substantial toxicities and/or the development of drug-resistant viruses. Previously, we engineered a LTR-specific recombinase (Tre-recombinase) that can effectively excise integrated HIV-1 proviral DNA from infected human cell cultures, suggesting that customized enzymes might someday help to eradicate HIV-1 from the body. Here, we provide an update on our recent and further analyses of Tre-recombinase in various HIV-1 infection models. Moreover, we discuss potential future strategies to deliver Tre-recombinase into infected subjects.

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Small Molecules Targeting Protein–Protein Interactions: A Promising Anti-HIV Strategy

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The development of multidrug-resistant viruses compromises the efficacy of anti-human immunodeficiency virus (HIV) therapy and limits treatment options. Therefore, new targets with a different mechanism of action with respect to anti-AIDS drugs so far in therapy need to be identified. In recent years, many examples of protein–protein interactions in the HIV life cycle and related inhibitors is growing rapidly (Busschots et al., 2009). Thus, protein–protein interactions (PPIs) provide an important new approach for the drug design against HIV infection. In our previous paper, a structure-based 3D pharmacophore model for potential inhibitors of the interaction between HIV-1-IN and its cellular cofactor LEDGF/p75 was developed and used for virtual screening of chemical databases, leading to the identification of interesting hits for further optimization (De Luca et al., 2009). Consequently, the rational design, synthesis and biological tests of some derivatives have been carried out. Our studies resulted in the discovery of compounds able to interfere with the IN-LEDGF/p75 interaction at micromolar concentration. Docking simulations were also performed with the aim to investigate the possible binding mode of our new compounds.

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A Cell Protection Screen Reveals Potent Inhibitors of Multiple Stages of the Hepatitis C Virus Life Cycle

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The hepatitis C virus (HCV) life cycle involves multiple steps, but most current drug candidates target only viral replication. Inability to systematically discover inhibitors targeting multiple steps of the HCV life cycle has hampered antiviral development. We describe a new screen for HCV antivirals based on the alleviation of a HCV-mediated cytopathic effect experienced by an engineered cell line—n4mBid. This approach obviates the need for a secondary screen to avoid cytotoxic false positive hits. Application of our screen to 1280 compounds, many in clinical trials or approved for therapeutic use, yielded >200 hits. Of the 55 leading hits, 47 inhibited one or more aspects of the HCV life cycle by >40%. Six compounds blocked HCV entry to levels similar to an antibody (JS-81) targeting the HCV entry receptor CD81. Seven hits inhibited HCV replication and/or infectious virus production by >100-fold, with one (quinidine) inhibiting infectious virus production by 450-fold relative to HCV replication levels. The described approach is simple and inexpensive, and should enable the rapid discovery of new classes of HCV life cycle inhibitors.

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Synthesis and Tissue Distribution Studies of Acyloxyalkyl Prodrug Derivative of an Anti-HBV Dinucleotide

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We have reported that phosphorothioate dinucleotides and trinucleotides are a new class of anti-HBV compounds with potent activity *in Vitro* and *in Vivo*. Recently, we had developed the acyloxyalkylester derivative as an oral prodrug of the model anti-HBV dinucleotide [R_p,S_p]-3′-dA-ps-$U_{2'OMe}$ (**1**). The current studies were undertaken to evaluate the distribution of the ^{35}S-labeled prodrug in the liver and other organs in rats. The ^{35}S-labeled prodrug was obtained as a solid in high specific activity (120 mCi/g; 84.9 mCi/mmol) by chemoselective *S*-alkylation of ^{35}S-1. The requisite ^{35}S-1 was synthesized using solid-phase phosphoramidite chemistry. Thus, controlled-pore-glass (CPG)-supported dA^{NBz} was coupled to 5′DMT-2′-OMe-uridine-3′-phosphoramidite to generate the intermediate dinucleoside phosphite. The sulfurization of the phosphite using ^{35}S-labeled 3H-1,2-benzodithiole-1,1-dioxide (independently synthesized) followed by deprotection of the CPG-bound dinucleoside phosphotriester, and HPLC purification gave ^{35}S-1. ^{35}S-1 was administered to rats, at a dose of 10 mg/kg, by intravenous (iv) and oral (po) routes. Radioactivity was readily detected in plasma at different time-points after both iv and po administration. Radioactivity concentrated in the liver and the ratio of liver to plasma concentration was as high as 2.9 (iv route) and 3.9 (po route) 1 h after dose administration. Other tissues – kidney, brain, spleen, and heart – contained minor amounts of radioactivity. The primary route of excretion of the radioactivity from the com-

pound after iv was in urine (50–60%), with 10–20% in feces. After po administration, radioactivity was excreted about equally in urine and feces, ~35% each in 24 h. Thus, acyloxyalkly derivatization represents a novel strategy for the oral delivery and liver-targeting of dinucleotide compounds and oligonucleotides.

