NIAID Influenza Antiviral Development Workshop: New Generation

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National Institutes of Allergy and Infectious Diseases (NIAID),
National Institutes of Health (NIH),
Department of Health and Human Services (DHHS)
and
Biomedical Advanced Research and Development Authority (BARDA),
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Welcome & Opening Remarks

Dr. Carole Heilman, Director, Division of Microbiology and Infectious Diseases (DMID), NIAID, welcomed the participants on behalf of Dr. Anthony S. Fauci, Director, NIAID, who was testifying before Congress.

Dr. Heilman provided an overview of influenza virus research and disease. The annual burden of seasonal influenza is 250,000 to 500,000 deaths globally. Annually, in the United States, there are 36,000 deaths and more than 200,000 hospitalizations with an economic cost of $37.5 billion related to influenza and pneumonia.

She noted that the influenza field has evolved into two sub fields: re-emerging disease (seasonal flu) and newly emerging disease (potential pandemic flu). It is important to recognize that knowledge derived from research in each of these areas informs the other area.

NIAID has always had a strong research effort in influenza and with the emergence of H5N1 viruses there has been a considerable increase in NIAID funding in this area. More than 50% of the NIAID influenza research effort is in the area of vaccines; there is also support for diagnostic and antiviral research as well as for a basic research program that informs the more applied efforts.

The currently available influenza antivirals have a number of limitations. There are only four licensed antivirals, two that are targeted to M2 (amantadine and rimantadine, which are delivered orally [PO]) and two that are targeted to the neuraminidase (oseltamivir (PO) and zanamivir [inhaled]). Additional agents that are at the investigational stage include one targeted to the RNA polymerase (T705 [PO]) and two others targeted to the neuraminidase (peramivir [intravenous] and CS-8958[inhaled]). There is limited data on the use of antivirals in vulnerable populations or in severe disease. Manufacturing of the antiviral products is complex, multiple doses are often required, and there are no licensed parenteral agents. Importantly, resistance has been observed to the licensed products.

Dr. Heilman mentioned some of the ongoing NIAID efforts in influenza drug development. These include combination studies; studies of the safety of oseltamivir in infants; studies of parenteral agents, of long-acting neuraminidase inhibitors, of broad-spectrum antivirals, of antibody therapeutics; evaluation of novel drug targets; and investigation of the antiviral effects of activating the innate immune system. She stated that the major challenges of antiviral development include: optimizing the dose and administration

schedules for vulnerable populations and for combination therapies; developing strategies to prevent and treat resistant viruses; developing more powerful, broadly active agents; lowering development costs; and developing longer acting, single-dose strategies.

Dr. Heilman concluded by indicating that the objectives of the workshop are to review the state of the science including: the use of antivirals in vulnerable populations, new approaches and technologies, strategies that target the host response to infection, and combination studies. It is anticipated that the group members will learn from one another, exchange information and ideas and challenge current paradigms.

Dr. Linda Lambert, Chief, Respiratory Diseases Branch (RDB), DMID, NIAID also welcomed the participants and indicated NIH's pleasure at hosting the meeting. She thanked the organizing committee for their efforts in assembling the impressive program. The organizers included Dr.Larisa Gubareva at the Centers for Disease Control and Prevention (CDC), Dr. Barbara Styrt at the U.S. Food and Drug Agency (FDA) and Drs. Amy Krafft, Helen Schiltz and Heather Greenstone at NIH.

Dr. Lambert indicated that a summary of the meeting would be prepared.

1. PRE-CLINICAL SESSION: APPROACHES TO NEW DRUG TARGET DISCOVERY AND MECHANISMS OF ACTION

Virulence determinants of the 1918 H1N1 and the H5N1 flu A viruses

Dr. Peter Palese, Mt. Sinai School of Medicine, New York, New York, described efforts to understand the virulence determinants of influenza viruses with a focus on the properties of the 1918 pandemic virus. Using a reverse genetics system, it was possible to make reassortants containing single genes from the 1918 H1N1 pandemic influenza virus and from H1N1 human Texas/91 virus and to identify genes important to virulence in animal models. Using this approach, PB1, HA and NA were shown to play a critical role in virus virulence in ferrets, mice and chick embryos. The PB1-F2 protein has been shown to induce apoptosis and immune cells and interacts with mitochondrial VDAC proteins. A single amino acid change in PB1-F2, S66N, has been shown to cause increased virulence and is present in the 1918 H1N1 pandemic virus and in the H5N1 virus from the HK/97 outbreak. PB1-F2 proteins with an S at position 66 cause increase cytokine levels in the lungs of infected mice, potentially accounting for the cytokine dysregulation seen in mouse and human infections.

Dr. Palese also addressed the concept of using transmission in a guinea pig as a model as a way to measure influenza virus virulence. A 1919 paper had reported that guinea pigs present at Camp Cody during the pandemic had died, suggesting that they might be used as a model system. A transmission cage apparatus was constructed which allowed for infected and uninfected guinea pigs to be placed in side-by-side cages in an environmental chamber in which humidity and temperature could be adjusted. Aerosol transmission of influenza virus was found to be most effective at low temperature and low relative humidity; this could be a partial explanation for the seasonality of influenza infection.

Studies were undertaken to determine if neuraminidase inhibitor-resistant viruses behaved differently in the guinea pig model system. Recombinant oseltamivir-resistant viruses were constructed in the A/Panama/2007/1999(H3N2) background. These viruses have a small-plaque phenotype that can be reversed with exogenous bacterial neuraminidase. Both resistant and sensitive viruses have similar in ovo growth rates. When tested in the guinea pig transmission model, the oseltamivir-resistant viruses did not transmit by aerosol at 20 ^{0}C/ 20% relative humidity. In studies of contact transmission, in which the infected and the naïve guinea pig were placed in the same cage, the oseltamivir-resistant viruses were

efficiently transmitted. This year's N1 clinical isolates were obtained from the NY State Department of Health and are were determined to be Tamiflu (oseltamivir)-resistant.

Studies in the guinea pig model also suggest that N1 viruses are less readily transmitted by aerosol than N2 viruses; however, they are transmitted by contact. Additionally, aerosol transmission was not seen in the guinea pig model when WT rVN 1203 H5N1 virus was tested.

During the discussion, it was noted it is not clear why the guinea pigs at Fort Cody had died. These animals might have been infected with a different viral strain from that used in the recent studies; they might have been fed a different diet or they may have had some co-morbid infections.

Systems biology approaches to flu infection

Dr. Ari Helenius' work at the, Swiss Federal Institute of Technology, ETH Zurich, Switzerland, has focused on the cell biology of virus infection. Dr. Helenius noted that there are complex interactions between the virus and host cell during the process that spans from virus entry to the completion of virus infection. Viruses clearly understand the language of cells and activate complex signaling pathways and host responses. He has used influenza to understand this process with the goal of studying the infectome, that is, the host genes critical for infection. These types of studies are feasible given that the human genome is available, siRNA silencing can be done to validate findings and high throughput technologies can be used to look at the various steps in virus replication. Dr. Helenius is using an assay that looks at NP expression (using indirect immunofluorescence and anti-NP antibody), and thus, focuses on the early steps in virus replication. The studies are using the H3N2 virus and the 7000 "druggable genes" for which there are siRNAs. The automated high throughput siRNA silencing screen includes a control for toxicity. Of the 7000 potential druggable genes, there have been about 270 hits; these hits fall into every functional category, e.g., receptor, kinase, ion channel, nuclear receptor, etc. The data are entered in to a bioinformatics program which searches the literature for evidence of protein-protein interactions. Using this approach, one can show clusters of factors, e.g., kinases, splicing, transcription factors.

Dr. Helenius noted that the critical issues in interpreting the results include: toxicity, off-target effects and false negatives; cell type differences; strain differences; differences in siRNA libraries; assay design differences (e.g. infection, full cycle, time of transfection.) To avoid errors in interpretation, there are: validation studies; screens for toxicity, subscreens to e.g., explore cell lines and virus strain effects; alternative techniques, such as, dominant negative constructs; parallel chemical screens; and comparative analysis with other infectomes.

Dr. Helenius showed examples of the types of data that are found including molecular signatures for functional molecules, e.g. those needed for virus entry. It is also possible to examine convergent adaptation which appears to be fundamentally different for enveloped versus non-enveloped viruses.

Dr. Helenius briefly described the process of influenza virus entry into cells and cited recent findings reported at a meeting in Germany which suggested that influenza virus uses the EGF receptor.

He also described the process of reducing the number of hits so as to make investigations of inhibitors and mechanisms feasible. Dr. Curnette's presentation later in the meeting focused on this issue.

Dr. Helenius summarized by noting that infection opens a window into the cell and provides a list of critical host factors; it reveals new molecular mechanisms; identifies overlaps between viruses (e.g. between viruses which infect the airways) which could lead to general/broad spectrum antivirals; it allows for functional classification of pathogens; it opens new approaches for perturbing infection and provides new targets for antiviral strategies.

During the discussion it was noted that about 17% of the hits are listed as receptors; these may be molecules that are on the cell surface, but which do not act as receptors. Alternatively, it is also possible that there is a redundancy in viral receptors.

Potential of sphingosine analogs to dampen the cytokine response during influenza virus infection

Dr. Hugh Rosen, The Scripps Research Institute, La Jolla, California, focused on the issue of whether the host immune response to influenza could be modulated so as to reduce the immunopathological response to influenza virus infection which contributes to the morbidity and mortality associated with influenza. His investigations focused on the sphingosine 1-phosphate (S1P) system which regulates the immune system at the level of T cells, dendritic cells (DCs) and endothelium. The S1P immunoregulatory axis includes $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$, each of which interacts with different cell types and/or organs. There are a variety of synthetic sphingosine analogs which have different targets and which can be used to dissect the basis of the immunopathological effects of influenza virus. Dr. Rosen described studies in which the chiral sphingosine analog (*R*)-2-amino-4-(4-heptyloxyphenyl)-2-methylbutanol (AAL-R) was used in a FLU-LCMV mouse model system. Intra-tracheal (IT) delivery of AAL-R was shown to inhibit the accumulation of virus-specific CD8+ T cells in lungs 6 days post infection. Further experiments showed that the AAL-R effect on the T cell response is local and is not a function of altering lymphocyte recirculation. AAL-R is rapidly phosphorylated to AAL(R)-phosphate (AFD(R)) in the lungs following IT delivery. Phosphorylation of AAL-R is needed for it to be effective in suppressing the T cell response. Immunosuppression is mediated through the activation of S1P receptors in the lungs.

Further studies of the impact of S1P receptor modulation showed that AAL-R reduces cytokine release and reduced lung leukocyte accumulation in AAL-R-treated mice. AAL-R treatment did not impair influenza-specific antibody production by infected mice nor did it affect class switching. In order to explore the immunological processes that were being modulated, imaging studies were undertaken. It was found that AAL-R inhibits dendritic cell (DC) activation in the lungs, inhibits the accumulation and activation of DC in the draining lymph node, and inhibits the clonal expansion of virus-specific CD8+ cells in the draining lymph nodes. Adoptive transfer of infected DCs that were treated *in vitro* with AAL-R hampered the T cell expansion ,*in vivo* again suggesting that AAL-R inhibits DC activation in the lungs. It is known that $S1P_2$ is not involved in these effects and additional studies suggested that $S1P_1$ and $S1P_3$ are also not involved. Thus, either S1P4 or $S1P_5$ may mediate these effects. Dr. Rosen indicated that further studies are needed to determine the potential for sphingosine analogs to complementing anti-viral chemotherapeutics with the goal of reducing the morbidity and mortality of influenza virus infection.

During the discussion, Dr. Rosen indicated that the effects described in his presentation are seen in all mouse strains and are not antiviral effects, but rather modulation of the host response.

Structural and functional basis for broad-spectrum neutralization of avian and human influenza A viruses

Dr. Wayne A. Marasco, Dana Farber Cancer Institute, Boston, Massachusetts, noted that control of influenza infection by vaccines has been hampered because of the emergence of antigenic variants. The vaccine antigens need to be updated annually and this choice is based on global influenza surveillance of circulating virus antigens. Unfortunately, these efforts are not always successful. Vaccines are based on induction of neutralizing antibodies which interfere with viral functions, such as viral entry, conformational changes needed for viral protein structure, etc. About 50% of anti-influenza antibodies are to the HA and are directed to the globular head of the HA. Dr. Marasco reviewed the phylogenetic relationships between different HAs of different influenza subtypes. He discussed the possibility for anti-

influenza monoclonal antibodies to be used in an outbreak situation for prophylaxis and acute treatment. He noted that the scientific barriers that have delayed the commercial development of neutralizing human antiviral monoclonal therapies include the antigenic variability of circulating strains and the ability of the virus to undergo genetic changes so as to escape neutralization. He reviewed the status of H5N1 influenza in humans and birds, and the particular epidemiological and medical issues associated with H5N1 influenza.

