Development and Use of Antivirals for Pandemic Influenza

Meeting Summary

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BACKGROUND AND INTRODUCTION

Dr. Bruce Gellin, Director of the National Vaccine Program Office of the US Department of Health and Human Services (HHS), welcomed participants to the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) meeting on development and use of antivirals for pandemic influenza. Attendees were members of research institutions, regulatory agencies, clinical centers, and public health organizations from the United States and abroad.

Dr. Gellin indicated that the mission of the Department of Health and Human Services and agencies within HHS is improving the health, safety, and well-being of the public. It is involved in global activities, planning emergency response activities at the federal, state and local level.

The HHS currently has approximately 36 million courses of antivirals on hand or on order; the goal is to have 81 million courses of antivirals. In the event of a pandemic, 6 million courses would be used to contain an initial U.S. outbreak and 75 million would be used to treat 25 percent of the US population.

He indicated that the main objectives of the meeting are to bring together experts on many facets of influenza research to review and discuss the current status of antiviral use, availability and future directions. In particular participants were asked to:

- Provide an overview of current anti-influenza antiviral research (basic to clinical studies);
- Identify critical scientific issues that must be addressed in the development of antivirals for influenza;
- Identify strategies for advancing development of products to treat and/or prevent influenza; and
- Identify steps that can be accomplished now to enhance the understanding of influenza antivirals and their potential uses for pandemic influenza.

MEETING STRUCTURE

Presentations from 21 speakers were interspersed with periods set aside for questions, comments, and open discussion. The meeting moderator, Dr. Arnold Monto, encouraged questions from the floor during the presentations.

The first day of the meeting included presentations and discussions on regulatory considerations, basic virology, antiviral drugs and drug targets, and human studies. The second day consisted of additional presentations and discussions on a variety of animal models, as well as an open discussion to address critical scientific issues and strategies.

REGULATORY PERSPECTIVE

Dr. Barbara Styrt from the Food and Drug Administration (FDA) discussed development of influenza antivirals in the context of regulation and approval. Historically, treatment effects have been studied in people who are otherwise healthy, while prophylaxis studies have occurred in settings where exposure has been documented.

The four antiviral drugs currently approved for influenza are amantadine, rimantadine, zanamivir, and oseltamivir. Table I summarizes the influenza types these drugs inhibit, the route of administration, the age groups for which the drugs are approved for treatment and prophylaxis, and the approval dates.

Table I. Antiviral Drugs for Influenza

	Amantadine	Rimantadine	Zanamivir	Oseltamivir
Influenza Types Inhibited	A	A	A and B	A and B
Route of Administration	Oral (tablet, capsule, syrup)	Oral (tablet, syrup)	Inhalation	Oral (capsule suspension)
Ages for which treatment is approved	≥ 1 year	Adults	≥ 7 years	≥ 1 year
Ages for which prophylaxis is approved	≥ 1 year	≥ 1 year	≥ 5 years	≥ 1 year
Approval date	1960's	9/17/93	7/26/99	10/27/99

Dr. Styrt briefly described some of the stages in the approval process. Initial review of proposals for new antiviral drugs and for new uses of existing drugs occurs in the FDA Division of Antiviral Products; this office serves as a point of contact for collaboration with other divisions and centers. It was noted that the first contact with FDA is usually a pre-Investigational New Drug (pre-IND) consultation, which is often used to gain feedback. The pre-IND could include *in vitro* and animal antiviral activity data, preliminary development questions, and plans for human studies; the pre-IND also may explore protocol concepts for new uses of existing drugs. The FDA reviews everything that is submitted and may allow the pre-IND to proceed, place it on hold, or offer advice to applicants.

Dr. Styrt described the next stage of the approval process, which is the IND submission. She mentioned that this occurred after identification of a compound or formulation by the sponsor and includes animal safety and *in vitro/in vivo* activity data, initial development plans, and a clinical trial protocol to be executed in the United States. The standard sequence is Phase I (pharmokinetics [PK] and tolerability), Phase II (additional safety and dose finding), and Phase III (larger pivotal studies to demonstrate safety and efficacy). She noted that there other components including a toxicity profile, information on the population, and treatment route. The type of protocol that would be approved would depend on the drug, the proposed use, and available information.

