that the compound functions as a chain terminator during viral RNA synthesis. NITD008 has good in vivo pharmacokinetic properties, and is biologically available through oral administration. Treatment of DENV-infected mice with NITD008 suppressed peak viremia, reduced cytokine elevation, and completely prevented infected mice from death. Our results have proved the concept, for the first time, that a small molecular inhibitor could be developed for clinical treatment of flavivirus infections.

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Small Molecule Agonists of the RIG-I Pathway and their Potent Antiviral Actions

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We report on the identification of five potent, drug-like small molecule agonists of the RIG-I innate immune pathway that demonstrate effective antiviral activity against both the hepatitis C and influenza viruses. Hepatitis C virus is a highly successful virus infecting nearly 200 million people worldwide and causing a chronic lifelong infection in approximately 75% of acutely infected subjects. Influenza A virus continues to be a major health concern despite seasonal vaccination programs, with 5–20% of the U.S. population contracting the infection every year leading to an average of 200,000 hospitalizations. Recent drug development efforts have focused on antiviral products that directly target key viral enzymes, but major improvements to the immune-modulating therapeutic backbone have received scant attention. Drugs that modulate and enhance innate immunity would display broad antiviral activity, immune-enhancing efficacy and an ability to overcome virus countermeasures, while remaining insensitive to the rapid evolution of drug resistance that plagues conventional small molecule therapies. A key pathway that is responsible for mediating the innate immune response to RNA virus infection involves activation of RIG-I and targeting this pathway has successfully lead to the identification of agonist molecules that are highly potent and broadly active antiviral molecules. We have identified five lead compound candidates that specifically agonize the RIG-I pathway, a key mediator of the innate immune response to virus infection. The compounds activate RIG-I responsive promoters by mediating nuclear translocation of IRF-3 and display highly potent antiviral activity against hepatitis C virus and influenza A virus. These molecules efficiently decrease the synthesis of viral proteins, the accumulation and spread of viral RNA, as well as the production of infectious virus. Ongoing studies will further define the mechanism of action of these RIG-I agonist molecules and utilize QSAR studies to optimize their antiviral and drug-like properties.

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The NF-κB-Inhibitor SC75741 Efficiently Blocks Influenza Virus Propagation by Retention of the Viral RNP Complexes in the Nucleus without the Tendency to Induce Resistant Virus Variants

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Influenza is still one of the major plagues worldwide. The appearance of highly pathogenic avian H5N1 viruses or swine-origin H1N1v influenza viruses in humans and increasing incidence of resistance to the currently available medication highlight the need for new and amply available antiviral drugs. We and others have demonstrated that influenza virus misuses the cellular IKK/NF-κB signalling pathway for efficient replication suggesting that this module may be a suitable target for antiviral intervention. Here we show that the novel NF-κB inhibitor SC75741 efficiently blocks replication of influenza A and B viruses, including A/H5N1 isolates and H1N1v strains in concentration that do not affect cell viability or metabolism. The underlying molecular mechanism of SC75741 action involves impaired expression of proapoptotic factors, subsequent inhibition of caspase activation as well as block of caspase-mediated nuclear export of viral ribonucleoproteins (RNPs). Besides this direct antiviral effect the drug also suppresses virus-induced overproduction of cytokines and chemokines, suggesting that it might prevent the so-called cytokine burst that is an important pathogenicity determinant of infections with highly pathogenic influenza viruses, such as the A/H5N1 strains. Most importantly the drug did not shown any tendency to induce resistant virus variants. Thus, a SC75741-based drug may serve as a broadly active non-toxic anti-influenza agent.

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The Triple Combination Antiviral Drug (TCAD) Regimen of Amantadine, Ribavirin, and Oseltamivir is Highly Efficacious Against Susceptible and Resistant Influenza Virus Strains in Mouse Treatment Models

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The triple combination antiviral drug (TCAD) regimen composed of amantadine (AMT), ribavirin (RBV), and oseltamivir (OSL) has been previously shown to be highly active *in vitro* and synergistic against a range of susceptible and resistant influenza viruses. Here we evaluated the TCAD regimen in mouse models of influenza A infection and compared the efficacy to monotherapy and double combinations using factorial design. In two separate studies, mice were infected with lethal doses of susceptible influenza A/Duck/MN/1525/81 (H5N1) or AMT-resistant novel influenza A/CA/04/09 (H1N1) virus. Treatments were initiated 24 h after infection via oral gavage and continued TID for 5-days. The dosing regimens (OSL 25 mg/kg/day; AMT 46 mg/kg/day; RBV 27 mg/kg/day) were selected to produce drug exposures in mice that approximate those in humans. Survival and body-weights were monitored for 21-days. TCAD was highly effective at treating mice infected with a lethal dose of A/H5N1 and novel A/H1N1 influenza viruses, producing survival rates of 90 and 95%, respectively. In contrast, monotherapy with OSL produced 0 and 20%

survival rates. The other monotherapy and double combination regimens provided no protection or only partial protection (0–60% survival) in each study. A comparison of the Kaplan–Meier survival curves showed that TCAD provided significantly better protection ($P < 0.05$) than all other regimens in both studies. TCAD produced survival rates in each study that were greater than the additive rates of each drug as a monotherapy, indicative of synergy. Moreover, TCAD reduced the magnitude of weight loss in infected animals significantly relative to all other treatments. Importantly, AMT contributed to the efficacy of TCAD against the AMT-resistant novel A/H1N1 virus. The TCAD regimen is highly active in two lethal mouse influenza treatment models against susceptible and resistant viruses. These results validate and build upon the previously demonstrated superior *in vitro* efficacy of TCAD versus monotherapy and double combination regimens and translate them into an *in vivo* model.