Dr. Marasco noted that the concept of passive immunotherapy with convalescent serum is an old one: passive immunotherapy was undertaken in 1907 with measles convalescent serum. During the SARS epidemic, his group developed a process for the rapid development and assessment of human scFv-phage display libraries to antigens of interest. Using this process, he was able to identify monoclonal antibodies which neutralized all three clades of H5. These antibodies were derived from a non-H5 immune library and are high affinity. These antibodies demonstrated good levels of protection in a mouse model when given either prophylactically or therapeutically.

Studies of the mechanism of neutralization showed that these antibodies inhibited membrane fusion and syncytial formation. Importantly, they did not bind the globular head of the HA. Comparative studies of one of these monoclonal antibodies, F10, with C179, a mouse monoclonal derived in 1993, suggested that they both reacted in the highly conserved stem region of the HA which is distant from the globular head. Structural studies of F10 binding suggests that it locks the HA into the pre-fusion configuration. Because this conformational change is prevented, the virus cannot exit in the endosome. The epitope is made up of regions of HA2, HA1 and the fusion peptide. Examination of the public databases suggest that this region of the HA is highly conserved among Influenza A viruses. The antibody bound to and neutralized all members of group 1, but not of group 2 viruses. It appears that the binding pocket is highly conserved in groups 1 and 2 of Influenza A viruses, but that the orientation is different in group 2 viruses, and thus, the reaction is different. Dr. Marasco indicated that the Crucell antibody, CR6261, also seems to react with this pocket. The antibodies that Dr. Marasco has identified do not show auto-immune reactivity.

Dr. Marasco proposed that anti-viral monoclonal antibodies could have a role in biodefense and in viral outbreak settings. They could be used prophylactically for first responders, heath-care workers, in high-risk exposed families and co-workers, and in patients with co-morbid disease. Uses for such antibodies also include the very young, the elderly, pregnant women and persons with compromised immunity. Monoclonal antibodies could also be used in the treatment mode in conjunction with other anti-viral and anti-microbial agents in lower tract disease and when there is resistance to small molecular inhibitors in the circulating virus strains. Costs for these types of products are decreasing as both pharmaceutical and biotechnology companies are producing them from cellular factories and in-production facilities.

During the discussion, it was noted that the pocket found by Dr. Marasco's group is different from that found by Dr. John Skehel's group.

Novel anti-influenza inhibitors from natural product extracts

Dr. Barry O'Keefe, National Cancer Institute (NCI), NIH, Frederick, Maryland, addressed the potential to identify novel anti-influenza inhibitors from natural product extracts. He noted that greater than 60% of the new antimicrobials and anti-cancer drugs come from natural products. The NCI has the largest collection of natural products in the world (more than 150,000 extracts). In terms of infectious agents, the NCI library has mostly been screened against HIV.

Dr. O'Keefe discussed several compounds that had anti-influenza activity. Cyanovirin-N (CV-N) is an 11 kDa protein with 101 amino acids that was isolated from *Nostoc ellipsosporum*. It inactivates diverse strains of HIV-1 and HIV-2 and has a unique fold family of mostly β-sheets. When the anti-influenza activity of CV-N was tested against a variety of Influenza A and B strains, it showed antiviral activity

against a range of strains, with neuraminidase inhibitor-resistant strains seeming to be more sensitive to CV-N, and PR-8 virus being resistant to CV-N. CV-N was shown to bind to HA in an oligosaccharide-specific manner and bound selectively to HA from sensitive influenza strains, but not to the HA from resistant strains. Mouse-passaged virus was found to be resistant to CV-N. Further studies indicated that the sugars on the HA determine CV-N sensitivity and resistance. Mice challenged with Influenza A/WSN/33-HAnc (D225G (H1N1), an engineered virus that retains CV-N sensitivity, and treated intranasally with CV-N demonstrated survival against lethal challenge when the drug was given 4 hours prior to challenge; there were also protective effects, although lesser, when drug was given at 6 or 12 hours post challenge. Reduced virus titers were also found in ferrets treated intranasally prior to and then after infection.

CV-N treatment of nasal wash specimens from volunteers who were experimentally infected with A/Texas/91 showed that Influenza A viruses glycosylated by human host cells were highly susceptible to CV-N. Likewise, the infectivity of viruses from nasal wash specimens from patients diagnosed with Influenza A during the 1994-98 seasons was completely neutralized. Thus, there is the potential for administering this type of molecule with an inhaler to prevent spreading of the virus.

Griffithsin (GRFT) is a 12.7 kDa protein with 121 amino acids that is highly active against HIV-1. It binds to envelope glycoproteins in a carbohydrate-dependent manner and can be produced on a large scale in tobacco plants. This is a multivalent molecule with 6-binding sites per unit. It has anti-influenza virus activity against a number of Influenza A virus strains. It is well-tolerated intranasally and also interacts with HCV, SARS and Ebola.

In summary, CV-N and GRFT target the HA of Influenza A viruses. A high mannose oligosaccharide at a conserved residue (Asn 94) of the HA1 subunit is the primary target. These molecules show *in vitro* activity against H5N1 strains.

Influenza A NS1 protein structures and functions that defeat innate immunity: Targets for antivirals

Dr. Robert M Krug, University of Texas, Austin, Texas, described work targeting the Influenza A NS1 protein (NS1A) of Influenza A/Udorn/72. NS1 is a multi-functional dimeric protein that participates in both protein-RNA and protein-protein interactions. The RNA-binding domain binds to dsRNA and the effector domain binds to p85β (leading to the activation of P13K) signaling to PKR (inhibiting PKR activation). NS1A also binds to CPSF30, a cellular factor required for the 3'end processing of cellular pre-mRNAs, including IFN-β pre-mRNA. Thus, NS1A can affect the formation of mature mRNAs for one of the cell's antiviral molecules. Additional studies showed that two of the zinc fingers (F2F3) of CPSF-30 are involved in its binding to the NS1A protein and that the complex of NS1A with F2F3 is a tetramer of two molecules of NS1A and two of F2F3. In the CPSF30 binding pocket for NS1A, there are 6 amino acids that are almost completely (>98%) conserved among Influenza A human isolates, including H5N1 viruses and the1918 virus. Studies with mutants at this binding site demonstrated that NS1A-CPSF-30 binding is responsible for the inhibition of production of IFN-β mRNA in cells infected by human Influenza A viruses. Additional studies demonstrated that two NS1A amino acids (F103, M106) outside the binding pocket participate in key hydrophic interactions that are needed to stabilize the tetrameric complex. Essentially all (99.6%) of human Influenza A viruses (H1N1, H2N2, H3N2, H5N1) contain F103 and M106 in their NS1A proteins. However, two prominent human Influenza A viruses have different amino acids at these positions: the 1997 pathogenic H5N1 Influenza A/Hong Kong/483/97 and the H1N1 Influenza A/PR/8/34, thus highlighting the importance of NS1A-mediated CPSF-30 binding for circulating human Influenza A viruses. The importance of this amino acid substitution was confirmed when a recombinant of the HK97 virus was made in which L103 was changed to F and I106 is changed to M; these substitutions resulted in increased virus replication and decreased IFN-β mRNA.

These amino acid changes also increased the virulence of the viruses in mice. Based on these findings, the NS1A protein appears to be a good antiviral target.

Another antiviral target in the NS1A protein is the RNA-binding domain, which binds to the A-form of dsRNA. There does not appear to be another structure like this in the database, which is encouraging for this being a potential site for antivirals. R38 appears to be the only critical residue for RNA binding. The binding cavity also appears to have conserved residues. The structure of NS1B also seems to be similar suggesting to that of NS1A; thus, there is potential for an antiviral to react with both molecules. Additional promising findings are that an Influenza A/Udorn/72 virus encoding an NS1A protein with an R38 mutation is attenuated and forms pinpoint plaques. An assay is available for screening for inhibitors that affect the binding of dsRNA. 20,000 compounds have been screened and a candidate has been found that shows a 20-40 fold reduction of the rate of replication of the Udorn virus in MDCK cells.

Exploring plant diversity for novel antiviral compounds

Dr. James S Miller, The New York Botanical Garden, Bronx, New York, addressed the potential of the botanical world to provide new classes of compounds. He noted that at least one-third of prescription drugs have a plant-derived ingredient. This is an underestimate as it does not take into consideration natural products that were lead compounds, situations where there is now a synthetic compound or where the natural product was used to describe the pharmacological mechanism that led to a drug.

Dr. Miller has been involved in plant surveys around the world and had a contract with the NCI to gather compounds for use as anti-cancer agents. These compounds were subsequently shared with NIAID and used for screening for anti-HIV activity.

The strategy for collection uses a separate bag for each different plant part, e.g., roots and stems, as each of these parts might have different active agents. Collection is made across the taxonomic spectrum so as to maximize the different types of plants surveyed. He stated that while the destruction of biodiversity is well-recognized, it is less often considered that there is a loss of oral tradition, and of treatment traditions and languages that are associated with the use of plant materials to treat disease. Investigators work with native healers in those situations where the native healers recognize diseases that are similarly described in western medicine.

Dr. Miller then summarized an exercise he had conducted a number of years ago to estimate the number of plant derived drugs that potentially exist and have yet to be recognized. The estimates for the number of plant species are between 330,000 and 546,000. He described the various factors that go into such a calculation and noted that there are about 2000 plant species that are described as new each year. He also noted that many of the plant materials have only been subjected to limited screens and have not been evaluated against multiple targets. He also stated that detection is limited by the concentration and potency of compounds that may be screened in crude extracts. Unfortunately, most screens have not employed fractionation and concentration, and thus, may have missed many potential drugs. There are other sources of biologically active materials, e.g., arthropods that have not been explored. Overall, he feels that we have only discovered 1 to 13% of the potential plant-derived drugs. Thus, the full range of biodiversity has not yet been explored. There is a need for large libraries of samples that could be used more effectively and efficiently. These samples need to be fractionated and concentrated prior to bioassay.

Later in the meeting, it was noted that Tamiflu originated from plant material.

Evolutionary genetic chemistry approach to generate small molecules targeting influenza

Dr. Pascal Longchamp, Evolva AG, Allschwil, Switzerland, provided an overview of Evolva's approach to develop small molecules targeting influenza. The underlying concept is that 52% of new drugs in the last 22 years have "originated" from nature (e.g. statins, taxols and most antibiotics). The majority of species have never been explored chemically. The main problem for industry in terms of developing natural products is that natural selection does not aim to make drugs and optimizes compounds on other criteria; moreover, natural selection does not aim to provide an industrial process. The goal of Evolva's system is to replace natural selection with "genetic chemistry systems" that capture the power of evolved genes to make appropriate structures while avoiding the problems of natural systems.

In the Evolva approach, genes that have interesting structures, functions, reactions or unexplored chemical diversity are collected from nature. These genes are placed randomly on artificial yeast chromosomes with inducible promoters which are transferred into yeast. Each cell thus has a different genetic make-up. A therapeutic target is then linked to the yeast. In response, new small molecules are produced from the yeast artificial chromosome (YAC). The individual yeast can be assayed for new molecules and can be selected with a fitness assay. Bioactive compounds can be isolated, structurally characterized, de-replicated and novel compounds developed.

Evolva has several U.S. government supported biodefense-related programs using this approach. This includes an antivirals program supported by the Defense Threat Reduction Agency (DTRA) to address Ebola, influenza and HIV and seeking broad spectrum compounds that block virus-host protein-protein interactions. Another DTRA supported program targets innate immunity and toll, Rigs, NOD and similar receptors with the goal of a broad spectrum approach addressing pandemics and nosocomial infections. An Army Research Office (ARO) program seeks to use bacterial biosensor approaches and microfluidic technology to address Gram-negative bacteria. Dr. Longchamp noted that Evolva has an anti-thrombobiotic compound and a nephrotidic compound in Phase I trials as well as a number of drugs in preclinical study.

Dr. Longchamp summarized the use of Evolva's approach in anti-infective applications. An Ebola targeted interaction is designed to develop an inhibitor to prevent budding of virus by preventing the protein-protein interaction between VP40 and TSF 101. If the YACS produce a compound that inhibits the interaction, a reverse yeast 2-hybrid (RY2H) assay will give a positive readout when the protein-protein interaction is disrupted. A secondary colorimetric reporter is used to confirm the primary hits. Dr. Longchamp showed data in a mouse model in which one of the nine candidate compounds tested, EV-063-1544, protected mice at the 90-100% level and appears to be non-toxic based on lack of weight loss by the animals.

Influenza targets of interest to Evolva include the polymerase subunits PA, PB, the matrix proteins M1, M2, and the nucleoprotein.

In terms of studying immunomodulators, the Evolva approach is to use mammalian cells in which genes at the end of the innate immunity signaling cascade are replaced by fluorescent or colorimetric reporter genes, and thus create a mammalian reporter cell. The yeast cells that are secreting potential products are layered over the mammalian cells and green fluorescent protein is visualized for yeast cells generating potential active compounds. After additional steps, single active cells can be identified and used to generate potential drugs. One such product, EV-075-9904, has a novel mode of action and provides for 80% survival in a mouse model of Ebola challenge. Also in a mouse model, this compound seems to be as effective as Tamiflu against influenza. This product will be moved forward.

