In vitro combinations of ANA598 with Interferon-α, 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.

doi:10.1016/j.antiviral.2010.02.397

88

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

Benjamin Petsch[1,*], Clarence R. Hurt[2], Beverly Freeman[2], Elisabeth Zirdum[1], Anupama Ganesh[2], Alexandra Schörg[1], Anatoliy Kitaygorodskyy[2], Yoko Marwidi[2], Olivier Ducoudret[2], Colm Kelleher[2], William Hansen[2], Vishwanath R. Lingappa[2], Christian Essrich[2], Lothar Stitz[1]

[1] *Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Immunology, Tübingen, Germany;* [2] *Prosetta Bioconformatics, San Francisco, USA*

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 $EC_{50} < 20\ \mu M$. 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 $EC_{50} = 10$ nM, $EC_{99} = 100$ nM with a CC_{50} of 2.5–10 μM. 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.

doi:10.1016/j.antiviral.2010.02.398

89

Novel Imino Sugars Potently Inhibit HCV Virion Secretion by Targeting Cellular Endoplasmic Reticulum α-Glucosidases

Xiaowang Qu[1,*], Xiaoben Pan[1], Jessica Weidner[1], wenquan Yu[2], Michael Xu[2,3], Timothy Block[1,2], Ju-Tao Guo[1], Jinhong Chang[1]

[1] *Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, USA;* [2] *Institute for Hepatitis and Virus Research, Hepatitis B Foundation, Doylestown, USA;* [3] *Enantigen Therpeutics, Inc., Doylestown, USA*

Imino sugars, such as deoxynojirimycin (DNJ), are glucose mimetics that competitively inhibit endoplasmic reticulum α-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_{50} values of 25 and 5 μM, respectively. Consistent with the inhibition of α-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 μM 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].

doi:10.1016/j.antiviral.2010.02.399

90

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

S. Rak[1,*], O. Pyankova[1], A. Chinarev[2], A. Tuzikov[2], N. Bovin[2], A. Agafonov[1], O. Demina[1], A. Ryzhikov[1]

[1] *FSRI SRC VB Vector, Koltsovo, Russia;* [2] *Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia*

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