Dr. Styrt continued her presentation with information on the definition of Emergency Use Authorization (EUA) and when it would likely be used. EUA would be appropriate to use in a life-threatening situation and only in a declared emergency. She mentioned that EUA was a relatively recently developed path for use of pharmaceutical products and does not replace the IND process or standard licensure pathways. She further described that unlike a bioterror event, which would be a finite occurrence, pandemic potential for influenza would continue for an unknown period and a New Drug Approval (NDA) or Biologics License Application (BLA) would be necessary. NDAs and BLAs would require adequate and well-controlled studies that support the conclusion that the drug had the stated effect. Typically, there would be two clinical trials for the indication, as well as supporting studies, which could be initial PK/tolerability assessments, cell culture and animal model activity data, experimental human challenge studies, and exploratory PK and safety data in varied populations.

Dr. Styrt discussed that data obtained in animal models has played an important role in development of influenza therapeutics. Toxicology/kinetics studies in animals performed early in the development process may affect the type and design of subsequent human studies in terms of range of *in vivo* activity, dose, and duration, time of initiation of treatment, and disease manifestations that would be explored. She

stated that the "Animal Rule" (21 CFR 314.600 or CFR 601.90) allows the FDA to rely on animal studies if human studies were not feasible or relevant, if the pathophysiology was reasonably well understood and endpoints were relevant, and if animal models were expected to predict human outcomes and dosing. Generally, human studies in influenza have been feasible and animal models were not uniformly predictive, largely because it was not known which viral strains would affect humans; therefore, although animal data continue to play an important role in influenza drug research, the Animal Rule has not been paramount for influenza drug development.

Dr. Styrt discussed regulatory procedures to be used in emergency situations and pointed out that in an emergency, most products will get rapid attention from the FDA. She reiterated that if the product is at the NDA stage there could be a priority review and if accelerated approval is necessary, surrogate endpoints may be considered for an accelerated approval. Dr. Styrt mentioned that this has been done for influenza drugs in the past. She continued and stated that if a product was not yet at the NDA stage but there was an emergency situation where the drug might be useful, other approaches could be taken, such as single patient INDs, treatment INDs, and similar kinds of protocols. However, she highlighted the fact that such measures would not greatly expand the amount of drug availability because investigational drugs would not be made in large quantities and that the goal of drug development should be marketing approval based on safety and appropriate use.

Dr. Styrt provided links for more information on this topic:
www.fda.gov/cder/drug/antivirals/influenza/default.htm,
www.fda.gov/cder/ode4/preind/emerging.htm,
www.fda.gov/cder/ode4/preind/default.htm

OVERVIEW OF INFLUENZA

Basic Virology

Basic influenza virology was discussed by Dr. Robert Lamb from Northwestern University. He presented information on a number of outbreaks of influenza A during the last century noting an H1 variant that caused the very severe flu pandemic of 1918 (Spanish flu). He mentioned that avian influenza (bird flu) has been recognized for many years and since the 1990's outbreaks have been caused by H5, H7, and H9 variants with rare transmission to humans. It was mentioned that aquatic birds provide a reservoir for influenza A and that humans could be infected by virus subtypes transmitted by domestic poultry and pigs.

Dr. Lamb provided a background on the virus and its structure. He described the forms of the virus stating that the virus often occurred as a spherical particle but that particles isolated from patients' throats were filamentous. Dr. Lamb emphasized that because the spherical form of the virus binds to a host cell receptor through the HA and the particle is endocytosed via a sialic acid link, the HA receptor would be an ideal target for an antiviral drug. In contrast, he stated that the full details of the mechanism for the filamentous form of influenza virus were not known.

He compared influenza A with influenza B and stated that the M2 channels present on influenza A virus allowed proton entry and acidification which were important for viral uncoating to occur and that the antiviral drugs amantadine and oseltamivir act by inhibiting viral entry into the host cell. In contrast, he stated that influenza B had an additional membrane protein of unknown function which is not inhibited by amantadine.

