GNA, NPA and CA; the procaryotic cyanovirin-N (CV-N)) and the GlcNAc-specific (i.e. the plant lectin UDA) CBAs efficiently abrogate the DC-SIGN-directed HIV-1 capture and subsequent transmission to T-lymphocytes. The aim of our study is to demonstrate the ability of CBAs to inhibit HIV-1 capture in M/M, and subsequent virus transmission to $CD4^+$ T-lymphocytes. Our results show that CBAs efficiently prevent the capture of a variety of HIV-1 laboratory strains and isolates, and HIV-2 in human primary M/M cultures. Moreover, we observed that pre-exposure of HIV-1 to CBAs is able to prevent syncytia formation in co-cultures of CD4+ T-lymphocyte C8166 cells and CBA-exposed HIV-1 infected M/M. Thus CBAs can efficiently target the glycans of HIV, blocking the virus-cell interaction and preventing the transmission of the virus from M/M to $CD4^+$ T lymphocytes. The potential of CBAs to impair M/M in their capacity of capture and transmission of HIV to T-lymphocytes might be an important property to be taken into consideration in the eventual choice to select microbicide candidate drugs to the clinical setting. For these reasons, CBAs represent promising compounds able to compromise the infectivity and transmission of HIV by M/M.

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Generation and Characterization of Fully Human Antibodies Against Orthopoxviruses

Tatynana Yun[1,*], Nina Tikunova[1], Ludmila Shingarova[2]

[1] FSRI SRC VB "Vector"; [2] Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry

The genus Orthopxviruses includes several species of well-known pathogens, e.g. variola, vaccinia, cowpox and monkeypox viruses. Reemergence of monkeypox as a serious human disease in Africa have fueled renewed interest in orthopoxviruses. Vaccinia virus (VACV) was used in the past as an effective vaccine against smallpox. Although, VACV is generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such complications can be treated by therapeutic administrations of human VACV immunoglobulin (VIG). However, their limitations include lot-to-lot variation, low content of specific antibodies and potential contamination by infectious agents. Recombinant fully human antibodies offer an obvious alternative to VIG and human antibodies from the traditional hybridomas technology.

Specific single-chain phage antibodies were selected from the synthetic phage display library of human scFvs antibodies biopanning procedure against VACV, strain Elstree. Positive clones were characterized and sequenced. One of the most promising scFv—1F4 was used for creation of fully human antibody. To generate this antibody the V genes from the 1F4 scFv were amplified by olgonucleotides specific for V genes with extensions including restriction-enzyme cleavage sites for cloning into modified pcDNA eukaryotic expression vectors carrying constant domains of human IgG1 for H-chain and L-chain correspondingly. The 293T human cells have been co-transfected with these constructs using Lipofectamine 2000 reagent. Fully human 1F4 antibody (fh 1F4) was purified from culture supernatant by affine chromatography.

Immunochemical properties of fh 1F4 obtained have been assayed by ELISA and Western-blot analysis. Specificities of the fh 1F4 were tested by ELISA using different orthopoxviruses such as VACV, cowpox virus, Ectromelia virus. Binding activity of the fh1F4 was assyed using subsequent dilutions of antigens and antibodies in ELISA, and affinity constant was calculated and compared with parental scFv. The fh 1F4 affinity constant was determined as $1.3 \times 109\,M^{-1}$, approximately 100 times more, than for the parental scFv. The fh Ab 1F4 did not neutralize vaccinia virus as a parental scFv.

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Susceptibility of German Porcine H3N2 Influenza A viruses Against Existing Antiviral Drugs

Katja Bauer[1,*], Christina Schrader[2], Jochen Suess[3], Peter Wutzler[1], Michaela Schmidtke[1]

[1] Institute of Virology and Antiviral Therapy, FSU, Jena, Germany; [2] Federal Institute for Risk Assessment, Berlin, Germany; [3] Friedrich Loeffler Institute, Jena, Germany

Influenza A viruses (FLUAV) of subtype H3N2 are circulating in the European human population as well as in pigs. As a "mixing vessel" of avian and human FLUAV pigs may contribute to interspecies virus transmission and reassortment of viral genes including those responsible for antiviral susceptibility. During this study, the susceptibility of selected porcine H3N2 FLUAV isolated in Germany between 1982 and 1999 against: (a) the M2 ion channel blocker amantadine and (b) the neuraminidase inhibitors (NAI) oseltamivir and zanamivir was examined. Plaque reduction assay was performed to examine the amantadine phenotype. The NAI susceptibility phenotype was determined in enzyme- and cell culture-based inhibition assays. Genotypes were examined by sequencing the viral matrix protein (M), hemagglutinin (HA) and neuraminidase (NA) genes. Additionally, agglutinating properties of these viruses were compared.

In the result of antiviral studies, only two of seven isolates were shown to be amantadine-susceptible. The amino acid substitution S31N in viral M2 protein, known to confer amantadine resistance, was found in all resistant virus strains. In neuraminidase enzyme-inhibition assays all isolates were susceptible against oseltamivir and zanamivir. Both compounds inhibited virus spreading, reduced the virus yields as well as plaque size at nanomolar concentrations. But, much higher drug concentrations are necessary to achieve reduction in plaque number.