Anti-influenza virus target discovery gene trap – siRNA: Identify and confirm

Dr. Bill O'Brien, Zirus, Buford, Georgia, noted that the need for anti-influenza drugs is great given the limited number of effective antivirals available, the challenges related to virus diversity and mutability,

the development of resistance, the emergence of viruses with pandemic potential, and the challenges to vaccine development because of the unpredictability of virus evolution.

The investigators at Zirus are seeking a paradigm change in antiviral development by using a platform to identify and validate cellular targets (rather than viral targets) for anti-influenza drug development. The underlying rationale is that influenza viruses are intracellular pathogens that require cells functions. The goal is to identify human cellular targets by using Gene-Trap®, to confirm the targets with siRNA and then assess the potential antiviral molecules (siRNAs) that inhibit the host gene products using murine models. A shuttle vector containing G418 (neo) is integrated randomly into susceptible cells. Following selection with G418 and infection with the pathogen, the surviving cells are expanded and the trapped gene is cloned and sequenced. Thus far, the Gene-Trap® has identified over 500 candidate host genes. siRNA treatment and infection of non-trapped target cells allows for independent confirmation of the requirement by the virus of candidate host genes.

Dr. O'Brien summarized the findings with two cellular factors (genes), Rab9 and ADAM-10 that are required for HIV and influenza virus infection. Their mechanisms of function with HIV have been characterized. Rab9 is a known GTPase. Dr. O'Brien described the criteria for selection of anti-influenza cellular targets that will be used for *in vivo* studies and summarized the overall steps in target-to-drug discovery. He stated that pathway targeting is the richest approach. He suggested that, once target genes have been optimized, short-term therapeutic approaches might involve the use of siRNA or of repurposing of existing drugs. More than 50 genes related to influenza virus have been identified so there is the potential for combination therapies.

The emerging conclusions from this work are that: viruses hijack host proteins to enable their replication; there is an apparent redundancy for many cellular functions such that down regulation of a cellular function can markedly affect virus growth without apparent harm to the cell; and the Gene-Trap® approach prevents the selection of genes that are required by the cell. Dr. O'Brien noted that even if there are toxicities associated with blocking a cellular factor, short-term inhibition of that factor may still be useful for targeting infections, such as pandemic influenza or Ebola infection, where chronic drug treatment is not needed.

Targeting the host: 3-V Bioscience's approach to antiviral drug development

Dr. John Curnutte, 3-V Biosciences, Palo Alto, California and Zurich, Switzerland indicated that his presentation will follow-up on Dr. Helenius' presentation. The concept underlying 3-V's approach is that there are numerous potential host targets that may provide therapeutic opportunities for previously intractable pathogens. Potential host targets may be active against existing and emerging strains, may be effective against unrelated pathogens, and may enable treatment of clinical symptoms in both the upper and lower respiratory tract. This approach is less likely to lead to resistance compared to targeting a single point mutation in a virus as the host is more genetically stable than a virus.

He summarized the experience with host cell genes which affect influenza virus. Of the 7000 druggable targets, 177 were found to be non-toxic using several approaches, elimination of duplicative targets left 100 genes for further study. These were further prioritized based on pathways, the spectrum of activity against different virus families, and the breadth of cells in which they interacted. A further reduction in the number of genes, and a way to jump start the process, was looking for known molecules with known inhibitors. There were 13 such genes and 5 of them were active against influenza. The group chose one target as a lead based on its potency and scope of action. There were two unrelated inhibitors, TVB-24 and TVB-26, for the target. The lead compound was also active against human rhinoviruses (HRV). Dr. Helenius had shown data with clinical influenza strains in human bronchial epithelial cells in which the IC_{50} and IC_{100} were in the low nanomolar range. When two avian H5N1 strains (one human and one duck) were studied with TVB-24, TVB-26 and Tamiflu, both TVB-24 and TVB-26 were more effective than

Tamiflu and were also effective against a Tamiflu-resistant duck isolate. Studies are now underway with collaborators using human lung organ culture. These studies have shown that with increasing doses of drug there is a decrease in the level of infection when compared to Tamiflu. Protection against lethal infection was also seen in a mouse model. These compounds are well -tolerated *in vivo* and the effect on virus is not due to cytotoxicity.

During the discussion, it was noted that the human bronchial epithelial cells are derived from multiple donors and so there does not seem to be a genetic diversity issue in terms of being able to demonstrate the antiviral effect across individuals.

Novel cellular and viral targets for anti-influenza virus drugs

Dr. Megan Shaw, Mt Sinai School of Medicine, New York, New York, discussed three approaches that are aimed at the discovery of novel small molecule inhibitors and new drug targets for influenza viruses. New cell-based assays were developed and used in high-throughput screens (HTS) to detect: small molecule inhibitors of influenza virus infection; small molecule inhibitors of influenza virus NS1 function, and cellular gene requirements for influenza virus infection and NS1 function.

Dr. Shaw described the HTS screen for monitoring influenza infection. A549 human lung epithelial cells are transfected with a luciferase reporter. Compounds to be tested are added 6 hours prior to infection with A/WSN/33 and the luciferase read out is done 18-20 hours later. More than 73,000 compounds were screened at the NSRB-ICCB Longwood Facility at Harvard Medical School and there was a counter screen for non-specific effects. Three lead compounds were identified from 47 hits. One of these, A3, is a potent inhibitor of polymerase function. The second compound, A35 acts at a post-entry step. The third compound, C2, inhibits virus entry/endocytosis.

A different screen is used to search for compounds targeting the anti-interferon function of the NS1 protein. This assay uses an MDCK IFNβ-luciferase cell line. The positive controls are infected with an rPR-8 –NS1 mutant, the negative controls are infected with PR8 and the screen cells are infected with PR8 and the potential inhibitor. In a collaborative effort with the NRSB, 84,000 compounds were screened and 264 hits found. The bioassay for antiviral response involves treating MDCK cells with the compound, infection with PR8 virus, removal of the supernatant at 18 hours post infection and UV inactivating the virus in the supernatant. Dilutions of supernatant are added to fresh MDCK cells which are then infected with VSV-green fluorescent protein. If IFN is induced, the VSV reporter is inhibited. One compound identified by this process, A2, inhibits the growth of influenza virus and induces IFNβ-luciferase. This assay can detect compounds targeting either viral or cellular proteins; the identified inhibitors may be influenza-specific or may have more broad-spectrum activity.

The third approach is to use whole genome siRNA screens to identify druggable cellular targets. This approach is a collaborative effort involving the Mt. Sinai School of Medicine, the Burnham Institute for Medical Research, the Genomics Institute of Novartis Research Foundation and the Salk Institute. This assay involved the generation of a luciferase-expressing influenza virus (WSN-Ren). On day 1, V549 cells are transfected with siRNAs; on day 3, the cells are infected with WSN-Ren virus and on day 4 the luciferase assay is read.

Studies using these three approaches are continuing.

Design and delivery of siRNA therapeutic cocktails for influenza

Dr. Patrick Y. Lu, Sirnaomics, Inc., Gaithersburg, Maryland, discussed the potential of RNAi technology as a new approach for antiviral drugs and noted that there are more than 15 trials involving RNAi

therapeutics. He stated that there are a number of advantages to RNAi therapeutics including: that they are an endogenous mechanism; that they can be highly specific, highly effective, have low toxicity and can be easy to make and formulate. siRNAs are non-biologics and regulated by the FDA Center for Drug Evaluation and Research (CDER). In terms of their antiviral properties, the siRNA can be designed to target viral RNA and not host RNA. Use of siRNA cocktails would allow for targeting to multiple hot spots. siRNAs can be targeted to both strands of nucleic acids. There a many examples of proof-of-concept studies with siRNA antiviral agents including ones targeted to HIV, HPV, HBV, HCV, HSV, RSV, CMV, EV71, Influenza A and SARS CoV.

Dr. Lu noted that the main challenge to use of siRNA therapeutics is delivery, an area in which Sirnaomics has a lot of expertise.

Dr. Lu described the experience of Sirnaomics with siRNA targeting SARS (SCV) virus. 48 siRNAs were made and based on a CPE assay and EM examination; two were selected for study in primates. Based on its properties, D5W was chosen as the lung delivery vehicle. The siRNAs were studied for delivery prior to infection, concurrent with infection or post-infection. The data showed relief from SCV-induced fever and protection from virus infection.

Dr. Lu noted that Sirnaomics has built a platform for rapid development of siRNA drug products and uses a proprietary algorithm for siRNA API design. They also have a process for eliminating host targeting.

Dr. Lu summarized the challenges posed by H5N1. In terms of vaccines, the H5 HA is poorly immunogenic compared to the HA of H3N2 or H1N1 viruses. There are already multiple clades of H5 circulating and manufacturing capacity is limited. The limited number of antiviral agents is compounded by the problem of drug resistance.

STP702, Sirflunib™, is an siRNA therapeutic proposed for therapeutic use in pandemic influenza. This product targets the NP and M2 proteins and was tested in MDCK cells. It would be delivered using a PEGylated nanoparticle, Snano 4. It is being evaluated in a mouse H5N1 (A/Vietnam/1194/04) challenge model where it has shown protection by intranasal and intratracheal delivery. There is also a system available for systemic delivery.

Tackling antiviral resistance of influenza viruses: Solutions lie within

Dr. Prakash Sambhara, Influenza Division, CDC, addressed the potential for targeting host functions as an antiviral strategy for dealing with antiviral resistance of influenza. Activating the innate antiviral system could block viral entry or block viral replication. It would take advantage of a system that has been tested through evolution. The innate system can very rapidly eliminate microbes that succeed in entering the host tissue and can instruct the cells of the adaptive immune system to eliminate the microbe if the innate system is unsuccessful. Dr. Sambhara described the pathogen sensors of the innate immune system that are present at multiple host levels and include factors that are extracellularly soluble, membrane bound, present in vesicles or present in the cytoplasm. He provided an overview of the signaling pathways that are triggered by viral or bacterial activation of the innate immune system.

He noted that there are many viruses that have a component that inhibits RIG-1-like receptor (RLR) signaling. The NS1 protein of Influenza A is such an inhibitor. He noted that RIG-1-based[1] activation of antiviral defenses occurs against drug-resistant human strains of influenza, highly pathogenic avian influenza viruses, the 1918 pandemic influenza and Ebola virus. He showed data on the reduction of plaques of these types of viruses using 5'PPP-RNA. He concluded that activation of RIG-1 generates pan-

[1] RIG-1 (retinoic acid inducible gene 1) is the receptor for intracellular ds-RNA and through the MAV adaptor protein activates NF-κβ and 1RFS.

antiviral effects that are independent of genetic make-up, drug-sensitivity status, pathogenicity and type of virus. It is possible to screen for small molecules that activate the RIG-1 pathway. He proposed that understanding of innate immunity can lead to the discovery of new anti-microbial agents, anti-inflammatory agents, molecular adjuvants for vaccines and other therapeutics.

2. CLINICAL SESSION: CLINICAL TRIALS OF LICENSED DRUGS IN VULNERABLE/SPECIAL POPULATIONS

Treatment of H5N1 influenza: Past experience, current work and future directions

Dr. Menno de Jong, University of Amsterdam, the Netherlands, addressed the challenges of treatment of H5N1 infections that cause a rapidly progressing pneumonia in humans with a case mortality rate of 60%. Dr. de Jong cited findings from H5N1 cases in Vietnam, Thailand, Turkey, Egypt and Indonesia. Some studies have found that fatal outcome is associated with high viral load and hypercytokinemia. H5N1 infection is also associated with an intense inflammatory response (high levels of certain cytokines and chemokines) that is greater than that observed in H3N2 virus infections or in seasonal disease. There is a correlation between the amount of viral infection and cytokines and both seem to contribute to disease and to mortality. Steroids seem to provide better survival outcomes, but do not control disease. Oseltamivir provides clinical benefit, but this benefit is limited; the drug benefit is greater when the drug is administered early in disease. Thus, early recognition and diagnosis are important; sensitive point-of-care diagnostics can help in this regard.

Dr. de Jong noted that the term "cytokine storm" has been introduced to describe the pathology that is observed with H5N1 infection and that many publications have focused on the innate immune response as being central to infection. He felt high levels of virus replication may be as or more important than the cytokine effects. If this assumption about the importance of virus levels is correct, then the mainstay of H5N1 treatment should be with antivirals.

Dr. de Jong addressed the issue of why drugs and immune modulators have thus far not been that effective in treating H5N1 disease. He suggested that it is possible that the levels of oseltamivir being used are not adequate as the standard dose is based on studies in uncomplicated influenza; there have not been many studies in severe influenza. Additionally, *in vitro* and animal studies suggest that higher doses may be needed to treat H5N1. Finally oral drug administration in an intubated patient is challenging. He noted that there are ongoing studies of higher doses of oseltamivir in severe influenza in Southeast Asia and that developing intravenous formulations of the drug is also desirable.