Dr. Lamb pointed out a few unusual features of influenza viruses and that the RNA is on the outside of the nucleoprotein and therefore not well protected from RNAase digestion. Another unusual feature he mentioned was that transcription and replication of viral RNA and maturation of the viral ribonucleoprotein complex occurred in the host cell nucleus. He highlighted the significance of this process since it required a complicated series of events during which viral proteins that were synthesized in the cytoplasm were translocated back to the nucleus for RNA synthesis, since viral polymerase is required for transcription and replication. This extrusion of the virus from the nucleus may be a possible site for antiviral drugs.

Current Antivirals and Potential Drug Targets

Dr. Frederick Hayden of the Global Influenza Program of World Health Organization (WHO) continued the overview of influenza viruses with a discussion of current antivirals and potential drug targets.

Table II, provided by Dr. Hayden, summarizes investigational agents now in or about to enter clinical development, their likely targets, the corporate sponsor, route of administration, and current phase of development.

Table II. Investigational Agents in Clinical Development

Agent	Target	Sponsor	Route	Phase of Development
Zanamivir	NA	GSK	Intravenous (IV)	Phase 1, 2a
Peramivir	NA	Biocryst	IV, intramuscular (IM)	Phase 1
CS8958	NA	Sankyo, Biota	Topical	Single dose Phase 1
T-705	Polymerase	Toyama	Oral	Pending
DAS181	HA receptor	Nexbio	Topical	Pending

Dr. Hayden discussed how NA inhibitors oseltamivir, zanamivir, peramivir, and A-315675 have different resistance profiles against oseltamivir-resistant variants, probably due to different interactions at the active site. He stated that promising results with high plasma drug levels and acceptable tolerance profiles have been shown with parenteral zanamivir and peramivir in human pharmacokinetic studies. Other drugs, the long-acting neuraminidase inhibitors (LANI) CS-8958 and FLUNET showed promise in animal model prophylaxis studies. He presented pre-clinical studies of T-705 which showed inhibition of influenza A, B, and C viruses *in vitro* and inhibitory activity when delivered orally in murine models. It was pointed out that the drug did not appear to affect host cellular RNA or DNA synthesis but that in preliminary studies, T-705 was observed to be superior to oseltamivir in reducing lung virus titers in H5N1-infected mice. Another drug under study, DAS181, was inhibitory for a range of influenza A and B viruses *in vitro* and active when delivered to mice or ferrets by intranasal dosing. He further discussed that combination antiviral therapy may be another approach that seemed to be effective in animal models and is currently being considered for a clinical study.

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Dr. Hayden mentioned that there seemed to be no statistical difference in virologic endpoints in a small study that provided nebulized zanamivir and oral rimantadine to hospitalized adults, although there seemed to be some suggestion of benefit from using the combination of drugs, compared to using rimantadine alone. He pointed out that more data were needed in this area.

Dr. Hayden outlined some current and proposed future directions for influenza antivirals. A multi-partner clinical trials network had been established in South East Asia to conduct prospective clinical studies. It was mentioned that antivirals with other mechanisms of action were entering clinical development and studies of injectable NA inhibitors (peramivir and zanamivir) were in progress. He placed an emphasis on the need to develop alternative formulations including parenteral formulation of current anti-influenza agents. In addition, he felt that more studies need to be performed including antiviral combination therapy and studies with immunomodulators.

For more information on the South East Asia clinical trials network: www.seaclinicalresearch.org

Clinical Features and Pathophysiology

Dr. Robert L. Atmar from the Baylor College of Medicine described the clinical presentation of influenza. He described that the incubation period for influenza is typically two to three days, with a range of one to seven days. Onset was abrupt and could involve a combination of respiratory symptoms (rhinorrea, nasal congestion, pharyngitis, dry cough) and systemic symptoms (fever, myalgia, malaise, anorexia, headache). He mentioned that in many populations there seemed to be a correlation between the magnitude of clinical symptoms and time of peak viral shedding, although he stated that in children 1-4 years of age neither fever nor cough was a predictor of influenza infection. Similarly, cough was not a predictor in patients with COPD (chronic obstructive pulmonary disease), and signs and symptoms of influenza infection seemed atypical in the elderly. Due to varying responses, he highlighted the need for rapid diagnostics that may serve as a tool to better diagnose influenza infection in different populations.