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CYSTUS052, a New Compound Against Seasonal and Pandemic Influenza Virus

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Influenza still represents a major threat to humans and several animal species. Beside vaccination, only two classes of drugs are available for antiviral treatment against this pathogen. The appearance of pandemic H1N1 and highly pathogenic avian influenza viruses of the H5N1 subtype being able to infect humans reveal the urgent need for new and efficient countermeasures against this disease. Even though several antiviral compounds have been developed against influenza virus, their long-term efficacy is often limited, because of their toxicity or the emergence of drug-resistant virus mutants. Moreover, it is also widely discussed that neuraminidase inhibitors the most common anti-influenza agents, are less effective against new H5N1 isolates and seasonal H1N1 strains. In this regard, we were able to show that a polyphenol rich plant extract from a special variety of Cistus incanus named CYSTUS052 exhibits antiviral activity against influenza viruses *in vitro* and in a mouse model and a randomized, placebo controlled clinical study. The recovery from clinical symptoms was 2.5 days faster in the CYSTUS052 group compared to patients from the placebo group. In addition, we investigated the antiviral potential of CYSTUS052 in comparison to oseltamivir against the swine origin influenza virus (SOIV) H1N1 and various H5N1 influenza viruses. Using an *in vitro* infectivity inhibition assay we found that during the first 24 h after infection a single treatment of CYSTUS052 was highly effective against these H5N1 viruses compared. Therefore, we conclude that CYSTUS052 might be an effective antiviral with prophylactic and therapeutic potential against influenza viruses including the current pandemic strain and A/H5N1.

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Lectins and T-20, but not Neutralizing Antibodies, Inhibit HIV-1 Env-mediated Syncytium Formation between Clone69t1RevEnv and Supt1 Cells Monitored by Fluorescence Microscopy

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Objectives: The HIV-1 envelope protein Env (gp120/gp41) mediates the fusion of the viral envelope with the host cell membrane. We developed an HIV fusion assay, using fluorescent Clone69T1RevEnv cells expressing Env, and highly CD4+ SupT1 cells. We examined whether previously established inhibitors of HIV-1 infection, including a peptide, lectins, and neutralizing antibodies, inhibit Env-mediated syncytium formation.

Methods: Clone69TRevEnv cells were induced to express Env by removing tetracycline from the medium. The cells were labeled with Calcein-AM Green, incubated for 3 h with SupT1 cells labeled with CellTrace™ Calcein red-orange, with or without the inhibitors, and observed under a Nikon inverted fluorescence microscope. Co-localization of the two fluorescent probes following syncytium formation resulted in orange fluorescence. Antibodies were obtained from the NIH AIDS Research & Reference Reagent Program, Polymun (2G12) and D. Dimitrov (m14; NIH). T-20 was from the AIDS Reagent Program.

Results: The lectins *Hippeastrum hybrid* agglutinin (HHA) and *Galanthus nivalis* agglutinin (GNA), and the peptide T-20, inhibited syncytium formation at 1 μg/ml. Antibodies to gp120 (IgG1B12, m14 IgG, F105 and 2G12), and gp41 (2F5 and 4E10) that inhibit HIV-1 infection had little or no inhibitory effect on syncytium formation.

Conclusions: The observation that antibodies that inhibit HIV infection are not effective against syncytium formation, suggests that the mechanisms of interaction of Env with cell membrane CD4 and co-receptors may be different in cell–cell and virus-cell membrane fusion, as suggested previously (J. Gen. Virol., 1995, 76, 669–679). These results also indicate that "neutralizing" antibodies may not be able to inhibit the spread of viral genetic material from infected cells to uninfected cells. This fluorescence assay can be adapted to screen novel inhibitors of membrane fusion in high-throughput assays.

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CYSTUS052, a Polyphenol Rich Plant Extract, Exerts Potent Antiviral Activity Against Influenza- and Rhinoviruses by Preventing Viral Attachment to Host Cells

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Infections with influenza A viruses (IAV) still pose a major threat to humans and several animal species. The appearance of highly pathogenic avian H5N1 viruses and new H1N1v swine-origin influenza virus in humans as well as the increasing incidence of resistance to the currently available medication highlight the urgent need for novel antiviral drugs for prophylaxis and therapy. Here we demonstrate that the polyphenol rich plant extract CYSTUS052 from a variety the Mediterranean plant *Cistus incanus* exerts a potent anti-influenza virus activity in cells infected with various influenza viruses including those of the H5N1 and H1N1v type. The extract is also highly active against different types of human rhinoviruses (HRV). CYSTUS052 did not exhibit apparent harming effects on cell viability and did not influence metabolism, proliferation or cell activation by extracellular ligands. Furthermore, viruses did not develop resistance to CYSTUS052 upon consecutive passaging. Mechanistically, the protective effect appears to be due to a binding of the CYSTUS052-ingredients to the virus surface, preventing virus-binding to cellular receptors. Since these plant extracts are already in use in traditional medicine for centuries without reports of side effects, local application of CYSTUS052 to the respiratory tract may be a promising approach for