Dr. de Jong reported on studies of oseltamivir that address aspects of the dosage issue. Studies in adult patients with severe influenza have shown that oseltamivir is adequately absorbed following nasogastric administration. For this study, oseltamivir from capsules was dissolved in water and administered through a nasogastric tube. Pharmacokinetic studies of high-dose oseltamivir in healthy volunteers showed that the high doses were well-tolerated and linear. Probenecid increased the AUC by 150%, and thus, could enable a 2/3 dose reduction. However, the increase of AUC was less pronounced in saliva. One subject showed reduced O-phosphate to O-carboxylate conversion; it is not clear if this represents a constitutional impairment of carboxylesterase activity.

Dr. de Jong also addressed possible viral issues that may impact the effectiveness of antiviral therapy. In persons infected with Vietnam clade 1 H5N1virus, rapid control of viral replication is associated with a beneficial outcome even when the treatment is instituted late. Good outcomes are found in Thailand and other countries with this approach. Clade 2 H5N1 viruses seem to be less susceptible to oseltamivir than clade 1 viruses. There is higher mortality with clade 2 virus infection, especially in Indonesia.

Dr. de Jong noted that the development of drug resistance is associated with poor clinical outcome even in situations where the drug is given early.

He suggested that alternative more effective antiviral strategies are needed, such as: parenteral neuraminidase inhibitors, sialidases, polymerase inhibitors, antibodies and combination therapies. In terms of combination therapies, there is the potential for synergistic/additive antiviral activity, for creating genetic barriers to resistance development and for using combinations of existing drugs. He noted that each of the existing neuraminidase inhibitors targets different aspects of the neuraminidase. He listed a number of studies, *in vitro*, in mice and in humans, that have been conducted in the last 40 years and that have used antiviral combinations for influenza. He cited unpublished data in which a triple combination of oseltamivir + amantadine + ribavirin showed greater synergy than double combinations against H1N1, H3N2 and H5N1 viruses.

Dr. de Jong concluded by noting that clinical research on H5N1 influenza is urgently needed and requires regional collaborations and the building of research capacity in affected countries. The Infectious Disease Clinical Research Network is a multilateral collaborative partnership between hospitals and institutions in Thailand, Vietnam, Indonesia, Singapore, the UK and US that is funded by the NIAID and Wellcome Trust and is making a number of efforts in influenza clinical research and capacity building. Accomplishments, ongoing and planned efforts of this network include: GCP capacity building in Vietnam, Thailand and Indonesia; development of international standard molecular diagnostic laboratory capacity, development of quality systems of routine laboratory tests, and promotion of training, workshops and short-term fellowships and a range of clinical studies to address the issues cited in Dr. de Jong's presentation.

Oseltamivir in infants - Collaborative Antiviral Study Group (CASG)

Dr. David Kimberlin, University of Alabama, Birmingham, Alabama, described studies being pursued to address the lack of information about the safety and efficacy of influenza antivirals in children, especially those under 1 year of age. Oseltamivir is not licensed for children less than one year of age. The NIAID Collaborative Antivirals Study Group (CASG) Protocol 114 has been developed with Roche Pharmaceuticals and has its primary objective: "to define the pharmacokinetics of oseltamivir and oseltamivir carboxylate in children with confirmed influenza less than two years of age." The secondary objectives include describing the frequency of adverse events, including neurological adverse events; to assess the clearance of virus and viral RNA as a function of drug pharmacokinetics; and to determine the potential for the development of resistance to oseltamivir as a function of pharmacokinetics and age. Enrollment was started with children 12-23 months of age, in which the drug is approved and has proceeded downward in age cohorts from older to younger children (9-11 [still enrolling] months, 6-8 [closed], 3-5 [still enrolling] and 0-2[not yet started]). Subjects will receive oseltamivir bid x five days (10 doses). The target AUC_{12} based on adult data is 3800ng*hr/ml. The DSMB and FDA review all safety and pharmacokinetic data from the preceding cohort prior to opening the next cohort for enrollment. This strategy allows for adapting the dose of drug; this has been done for cohort 2 (9-11 month group). Dr. Kimberlin described the dose adjustment schema for the various cohorts and the inclusion and exclusion criteria for study participants. Inclusion criteria include previously healthy children with a confirmed laboratory diagnosis of influenza by viral culture or rapid influenza diagnostic test within 96 hours prior to study enrollment and duration of influenza symptoms for ≤96 hours. Children with chronic illness or any neurological problems are excluded. As of March 18, 2009, a total of 35 subjects have been enrolled. The mild influenza season has somewhat impacted enrollment and so it will be necessary to continue enrollment into the next influenza season. Based on CDC's interim treatment recommendations, a study amendment in December 2008 allowed for the concomitant use of amantidine, if needed. Of the 55 AEs, the majority have not been designated by the site investigator as drug-related. The AEs related to drug thus far consisted of 5 vomiting events. There was only one SAE that designated as drug-related and this was a hypersensitivity event. As the younger cohorts are assessed, the AUC_{12} gradually increases

suggested that there may be slower drug clearance in younger children. The Glasgow Coma score is being used as a way to measure neurological events; it measures alertness. Thus far, there has not been any evidence of depressed scores. Preliminary analysis of viral load suggest that PCR and culture of nasal wash samples show similar titers. There is a correlation of the decline of viral load and days on drug. Studies of virus type show that type B influenza is slower to clear than type A, and that there is more rapid clearance of H1N1 virus than of H3. There did not appear to be a correlation of viral load and illness severity (measured by the number of symptoms). If two apparent outliers are removed, the higher concentrates of drug correlate with a larger decline in viral load as detected by PCR or culture.

Influenza pathogenesis and viral dynamics: A focus on immunocompromised patients

Dr. Matthew Memoli, NIAID, NIH, Bethesda, Maryland, discussed the dynamics of the evolution of viral pathogenesis with a focus on immunocompromised patients. Dr. Memoli noted that while the focus is often on the surface glycoproteins when one thinks about influenza evolution, the other segments of the influenza genome need to be considered as well. In an analysis of 1300 complete influenza seasonal genomes, Dr. Jeffery Taubenberger and a number of collaborators found that antigenic drift occurs between seasons, but that there are also other events that occur. There are frequent subtypic intragenomic reassortments that occur as well as selective intragenomic sweeps and extinctions. An example of this relates to the 2003-04 season during which there was the emergence of the Fujian-like viruses (H3N2). What normally happens is that there is one dominant circulating clade and other non-dominant clades co-circulating. A previously non-dominant strain donated its HA to a previous dominant strain, and thus, formed a new strain, Fujian virus. This is an example of an intrasubtypic reassortment event. It resulted in increased morbidity and mortality due to influenza and pneumonia because of a vaccine mismatch.

In the subsequent 2004-05 season, there was also an increase in mortality from influenza and pneumonia. There were multiple co-circulating clades. A new clade emerged from the clade that had donated its HA. There was also a small amount of antigenic drift from the Fujian virus to the California-like virus. There were also a small number of changes in the internal genes which may have resulted in this virus becoming dominant. This seemed to be a genomic sweep in which the majority of circulating viruses came from this one clade, unlike in previous years. This virus grew to higher titer in tissue culture and in ferrets lungs and nasal washes and caused more pathology than did the previous strains. The investigators sought to define the selective pressures for these changes. The HAI assay showed only small changes in HA which did not appear to account for the changes in virus biology. Examination of the four internal segments (PB1, PB2, PA, NP) in a polymerase reporter assay found that the virus had an increased polymerase activity; sequences in the PA segment were identified that seemed to account for some of this increased activity. Dr. Memoli noted that this type of finding highlights the importance of intrasubtypic genomic reassortment events and selective sweeps in evolution and suggests that the internal gene segments along with antigenic drift may be important for this evolution. Together, these may affect replicative fitness of the virus. This is relevant to drug resistance as well because every replicative cycle creates a possibility for a mutation arising that could contribute to drug resistance. Thus, there are questions about how the use of antivirals may contribute to the evolution of influenza.

Dr. Memoli noted that immunocompromised individuals are impacted by influenza and the course of influenza disease in immunocompromised individuals could potentially impact influenza evolution. A low estimate of the number of immune-deficient individuals in the U.S. is 10 million persons. In seasonal influenza, many of the deaths occur in vulnerable populations such as children, the elderly and immunocompromised patients. There is a high incidence of influenza and pneumonia in persons who have received stem cell and solid organ transplants; and a prolonged course of illness and virus shedding in immunocompromised persons. Additionally, two subtypes of virus have been isolated from individual immunocompromised patients as have drug-resistant viruses. Dr. Memoli cited a recently published case of an H3N2 oseltamivir-resistant virus that was isolated from a child with SCID who had received a stem cell transplant. There was a deletion from amino acids 245 to 248 in the neuraminidase gene which lead

to the oseltamivir resistance after 107 days of drug therapy. It is not known how common these events are, how rapidly they occur, whether the altered viruses are transmitted, and what the implications for influenza evolution are from such altered viruses.

Dr. Memoli has a protocol in place at NIH. Over the last two years, he has collected H3N2 and H1N1 viruses from immunocompromised patients. He has found that 70% of immunocompromised patients have fever on presentation; this is in contrast to 34% of challenge studies in normal hosts. Virus shedding is 6.9 days versus 4.8 days in challenging studies. 90% of the patients were imaged within one day of diagnosis; 37% had an acute change that could be seen on imaging. Last year, there were 2 cases of resistant virus; this year there have been 9 resistant viruses and all of which have the H274Y change (H1N1).

Dr. Memoli provided more detail about one of the patients from his study population who had H3N2 oseltamivir-resistant virus. This patient was a 43-year old male with mantle cell lymphoma who had undergone an allogeneic stem cell transplant about 2-years ago and who had had a number of complications. During the course of treatment for an abscess, he developed influenza-like symptoms; a nasal wash confirmed the presence of influenza A virus. Nasal washes were done and there was virus shedding for 12 days. The patient received oseltamivir starting on day 1 post-diagnosis. The viruses found over the 12 day period were sequenced. Between days 1 and 4, the neuraminidase gene of the virus was wild type; by day 6, there was a deletion from amino acids 245-248 in the neuraminidase gene, similar to that found in the child with SCID; this deletion was present in all of the subsequent viruses. The virus isolated on day 1 was shown to be oseltamivir-sensitive; by day 8, it was resistant. Future studies will continue in order to determine if drug-pressure plays a role in the development of resistance and to determine the fitness and transmissibility of the virus and whether it is resistant to drugs other than oseltamivir. Dr. Memoli and his colleagues will continue to compile clinical data in this patient population and to try to assess how or if the viruses from this population affect influenza evolution.

Evaluation and management of influenza in immunocompromised and hospitalized adults

Dr. Michael Ison, Northwestern University, Evanston, Illinois, noted that influenza infection in both solid organ transplant (SOT) recipients and human stem cell transplant (HSCT) recipients follows the same seasonality as that of the general population. Influenza prevalence is 1-3% of stem cell transplant recipients and 3-12% in solid organ transplant recipients. In the immunocompromised populations, there is an increased risk of disease progression to the lower respiratory tract, of secondary infection, of hospitalization and of mortality (25-28% in HSCT; 25% in lung transplant). Among solid organ transplant recipients with influenza infection, lung transplant recipients have the great risk of acute organ rejection; rejection is related to the upregulation of local and systemic inflammation, lymphocyte tracking to the infected site and local mucosal damage. Chronic rejection occurs in about 36% of lung transplant recipients and the greatest risk is in those with lower tract involvement. In terms of symptomotology, the more severe cases of respiratory virus infections were associated with infections that occurred early post-transplant and with a higher degree of immunosuppression; however there was often few fever symptoms in the immunocompromised patients.

In terms of management of influenza in transplant recipients with antivirals, there are no prospective data and most information is retrospective data with M2 and neuraminidase inhibitors. Overall, there is reduced mortality, reduced virus shedding and lower rates of progression to pneumonia; however, with M2 inhibitors resistance frequently occurred which can limit the utility of these antivirals. The knowledge gaps in this area include: that there is little epidemiological knowledge in transplant recipients other than HSCT and lung recipients and these data are not prospective. There is not enough information about: the optimal treatment in terms of dose, duration, use of combination treatments; the risk factors and rapid detection of the emergence of resistance; and the impact on pulmonary function over the course of disease and treatment. The challenges to study design in transplant recipients include the types of controls that are

feasible and the limitations of the endpoints. The feasible approaches include dose ranging studies and exploratory studies, such as natural history. The February 2009 FDA draft guidance on development of drugs for treatment and prophylaxis of influenza covers some of these study design issues.