Dr. Atmar also described features of the acute respiratory syndromes caused by interpandemic influenza including the common cold, upper respiratory illness, pharyngitis, laryngitis, tracheobronchitis, and pneumonia. He also mentioned there are other non-respiratory complications include otitis, sinusitis, viral and bacterial pneumonia, encephalopathy, and Reyes syndrome.

Dr. Atmar specified that the clinical presentation of avian influenza could be atypical. He noted that conjunctivitis was a frequent symptom during the 2003 influenza A/H7N7 outbreak in the Netherlands. However, he indicated that in patients infected with H5N1, upper respiratory symptoms were often absent. Instead patients seemed to have extensive lower respiratory symptoms that progress to pneumonia, acute encephalitis, diarrhea, vomiting, and abdominal pain. He continued to mention that unlike seasonal flu, the H5N1 viral load in the oropharynx is higher than in nasal samples and virus or viral RNA has been detected in blood, stool, and CSF (cerebral spinal fluid). He indicated that hypercytokinemia may play a role in H5N1 pathogenesis and molecular analysis of the immune response may reveal potential targets for therapeutics. Finally, he mentioned that routes of avian influenza transmission appear to include small droplets, large droplet aerosols and direct contact with contaminated poultry.

Summary of Discussion of Influenza Virus

The overview on influenza viruses highlighted the unique features of the virus as compared to other RNA viruses. Namely, the lack of fidelity of the virus's transcription and replication process allows for many

mutations resulting in minor changes in the viral subtype and the need for yearly influenza vaccination. The success of the virus in infecting individuals is thought to be a balance between the pathogenesis of the virus and the host's immune response. Influenza infection can range from minor illness of cold-like symptoms to severe infection like pneumonia and death. Although influenza infections have normally been associated with respiratory illnesses, recent avian influenza infections have also involved the central nervous system, gastrointestinal tract and one case of systemic involvement in which virus was detected in the blood. With this in mind, there is a need to continue gathering more data on the effectiveness of current drugs on novel strains of influenza viruses as well as data from clinical trials involving novel therapeutic agents including identifying surrogate markers for effectiveness.

CLINICAL STUDIES

Review of Data from Antiviral Studies Performed during Seasonal Influenza

Dr. Hayden presented a review of data from antiviral studies performed during outbreaks of seasonal influenza. He showed that M2 inhibitors have proven efficacy for prophylaxis and constitute a cost-effective measure if the circulating virus was susceptible to them. In contrast, he stated that neuraminidase (NA) inhibitors seem highly effective for both seasonal and post-exposure prophylaxis (PEP). He pointed out that because secondary transmission generally occurred in the first few days after index cases were recognized in households, socially targeted antiviral prophylaxis (PEP) would be an effective strategy but that rapid initiation was essential for effectiveness. On the other hand, he notes that long-term prophylaxis would not be an efficient use of limited drug supplies in a pandemic. He stated that in aggregate, prophylaxis studies showed that antivirals could provide reasonable protection in a high-risk environment such as a nursing home, although antiviral resistance emergence and transmission may be a limiting feature with M2 inhibitors.

Dr. Hayden showed results of treatment studies in North America, Europe, Japan, and elsewhere which indicated that early treatment with an NA inhibitor reduced functional disability and lower respiratory tract (RT) complications in seasonal influenza. He mentioned that treatment with oseltamivir appeared to reduce all-cause hospitalizations and mortality. He highlighted that oseltamivir treatment of ill index cases in households may reduce transmission of virus but that this required further study. Finally, he mentioned that inhaled zanamivir did not appear to reduce transmission.