Genotyping revealed several substitutions in the NA and HA proteins including substitutions that were suggested to affect NAI susceptibility. However, neither R249K in NA nor T155Y and Q226L in HA impaired NAI susceptibility. Two isolates that

differ in glycosylation pattern of viral HA possessed markedly reduced drug susceptibility against NAI in cell culture based assays.

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Activation of GS-7340 and Other Tenofovir Phosphonoamidate Prodrugs by Human Proteases

Gabriel Birkus*, Nilima Kutty, Gong-Xin He, Andrew Mulato, William Lee, Martin McDermott, Tomas Cihlar

Gilead Science, 333 Lakeside Drive, Foster City, CA 94404, USA

GS-7340 is an isopropylalaninyl phenyl ester prodrug of a nucleotide HIV reverse transcriptase inhibitor tenofovir (TFV; 9-[(2-phosphonomethoxy)propyl]adenine) exhibiting potent anti-HIV activity and enhanced ability to deliver parent TFV into peripheral blood mononuclear cells (PBMCs) in vivo. The present study focuses on the intracellular metabolism of GS-7340 and its activation by a variety of cellular hydrolytic enzymes. Incubation of human PBMCs in the presence of GS-7340 indicate that the prodrug is more efficiently hydrolyzed to an intermediate TFV-alanine conjugate (Met X) in quiescent PBMCs compared to activated cells. In contrast, the conversion of Met X to TFV and subsequent phosphorylation to TFV-diphosphate occur more rapidly in activated PBMCs. The activity of GS-7340 hydrolase producing Met X in PBMCs is primarily localized to lysosomes and is sensitive to inhibitors of serine hydrolases. Cathepsin A, a lysosomal serine protease has recently been identified as the primary enzyme activating GS-7340 in human PBMCs. Result from the present study indicate that in addition to cathepsin A, a variety of serine and cysteine proteases cleave GS-7340 and other phosphonoamidate prodrugs of TFV. The substrate preferences displayed by the tested proteases towards a series of TFV amidate prodrugs is nearly identical to their relative activities displayed against peptide substrates, indicating that GS-7340 and other amidate derivatives can be considered peptidomimetic prodrugs of TFV.

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NIM811, A Cyclophilin Inhibitor, and NM107, An HCV Polymerase Inhibitor, Synergistically Inhibits HCV Replication and Suppresses the Emergence of Resistance In Vitro

Joanna Boerner*, Sue Ma, Teresa Compton, Kai Lin

Novartis Institutes for Biomedical Research, 500 Technology Sq., Cambridge, MA 02139, USA

More effective and better tolerated therapies are needed for chronic hepatitis C which affects 170 million people worldwide. Because of the high heterogeneity and mutation rate of hepatitis C virus (HCV), future therapies are likely to consist of multiple drugs to maximize antiviral efficacy and to prevent resistance. We are taking a two-prong approach to develop novel therapeutic agents for HCV. The first strategy is to target viral proteins such as the NS5B RNA polymerase directly. One such inhibitor, NM283 (valopicitabine), is currently in Phase II clinical trials. The second and a complementary strategy is to target host factors that are also essential for viral replication. NIM811, a cyclophilin inhibitor with potent in vitro antiviral activities, represents such an approach and is under clinical investigation in HCV patients. Here, the combination of NIM811 and NM107 (the active moiety of NM283) was evaluated in vitro using the HCV replicon model as the first step to explore the possibility of using such a combination in patients. HCV replicon cells were treated with various concentrations of the two compounds either alone or in combination. There was a concentration- and time-dependent inhibition of HCV replicon with NIM811 and/or NM107. Importantly, the combination always led to a stronger antiviral effect than either agent alone with no significant increase of cytotoxicity. Moreover, the effect of combination was determined to be synergistic as analyzed in a mathematic model. In addition, drug-resistant clones were generated, and there was no cross-resistance between these two inhibitors of different mechanisms. Furthermore, the frequencies of resistance were determined with the compounds at various concentrations. The barrier to resistance was greatly increased when NIM811 and NM107 were used in combination. In summary, these in vitro results illustrate the significant advantages of combination therapies and warrant exploration of this specific combination in further studies.

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Identification of Novel Low Molecular Weight HIV-1 gp41 Fusion Inhibitors Using A New Quantitative High Throughput Fluorescence Intensity Assay

Miriam Gochin [1,2,*], Lifeng Cai [1]

[1] Department Basic Sciences, Touro University—California, Vallejo, CA 94592, USA; [2] Department Pharmaceutical Chemistry, UCSF, San Francisco, CA 94143, USA

A new high throughput screening assay for HIV-1 gp41 inhibitors has yielded novel low molecular weight fusion inhibitors from a peptidomimetic library. Both the assay and the new compounds are described. The assay is performed by mixing two designed peptides with compounds arrayed in multi-well plates, and measuring fluorescence intensity. It can be readily applied to screen large chemical databases for identification of HIV-1 fusion inhibitors. Inhibitors can be detected quantitatively from the assay in three simple steps: (1) a high throughput screen to identify possible positive hits by fluorescence intensity enhancement; (2) a control high throughput screen to eliminate false positives; (3) serial dilution of true positive hits to obtain high throughput dose–response curves for determination of inhibition constants (K_i). The HTS assay has a Z' factor of 0.88 and can rank order inhibitors at 10 μM concentration with K_i's in the range 0.2–30 μM, an ideal range for drug discovery. The assay was validated using known gp41 inhibitors. The applicability