

*In vitro* combinations of ANA598 with Interferon- $\alpha$ , ANA773 (an oral TLR7 agonist), the HCV NS3/4A protease inhibitor telaprevir, the NS5B polymerase nucleoside inhibitor PSI-6130 (active moiety of R7128), an NS5A inhibitor, and other non-nucleoside inhibitors of NS5B polymerase were conducted in Huh-7 cells containing either the wild type replicon or replicons bearing common palm site mutations (e.g., M414T or G554D). We have previously demonstrated that there is no overlap in viral mutations conferring resistance to NS5B palmsite inhibitors and agents acting at distinct polymerase sites or against the HCV NS3/4A protease *in vitro*. No cytotoxicity was observed for any of the combinations tested. The inhibitory activity of the two agents in combination was compared to the dose response of each agent alone and analyzed assuming Loewe Additivity or Bliss Independence. For each combination evaluated, the antiviral effect between the compounds was determined to be additive to synergistic.

The *in vitro* combination studies (see also Thompson et al. ICAR 2010 abstract) suggest that such combinations may produce a greater viral load reduction and potentially delay the emergence of drug resistance *in vivo*. Collectively, the results provide support for clinical exploration of combination regimens that include ANA598.

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#### Discovery of Novel Small Molecule Inhibitors of Multiple Influenza Strains in Cell Culture

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Efficacy of drugs currently used in anti-Influenza A therapy is decreasing because of emerging viral resistance. Prosetta has discovered novel antiviral compounds, which inhibit the propagation of influenza A virus, by employing a unique moderate throughput screen based upon the interaction of viral proteins with cellular host-factors. Screening of a small molecule chemical library identified 17 distinct chemical series whose activity was validated against Influenza A H7N7, (fowl plague virus (FPV), (Bratislava)) in MDCK cells. In this live virus cell culture assay, six chemical series showed EC<sub>50</sub> < 20  $\mu$ M. From these early hits, one chemical series was selected for optimization. The synthesis of a small diversity set surrounding this series indicated a robust structure activity relationship existed and the series is currently undergoing optimization for potency, ADMET, and safety profiles. The series has produced multiple compounds with improved anti-viral drug characteristics from the initial screening hit. For example, one compound from this series shows EC<sub>50</sub> = 10 nM, EC<sub>99</sub> = 100 nM with a CC<sub>50</sub> of 2.5–10  $\mu$ M. Early pharmacokinetic studies in mice have shown promise, with a mean residence time > 24 h and 31% bioavailability after oral application at 3.6 mg/kg. Compounds in the series have been tested on an Influenza A H1N1 (Puerto Rico 8) strain and they possess activity comparable to that against FPV. Further optimization of potency, ADMET and safety profiles are underway as this series will soon enter animal efficacy studies.

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#### Novel Imino Sugars Potently Inhibit HCV Virion Secretion by Targeting Cellular Endoplasmic Reticulum $\alpha$ -Glucosidases

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Imino sugars, such as deoxynojirimycin (DNJ), are glucose mimetics that competitively inhibit endoplasmic reticulum  $\alpha$ -glucosidases I and II, which are essential for glycan processing and folding of viral glycoproteins and are the validated antiviral targets of many enveloped viruses. In our efforts to improve the antiviral efficacy of imino sugars, we discovered recently that *N*-pentyl-(1-hydroxycyclohexyl)-DNJ (OSL-95II) and its derivatives containing modified terminal ring structures, such as PBDNJ0804, demonstrated 25–1000-fold improved antiviral activity against dengue virus than a classical imino sugar NB-DNJ (Chang et al., 2009). In the study reported herein, employing a system for production of infectious HCV particles in cell culture, we found that OSL-95II and PBDNJ0804 also had a superior antiviral activity against HCV with EC<sub>50</sub> values of 25 and 5  $\mu$ M, respectively. Consistent with the inhibition of  $\alpha$ -glucosidases, both OSL-95II and PBDNJ0804 did not affect the levels of intracellular HCV RNA and non-structural proteins, but efficiently inhibited glycan processing of HCV E2 glycoprotein and induced its degradation. Consequentially, secretion of HCV infectious virions was reduced by 75 and 10,000-fold in the presence of 100  $\mu$ M of OSL-95II and PBDNJ0804, respectively. Moreover, pharmacokinetics studies showed that the novel imino sugars had good oral bioavailability and were well tolerated by mice and rats *in vivo*. The superior antiviral efficacy and low likelihood of drug resistant virus emergence hold promise for the novel glucosidase inhibitors to be developed as therapeutic agents against HCV infection, especially as components of combination therapies with IFN or inhibitors of viral proteases and RNA polymerase [Chang, J., Wang, L., Ma, D., Qu, X., Guo, H., Xu, X., Mason, P.M., Bourne, N., Moriarty, R., Gu, B., Guo, J.T., Block, T.M., 2009. Novel imino sugar derivatives demonstrate potent antiviral activity against flaviviruses. *Antimicrob. Agents Chemother.* 53(4), 1501–1508].