Vulnerable Populations

Residents of Long-Term Care Facilities

Dr. Arnold Monto from the University of Michigan discussed the results of 1989-90 and 2001-2 studies performed in multiple nursing homes in Southern Michigan. He showed that in both studies, the percent of residents vaccinated correlated with protection; however, even at sites with higher vaccinations rates among the residents, the rate of flu was high. The source of infection may have been members of nursing home staffs, who tend to be vaccinated at low rates.

It was mentioned that treatment and prophylaxis for residents of long-term care facilities (LTCF) often occurred late, possibly because this population was often under the care of multiple physicians. Dr. Monto commented that diagnosis of influenza among nursing home residents was often difficult because fever occurred less frequently in elderly individuals than in younger patients. In addition, he states that

older individuals shed less virus than younger people, as shown by nested PCR, which may make it more difficult to establish the cause of outbreaks in LTCF.

Elderly Patients

Dr. Carolyn Bridges of the Centers for Disease Control and Prevention (CDC) continued the discussion on the use of influenza antiviral medications in nursing home patients. She noted that most information about influenza antivirals in the elderly was derived from nursing home outbreaks primarily due to the high rate of complications in this population in which controlled trials were difficult.

Dr. Bridges further mentioned that nursing home residents were at high risk for influenza for a number of reasons stating that they often were weakened by chronic medical conditions or general debility; they had decreased immune responses making vaccines less effective; the closed setting facilitated rapid spread of infections. She mentioned that in uncontrolled outbreaks, mortality of LTCF residents was greater than 10 percent. She emphasized that the best prevention and control measure is high vaccine coverage of both residents and staff. However, she mentioned that other measures were valuable in reducing the impact of influenza on LTCF residents, primarily early detection which could identify sick residents who could then be placed in separate rooms, away from healthy residents; droplet control measures could be implemented; antiviral treatment could be provided to all residents; staff could be vaccinated; group activities and staff floating could be limited and visitors restricted.

Dr. Bridges highlighted that antiviral medication could reduce the spread of infection when used for treatment and prophylaxis. However, she noted that the use of rimantadine and amantadine have been complicated by the emergence of antiviral resistant strains. In addition, patients have had adverse reactions to amantadine, notably agitation and confusion. She also mentioned that although the use of Zanamivir has been found to be an effective treatment, its use was limited primarily because it was not easily administered as it is an inhaled medication. Dr. Bridges stated that presently, oseltamivir seems to be the most commonly used antiviral. Compared to other drugs, the dosing regimen for oseltamivir was less complex and there seemed to be a lower risk that resistant strains would emerge. In addition, oseltamivir did not cause neurological side effects although a low level of nausea and vomiting has been observed. A study by Bowles et al. supports use of oseltamivir in this population (Bowles et al, Use of Oseltamivir during Influenza Outbreaks in Ontario Nursing Homes, 1999-2000. *J Am Geriatr Soc*. 2002 Apr;50(4):608-16).

After the presentation by Dr. Bridges, participants highlighted the importance of the time of treatment with oseltamivir and the importance of vaccinating LTCF staff.

Immunocompromised Pediatric Patients

The discussion of vulnerable populations was continued by Dr. Janet Englund from the University of Washington, Seattle, who described results of studies on immunocompromised pediatric patients.

She highlighted that in an immunocompromised host, influenza infection may entail months of symptoms and virus shedding as well as a high frequency of pneumonia and death. She mentioned that although children with cancer may not have a higher rate of infection, once infected, they have a high rate of complications. Such complications could include the need for ventilator assistance and delay of chemotherapy. Children who were infected with HIV may also have a high rate of complications following infection with influenza, as do other vulnerable populations including immunosuppressed patients with rheumatoid arthritis and those patients with chronic obstructive pulmonary disease. Dr. Englund further mentioned that when rimantadine and oseltamivir were given early to patients who received stem cell transplants (SCT), they were protective against progression of flu to pneumonia.

Unfortunately, SCT recipients who survive influenza infection seem to have an overall poorer outcome than those who were not infected with the virus.

