optimal post-exposure dose of ST-246 to effectively treat rabbits using the intranasal RPXV challenge model.

Methods: Two sets of thirty 9-week-old NZW rabbits divided into 5 groups of 6 rabbits each were challenged intranasally with 1×10^5 PFU of RPXV, Utrecht strain. At 48 or 72 h post-infection (hpi) once daily oral treatment was initiated in each set of 4 groups at doses of 40, 20, 10 and 5 mg/kg, respectively for 14 days. The remaining group received vehicle only. Animals were monitored daily for clinical signs, body weight and temperature. Viral load in the blood and tissue was measured by quantitative PCR.

Results: ST-246 at a dose of 40 mg/kg given at 48 and 72 hpi provided 100 and 83% protection, respectively, despite a transient increase in temperature and moderate initial weight loss. Treatment doses of 20 mg/kg or 10 mg/kg ST-246 when given 48 hpi provided 50% protection against severe RPXV disease, whereas, only 17% protection was achieved when the same doses were given a day later. Protection conferred by ST-246 was associated with suppression of viremia in a dose-dependent manner and suppression or clearance of RPXV in the lung, liver and spleen. By contrast, all non-survivors developed typical signs of rabbitpox disease including nasal and ocular discharges, respiratory distress, pyrexia, and anorexia. Finally, viral loads in non-survivors ranged between 6 and 9 logs genome copies/mL with mean time-to-death of 6.5 days.

Conclusions: ST-246 demonstrated dose and time dependent protection against lethal RPXV disease. These data further support the advancement of ST-246 as a promising therapeutic for smallpox. This work was funded by NIAID-DMID contract N01-AI-30063.

doi:10.1016/j.antiviral.2010.02.345

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Evidence for Host Drug Targets Essential for Dengue Virus Capsid Formation

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By analogy to previous studies for hepatitis B virus, HIV, and hepatitis C virus, we established a cell-free system involving *de novo* protein biogenesis that appears to faithfully carry out critical steps in the assembly of Dengue virus capsids. The protein synthesis-linked capsid assembly system was converted into an ELISA-based screening platform for identification of small molecules that interfere with proper Dengue virus capsid formation. This screen potentially can identify molecules acting either directly or indirectly, via interference with essential host factors, anywhere in the assembly pathway. A number of small molecules conforming to Lipinski's rules were identified as hits likely acting at diverse steps in the capsid assembly pathway and by different mechanisms. This hypothesis is based on evidence to be presented that the activity of some of these molecules results in aberrant capsids by several different criteria including resistance to digestion by proteases and changes in buoyant density, compared to non drug-treated controls. When tested against live Dengue virus in cell culture, a number of these compounds were found to be robustly active, resulting in multilog drop in plaque forming unit (pfu) titer in the nanomolar to low micromolar range. These active molecules were sorted by chemical class, activity, and toxicity. A total of 11 chemical classes (pharmacophores) were found to be potent ($EC_{50} < 7.5\ \mu M$) and non-toxic (TI > 10). These findings provide strong support for the hypothesis that critical steps in Dengue virus capsid formation are faithfully re-created in the cell-free system. The targets of those drugs not acting directly on the capsid protein are promising candidates for essential host factors in the Dengue virus life cycle.

doi:10.1016/j.antiviral.2010.02.346

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An Adenosine Nucleoside Inhibitor of Dengue Virus

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Dengue virus (DENV), a mosquito-borne flavivirus, is a major public health threat. The virus poses risk to 2.5 billion people world-wide and causes 50–100 million human infections each year. Neither vaccine nor antiviral therapy is currently available for prevention and treatment of DENV infection. We have developed a novel nucleoside NITD008, (2R,3R,4R,5R)-2-(4-amino-pyrrolo[2,3-d]pyrimidin-7-yl)-3-ethynyl-5-hydroxymethyl-tetrahydro-furan-3,4-diol, that potently inhibits DENV both in vitro and in vivo. Besides the four serotypes of DENV, NITD008 inhibits other flaviviruses, including West Nile virus (WNV), yellow fever virus (YFV), and powassan virus (PWV). The compound also suppresses hepatitis C virus (HCV), but it does not inhibit nonflaviviridae, such as Western equine encephalitis virus (WEEV) and Vesicular stomatitis virus (VSV). A triphosphate form of NITD008 directly inhibits the RNA-dependent RNA polymerase (RdRp) activity of DENV, indicating

that the compound functions as a chain terminator during viral RNA synthesis. NITD008 has good in vivo pharmacokinetic properties, and is biologically available through oral administration. Treatment of DENV-infected mice with NITD008 suppressed peak viremia, reduced cytokine elevation, and completely prevented infected mice from death. Our results have proved the concept, for the first time, that a small molecular inhibitor could be developed for clinical treatment of flavivirus infections.

