resulted in tumor growth suppression. EBNA1 plays a key role in EBV's gene transcription and since EBV is maintained in the nucleus of the infected cell, EBNA1 has access to host genomic DNA, as well. Therefore, we examined whether it may bind to cellular sequences. Here we report testing this hypothesis in EBV-free cell lines which are engineered to express EBNA1. Initially, microarray analysis demonstrated changes in gene expression as a reflection of EBNA1's expression. Subsequently, an anti-EBNA1 antibody was used to produce chromatin immunoprecipitation DNA from these cell lines. These DNA samples were hybridized to human promoter arrays to reveal that host genomic sites had been complexed with EBNA1. These genomic binding sites consisted of coding and non-coding sequences from both DNA strands. We have managed to demonstrate the presence of the promoters for some of the genes depicted by the microarray expression analysis, and to confirm it by QR-PCR. Subsequently, we have identified an EBNA1 consensus sequence within the engaged cellular promoters. This sequence shows no similarity to the viral DNA binding sequence of EBNA1 or to any transcription factor. This dual transcription capacity improves the virus' ability to coexist within the infected cell by monitoring viral gene expression while actively and directly manipulating host gene expression. Our studies corroborate evidence of the crucial role EBNA1 plays in EBV etiology and neoplasm and suggest that a genomic approach is needed to monitor drug design directed to target EBNA1 as it is involved in various cellular gene expression changes. Currently, the lack of specific anti-EBV therapies generates adverse side effects for patients undergoing chemotherapy.

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## Oral Session 5: Respiratory Viruses, Emerging Viruses and Biodefense

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### Plenary: Filoviral Minigenome systems and iVLPs as tools for antiviral research

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### Identification and Mechanistic Studies on a Novel Class of Influenza Virus Fusion Inhibitors

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We here report on a new class of inhibitors of influenza virus hemagglutinin (HA)-mediated fusion, with a similar backbone structure as some reported fusion inhibitors, consisting of an aromatic cyclic system linked to a non-aromatic cyclic system via an amide bridge [Luo et al., Virology, 226:66–76 (1996)]. In Madin–Darby canine kidney (MDCK) cells infected with influenza virus A/H3N2 (X-31), the 50% effective concentration of the lead compound [4M] was 3.4 μM, as determined by microscopic examination of the viral cytopathic effect and MTS cell viability assay. The concentration producing 50% inhibition of cell proliferation was 89 μM. Similar activity was seen for other A/H3N2 strains, whereas no activity was noted for influenza A/H1N1 and B viruses. At 20 μM [4M], virus yield was reduced by 3 logs. In time-of-addition studies, [4M] lost activity when added 1 h or later post-infection, showing that [4M] inhibits an early step in virus replication. Definite proof for HA-mediated fusion as the antiviral target was provided by the inhibitory effect of [4M] on virus-induced red blood cell hemolysis at low pH. [4M]-resistant mutants, selected after three passages in MDCK cells in the presence of 20–150 μM [4M], were plaque-purified and sequenced. The two main amino acid substitutions associated with [4M] resistance were R220S and E57K, located in the HA1 and HA2 domain, respectively. This suggests that [4M] may bind to the same pocket of the influenza virus HA as the structurally unrelated H3-specific fusion inhibitor tert-butyl hydroquinone [Russell et al., PNAS, 105:17736–41 (2008)]. The mutants showed uncompromised fitness, with similar efficiency for binding and replication as wild-type virus. However, in the hemolysis assay, the mutants displayed an increased fusion pH. Molecular modelling of [4M] within the HA structure will help to design new [4M] derivatives with improved activity. Also, the activity against other virus subtypes (e.g. A/H5N1 and A/H7N7) is under investigation.

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### Novel Broad-Spectrum Biopharmaceuticals: From HIV-1 to Pandemic Influenza A Virus

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Recently, my laboratory has reported the identification of the most potent endogenous furin-directed inhibitor, Spn4A [$K_i$: 13 pM (Richer M. et al. PNAS 2004)]. Because of our previous success with the bio-engineered serpin $\alpha_1$-PDX [$K_i$; 0.6 nM; (Jean F. et al. PNAS 1998)] as a protein-based therapeutic (Jean F. et al. PNAS 2000), we hypothesized that strategic manipulation of the furin-like cellular subtilase levels by Spn4A and Spn4A-engineered variants might provide a means of effectively inhibiting the subtilase-dependent proteolytic cleavage of viral envelope precursor glycoproteins in the host secretory pathway, a critical cellular event required for production of infectious progeny (e.g., HIV-1, highly pathogenic H5N1 influenza A virus, West Nile virus, Dengue virus).

In this study, we report our recent original work in the exciting field of protein-based inhibitors as broad-spectrum biopharmaceuticals. First, we describe the anti-proteolytic activities and anti-HIV properties of our novel recombinant adenovirus (Ad−) expressing Spn4A variants (Ad-Spn4A) in the host cell secretory pathway. We demonstrated that expression of Ad-Spn4A in MAGI-CCR5 cells completely inhibited the subtilase-dependent processing of the HIV-1 envelope precursor gp160 and resulted in a complete reduction of productive HIV-1 infection as determined by HIV-1 Tat-driven β-galactosidase activity and syncytia formation assays. Second, we demonstrated that our novel Ad-Spn4A variants also completely block the subtilase-mediated cleavage of the hemagglutinin H5 encoded by pandemic influenza A viruses (HK/97) and resulted in a complete block of syncytia formation in human A549 epithelial cells. The detailed cellular mechanism of action of our novel serpin-based antiviral strategy and the impact of our findings for developing a novel generation of broad-spectrum protein-based

biopharmaceuticals for antiviral therapeutic intervention are discussed.