Dr. Englund emphasized that pregnant women, infants, schoolchildren, and toddlers constitute vulnerable populations who have high attack rates of influenza and may suffer severe complications of disease, but that there were few data regarding antiviral treatment in those patients. She noted that children less than 6 months of age have the highest rate of hospitalization of any age group but there was no licensed influenza drug for this population. In light of this, she mentioned that in emergency situations, physicians typically prescribed oseltamivir off-label for infants, raising a need for data on dosage and duration of treatment in this group. In older, otherwise healthy children, it has been shown that treatment with oseltamivir hastened the resolution of illness by approximately 1 day and significantly reduced viral shedding, which is important for transmission. In certain cases, the prompt use of oseltamivir significantly reduced complications such as otitis media and conditions that involved the use of antibiotics.

Dr. Englund stated that as a means of prophylaxis, oseltamivir had a good safety and efficacy profile in otherwise healthy people of all ages above 1 year. When oseltamivir was used for prophylaxis in pre- and post-SCT adults, an outbreak in a group living facility was derailed. She noted that despite this, oseltamivir is not part of the current guidelines for preventing opportunistic infections in SCT recipients from the CDC, the Infectious Disease Society of America (IDSA), and the American Society of Blood and Marrow Transplantation (ASBMT).

With respect to the topic of drug resistance, Dr. Englund referred to a study of naturally occurring influenza infection in children in which resistance to oseltamivir was seen in 9 of 105 patients (8.6 percent) aged 1-12 years (Whitley, PIDJ 2001;20: 127). She also stated that other studies have found higher levels of resistance using different methods of testing (Kiso et al, Lancet 2004; 364:759).

Dr. Englund concluded her presentation stating that oseltamivir was generally well tolerated, with the exception of occasional gastrointestinal distress. Nonetheless, a study in neonatal rats has raised safety concerns due to deaths in those animals.

Oseltamivir, M2 Inhibitors and Development of Resistance

Dr. Hayden discussed use of oseltamivir and M2 inhibitors and development of resistance. He stated that in adult outpatients, the appearance of drug resistant strains after treatment with oseltamivir occurred at a low rate, 0.4 percent, compared with a resistance rate of 30 percent after treatment with an M2 inhibitor. He noted that in outpatient children, appearance of resistant strains occurred in 5.5 percent of cases after oseltamivir treatment and 30 percent of cases after treatment with an M2 inhibitor. However, he pointed out that the rate of appearance of resistant strains may be dependent on the technique used, especially those which could recognize resistant clones in mixed populations. Using sensitive methods in hospitalized children, he stated that resistance was detected in 18 percent after treatment with oseltamivir and 80 percent after treatment with an M2 inhibitor.

In 2003, Dr. Hayden mentioned that there was an increase in the frequency of strains resistant to M2 inhibitors, apparently due to a serine-to-asparagine mutation at position 31 of the viral M2 protein, a proton channel located on the virus membrane. Dr. Hayden emphasized that the rate of resistance to oseltamivir is low, despite substantial use of the drug, especially in Japan. Nonetheless, oseltamivir resistant strains that contain a histidine-to-tyrosine mutation at position 274 of the N1 variant of the viral NA protein have emerged. In contrast, Zanamivir was effective against strains with this mutation.

Dr. Hayden expressed that because of the potential problem of oseltamivir resistance, a need exists for alternative agents and data on antiviral combinations. Dr. Hayden expressed his personal view that concerns about resistance to NA inhibitors should not be a deterrent to stockpiling decisions.

Summary of Discussion of Clinical Studies in Vulnerable Populations

Influenza infection results in more than 36,000 deaths each year with significantly more hospitalizations in vulnerable populations including young infants, elderly, immunocompromised individuals and individuals at high risk for complications due to underlying disease. The currently licensed antiviral medications available are effective in reducing the spread of infection when used early for treatment and prophylaxis. However, the effectiveness of the drug is complicated due to underlying disease, the emergence of antiviral resistant strains, adverse reactions to some drugs, and limited formulations. In infants less than one year of age, there are no licensed drugs, and off label use has occurred in emergency situations. Thus more data is needed on dosage and duration of treatment in this age group. Other factors which limit effectiveness of antiviral medications include the vulnerable population themselves. There may be a longer duration of symptoms and virus shedding in the immunocompromised host and this group tends to have higher frequency of health complications. For these reasons, there is a need for the development of novel antiviral agents, alternative formulations, and data on using antiviral combinations in these populations.