Dr. Ison also addressed influenza in hospitalized adults, where the prevalence is between 2 and 20%. Such persons tend to be older, have an underlying chronic disease and many have been vaccinated. There are data to suggest that treatment of these patients results in decreases in mortality and that there is benefit even if therapy is started 48 hours after the onset of symptoms. Duration of viral shedding in hospitalized patients is longer than in ambulatory adults.

Dr. Ison summarized the results from two prospective studies in hospitalized patients, a CASG study and the BCX1812-20. The CASG study was a two-arm randomized, double-blind placebo-controlled study comparing rimantadine + placebo with rimantadine + zanamivir. The study subjects had confirmed severe Influenza A or B, less than 4 days of symptoms, and evidence of lower respiratory tract involvement. The results showed that there was a trend to clear virus more rapidly when two drugs were used compared with one drug. M2 resistance was only seen in monotherapy and was not found in the combination therapy.

The BCX1812-20 study was a randomized, double-blind, double dummy in which oseltamivir-75mg was compared with Peramivir-200 mg vs. Peramivir-400 mg. The subjects in this study were similar to those in the CASG study. The endpoint was time to clinical stability and the only measure that showed statistical differences was fever. There was a clear improvement in viral titer changes from baseline for those subjects with Influenza B infections.

Dr. Ison summarized the knowledge gaps for influenza infection in hospitalized patients. There are limited prospective studies of persons with severe influenza and limited knowledge about the criteria for admission. It is not known whether viral data correlates with clinical outcomes. Challenges to study design include the selection of endpoints and outcomes and the selection of patients and types of controls. The effect of treatment on patient discharge and occurrence of complications is not clear. The February 2009 FDA draft guidance also covers some of the issues in studies of hospitalized individuals.

Risk-benefit assessment for special populations in influenza drug development: A regulatory perspective

Dr. Wendy Carter, FDA, CDER, Office of Antiviral Products, Silver Spring, Maryland, noted that there is a February 2009 draft guidance on "Influenza: Developing Drugs for Treatment and/or Prophylaxis" (http://www.fda.gov/cder/guidance/7927dft.pdf) which covers issues related to special populations.

The special populations that she addressed were: hospitalized patients with serious infection; patients with underlying conditions (e.g. pulmonary disease, cardiac disease, immunosuppressive conditions), the elderly, pediatric patients, and pregnant women. For drug-development in high-risk populations, the concerns may vary by drug and placebo-controlled trials, may be controversial, unethical or unfeasible. Alternative trial designs may include: dose-response superiority versus active control or current standard of care, or superiority of add-on to the standard of care. The high-risk groups may differ from the general population in disease outcomes, incidence of adverse event, and overall risk-benefit profile. These differences need to be planned for during study development.

In terms of hospitalized patients with serious influenza, there have been few studies to date. Alternatives to placebo-controlled trials include randomized dose-response trials showing a significant dose response and superiority add-on trials. Elderly patients and patients with underlying medical conditions are more likely to be hospitalized as a result of influenza infection. Data from these groups may give useful insight about possible events in a pandemic setting. Dr. Carter noted that Rapid Antigen Testing for screening in

this patient population has variable sensitivity which could introduce a selection bias. Alternative enrollment criteria may need to be considered.

Dr. Carter noted that the Pediatric Research Equity Act requires pediatric assessment for any new drug, dosage form, route of administration, indication, or dosing regimen if the drug represents a meaningful therapeutic benefit over existing treatment and will be used in children. Pediatric exclusivity under the Best Pharmaceuticals for Children Act (BPCA) may also be available on written request. When there is a desire to extend treatment and/or prophylaxis to pediatric groups, it is important to have early discussions of the trial with the Division of Antiviral Products. Additional trials will be likely for proposed pediatric usage. Antiviral drug efficacy for influenza in children cannot be extrapolated from studies of adults because of important clinical differences including: prior exposure and immunity; manifestations of illness; rates of complications; and viral shedding and resistance.

In pregnant women, influenza infection is associated with higher risks for complications, including pneumonia and preterm delivery. Historically, there is increased mortality observed in pregnant women during influenza epidemics. Antiviral drug use during pregnancy depends on individual assessment. Inactivated vaccine is recommended in pregnant women. Because of physiological changes in pregnancy which occur mostly in the first and second trimester, it is not possible to extrapolate PK data from non-pregnant adults. The risk-benefit in influenza drug development in pregnant women is influenced by a number of factors including: the product profile, the current public health need, the severity of the influenza epidemic and virulence of circulating strains, and the availability of vaccines. Non-clinical data needed prior to study in pregnant women includes toxicity and genotoxicity studies. There are ethical concerns that must be addressed as well.

Overall, studies in special populations should identify the unique differences of these populations as well as of the study drug and consider them in protocol design. Guidances for industry are available for many aspects of study design and program development. Early and frequent communication with DAVP during development is important. Pre-IND program information is available at: http://www.fda.gov/cder/ode4/preind/getting.htm.

Pandemic influenza complications: Secondary bacterial infections

Dr. Jeffery Taubenberger, NIAID, NIH, Bethesda, Maryland, provided an overview of past influenza pandemics and how they might be predictive of future ones. The 1918 "Spanish" H1N1 pandemic resulted in 675,000 deaths in the U.S. There were 70,000 deaths in the US as a result of the 1957 H2N2 "Asian" flu and 30,000 deaths from the H3N2 "Hong Kong" flu. There is no way to know when, where, what subtype and how virulent the next influenza pandemic might be. The 1918-19 pandemic caused an estimated 50 million deaths world-wide, with a case fatality rate of 2.5%; the pandemic course was similar in all countries. Nevertheless, 97.5% of cases had a self-limited disease with full recovery in the absence of vaccines, antivirals or antibiotics. There was a unique epidemiological pattern of influenza and pneumonia deaths seen in the 1918 pandemic compared with other previous pandemics (1889) and subsequent (1957) pandemic and with seasonal influenza from 1911-1917. In addition to the high death rates in the very young and the elderly (which form a U shaped curve in a plot of specific death versus age group), there was also an unexplained high mortality in the 1918-19 pandemic among persons in the 15-44 age group with a peak of mortality in the 25-34 age group. There was lower pneumonia and influenza mortality in the elderly in 1918 than in 1911-1917. The lower rate among the elderly may have been related to protection from prior pandemics in the mid to late 1800's. The current hypothesis about the viral genetic basis for the pathogenicity of the 1918 influenza is that the HA and NA proteins may have contributed to virulence through increased tropism in the respiratory tree and through inducing acute inflammatory pathway genes. In order to study this issue, investigators have compared the pathology of wt virus and viruses that were isogenic with the 1918 pandemic strain but had mutations in the receptor binding domain of the 1918 HA and found them to all be highly virulent, suggesting that sialic acid

specificity is not the controlling factor in mouse virulence and that virulence is outside of the receptor binding domain. Studies using the human 1918 virus and Swine/Iowa/1931 virus showed that these viruses had similar virulence in mice, ferrets and swine. Similar types of studies in macaques also did not demonstrate distinct pathological lesions. Thus, it is difficult to relate the findings in the animal experimental models of disease to the influenza disease observed in humans in 1918.

Dr. Taubenberger noted that there was a large number of careful postmortem studies performed and published in the aftermath of the 1918-19 pandemic. The common features observed in these studies were that the histopathological changes were asynchronous and focal; although the features of primary viral infection could be teased out, the features of secondary bacterial pneumonias predominated. Evidence of repair of the primary viral process was commonly seen, supporting the role of secondary pneumonias in fatal infection. The histological features seen in 1918 were also confirmed in the 1957 and 1968 pandemics. The contemporaneous 1918 investigations concluded that bacterial pneumonia was the primary cause of mortality. A recent review of 8398 published autopsy records also supports this conclusion. The 1918 virus became the seasonal virus in subsequent years, and in the 1921-22 influenza season, the age-related mortality curve assumed its classical U shape. It remains unclear what happened to the 1918 virus in subsequent years: was there mutation(s) in the virus, loss of susceptibles in the population or emergence of population immunity? There remain many questions about the viral basis for pathogenicity in animal models and the significance of the findings there to human disease. The roles of cytokines and host factors remain unanswered as does the question of how the availability of antibiotics would have impacted the pandemic. In terms of the current situation, the prevalence of antibiotic-resistant bacteria and antiviral-resistant influenza needs to be considered in the context of stockpiling of antibiotics and vaccines. There is the potential that a future pandemic strain with antiviral resistance could emerge via reassortment or de novo.

3. PRE-CLINICAL SESSION: PASSIVE IMMUNOTHERAPY DEVELOPMENT

Polyclonal IVIG for H5N1

Dr. John Beigel, Macrogenics, Rockville, Maryland, presented work that he had done while at NIH on the use of Polyclonal IVIG for H5N1. He noted that the concept of passive antibody is a long standing one in infectious diseases. His studies began in the context of SARS as an inexpensive and rapid therapeutic approach. The use of convalescent-derived polyclonal IVIG in that context showed reduced mortality and increased hospital discharge rates. However, there are complex collection and regulatory issues related to the development of this type of approach (imported convalescent sera) in the U.S.

Dr. Beigel described studies to derive H5N1 IVIG through the plasmaphoresis of hyperimmunized individuals. Studies by Dr. John Treanor had demonstrated that higher titers were obtained from individuals who received multiple vaccine doses. Dr. Beigel has conducted studies to determine the optimal antigen dose, number of doses, and site of administration (deltoid versus buttock). He found that there was no increase in HA inhibition (HAI) or microneutralization (MN) with increasing antigen dose; increasing the number of doses did increase titers, but mainly for persons whose titers were mid-range. There was a suggestion towards higher HAI when persons were vaccinated in the deltoid region. Ten of 126 subjects met the criteria for plasmaphoresis and for manufacture of IVIG. 41 units of plasma were obtained and manufactured into 9 units of IVIG. It was disappointing that the titer did not increase significantly from the original plasma units to the final IVIG product.

Future plans are to manufacture additional H5N1 IVIG, conduct proof-of-concept studies in animals for development of human polyclonal IVIG as a therapeutic for H5N1 and conduct of long term (1 year) follow-up post vaccination. Dr. Beigel concluded by enumerating some of the advantages of polyclonal antibody therapies over monoclonal antibodies including: the more rapid speed of production and less

developmental expense. Alternatively monoclonals have less heterogeneity and the potential to be more potent. It is not clear if there is an advantage to one of these approaches in terms of decreasing the development of resistance.

During the discussion it was noted that there is person-to-person variability in terms of the generation and amount of HAI and MN titers as well as poor correlation between HAI and MN titers within an individual. The effect of prior influenza immunization on the immune response in these subjects has not yet been assessed.

Therapeutic efficacy of anti HA monoclonal antibodies in animal models

Dr. Richard Webby, St. Jude Children's Research Hospital, Memphis, Tennessee, summarized studies designed to obtain the most potent anti-H5 monoclonal antibodies rather than to search for cross-reactive antibodies. He noted that antibodies to the globular head of the HA are the most potent antibodies. The approach that was used was to immortalize B cells from PBMCs persons who took part in studies with experimental H5N1 vaccines (inactivated Vietnam/1203 strain). Most people had poor titers. When he looked at people who had good responses, they had taken part in the H5/97 vaccine studies. He noted that cross-clade antibodies were found in some of these individuals who had been primed by this prior immunization. Hybridomas were developed from these individuals by using IFA and HAI screens using the vaccine antigen. The monoclonals were shown to have cross-reactivity between the priming and boosting antigens. Based on these findings it is likely that a therapeutic would consist of a cocktail of antibodies.

Dr. Webby described studies demonstrating the prophylactic efficacy of human monoclonal antibodies against the Vietnam/1203 strain in mice and noted that these antibodies were also effective in ferrets. In terms of treatment, the data suggested that a single dose could protect against mortality, but not morbidity, out to about day 3 post infection. Studies of viral titers in the brain suggested a good correlation between systematic spread and mortality. Studies also suggested reasonable protection of mice against heterologous challenge with Clade 2 virus.

Dr. Webby noted that escape mutants were generated and found two types, serine 145 and lysine 144 that were more frequent than others. Nevertheless, the monoclonal antibodies provided reasonable protection against the escape mutants.

In terms of developing and testing monoclonals, Dr. Webby noted that *in vitro* models can do only so much and that extra-pulmonary virus may be the key issue in H5N1 mouse models. He cautioned that mice are generally easy to protect against influenza. He stated that multiple sources of antibodies may be optimal for developing a therapeutic as different individuals respond differently to a given vaccine and different vaccines may elicit different antibody responses.

During the discussion, it was noted that the weight-loss in mice in the prophylactic studies was minimal, but that there was a lot of weight loss in mice in the therapeutic models. Dr. Webby indicated that he would like to do more studies on routes of antibody administration in ferrets as well as study affinity maturation of antibodies.

