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

prophylaxis and therapy of respiratory virus infections cause by IAV or HRV.

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Development of Resistance to the Natural HIV-1 Entry Virus Inhibitory Peptide (VIRIP)

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The virus inhibitory peptide (virip) was identified as a component of human hemofiltrate and shown to have anti-HIV activity through the inhibition of HIV-1 gp41-dependent fusion. We confirmed the anti-HIV activity of virip and the optimized virip-derived peptide vir353 in lymphoid cells. Virip and vir-353 showed a dose-dependent activity with 50% effective concentrations of 16 and 0.7 mM respectively and a time of addition experiment showed that virip and vir353 target a time/site of action that corresponds to gp41-dependent fusion. Sequential passage of HIV-1 NL4-3 in lymphoid MT-4 cells in the presence of increasing concentration of different anti-HIV drugs led to the generations of virus resistant to nevirapine (10 days), the entry inhibitor BMS-155 (30 days), the fusion inhibitors, enfuvirtide (90 days), sifuvirtide (180 days) and vir-353 (260 days) suggesting a high genetic resistance for the virip-related compound. The resulting vir-353 resistant virus was completely cross-resistant (>200-fold) to virip but remained sensitive to the fusion inhibitors enfuvirtide and C34 as well as other HIV inhibitors targeting virus entry (AMD3100) or reverse transcriptase (AZT, nevirapine). Recombination of gp41 of the virip resistant virus into a wild-type HxB2 backbone partially recovered the resistant phenotype but both resistant gp120 and gp41 were necessary to recover full resistance to virip. Mutations were found in both gp120 and gp41 of the virip-resistant virus. However, no mutations were found in the fusion peptide of gp41 the alleged target of virip. The time needed to generate a virip resistant virus and the position of mutations found suggest that virip may target an essential part of gp41 and highlight possible interactions between gp41 and gp120 required during the fusion process.

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Single-dose Intranasal Delivery with DEF201 (Adenovirus Vectored Mouse Interferon-α) Protects Against Phlebovirus and Sars Coronavirus Challenge

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Interferon (IFN)-α is an effective and safe recombinant human protein with broad clinical appeal. While recombinant IFN-α has great therapeutic value, its utility for biodefense is hindered by its short *in vivo* half-life and costly production. Here we describe the use of Ad5-mIFN-α (DEF201) to address these limitations as a prophylactic countermeasure in two murine viral challenge models, Punta Toro virus (PTV; *Bunyaviridae*, *Phlebovirus*) and SARS coronavirus (CoV). Significant protection ($p < 0.001$) against PTV and SARS-CoV infections was observed in mice from a single dose of DEF201 administered 1 day to 3 weeks prior to challenge. DEF201 was delivered intranasally to stimulate mucosal immunity at the probable site of infection and bypass any preexisting immunity. Intramuscular inoculation with DEF201 rapidly increased (~3 h) IFN-α concentrations in unchallenged mice and persisted for extended periods of time. In contrast, a control Ad5 construct elicited only small amounts of IFN-α that were short-lived. Studies investigating the kinetics of mucosal and systemic IFN-α levels following intranasal administration of DEF201 are underway. Effective medical countermeasures that are highly stable, easily administered, and elicit long lasting protective immunity are much needed. The DEF201 technology has the potential to address all of these issues and serves as a broad-spectrum approach to enhance host defense against a number of viral pathogens.

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Evaluation of the Contribution of Amantadine, Ribavirin, and Oseltamivir in a Triple Combination Antiviral Drug (TCAD) Regimen to Suppressing the Emergence of Resistance using a Novel Quantitative Approach

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Background: We have previously demonstrated that a triple combination antiviral drug (TCAD) regimen comprised of amantadine, ribavirin, and oseltamivir carboxylate was highly active and synergistic against susceptible and resistant influenza A viruses *in vitro*. To determine the contribution of each drug in TCAD to preventing the emergence of resistance, we have developed a novel assay to quantify the development of resistance following serial passage under drug pressure.

Methods: MDCK cells in 96-well plates were infected with influenza A/Hawaii/31/2007 (H1N1) in the presence of a clinically achievable, fixed concentration of two drugs alone, or in triple combination with varying concentrations of the third drug, using 12 replicates for each condition. Following 5 serial passages, the percentage of wells for each condition having virus breakthrough (>50% cytopathic effect) and the presence of resistance-associated mutations (>1% total population of variants bearing the V27A, A30T, and S31N substitution in M2, and the H274Y substitution in neuraminidase) was determined by neutral red staining and mismatch amplification mutational analysis, respectively.

Results: Treatment of infected cells with any double combination resulted in virus breakthrough in up to 12 of 12 wells (100%) and virus resistance in up to 10 of 11 wells (91%). Addition of each third drug (TCAD) resulted in concentration dependent reductions in the percentage of wells with virus breakthrough and virus resistance. Importantly, the contribution of each drug in preventing the emergence of resistance was shown by a statistically greater ($P < 0.05$) reduction in virus breakthrough and/or emergence of influenza resistant variants compared to all double combinations at clinically achievable concentrations.