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## Recruitment of the TSG101/ESCRT-I Machinery in Host Cells by Influenza Virus: Implications for Broad-Spectrum Therapy

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Many different viruses recruit or hijack normal host cells processes to facilitate viral propagation. Prominent among these mechanisms is the recruitment of Tsg101 and other components of the ESCRT-I machinery. Tsg101 is normally a resident to the cytoplasm, where it mediates intracellular transport. Upon infection with certain viruses that bud at the plasma membrane Tsg101 redistributes to the cell membrane where it plays and essential role in viral budding and release. Using flow cytometry we provide novel evidence that Tsg101 is uniquely exposed on the surface of cells infected with multiple and different strains of seasonal and pandemic influenza viruses. Using flow cytometry analyses, Tsg101-specific antibodies detected selective exposure of Tsg101 on the surface of infected cells, but not matched controls. The relocalization of Tsg101 to the plasma membrane correlates with the time course of viral release. Importantly, the comparable findings were obtained in different cell types and with multiple and different strains of influenza virus, including both seasonal (H3N2, H1N1, H2N2) and pandemic (H5N1) strains. We have also demonstrated that Tsg101 monoclonal antibodies reduced viral release from infected cells, suggesting an essential role for Tsg101 in the viral life cycle and provide an opportunity for therapeutic intervention. The finding of a cellular factor involved in budding not only increases our understanding of influenza virus by may provide opportunities to develop a broad spectrum measures to prevent or treat influenza virus infection.

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## Role of NA mutations Conferring Resistance to NA Inhibitors on Viral Fitness and Pathogenicity in A/Turkey/15/06 (H5N1) Influenza Virus

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Highly pathogenic H5N1 influenza viruses of clade 2 were recently found to be 15–30 times less susceptible than clade 1 viruses to neuraminidase (NA) inhibitor oseltamivir carboxylate *in vitro*. The molecular basis of their decreased sensitivity is poorly defined. In this study, we evaluated the role of NA residues at the active site in altering the susceptibility of clade 2.2 H5N1 virus to oseltamivir carboxylate. We used reverse-genetics technique to generate recombinant A/Turkey/15/06-like (H5N1) viruses carrying various NA mutations (V116A, I117V, E119A, K150N, Y252H, H274Y, and N294S) and investigated their susceptibility profiles to NA inhibitors, NA enzyme kinetic, viability, genetic stability and viral pathogenesis. NA enzyme inhibition assay showed that most of the NA mutations resulted in the resistance to oseltamivir carboxylate ($IC_{50}$s decrease, from 7- to 1212-fold), whereas resistance to zanamivir was found only with substitutions at V116A and E119A residues ($IC_{50}$s decrease, >30-fold and >1900-fold, respectively). In contrast, Y252H NA change contributed for increased susceptibility of H5N1 virus for oseltamivir carboxylate ($IC_{50}$ increase, 16-fold). All recombinant A/Turkey/15/06-like (H5N1) viruses demonstrated viable and genetically stable phenotype in MDCK cells. Enzyme kinetic parameters ($V_{max}$, $K_m$ and $K_i$) of avian-like NA glycoproteins correlated with their $IC_{50}$s data. The pathogenesis of A/Turkey/15/06-like (H5N1) viruses varied in a ferret model and was dependent on the location of NA mutation. Our results suggest that highly pathogenic H5N1 variants carrying mutations within or near the NA active site have decreased susceptibility to NA inhibitors and retain viral fitness in mammalian species. Although the clinical relevance of a ~10-fold decrease in the susceptibility to oseltamivir carboxylate *in vitro* is currently unresolved, the possibility that their *in vivo* susceptibility to anti-NA drugs could be decreased cannot excluded. Our results highlight the significance of continued characterization of all H5N1 isolates for their susceptibility to NA inhibitors.

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## In Vivo Efficacy Evaluation of Vaccines Against H5N1 Influenza Virus

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Various pharmaceutical and research organizations are developing vaccines against H5N1 influenza virus. A major process in the vaccine development before clinical trials is the animal model evaluation of the vaccine. This paper describes efficacy of a few of these vaccines against A/VN/1203/04 and A/Indo/05/05 in ferret and mice animal models. The strains of H5N1 were amplified in embroynated eggs and characterized for lethal dose in mice and ferrets. Various prophalytic and therapeutic drugs and vaccines were tested for their efficacy against challenge by 10MLD50 or 10FLD50 of the virus strains in mice and ferrets, respectively. The results of the efficacy studies are discussed with respect to weight and temperature changes, clinical signs, immunological responses and viral load in tissues. Kaplan–Meier survival curves are compared. The H5N1 virus like particles (VLP) vaccine protected ferrets following lethal challenge with wild type H5N1 viruses. The immunized ferrets shed less virus from nasal washes collected post-challenge. The study data indicates that other immunological measures besides broadly accepted HAI and neutralizing antibody responses could be important in predicting immunity against H5N1 virus illness. A vaccine which was developed by incorporating multiple antigens from both avian and Spanish influenza viruses into complex recombinant adenovirus vectors was also found to induce protection against lethal A/VN/1203/04 and A/Indo/05/05 virus challenge. Unvaccinated control animals were hypoactive and showed significant weight loss as compared to the vaccinated animals. Vaccine based upon inactivated virus yielded no weight loss or temperature change in ferrets. Viral load in nasal wash and lung samples was high in non-vaccinated controls but were below limit of detection in vaccinated groups. Monoclonal antibodies were also tested for their efficacy. Non-infectious HA proteins with or without adjuvant were tested in mice and showed positive results. Various vaccines efficacy variables are provided in tabulated format. The studies give the general directions in which the ongoing development of vaccines should be concentrated to yield quick and positive results. The efficacy studies show promising vaccine candidates which can act against the avian influenza.

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