Generation of combinatorial antibody libraries from bone marrow of H5N1 survivors in Turkey (MAbs)

Dr. Arun Kashyap, Sea Lane Biotechnology, Menlo Park, California, described efforts to generate combinatorial antibody libraries from the bone marrow of individuals who were infected with and survived H5N1 infection in Turkey between 2005 and 2006. Using bone marrow from 6 survivors, his

group was able to generate a broad spectrum of over 300 unique antibodies to H5 and H1. These can neutralize both H5 and H1 and have the potential to be used for immunotherapy as well as to guide vaccine design. The rationale for this approach is that human antibody libraries could provide better and broader immune profiles, a faster path to the clinic, and that the use of bone marrow cells as the source of the libraries also provided an opportunity to study a deeper repertoire of antibodies than that available from the peripheral circulation and provides access to the memory compartment. To avoid the problems associated with dominant clones found in phage panning, the investigators have developed a sub-library system.

The investigators had available both bone marrow and matched serum from these patients. These were tested for reactivity against the HA protein of the Vietnam/1203/04 strain, although the patients had been infected with the Turkish isolate. The results showed reactivity to the HA of Vietnam.

Dr. Kashyap described the process of library construction. He indicated that separate light chain libraries can be made and that Sea Lane Biotechnology has a proprietary method of bar coding the antibodies so that donor-specific panning studies can be done.

In doing "jack-pot" sequence analysis of the heavy chain, the investigators found that CDR3 region had little diversity; usually this region shows changes. Studies also showed multi-codon usage which suggested that there was independence of the origins for the anti-H5 antibodies.

The libraries were made into IgG. Plaque neutralization studies showed that there was neutralization of multiple H5N1 clades as well as of contemporary H1N1 strains. When the region of antibody binding was studied, it was shown to be non-reduced and uncleaved HA0. Dr. Kashyap suggested that this type of antibody could be developed and stockpiled for an outbreak or for treatment of oseltamivir-resistant infections. He indicated that the investigators would like to identify the epitopes and then engineer an antibody that might cross-react and neutralize other HA types such as H5, H1, H3, H9, and H7.

Broadly cross-neutralizing human monoclonal antibody against a highly conserved region in HA

Dr. Robert Friesen, Crucell B.V., Leiden, The Netherlands, described the efforts at Crucell to develop a passive antibody treatment for vulnerable populations infected with influenza. The current treatment options for this group of individuals is limited and they represent the group in which influenza infection results in the greatest morbidity, mortality and health-care costs. The challenge in this effort is the changing hemagglutinin subtypes. The goal of Crucell is to obtain an antibody that recognizes all subtypes of hemagglutinin. Dr. Friesen cited a recent publication by investigators at Crucell showing heterotypic neutralizing monoclonal antibodies that were cross-protective against H5N1 and H1N1 and that were recovered from human IgM memory B cells. Modeling studies showed that this antibody, designated CR6261, bound to the stem loop of hemagglutinin, a site that is distant from strain-specific antibody binding. Crystal structure studies show that only the H chain of the antibody makes contact with the viral protein and that about 60% of the binding is to HA2 (residues are mostly hydrophobic and part of an alpha helix) and 30% to HA1 (residues are mostly polar). Dr. Friesen suggested that the small contact surface might lead to broad cross-reactivity and also might lessen the likelihood of escape mutants. A possible reason for sub-type cross reactivity is that alpha-helical regions are universally conserved across subtypes. Dr. Friesen reported that CR62661 neutralizes H1, H2, H5, H6, H8, and H9. The antibody does not bind to H3 and H7. However, there is a glycosylation site in this region at H3 and H7, thus explaining why there is no binding.

Dr. Friesen presented data in BALB/c mouse lethal challenge studies using (H1N1 and H5N1 challenges) which indicated that CR6261 was protective when used either prophylactically or therapeutically.

Dr. Friesen noted that the viral epitope that the antibody binds to is involved in membrane fusion; and thus may be conserved because of its function. It appears that the basis for the prophylactic efficacy of the CR6261 antibody is that it inhibits the pH-induced conformational changes in the H1 and H5 hemagglutinins that are needed for virus fusion activity.

Dr. Friesen stated that a key issue in the application of monoclonal antibodies is the ability to manufacture them. He indicated that Crucell has a propriety cell line and process for the manufacture of antibodies. The properties of these antibodies suggest the potential for a universal therapeutic. Dr. Friesen noted that Dr. Marasco's group had found a similar type of antibody.

Monoclonal antibodies (MAbs) to neuraminidase

Dr. Vidadi Yusibov, Fraunhofer Center for Molecular Biotechnology, Newark, Delaware, described studies in which mouse monoclonal antibodies to influenza neuraminidase and hemagglutinin were induced in mice as hybridomas by proteins that were produced in plants.

Dr. Yusibov stated that neuraminidase was chosen for study since a number of anti-influenza drugs are targeted to the neuraminidase. Antibodies were sought that could inhibit neuramindase activity in egg-produced virus. Electron microscopic studies demonstrated that these antibodies reacted to the virus cell surface, and thus, had the potential to be used for diagnostics as well as for treatment either pre- or post-exposure. Dr. Yusibov noted that anti-neuraminidase antibodies limit virus spread and do not neutralize virus.

The antibodies were sent to the CDC for testing and were shown to inhibit homologous strains and also some drug-resistant strains. The 2B9 monoclonal antibody to the N1-neuraminidase has broad cross reactivity against strains from Clades 1, 2a and 2b.

In vivo studies examined the survival of mice that were challenged with the homologous Vietnam 1203/04 strain. Fifty percent of the mice survived when given antibody for 5 days beginning at one-hour pre-challenge. A 50% survival rate was also seen in ferrets. Using a proprietary platform, the investigators humanized their mouse monoclonal antibodies and found similar results. Dr. Yusibov indicated that the technology used in this work is flexible and low cost.

The Fraunhofer Center investigators also studied anti-H5 hemagglutinin monoclonal antibodies produced by the same process. These monoclonals react with the viral cell surface. They plan to combine both types of monoclonal antibodies to determine if they can achieve complete protection against the homologous virus strain.

Dr. Yusibov concluded his study by briefly describing the GMP facility that Fraunhofer is building in Delaware and the plant expression system that was developed with DARPA support as a rapid and cheap methodology for monoclonal antibody production. The system uses vacuum infiltration for vector delivery and has a hydroponic system which allows for contained biomass generation. He indicated that one can start with an unknown gene and in less than 12 days provide a 98% pure product.

A unique class of protective human anti-M2 mAbs for pandemic influenza

Dr. Matthew Moyle, Spaltudaq Corporation, Seattle, Washington, described efforts to generate monoclonal antibodies to the M2e protein. This transmembrane antigen is present on the surface of virus and infected cells. It is accessible to antibody, but is also highly conserved in most strains. M2e is a proton channel that is essential for release of viral genetic material during infection, thus minimizing the potential for escape mutants. He noted that the amantadine target is in a different domain of the protein.

A number of companies have sought to make M2e-based vaccines, and thus far, protection by such vaccines seems to be due to antibody-mediated killing of infected cells. The elicited antibodies bind a linear M2e epitope present on the surface of infected cells, but do not bind to virus; thus, there is not a direct viral clearance mechanism. Additionally, the antibodies lack broad cross-protection. The approach at Spaltudaq Corporation was to seek naturally expressed human antibodies which might have been optimized by natural selection for target, epitope, affinity and effector function. To find such antibodies, the investigators used a proprietary discovery platform, I-STAR™. The donor population was normal healthy individuals in which B-cells were cultured in single-cell wells without the immortalization of the cells. Antibodies were selected using a screen for binding and function. The investigators felt that in contrast to selection through phage and yeast display, the Spaltudaq process would provide for native H and L chain pairing and would be less likely to generate H and L cell incompatibility or other undesirable properties. Although the initial screen with a peptide ELISA provided no hits in 120 donors; a cell-based screen with stably transfected 293-cells provided 12 positive donors. Two of these were used for B-cell activations. By this process, the investigators obtained two human anti-M2e monoclonal antibodies that bound infected cells and virus. The Spaltudaq antibodies cross-reacted with several high pathogenicity strains, Vietnam/1203, FW/1/50 and HK/483. These monoclonal antibodies bound to >99% of the M2e variants catalogued in the NCBI Influenza database. They have been tested against H1, H3, H5, H6, H7 and H9.

In studies in BALB/c mice involving lethal H5N1 challenge, there was protection when the antibodies were used prophylactically. In mouse studies in which the humanized monoclonal was used therapeutically against a Vietnam 1203/04 challenge, there was protection by the humanized antibodies; oseltamivir was not therapeutically effective in this type of experiment.

Dr. Moyle proposed that the human monoclonals used in this work might have better properties than traditional monoclonal antibodies in terms of epitope conformation, conservation across strains, binding to virus, clearance of virus by opsonophagocytosis, broad cross reactivity and protection *in vivo*. These types of antibodies can be stockpiled. It is anticipated that they will enter human testing in about 18 months.

Dr. Moyle noted that although these antibodies are not an answer for pandemic influenza, they might be useful to inform vaccine design. These antibodies make contact at the N terminal conserved sequences of the M gene which may lessen the likelihood of escape mutants. Most of the published anti-M antibodies make contact at the more variable region of the M gene.

To facilitate screening of native antibodies, Spaltudaq has developed an antibody microarray screening system on a microscope slide. Dr Moyle noted that there is the potential to make HA and M2e antibody cocktails.

4. CLINICAL SESSION: CLINICAL TRIALS OF NEW AGENTS

Peramivir advanced clinical development program

Dr. William Sheridan, Biocryst, Cary, North Carolina, described the clinical development of peramivir which was discovered at Biocryst through structure-based drug design and was designed to fit the influenza neuraminidase active site. Peramivir has broad-spectrum activity against influenza A and B, including activity across H3N2, H1N1 and B isolates. In a mouse model, intramuscularly administered peramivir is effective against lethal challenge with H5N1 viruses. Even a short administration rescued many mice; a long administration rescued all mice.

Given the rapid emergence of resistance to oseltamivir, peramivir would be useful in H1 infection *in vivo*. Mutations in the neuraminidase affect the dosage of peramivir needed, but seem to do so to a lesser extent than for oseltamivir. Currently, the dose and efficacy *in vivo* are not known, but are being based on the *in vitro* data.

In terms of the key events for peramivir clinical development, Dr. Sheridan summarized two Phase 2 projects. Randomized double-blinded placebo-controlled Phase 2 studies in Japan were undertaken in uncomplicated influenza in non-hospitalized individuals and used two doses of peramivir. The primary endpoint was Time to Alleviation of Symptoms (TTAS) and the results showed that there was a significant reduction in symptoms and in resolution of symptoms compared to placebo. A patient diary code was used to measure the symptoms. There was also an improvement in time to resolution of fever and a reduction in viral load in peramivir versus controls. Given the use of patient-scored outcomes, there is a potential for effects of cultural factors. In terms of safety, there are data on over 1300 patients involving several doses up to 600mg that will be used in the U.S. studies. The level of AE's is similar to that found in other studies of experimental products versus placebo.

The 2008 Biocryst Phase 2 study in the U.S. is a placebo control double-blinded multi-center randomized trial in uncomplicated influenza and uses a dose of 600mg IM and rapid antigen tests and symptoms to define subjects. The endpoint is also TTSA. There are a number of secondary endpoints and the study is ongoing.

During the discussion, there was the suggestion that the concentration of the drug in the nasopharynx might be more relevant and might be lower than the plasma levels. It may be possible to look at salivary levels as a way to assess this issue. It was also noted that pediatric studies for peramivir in uncomplicated disease are being considered.

Long-Acting Inhaled Neuraminidase Inhibitors (LANI), potent influenza antiviral

Dr. Jane Ryan, Biota, Melbourne, Victoria, Australia, presented information about CS-8958 which is being developed by Biota in partnership with Daiichi-Sankyo as a long-acting inhaled neuraminidase inhibitor (LANI) for both treatment and prophylaxis. A single inhaled dose is planned for treatment and a weekly dose for prophylaxis. The product is a dry powder that is delivered through an easily used and disposable inhaler. Only a small amount of product is used and the material is felt to be suitable for stockpiling. CS-8958 is a lead compound and the pro-drug of R-12589. The pro-drug is long lasting in the lung, thus reducing the number of doses needed; the pro-drug needs to be hydrolyzed for activity.

In vitro, R-12589 inhibits neuraminidase and viral replication in cultured cells and is effective for N1 through N9 as well as for avian H5N1. It is also active against oseltamivir-resistant mutants and has a zanamivir-like activity. In comparative studies in mouse infection/survival models, topically administered CS-8958 was similar in efficacy in survival outcomes to multidoses of zanamivir when given 7 days prior to virus infection and in the treatment context when given one day after virus infection. One topical dose of CS-8958 was found to be as effective as a multi-dose oseltamivir regimen in reducing mortality when given 7 days prior to infection. In terms of reduction of viral titer, CS-8958 was as effective as zanamivir (IN) and oseltamivir (PO).

