treatment, serum was collected for the determination of ALT and anti-HBsAg antibodies. Splenocytes were incubated with HBsAg for 48 h to allow for T-cell activation. These cell culture supernatants were tested for interferon-gamma as a surrogate marker for CD4 and/or CD8 T-cell activation. Two vaccine injections consisting of HBsAg/JVRS100 administered intramuscularly (IM) or a combination of HBsAg/JVRS100 administered IM and JVRS100 administered intravenously (IV) broke tolerance as evidenced by significantly ($P < 0.001$) increased HBsAg-specific IgG total, IgG1 and IgG2c, and IFN-gamma. The other treatment groups (JVRS100 and HBsAg) were not statistically different from the non-treatment group. The combination of HBsAg/JVRS100 administered IM and JVRS100 administered IV resulted in statistically increased serum ALT and decreased serum HBsAg. These data indicate that CLDC-adjuvant may be informative as to the potential for efficacy of a therapeutic HBV vaccine in human clinical trials.

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Pro-drugs of Strand Transfer Inhibitors of HIV-1 Integrase: Inhibition Data, Structure–Activity Analysis and Anti-HIV Activity

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HIV integrase is encoded at the 3′-end of the pol gene of HIV and catalyzes the integration of viral DNA into the host cell genome in two key steps, which are 3′-processing in the cytoplasm and strand transfer in the nucleus. HIV integrase is essential for the replication of this virus and is a significant biochemical target for the development of anti-HIV therapeutic agents. At present, there is only one FDA-approved integrase inhibitor, Raltegravir, for the clinical treatment of HIV-AIDS. As resistance and toxicity are issues that are regularly encountered with anti-HIV drugs targeted at various viral replication points of intervention, the discovery of new classes of integrase inhibitors remains a significant scientific challenge. This presentation will focus on the discovery of integrase inhibitors assembled on modified nucleobase scaffolds that were found to be potent inhibitors of the strand transfer step of HIV-1 integrase ($IC_{50} \leq 10$ nM). However, while the integrase data were compelling, a significant disconnect existed between the enzyme inhibition data and the cell culture data for anti-HIV activity. A possible explanation of this disconnect may be issues of cellular permeability. Thus, a pro-drug investigation was undertaken to enhance cell permeability. Pro-drug SAR will be explained and illustrated. For example, an active integrase inhibitor of this investigation had an enzyme IC_{50} of 6 nM. Its pro-drug showed an EC_{50} in cell culture of 9 nM and a CC_{50} of 135 μM. These and other data will be presented.

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Triple Combination Antiviral Drug (TCAD) Regimen Composed of Amantadine, Ribavirin, and Oseltamivir Imposes a High Genetic Barrier to the Development of Resistance Against Influenza A Viruses *In Vitro*

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Background: Virtually all circulating influenza A viruses are resistant to one of the two classes of approved antivirals. The continued use of antivirals as monotherapy could result in the emergence of strains resistant to both classes of approved drugs. Here, we evaluated the effects of a triple combination antiviral drug (TCAD) regimen composed of amantadine (AMT), ribavirin (RBV), and oseltamivir carboxylate (OSC) on the emergence of resistance in vitro.

Methods: Influenza A viruses were serially passaged in MDCK cells in the presence of fixed, clinically relevant concentrations of AMT and OSC as single agents and in double combination, and the TCAD regimen, or under escalating concentrations of each drug regimen. The emergence of genotypic resistance was determined by mismatch amplification mutational analysis for the M2 (codons 27, 30, and 31) and neuraminidase (NA; codon 274) genes, or by Sanger sequence analysis for the M2, hemagglutinin, and NA genes.

Results: Serial passage of influenza at fixed concentrations of AMT or OSC alone or in double combination resulted in the early breakthrough of viruses with resistance-associated mutations, with the resistant variants rapidly becoming predominant (>90% by passage 3). In contrast, treatment with the TCAD regimen resulted in sustained suppression of the resistant virus population (<35% at passage 5). Under escalating concentrations, the TCAD regimen imposed a high genetic barrier to the development of resistance, inhibiting virus replication at concentrations below the EC_{50} of each drug for up to 31 days in culture. For the double combination and single agents, the presence of resistance-associated mutations enabled virus replication at concentrations of up to 275-fold greater than the EC_{50} of each drug.

Conclusion: These data demonstrate that the TCAD regimen composed of AMT, RBV and OSC imposes a high genetic barrier to resistance and suppresses the replication of resistant influenza viruses in vitro, and support the use of TCAD therapy for the treatment of influenza A infection.

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Inhibition of Hepatitis C Virus Replication by Semisynthetic Derivatives of Glycopeptide Antibiotics

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Glycopeptide antibiotics (teicoplanin and eremomycin) are used as antibacterial agents. We here report on the anti-HCV activity of hydrophobic teicoplanin and eremomycin derivatives. Analogue LCTA-949 resulted in the most selective anti-HCV activity three dif-