Data from Treatment of Novel Strains: Amantadine Experience in H3N2 Emergence and H1N1 Re-emergence

Dr. Raphael Dolin from Harvard Medical School discussed data from treatment of novel influenza strains with a focus on the use of amantadine for H3N2 and H1N1. He stressed that because fever and respiratory indications were features of many illnesses, laboratory analysis was necessary to confirm the presence of influenza virus in studies involving natural infections. He mentioned that in placebo-controlled trials that utilized laboratory analysis, both amantadine and rimantadine were shown to be effective against pandemic H3N2 and H1N1 strains; the drugs were more effective in preventing clinical illness than in combating infection. He stated that treatment with amantadine or rimantadine reduced the duration of illness by about a day. Two days after administration of either drug, shedding of virus was reduced in the majority of subjects. The drugs were found to have modest therapeutic effect in uncomplicated illness in the above pandemics. He emphasized that the effectiveness of these drugs against severe or complicated disease was unknown.

There were some discussions regarding whether the ability of antivirals to reduce subclinical illness could result in more people who were transmitting virus. Dr. Dolin pointed out that there seems to be a correlation between titers and symptoms with those who were asymptomatic having lower titers. Therefore, if there were no symptoms and low viral titer, the risk of transmission would probably be low. It was also pointed out that this was not limited to antivirals and that there was often infection in vaccinated individuals.

Lessons from SARS: Preparing for Assessment of Intervention Effects in Outbreak Situations

Dr. Allison McGeer from Mount Sinai Hospital described lessons learned during the 2003 outbreak of SARS in Ontario. She mentioned that although randomized clinical trials (RCTs) during a disease outbreak could provide the biomedical community with a means to assess interventions, in the case of the Toronto SARS outbreak a clinical trial did not begin until weeks after the initial outbreak, when the majority of cases had been resolved. Dr. McGeer mentioned that in the setting of an outbreak, time

would be a critical issue, especially if investigators, staff, or IRB members were infected or quarantined. She highlighted that questions of ethics review and safety issues for clinical trials staff must be resolved prior to an outbreak. In addition, study design was fundamental to the problem. Investigators did not have pre-clinical data on which to base an intervention or information on which to base outcome measures. Case definition was non-specific and changed over time as basic information about the SARS virus was developed, such as the fact that it was a coronavirus. Dr. McGeer stressed the importance of detailed planning for an outbreak or pandemic before it occurred.

Data from Use in Recent Avian Influenza Experiences

Dr. Tim Uyeki (CDC) discussed human infections with highly pathogenic avian influenza A (H5N1) viruses. He mentioned that H5N1 viruses were currently circulating among poultry in two antigenically distinct, geographically different groups. Clade 1 viruses have been circulating among poultry in Thailand, Vietnam, Cambodia, and Laos and have infected people in Vietnam, Thailand, and Cambodia. Clade 2 viruses circulating among poultry consist of six subclades, while virus strains in three of these six subclades have infected humans to date: subclade 1 (Indonesia), subclade 2 (the Middle East, Europe, and Africa), and subclade 3 (China). H5N1 viruses have evolved genetically and antigenically since the emergence of human infections in 1997 and re-emergence in 2003. He mentioned that there have been more than 100 confirmed cases of H5N1 in 2006 and the case fatality was approximately 68 percent.

Dr. Uyeki stated that the transmission of H5N1 viruses was essentially avian-to-human and the behavior that entails the most risk was directly touching sick or dead poultry, although how H5N1 viruses infect the lungs was unknown. In addition, there was epidemiological evidence for a few incidents of limited non sustained person-to-person transmission. Dr. Uyeki mentioned that family clusters of H5N1 cases among blood relatives have occurred in Hong Kong, Vietnam, Thailand, Indonesia, China, Turkey, Iraq, Azerbaijan, and Egypt, including a large family cluster in North Sumatra, Inodonesia in 2006. He noted that possible interpretations of family clusters were common exposures with different incubation periods, different exposures, genetic susceptibility, and limited non-sustained person-to-person transmission.

