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Protection Against Genital HSV-2 in Guinea Pigs with Subunit Vaccines Containing gD2, gB2, and gH/gL Glycoprotein Antigens Complexed with CLDC Adjuvant

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An effective vaccine for genital herpes simplex type 2 (HSV-2) remains a priority. We previously showed that the CLDC adjuvant enhanced protection against genital HSV-2 in guinea pigs when combined with 5 μg gD2 antigen. To further evaluate other HSV-2 candidate subunit vaccines, we compared the following groups: no vaccine, gD2-FL (full length, 1–306aa) + CLDC, gD2-T (truncated, 1–285aa) + CLDC, gD2-T + gB2 (31–726aa) + gH2/gL2 (21–802aa) + CLDC, and gB2 + gH2/gL2 + CLDC. Guinea pigs were immunized SC twice at 3 week intervals, and challenged intravaginally 3 weeks later with 1×10^6 pfu HSV-2 (MS strain). All of the vaccinated groups had significantly reduced acute lesions and virus titers at 2 and 5 dpi compared to the no vaccine group ($p \leq 0.002$) whereas no significant differences were observed between the vaccinated groups. Vaccination with any of the vaccines significantly reduced recurrent HSV-2 disease ($p \leq 0.002$) compared to the no vaccine group. For all of the vaccination groups, recurrent mean lesion scores (<1.0 vs. 7.5) and recurrent mean lesion days (<1 day vs. 9 days) were significantly reduced compared to the no vaccine group. PCR analysis of latent viral DNA in the DRG after 63 dpi demonstrated a significant difference ($p < 0.01$) in the number of animals with detectable HSV-2 DNA following first round PCR for groups gD2-T + CLDC, gD2-T + gB2 + gH2/gL2 + CLDC, and gB2 + gH2/gL2 + CLDC compared to the no vaccine group while the gD2-F + CLDC group was not significantly different. Following nested PCR, all groups had > 80% of the DRGs positive for HSV-2 DNA, suggesting subtle differences between latent viral load between the vaccination groups. Analysis of recurrent vaginal shedding and antigen-specific antibody is currently underway. Taken together, these results indicate that all the vaccines were effective but that study design alterations, such as reducing the antigen dose or increased time between vaccinations, will be necessary to distinguish between the vaccine candidates [Supported by Contract NO1-AI-15438 from the Virology Branch, NIAID, NIH].

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In Vitro and *In Vivo* Activities of the Novel Anti-cytomegalovirus Compound AIC246

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Background: CMV remains an important pathogen for immunocompromised individuals including transplant recipients, AIDS patients, and newborns. To date, all drugs licensed for the treatment of CMV infection and disease target the viral DNA-polymerase. Although effective, there are several disadvantages associated with the use of these drugs including toxicity and emergence of drug resistance. Hence, safe and improved antivirals with novel molecular targets are urgently needed. Here we report on the antiviral properties of AIC246, a novel small molecular weight compound that is currently undergoing clinical phase II trials.

Methods: The anti-CMV activity of AIC246 was evaluated in vitro and in vivo using different cell culture assays and in an engineered mouse xenograft model. In addition, antiviral properties of the drug in comparison to Ganciclovir were characterized by the use of different virus strains, different cell types, increasing MOIs and "time of addition" experiments.

Results: AIC246 exhibited excellent in vitro inhibitory activity against HCMV (EC_{50} ~4 nM) and potent in vivo efficacy in a xenograft mouse model (ED_{50} ~3.4 mg/kg/day). The efficacy of the drug was not significantly affected by cell culture variations or MOI. Time of addition experiments revealed that the drug acts "late" in the virus replication cycle.

Conclusion: AIC246 represents a novel HCMV inhibitor with potent antiviral activity in vitro and in vivo and acts via a mode of action that is different to Ganciclovir.

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A Chimeric Guinea Pig Cytomegalovirus (GPCMV) Encoding Wild Type or Mutant HCMV UL97 Renders GPCMV Susceptible or Resistant to Ganciclovir while Retaining an Ability to Disseminate in the Animal Host

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The species specific constraints of human cytomegalovirus (HCMV) precludes direct study of this virus in animal models. GPCMV is uniquely useful for studies related to congenital CMV infection but is not sensitive to HCMV antivirals ganciclovir (GCV) and maribavir (MBV). Although the modes of action of these antivirals are different they act via the HCMV viral protein pUL97. Strains of HCMV can emerge with resistance to GCV or MBV. These resistant strains are due to specific pUL97 codon changes which modify the susceptibility of the virus to the GCV or MBV. We previously demonstrated the ability to improve the susceptibility of GPCMV to antivirals MBV and GCV by the generation of a chimeric GPCMV encoding UL97 in place of GP97, the homolog gene (McGregor et al., 2008). Not only did this chimeric virus have GCV sensitivity similar to HCMV clinical isolates but it retained an ability to be pathogenic in vivo, where animals treated with GCV therapy had reduced mortality and viral dissemination. We hypothesized that the generation of chimeric GPCMV containing specific UL97 mutations found in resistant strains of HCMV would result in the generation of chimeric GPCMV capable of normal viral replication but resistant to MBV or GCV, dependent on their specific mutations. Five chimeric mutant UL97 GPCMV were generated that carried individual mutations found in HCMV resistant strains. The pUL97 codon modifications were M460I, H520Q, A594V, L595S or a deletion (591–594). Additionally, two MBV UL97 mutant chimeric GPCMV were generated that carried pUL97 codon changes V353A or T409M. These chimeric mutant UL97 GPCMV exhibited growth kinetics similar to wild type GPCMV. A sixth GCV mutant UL97 chimeric GPCMV was generated that incorporated 3 specific mutations (M460I, H520Q and 591–594 deletion), designated v3xUL97. This chimera exhibited growth kinetics similar to wild type GPCMV as well as increased resistance to GCV in comparison to the wild type UL97 chimeric GPCMV. The highest level of resistance to GCV was exhibited by

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.

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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.

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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.

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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