doi:10.1016/j.antiviral.2010.02.347

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Small Molecule Agonists of the RIG-I Pathway and their Potent Antiviral Actions

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We report on the identification of five potent, drug-like small molecule agonists of the RIG-I innate immune pathway that demonstrate effective antiviral activity against both the hepatitis C and influenza viruses. Hepatitis C virus is a highly successful virus infecting nearly 200 million people worldwide and causing a chronic lifelong infection in approximately 75% of acutely infected subjects. Influenza A virus continues to be a major health concern despite seasonal vaccination programs, with 5–20% of the U.S. population contracting the infection every year leading to an average of 200,000 hospitalizations. Recent drug development efforts have focused on antiviral products that directly target key viral enzymes, but major improvements to the immune-modulating therapeutic backbone have received scant attention. Drugs that modulate and enhance innate immunity would display broad antiviral activity, immune-enhancing efficacy and an ability to overcome virus countermeasures, while remaining insensitive to the rapid evolution of drug resistance that plagues conventional small molecule therapies. A key pathway that is responsible for mediating the innate immune response to RNA virus infection involves activation of RIG-I and targeting this pathway has successfully lead to the identification of agonist molecules that are highly potent and broadly active antiviral molecules. We have identified five lead compound candidates that specifically agonize the RIG-I pathway, a key mediator of the innate immune response to virus infection. The compounds activate RIG-I responsive promoters by mediating nuclear translocation of IRF-3 and display highly potent antiviral activity against hepatitis C virus and influenza A virus. These molecules efficiently decrease the synthesis of viral proteins, the accumulation and spread of viral RNA, as well as the production of infectious virus. Ongoing studies will further define the mechanism of action of these RIG-I agonist molecules and utilize QSAR studies to optimize their antiviral and drug-like properties.

doi:10.1016/j.antiviral.2010.02.348

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The NF-κB-Inhibitor SC75741 Efficiently Blocks Influenza Virus Propagation by Retention of the Viral RNP Complexes in the Nucleus without the Tendency to Induce Resistant Virus Variants

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Influenza is still one of the major plagues worldwide. The appearance of highly pathogenic avian H5N1 viruses or swine-origin H1N1v influenza viruses in humans and increasing incidence of resistance to the currently available medication highlight the need for new and amply available antiviral drugs. We and others have demonstrated that influenza virus misuses the cellular IKK/NF-κB signalling pathway for efficient replication suggesting that this module may be a suitable target for antiviral intervention. Here we show that the novel NF-κB inhibitor SC75741 efficiently blocks replication of influenza A and B viruses, including A/H5N1 isolates and H1N1v strains in concentration that do not affect cell viability or metabolism. The underlying molecular mechanism of SC75741 action involves impaired expression of proapoptotic factors, subsequent inhibition of caspase activation as well as block of caspase-mediated nuclear export of viral ribonucleoproteins (RNPs). Besides this direct antiviral effect the drug also suppresses virus-induced overproduction of cytokines and chemokines, suggesting that it might prevent the so-called cytokine burst that is an important pathogenicity determinant of infections with highly pathogenic influenza viruses, such as the A/H5N1 strains. Most importantly the drug did not shown any tendency to induce resistant virus variants. Thus, a SC75741-based drug may serve as a broadly active non-toxic anti-influenza agent.

doi:10.1016/j.antiviral.2010.02.349

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The Triple Combination Antiviral Drug (TCAD) Regimen of Amantadine, Ribavirin, and Oseltamivir is Highly Efficacious Against Susceptible and Resistant Influenza Virus Strains in Mouse Treatment Models

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The triple combination antiviral drug (TCAD) regimen composed of amantadine (AMT), ribavirin (RBV), and oseltamivir (OSL) has been previously shown to be highly active *in vitro* and synergistic against a range of susceptible and resistant influenza viruses. Here we evaluated the TCAD regimen in mouse models of influenza A infection and compared the efficacy to monotherapy and double combinations using factorial design. In two separate studies, mice were infected with lethal doses of susceptible influenza A/Duck/MN/1525/81 (H5N1) or AMT-resistant novel influenza A/CA/04/09 (H1N1) virus. Treatments were initiated 24 h after infection via oral gavage and continued TID for 5-days. The dosing regimens (OSL 25 mg/kg/day; AMT 46 mg/kg/day; RBV 27 mg/kg/day) were selected to produce drug exposures in mice that approximate those in humans. Survival and body-weights were monitored for 21-days. TCAD was highly effective at treating mice infected with a lethal dose of A/H5N1 and novel A/H1N1 influenza viruses, producing survival rates of 90 and 95%, respectively. In contrast, monotherapy with OSL produced 0 and 20%