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-

ferent HCV subgenomic (genotype 1b) replicon systems with a 50% effective concentration (EC_{50}) of 1–5 μg/ml. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells was >30 μg/ml thus resulting in a selectivity index ~25. The anti-HCV activity observed in Huh 5-2 was corroborated by means of RT-qPCR. LCTA-949 inhibited also efficiently the replication of the HCV_{cc} (JFH/J6 chimera) as assessed by RT-qPCR and by monitoring expression of viral antigen. Unlike various selective HCV inhibitors, LCTA-949 is very efficient in clearing cells from HCV replicons. The fact that the compound inhibits subgenomic replicon replication at concentrations that are similar to those that inhibit HCVcc replication, indicate that the compound inhibits intracellular RNA replication. At the ultrastructural level, treatment of either uninfected or infected cells with LCTA-949 results in the formation of multilamellar bodies (MLB). The potential effect of MLB formation on the HCV replication is currently being studied. Semisynthetic hydrophobic derivatives of glycopeptides antibiotics may thus be an interesting route to explore novel antiviral strategies against HCV.

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Eradication of Persistent Bovine Viral Diarrhea Virus Infection in Cell Culture by Antiviral Treatment: How to Get Ahead of the Viral Evasion Strategy

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The bovine viral diarrhea virus (BVDV) is a member of the family of Flaviviridae. BVDV exists as two biotypes, i.e. cytopathogenic (cp) and non-cytopathogenic (ncp). The ncp variant can establish a persistent infection in live stock as well as in cell culture by eluding the host innate immunity. Here we report on how such a persistent BVDV infection can be completely eradicated from mammalian cells. To this end the persistently infected cells were treated for a number of consecutive passages either with interferon alpha, the interferon inducer polyIC or the small molecule pestivirus inhibitor BPIP [Paeshuyse et al., 2006. J. Virol. 80, 149–160] or a combination thereof. Afterwards the cells were passaged two more times in cell culture medium without inhibitors. For each passage the presence of intracellular and extracellular viral RNA was monitored. An initial experiment resulted in a rapid decline of viral RNA for all inhibitors studied. Combined these data enabled the design of different drug regimes that resulted in the total eradication of BVDV ncp in cell cultures. The results obtained could help better understanding how pestiviruses establish persistent infections and how persistently infected cells can be cleared from such an infection. The latter can be of value for sanitation of precious cell lines that are contaminated by an ncp BVDV infection. Furthermore it is being investigated whether prolonged antiviral treatment of persistently infected cells can restore the innate immunity.

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N,N′-Bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine Targets the HIV-1 Retroviral Nucleocapsid Zinc Fingers

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From an extensive structure-activity relationship study we have identified *N,N′*-bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine (NV038) that efficiently blocks the replication of various strains of HIV-1, HIV-2 and SIV. NV038 inhibited the replication of HIV-1 at a 50% effective concentration of 17.3 μM and was not toxic for the host cells up to 300 μM tested, resulting in a selectivity index greater than 17. The compound was equipotent against several drug resistant virus strains. Time-of-addition experiments indicate that NV038 interferes with an event of the viral replication cycle following the viral entry but preceding or coinciding the early reverse transcription step, pointing towards an interaction with the viral nucleocapsid protein (NCp7). NCp7 is a small protein with two 'CCHC' zinc fingers flanked by basic residues, where both determinants are required for high affinity binding to RNA. The anti-HIV activity of NV038 decreased in the presence of Gag containing VLPs, suggesting its inhibitory effect is caused by an interaction with one of the Gag structural proteins. In fact, *in vitro*, NV038 efficiently chelates the zinc ions and depletes zinc from NCp7, which is paralleled by the inhibition of the NCp7-induced destabilization of cTAR. A chemical model suggests that the two carbonyl oxygens of both esters present in NV038 are involved in the chelation of the Zn^{2+}-ion. Besides the structural features required for zinc chelation other structural elements prove to be crucial for specific target recognition. This new lead and our mechanistic study provide insight into the design of further derivatives against this target with improved efficacy and selectivity.

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In Vitro Combination Studies of ANA598 with Anti-HCV Agents Demonstrate Enhanced Anti-viral Activity

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ANA598 is a potent direct-acting antiviral inhibitor of HCV NS5B polymerase that is currently in a Phase II clinical trial in combination with SOC. ANA598 exhibits subnanomolar potency against genotype 1 NS5B polymerase enzymes and dissociates slowly with a $t_{1/2}$ of ~2 h. Nanomolar *in vitro* potency was observed for clinical isolates tested with a mean EC_{50} for genotype 1b of 2.8 nM ($n = 10$) and 27 nM for genotype 1a ($n = 9$). Due to the potential for rapid emergence of resistance mutations to any single direct antiviral used as monotherapy in hepatitis C, future treatment of chronic HCV infection is expected to be in combination with SOC or with complementary direct antivirals. We describe here the *in vitro* assessment of ANA598 in combination with SOC, and several other classes of clinically advanced direct acting antiviral agents.