When given IN or IT to the lung, CS-8958 is rapidly converted to RS-12589 in the lung and remains there. Dr. Ryan stated that non-clinical safety studies have shown an excellent safety profile.

Dr. Ryan summarized the status of clinical studies with this product. In Japan, a Phase 2, multi-center, randomized trial in complicated influenza has been completed in which CS-8958 was used as a single inhalant and the outcome was measured as time to resolution of symptoms and resolution of fever. Phase 3 clinical studies are going on in Asia in the 2008-2009 timeframe. NIH-funded Phase 1 clinical

studies are going on in the United Kingdom (UK) using a single dose of drug in healthy adults 18-55 years of age and a single dose in elderly persons 65 years of age and older. Thus far, there is an excellent safety profile and the PK is consistent with the findings in Japan and is consistent with long-term residency of the drug in the lungs. The UK studies complement those in Japan and provide data in a non-Asian population. The UK studies also incorporate spirometry and address potential mood issues.

During the discussion, it was noted that the reports of suicide events related to Tamiflu in Japan have not been observed in western countries. It was also noted that virus infection itself has been associated with CNS effects and that the suicide-association reported in Japan is not a clear one. It was further indicated that Roche Pharmaceuticals did a careful analysis of CNS effects and did not find any firm evidence for them. It was suggested that there is a possibility for non-conversion of pro-drug in teenagers.

There was also discussion of ways to measure the mean residence time of drug in the lung; it appears that the active compound is tightly associated with tissue and so it is not clear if bronchioaveolar lavage (BAL) would be suitable for assessing this. It was noted that drug efficacy will be the proof of sufficient levels of drug for resolving infection and for inhibiting resistant virus.

It was also noted that there are plans to study the CS-8958 in persons with underlying diseases. Dr. Ryan indicated that the use of a single dose reduces the possibility of bronchospasms. During the discussion, it was noted that pediatric studies are being considered for CS-8958; these studies would occur outside of Japan.

T-705 (Favipiravir): A novel anti-influenza viral agent

Dr. Yousuke Furuta Toyama Chemical Co., Tokyo, Japan, reported on studies with T-705 (Favipiravir), which was found by an in-house screen and selected based on potent *in vitro* antiviral activity, excellent oral bioavailability, and excellent *in vivo* efficacy. It was initially evaluated with NIAID assistance in 2005 and tested in Phase 1 studies in Japan and the U.S. in 2007 and Phase 2 studies in Japan in 2008.

T-705 has *in vitro* activity against H5N1, H1N1, H2N2 and H3N2 viruses as well as against type B and type C influenza; it also has activity against oseltamivir- and amantadine-resistant influenza viruses.

T-707 has 97% bioavailability in mice. Its therapeutic efficacy measured as survival in mice in high viral dose challenge with A/PR8/8/34 H1N1 was much better than that of oseltamivir. 100 percent protection of mice was seen with H5N1 A/Duck/MN/1525/81 challenge; by comparison, there was only 20% survival of mice receiving oseltamivir. Good therapeutic efficacy was seen even when treatment was started 96 hours after challenge with H5N1 virus.

Mechanistically, T-705 is converted to the ribosyl triphosphate active form which inhibits the viral RNA polymerase in a dose-dependent manner. There was a relationship between PK parameters and lung viral load in mice inflected with A/Osaka/5/70 (H3N2). The AUC showed a correlation with efficacy.

In Japan, Phase 1 studies are completed and Phase 2 studies with seasonal influenza are underway; Phase 3 studies are being planned for initiation as soon as Phase 2 studies end. Phase 1 studies of T-705 are currently underway in the U.S. with Phase 2/3 studies being planned for this year in the U.S. and/or European Union and Southeast Asia. The results from PK studies in the U.S. and Japan are comparable. There have also been multi-dose PK studies done. No serious AEs have been found.

A Phase 2 clinical efficacy study comparing high dose (600 mg) and low dose (400 mg) groups of T-705 with oseltamivir (75 mg bid 5 days) has finished the treatment stage and analysis of the data is underway.

During discussion, it was noted that the PD could be different in mice and humans. There was also a suggestion that given its proposed mechanism of action, the drug be tested for its effects on other RNA viruses and DNA viruses where it might also be active.

Inhaled Cationic Airway Lining Modulators for the Treatment and Prevention of Influenza: Advancing a new paradigm for the treatment and control of infectious and progressive respiratory

Dr. Robert Clarke, Pulmatrix Inc., Lexington, Massachusetts, presented the use of inhaled cationic airway lining modulators (iCALM) for influenza prophylaxis and treatment. iCALM are specific cations that show dose-responsive effects and can be delivered as a simple liquid aerosol, a concentrated liquid aerosol or as a dry powder. They target the airway lining fluid and have multiple effects. Rheological effects are at the interfacial surface and reduce the pathogen's ingress; osmotic effects enhance mucus clearability and alter pathogen homeostasis; mechanical effects improve MCC. Both innate and induced immunity have been shown to be enhanced by them. Patents are currently being filed for this product which is a 4 micron particle that does not directly inactivate the virus.

In vitro investigations have shown that iCALM reduces the penetration of influenza and of gram+ and gram- bacteria through a rheological mucus mimetic. iCALM induces a dose-dependent reduction in influenza virus titer in an air-liquid interface cell surface model. A combination of iCALM and a proprietary neuraminidase inhibitor showed synergy in reduction of viral replication compared to either treatment alone. This effect was dose dependent.

Dr. Clarke suggested that this approach has demonstrated efficacy as a "one-drug, multiple-bug" technology to treat, prevent and reduce transmission of a broad spectrum of respiratory pathogens. Dr. Clarke summarized results from animal studies and showed data indicated that iCALM inhaled only through the ferret nose significantly reduced the clinical signs associated with influenza (e.g. body temperature, weight loss, inflammatory cell counts). In swine models, iCALM treatment reduced clinical signs and lung pathology. In the transmission studies in which uninfected swine breathe air exhaled from infected animals, a reduction of airborne influenza transmission was observed. There was no evidence of illness in the exposed animals, but there was seroconversion, demonstrating that they had been exposed to the virus.

In terms of clinical development, Phase 1 studies have been completed in the U.K. There were no serious AEs observed; the mild AEs were not dose-related. Additional studies will be done in persons with asthma. There will also be placebo-controlled treatment studies in the UK with experimental infection. The investigators also hope to do a multi-site family study with household contacts.

The investigators anticipate that this type of product has a reduced likelihood of generating resistance and can be used for multiple pathogens. The current plan is in the direction of a dry powder. Dr. Clarke stated that for regulatory purposes, the product will be treated as a drug by the FDA.

During the discussion, there was a question of how the product might work in terms of blocking cell to cell transmission of virus. There is consideration being given to using iCALM in the context of cystic fibrosis and for studies to assess the effects on secondary bacterial infection in the context of influenza.

Development of DAS181 (Fludase®) for seasonal and pandemic influenza

Dr. Fang Fang, NexBio, Inc., San Diego, California, spoke about the properties of DAS181 (Fludase®). DAS181 is a fusion protein with a sialidase domain and an anchoring domain. It functions as an influenza virus receptor inactivator, targeting human and avian influenza viruses. DAS181 has demonstrated efficacy against more than 40 influenza virus strains *in vitro* and and *in vivo* and efficacy against H5N1

virus in mice and ferrets. DAS181 is also an inhibitor of parainfluenza viruses, which currently have no treatment options. This dual activity would facilitate empiric treatment of patients. DAS181 also has an anti-asthmatic effect which makes it a good choice for patients with underlying lung disease. The drug is formulated as powdered microparticles, an inexpensive format, and is stable for more than 2 years.

Studies have been done comparing the effects of Tamiflu, Relenza and DAS181 with clinical influenza isolates. Most recent virus isolates are Tamiflu-resistant and Relenza-sensitive. DAS181 was effective against neuraminidase-resistant clinical isolates.

Dr. Fang described selective-pressure passage studies to determine if DAS181-resistant viruses could be generated and to define the mechanism of resistance of such viruses. She stated that no meaningful resistance in breakthrough viruses was found from DAS181-treated MDCK cells, Human airway epithelia (HAE) and ferrets. Mild resistance developed in only two of the six influenza virus strains following up to 30 passages under increasing DAS181 selective pressure. She presented data on DAS181-resistant B/Maryland/1/59 in mice. Compared to wild type, the resistant virus showed low virulence in terms of causing mortality, body weight loss and virus titer. The resistant viruses grew slower and had smaller plaque size *in vitro*. The B/Maryland- DAS181-resistant virus was found to bind to the cell receptor with about 6 times higher affinity than the parental strain. The neuraminidase protein level in resistant virus was reduced by about 60%. Dr. Fang stated that the diminished virulence seems to be due to the HA/NA imbalance. Studies of DAS181-resistant A/Victoria/3/75 also showed reduced plaque size and reduced *in vivo* virulence. There was increased affinity to α 2,6-linked sialic acid due to a S186I mutation in the HA. This virus remains sensitive to the standard *in vivo* DAS181 treatment dosage. The DAS181-resistant phenotype is unstable and reversible within 2 passages after DAS181 withdrawal. This makes it unlike other types of drug-resistant influenza virus phenotypes.

Mouse studies suggested that DAS181 treatment prevented secondary pneumonia. In this model, animals not treated with DAS181 lost weight and died.

Phase 1 studies with DAS181 showed that it is well-tolerated and does not affect pulmonary function or oropharyngeal bacterial colonization. A Phase 1B clinical trial will be started in April 2009.

Poly-ICLC

Dr. John Beigel, Macrogenics, Rockville, Maryland, presented work that he had done while at NIH on the use of Polyinosinic-Polycytidylic acid (Poly-ICLC) as a possible influenza antiviral. Poly-ICLC is a dsRNA that is stabilized with poly-lysine and carboxymethylcellulose. It induces interferon through TLR-3 and induces antiviral enzymes. A number of findings by others had shown the protective prophylactic usage of Poly-ICLC in mouse challenge models of a number of infections agents including influenza, respiratory syncytial virus, SARS and anthrax. However, studies of this agent in humans in the late 1980's in the context of influenza infection and intranasal delivery had found unacceptable levels of nose bleeds and nasal ulcers. Studies in Australia showed protection of family members exposed to an index case of rhinovirus infection.

Dr. Beigel felt that Poly ICLC was worth revisiting because of its broad reactivity, which on a practical level would allow usage by physicians even without knowledge of the infectious agent, a feature that is appealing for both influenza and biodefense uses. The effects of Poly-ICLC in animals are long lasting and the agent is inexpensive to manufacture. There is a record of safety of Poly ICLC in oncology studies.

Dr. Beigel briefly described a Phase I study that he organized and that started in May 2008 and finished in February 2009. Fifty-six subjects participated and were accrued in cohorts of escalating doses. Although the results are blinded, the overall safety data indicates that there were no SAE. Two of the 4 AE events observed are unlikely to be related to the drug. The other AEs include rhinorhea and headache, common

AEs to most Phase I studies and taste alteration and sinus congestion, which may be drug related. There were 3 reports of nasal bleeding, but none of ulceration.

Dr. Beigel noted that uncertainties about Poly-ICLC include the safety profile with repeated doses, whether Poly-ICLC has a different profile than interferon in influenza and whether the results are as long lasting in humans as they are in mice. Despite these uncertainties, Dr. Beigel felt that further studies were warranted.

During the discussion it was noted that the 4-5 previous human interferon studies were all associated with mucosal inflammation and that interferon efficacy in humans was only shown for rhinoviruses. It was suggested that for other respiratory agents, it may be necessary to get drug delivery to the lower respiratory tract.

5. PRE-CLINICAL/CLINICAL SESSION: COMBINATION THERAPY

General perspective on Chinese combination therapy to combat influenza

Dr. Nanshan Zhong, Guangzhou State Key Laboratory of Respiratory Diseases, Guangzhou, China, noted that two of the three influenza pandemics of the 20th century began in Asia. WHO has confirmed 412 cases of H5N1 influenza in humans; of these 256 have died. He indicated that the traditional view of translational medicine is from bench to bedside. In China, translational medicine started to move toward taking empirical medicine to evidence-based medicine and that the further path should be moving these findings to the bench and then the bedside.

Dr. Zhong stated that the traditional approach to treatment of influenza in Chinese medicine is to remove the heat, chill and malaise of the acute stage. The integrated approach is to use herbs to treat the acute stage and then to strengthen the body's resistance ("Fu Zheng"). Dr. Zhong summarized the use of integrative therapy and treating of SARS patients in which a combination of herbs was used to treat the acute fever stage with the goal of cleaning the heat and relieving exterior syndromes. During the progression stage a different set of herbal treatments was used to supplement the Qi, nourish the Yin and strengthen the body resistance. Another group of patients received an integrated therapy which was designed to maintain the oxygen saturation and which provided better relief of symptoms, such as malaise and dyspnea, and which accelerated the resolution of pulmonary infiltrates.