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In Vitro and *In Vivo* Efficacy of Combinational Therapy with Favipiravir (T-705) and Oseltamivir Against Influenza A/CA/04/09 Pandemic H1N1 Virus

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Serious disease caused by emerging pandemic influenza A (H1N1) viruses and the possibility of drug resistance underscores the need for different approaches for treating influenza infections. The augmentation of current monotherapies with newly developed agents targeting alternative functions of the influenza virus replication cycle may be a solution. The current studies demonstrate the efficacy of using a combination of two drugs with different mechanisms of action to enhance total anti-H1N1 influenza A activity compared to each compound alone. Combinations of oseltamivir, a currently used, clinically approved neuraminidase inhibitor and favipiravir, an experimental viral RNA polymerase inhibitor, were evaluated alone and in combination for *in vitro*/*in vivo* efficacy against a pandemic H1N1 influenza A virus. *In vitro* combination studies revealed synergy with 0.032–1.0 μM oseltamivir combined with 0.32–10 μM T-705. In an H1N1 lethal mouse model it was found that: (1) orally administered combinations of favipiravir at 30–0.3 mg/kg/day and oseltamivir at 3 mg/kg/day resulted in significant protection against death ($P < 0.001$) and in total survivors ($P < 0.05$–0.01), (2) the combinations of favipiravir and oseltamivir at higher doses ameliorated the weight loss attributable to virus infection, (3) the combinations of favipiravir and oseltamivir at higher doses were highly synergistic, and (4) the use of favipiravir at 30 mg/kg/day or higher may permit the use of lower doses of oseltamivir to achieve efficacy against pandemic H1N1 viruses. The results suggest that these two compounds could be used in combination to treat serious infections in humans caused by pandemic H1N1 viruses.

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Antiviral Activity of Leflunomide Against Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is a major cause of often serious, fatal respiratory disease in infants and young children, organ transplant recipients, patients suffering cystic fibrosis or congenital heart disease, and the elderly. Severe RSV disease is characterized by a disproportionately intense pulmonary inflammatory response resulting in bronchiolar injury and compromise of airway function. Current treatment options are limited to ribavirin, which must be administered by small-particle aerosol for 12–18 h per day for 3–7 days, and passive immunoprophylaxis with monoclonal antibody specific for the RSV fusion protein (palivizumab), neither of which has been shown to reduce mortality. Leflunomide is an orally bioavailable anti-inflammatory drug approved for treatment of rheumatoid arthritis and currently in clinical trials as an immunosuppressant in transplant recipients. We have previously demonstrated that leflunomide exerts potent antiviral activity against CMV, HSV, and polyomavirus BK. We now report on the antiviral activity of this agent against RSV. Phase contrast microscopy and immunohistochemical staining demonstrated nearly complete attenuation of RSV-induced syncytia formation in infected human airway epithelial cell cultures treated with A77 1726, the active metabolite of leflunomide. Plaque assay of virus yield in RSV-inoculated cultures demonstrated potent, dose-dependent A77-mediated reduction in virus production. Likewise, pulmonary viral loads in RSV-inoculated cotton rats were reduced by >3 logs by leflunomide compared with vehicle-treated controls, even when leflunomide treatment was delayed until day 3 post-inoculation. Real-time rt-PCR demonstrated A77-mediated inhibition of viral genomic RNA synthesis and inhibition of transcription of several viral genes. Data generated by these experiments implicate leflunomide as a unique bifunctional agent with potential to both reduce viral load and, by virtue of its well-documented anti-inflammatory activity, attenuate the destructive inflammation associated with RSV disease. Sidwell and Barnard have stated that effective therapeutic intervention for severe RSV disease must include both antiviral and anti-inflammatory components. Leflunomide, it seems, effectively meets these criteria.

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Small-molecule Inhibition of Respiratory Syncytial Virus Fusion: *It Takes Two to Tango*

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Six-helix bundle (6HB) formation is an essential step for many viruses that rely on a class I fusion protein to enter a target cell and initiate replication. Because the binding modes of small molecule inhibitors of 6HB formation are largely unknown, precisely how they disrupt 6HB formation remains unclear, and structure-based design of improved inhibitors has thus been very speculative. It is currently believed that such inhibitors completely prevent 6HB formation by binding in a hydrophobic pocket composed of amino