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#### Antiviral Activity of Attachment Inhibitor Against the Pandemic Influenza A (H1N1) Virus

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The emergence and spread of novel swine-origin influenza A (H1N1) virus in humans highlights the urgent need for new effective therapeutics. An attractive approach for the prevention of influenza infection involves inhibition of virus attachment to susceptible cells by synthetic analogs of cellular receptors. Influenza virus attachment is mediated by the interaction of the viral surface glycoprotein hemagglutinin with host cell surface receptors containing sialooligosaccharides. The goal of this investigation was to study the antiviral effect of low-molecular polyvalent inhibitor

of influenza virus attachment, containing sialooligosaccharide 6-sialyl(*N*-acetylactosamine) (6'SLN), on the pandemic influenza A (H1N1) virus. The antiviral activity of attachment inhibitor against pandemic influenza virus A/California/07/2009 (H1N1) was examined in the inhibition assay of infectious focus forming in MDCK cells. To characterize efficacy of inhibitors *in vivo* we have investigated a mouse model, based on measuring the value of 50% respiratory infectious dose for mice. The inhibitor of influenza virus attachment exhibits potent inhibitory activity against pandemic influenza A (H1N1) virus. The value of 50% inhibiting concentration (IC<sub>50</sub>) of attachment inhibitor obtained in MDCK cells was 0.07 ( $\pm 0.005$ )  $\mu$ M. Intranasal administration of attachment inhibitor (2 mg/kg) completely protected mice from influenza virus A/California/07/2009 (H1N1) infection. Low-molecular inhibitor of influenza virus attachment did not negatively interfere with the proliferative and metabolic capacity of cells as determined in MTT assay. The results of this study suggest that low-molecular polyvalent inhibitor of influenza virus attachment represents a potential therapeutic agent for prevention and treatment of the pandemic influenza A (H1N1) virus.

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#### Therapeutic Response Guided Interferon Therapy Among Patients Chronically Infected with Hepatitis C Virus

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In Pakistan current standard of care for patients chronically infected with hepatitis C virus is combination therapy with interferon-alpha 2b (IFN-alpha-2b) and ribavirin. Treatment outcomes vary patient to patient and are associated with several multiple factors like genetic predisposition of host and viral genotype. In Pakistan, HCV genotype 3a is most prevalent and a viral response with standard therapeutic regimen (STR) varies and ultimately decides about the sustained virological response (SVR). We performed a prospective study to correlate SVR with rapid virological response (RVR) and early virological response (EVR) among individuals chronically infected with HCV confirmed through ELISA and PCR with primers specific for HCV genotype 3a. A total of 1471 (550 males and 732 females) individuals received 300MU IFN-alpha-2b (thrice a week) and 800–1200 mg ribavirin (adjusted to patient's weight) daily. The RVR, EVR and ETR (end treatment response) values are based on post-treatment HCV RNA determination through real time PCR at weeks 4, 12, and 24. Among 1471 participants 452 (30.7%) did not respond to STR. In this cohort, 637 patients achieved RVR whereas 575 patients had revealed an EVR. Individuals with EVR having higher compliance in their treatment (83% and above) showed an SVR of 68.5%. Group with RVR and higher compliance in their treatment had an SVR of 96%. The data suggests that individuals in compliant with their treatment having higher RVR significantly influence SVR towards better remission. Such individuals can be treated with short duration with standard of care treatment; whereas patients achieving a partial EVR have lower rates of SVR (58%) need prolonged treatment of 48 weeks. There was no gender bias in treatment outcomes but in females EVR was significantly correlated with early normalization of ALT. Our research data strongly suggest importance of RVR and EVR in

deciding the treatment outcomes and duration of treatment with INF and ribavirin among individuals chronically infected with HCV.

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#### The NF-KappaB-Inhibitor SC75741 Efficiently Blocks Influenza Virus Propagation *In Vitro* and *In Vivo* Without the Tendency to Induce Resistant Virus Variant

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Influenza remains a formidable foe throughout the world. The appearance of pandemic H1N1 and highly pathogenic avian H5N1 viruses in humans and the emergence of resistant seasonal H1N1 variants against neuraminidase inhibitors highlight the need for new and amply available antiviral drugs. We and others have demonstrated that influenza virus misuses the cellular IKK/NF-kappaB signalling pathway for efficient replication suggesting that this module may be a suitable target for antiviral intervention. Here we show that the novel NF-kappaB inhibitor SC75741 efficiently blocks replication of influenza A and B viruses, including avian and human A/H5N1 isolates *in vitro* in concentrations that do not affect cell viability or metabolism. In a mouse infection model with highly pathogenic avian influenza viruses A/H5N1 and A/H7N7, we were able to demonstrate reduced clinical symptoms, and survival of SC75741 treated mice. Moreover, influenza virus was reduced in the lung of drug-treated animals. Besides this direct antiviral effect the drug also suppresses H5N1-induced overproduction of cytokines and chemokines in the lung, suggesting that it might prevent hypercytokinemia that is discussed to be associated with pathogenesis after infections with highly pathogenic influenza viruses, such as the A/H5N1 strains. Most importantly the drug did not show 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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#### Trypsin Digestion of Hepatitis C Virus NS5B Polymerase Exposes a Hinge at the Active Site

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Tryptic protease digestion of the hepatitis C virus RNA-dependent RNA polymerase NS5B exposes the effects of inhibitors, RNA template, RNA template/primer, and NTP binding on enzyme conformation. In the absence of inhibitors or substrates, all regions of NS5B are equally resistant to protease treatment and no definitive cleavage products form. Binding of an inhibitor to the thumb-finger site defined by proline 495 resistance substitutions (P495 site) induces a change in NS5B conformation and the formation of a specific trypsin cleavage product. Edman sequencing of the product revealed the trypsin cleavage site is adjacent to the active site in NS5B. A similar pattern of trypsin cleavage was detected for NS5B in the presence of RNA template, but NS5B in the presence of template/primer or in the presence of high concentrations of NTP was more resistant to trypsin cleavage than