Dr. Zhong summarized the findings of a double-blind study comparing Ganmao capsules (which contained a mixture of herbal medicines) and Amantadine for prophylaxis and treatment of influenza during an outbreak in Tianjin in 1998. The findings showed better efficacy for therapy (time to symptom relief) and prophylaxis in the Ganmao group; this approach was found to be cost-effective and had no adverse events [Xue E. Dong Ze, Tianjin J Trad Chin Med. 1999; 16(4): 13-15].

Dr. Zhong also summarized the results of a three-arm trial study of the antipyretic effect of Mao-to, a Japanese herbal medicine, compared to oseltamivir alone or in combination for the treatment of type A influenza infection in children [Tomohiro Kubo, Hidekazu Nishimura, Phytomedicine 14 (2007): 96-101]. The study concluded that orally administered Mao-to was more effective than oseltamivir in the control of fever due to type A influenza infection in children.

Dr. Zhong summarized studies done to assess the antiviral activity of Isatis tinctoria L extract in influenza (A/PR/8/34 (H1N1)) infected MDCK cells. These studies showed that the extract from this root could inhibit cell death *in vitro*. Other data suggest that Isatis tinctoria L. may interact with the HA activity of H1N1 and block RNP expression. A double-blind placebo control trial is planned to evaluate the clinical efficacy of this extract.

Dr. Zhong noted that the evidence currently available to support the use of Chinese medicinal herbs for preventing and treating influenza is not sufficient and that more randomized controlled trials are needed in this area.

Dr. Zhong described his experience in using convalescent plasma or plasma from vaccinated individuals in the management of human avian influenza. The first patient, a cargo truck driver, presented with fever, cough, and dyspnea in June 2006. His condition deteriorated to multi-organ failure and he was confirmed as having avian influenza. He received convalescent plasma with a neutralizing antibody titer of 1:80 obtained from a patient who had recovered from H5N1 infection in February 2006. Three 200 ml infusions were given on day 12 after the onset of his illness. His virus load dropped from 1.68×10^5 copies per mil to 1.42×10^4 copies per ml during the first 8 hours of infusion. The patient showed remarkable improvement. His heart and renal function returned to normal and he recovered from his illness.

A second patient appeared to have been infected by person to person transmission. The onset of his illness was December 3, 2007. On December 7, the patient received two 200ml transfusions of plasma from a 30-year old woman who had received two doses of an inactivated H5N1 vaccine. The plasma was obtained 280 days after the second vaccine dose and was heat inactivated. The plasma had a titer of 1:40 against the clade 1 vaccine strain A/Vietnam/1194/2004-RG and 1:20 against the clade 2 virus strain A/Jiansu/2/2007. The patient recovered on December 10 and was discharged on December 26.

During the discussion, it was noted that traditional drugs are formulated differently by different doctors and may be given in different regimens. The fact that these traditional drugs have been used for thousands of years suggests that they are effective. It was also noted that there may be synergy between the multiple compounds in the herbal mixtures.

Method and strategy for influenza protection and treatment with Traditional Chinese Medicine (TCM)

Dr. Chuanjian Lu, Guangdong Provincial Hospital of TCM, Guangdong, China noted that there are many difficulties in the prevention and control of influenza which are related to a number of factors including the rapid virus mutation and relatively slow progress of vaccine development, the variety of virulent strain subtypes for which there is a lack of immunity in the general population, the fact that organ or tissue injury may occur as a result of an aberrant innate immune response, and the lack of sufficient antiviral drugs which is further complicated by the occurrence of drug resistance. WHO and national governments are developing strategies for the prevention and control of influenza in which traditional Chinese medicine will play a part.

Dr. Lu provided an overview of influenza from the perspective of Chinese medicine. She noted that over a 2000 year period there have been about 321 influenza pandemics in China which have been successfully controlled and limited to small regions and in which traditional Chinese medicine played a role in prevention and control. The potential approaches of Chinese medicine for influenza include: prevention of disease, reduction of the severity of symptoms, shortening of the duration of illness, treatment of complications and inhibition of aberrant innate immune responses. Prevention before an outbreak involves achievement of the balance of Yin and Yang by physical exercise, strengthening of the vital Qi, diet adjustment, life-habit regulation, emotion adjustment, pathogen evasion and taking Chinese herbal medicines. The principles of Chinese medicine relating to the treatment of influenza include: taking a holistic view of the human body and nature; differentiating the syndromes and treatment of the syndrome; strengthening the vitality and pathogen evasion which includes adjustment and restoration of the harmonious physical condition and activating the body's immunity; relieving of the primary and

secondary symptoms; and applying therapeutic measures in line with factors such as the season, local conditions and individuality.

Dr. Lu provided examples of classic decoctions used to treat influenza. She noted that modern research on pharmacologic mechanisms of TCM could demonstrate a range of ways in which TCM agents improve the body's ability to handle influenza infection including: antiviral effects, enhancement of effective immune responses and reduction of immunopathological responses and of secondary infection. Among the advantages of TCM are the focus on strengthening of the host as a prevention strategy and the ability to treat multiple organ systems. Dr. Lu noted that more randomized clinical trials need to be done in order to provide clinical evidence and to form clinical guidelines for use of TCM approaches and that there are opportunities for international collaborations and exchange in such endeavors.

Dr. Lu concluded her presentation by providing a brief introduction to the history, clinical practice, collaborations and facilities of the Guangdong Provincial Hospital of Chinese Medicine.

Pharmacodynamics for influenza drugs: Viral suppression and resistance emergence/suppression

George L Drusano, Ordway Research Institute, Inc., Albany, New York, discussed the implications of the pharmacodynamics (PD) of antiviral compounds for the development of drug-resistant influenza viruses. He showed data to demonstrate that amantadine shows a clear dose-response, but resistance emerges quickly in the hollow fiber infection model (HFIM) and in man. Dose response and resistance emergence occur both in continuous infusion and once-daily administration modes. Continuous infusion exposures of 6mg/L (AUC_{0-24} 144mg*h/L) did not suppress resistance and showed no trend to resistance suppression. Daily administration did not suppress, but did show a trend (Peak/EC_{50} suppresses resistance). As 660 mg is 3.3 times the normal daily dose and as amantadine has neurotoxicity limitations, amantadine cannot be dosed to suppress its own resistance.

Studies with two inhibitors of influenza neuraminidase, oseltamivir and peramivir, indicated that it is possible to identify the PD-linked variable for neuramindase inhibitors in a HFIM of influenza. The AUC/EC_{50} ratio is linked to viral suppression. The same outcome is found in man for peramivir. This implies that oseltamivir may be able to be administered once-daily in an influenza outbreak situation (150mg daily); however, this needs clinical validation. With respect to inhibitors of influenza A neuraminidase, it is possible to link exposure to effect in a human challenge model. Other than drug AUC, only baseline viral titer is important. Knowing the breakpoint exposure that will clear the nasal secretion of virus by a specific time is vitally important in containing influenza outbreaks by treating index cases and contacts. Even if the nasal viral titer is not gone, the transmission probability is lowered as the titer is lowered. Thus, understanding the PD of a drug is important to its clinical and epidemiological usage.

Statins as anti-influenza therapies?

Dr. Dale Barnard, Institute for Antiviral Research, Utah State University, Logan, Utah, described studies in BALB/c mice to determine the therapeutic effect of simvastatin on influenza infection. The underlying concept is that the anti-inflammatory properties of simvastatin would reduce the severity of a lethal infection when used in combination with a potent antiviral drug.

Studies with the A/NWS/33 (H1N1) strain at different doses of statin did not show a treatment effect under conditions in which ribavirin was effective. No protective effect in terms of survival or decrease in viral titers was seen against H3N2 infection in a BALB/c. Other measures that did not show benefit were lung weight or survival times.

Additional studies were undertaken on the efficacy of simvastatin, alone or in combination with T-705, in an H5N1 avian influenza BALB/c mouse model. Simvastatin alone was not effective. Simvastatin appeared to have some amelioratory effect on lung weights when used in combination with T-705. As expected T-705 effectively reduced virus lung titers, number of deaths due to infection and increased survival time. There did not seem to be significant synergy between the combinations. Combination studies of simvastatin and ribavirin are not yet completed, but there is a suggestion that the time of death was delayed.

Dr. Barnard summarized by stating that thus far, simvastatin did not seem to have a sufficient effect on the lethal challenge. During the discussion, it was suggested that a stronger statin might have a stronger effect. It was also noted that the anti-inflammatory effects of statins might be different in mice than in humans.

Regulatory perspectives on combination therapies

Dr. Barbara Styrt**,** FDA, CDER, Office of Antiviral Products, Silver Spring, Maryland, provided an overview of regulatory issues related to combination therapies. She indicated that there are a number of definitions that are used to define a combination therapy. She stated that there are three main FDA divisions with regulatory oversight for the kinds of therapeutics that were being discussed at this meeting. The Center for Drug Evaluation and Research (CDER) regulates products such as small molecule drugs, therapeutic proteins and monoclonal antibodies. The Center for Biologics Evaluation and Research (CBER) regulates products such as polyclonal immunoglobulins and antisera, vaccines and challenge pathogens. The Center for Devices and Radiobiological Health (CDRH) regulates products such as administration devices, *in vitro* diagnostics and personal protective equipment. The Division of Antiviral Products in CDER reviews proposals and data for new antiviral drugs or new uses of existing drugs; reviews drug products proposed as immunomodulators for viral infections; and reviews antiviral therapeutic proteins, monoclonal antibodies, and cocktails containing more than one monoclonal antibody. Overall, a proposed combination would be regulated based on the types of products and proposed approach.

Since there are some situations that are ambiguous as well as exceptions to this division of regulatory oversight, it is important for investigators and corporations to submit questions in advance. The FDA has a process for adjudicating jurisdiction within the Agency. There are also standards of evidence that apply to each type of product. Products need to be demonstrated to be safe and effective and shown to have the purported effects. There are internal Agency processes for obtaining expertise from other Divisions when that is needed to assess the product.

Dr. Styrt cited sections of the Code of Federal Regulations (CFR) that are applicable to combination therapies. Combinations are defined under 21 CFR 3 as are points related to jurisdiction. Standards of evidence are defined in 21 CFR 300.50. There are also Agency guidance documents (http://www.fda.gov/cder/guidance/index.htm and http://www.fda.gov/cber/guidelines.htm) that should be helpful to investigators. She also noted that there is a website for pre-IND interactions and contact information ((http://www.fda.gov/cder/ode4/preind/default.htm and links related to emerging infections (http://www.fda.gov/cder/ ode4/preind/emerging.htm).

Dr. Styrt presented several fictional cases of combination products and discussed which FDA division might have primary oversight and how other divisions or experts from other parts of the Agency might be asked to participate in assessing the submitted materials.

Dr. Styrt presented several issues that are specifically related to influenza, particularly the continually changing and sometimes unpredictable strain changes in the host-pathogen system. This creates the potential for unanticipated consequences, such as the Guillian-Barre Syndrome, that occurred during

swine influenza vaccination. She noted that it could be challenging from a regulatory perspective if an efficacy study is done with a monoclonal antibody and the manufacturing process is subsequently changed. She also noted that if a device such as an inhaler is changed, one cannot assume the equivalence of the new device.

She stressed that early contact with the Agency is important in all cases and allows for the agency to address adjudication and special expertise needs early in the process so as to facilitate interactions and speed action. Pre-IND consultations are encouraged. These do not need to be formal visits, but could just involve the submission of written information and the provision of written feedback by the Agency.

SUMMARY

The workshop participants provided an overview of the state of the science in the development of a new generation of influenza antivirals. A number of antivirals with novel mechanisms of action are being pursued. Systems biology, novel screening assays, and genetic chemistry approaches are being applied to identify novel targets. Targets include not only the viral genes themselves, but also approaches to enhance host immunity or to modulate those host responses that lead to immunopathology. Host signaling pathways have alternative or redundant pathways that allow the host to overcome inhibition of a particular pathway; however, certain pathways may be specific and critical to influenza virus replication and thus may provide novel antiviral targets which have little host toxicity. Traditional medicine and the study of natural products may also provide insights into novel therapeutic approaches and products. Broader scale studies of fractionated materials would be important in this context and allow for the detection of products that are present in low concentrations. The participants noted that there are gaps in our knowledge about the use of antivirals in vulnerable populations and about the potential for using combination therapies to enhance antiviral efficacy and reduce the potential for the emergence of resistance. Presentations from FDA staff provided an overview of regulatory issues and processes related to licensure of combination therapeutics and to the regulatory risk-benefit assessment for products that are proposed for use in vulnerable populations.

During their presentations, the speakers acknowledged a number of collaborative efforts among investigations in academia, industry and government which had facilitated progress and had allowed for the types of interdisciplinary and multidisciplinary efforts needed for providing the basic knowledge underlying the development of influenza antivirals and for the application of that knowledge to the development and testing of new drug products.

Dr. Krafft thanked the participants in the workshop and noted that the finalized slides from the workshop would be available on a CD-ROM to persons requesting them.