Dr. Uyeki discussed clinical data on H5N1 cases and said that they were limited. Most cases have occurred in children and young adults and most H5N1 patients have presented to hospital with severe respiratory disease. Viral pathogenesis and complications included cytokine dysregulation ("cytokine storm"), pulmonary, renal, and liver failure, septic shock, encephalitis, and gastrointestinal involvement. Viremia was associated with fatal outcomes. He mentioned that the virus could replicate in the lower respiratory tract and viral RNA could be detected in rectal swabs, and mRNA was detected in the gastrointestinal tract in one autopsy study suggesting that H5N1 viruses may also replicate in the GI tract. He continued to say that the average time from disease onset to hospital admission was four days. Although there have been no clinical trials, WHO recommended oseltamivir treatment for H5N1 patients. Although the optimal dose and duration of oseltamivir treatment was not known, Dr. Uyeki mentioned that the WHO recommends 5 days of treatment with the same dose as for human influenza A.

It was cited that oseltamivir resistant clade 1 viruses could develop, although data on this subject was only observational and did not come from controlled clinical trials. He suggested that among eight oseltamivir-treated cases in Vietnam, four fatal cases had high viral loads and four survivors had low viral loads, suggesting that oseltamivir may decrease viral shedding but that oseltamivir resistance could develop during treatment and was associated with high viral load and fatal outcomes. In contrast, he referred to an example where an oseltamivir resistant virus isolated from a respiratory sample taken from a 14-year old Vietnamese girl after three days of oseltamivir treatment contained a histidine-to-tyrosine substitution at position 274 in the NA protein; the virus was sensitive to zanamivir and the patient recovered.

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Dr. Uyeki mentioned that in the current situation WHO recommended that oseltamivir be provided as chemoprophylaxis to high risk groups, defined as household members, family and close contacts of a strongly suspected or confirmed H5N1 patient. The recommendation extended to pregnant women who have had contact with an H5N1 patient. He highlighted that many questions about antiviral treatment and chemoprophylaxis of H5N1 remain unanswered and that much more epidemiological and clinical data about human infection with H5N1 viruses were needed.

Summary of Discussion on Clinical Studies

The main focus of discussion was on the relationship between virologic and clinical outcomes in various clinical settings, specifically what are the best means for determining effectiveness of a particular treatment? Serologic markers and viral titers have traditionally been used as a measure of infection; however, treatment endpoints have moved toward more objective measures of infection including viral isolation in correlation with reduction in clinical symptoms like fever (termed functional recovery). Viral isolation is considered to be very important because it not only confirms causality but provides an opportunity to assess the emergence of drug resistant viruses.

Antivirals that produce symptom benefit have been shown to also reduce viral load. However, the correlation is complicated by the fact there are differences in influenza virus types. For example, the IC50 of oseltamivir is higher for influenza B virus compared to influenza A viruses. More data are needed to further evaluate type and strain differences. Furthermore, an individual patient's symptoms will vary. It was noted that hospitalized patients are heterogeneous and finding uniform endpoints is challenging. This is further complicated by the fact there are more data currently available for some groups of patient populations than others. The overarching need identified was to obtain more clinical data in various patient populations during seasonal influenza outbreaks, which could identify key measurements of antiviral effectiveness ahead of the next influenza pandemic included more placebo/quasi-placebo controlled clinical trials, antiviral combination studies, length of treatment studies, epidemiological studies, studies to evaluate the development of resistance, and prophylaxis profiling.

HUMAN CHALLENGE STUDIES

Use of the Human Challenge Model in Influenza Studies

Dr. John Treanor from the University of Rochester provided a review on the use of the human challenge model to study efficacy of new therapies. He described that volunteer subjects were infected with a laboratory preparation of virus under controlled conditions. Viruses that have been studied successfully using the human challenge model include influenza, rhinovirus, respiratory syncytial virus, parainfluenza virus, rotavirus, and norovirus.

He brought up that the system had a number of advantages for Phase I/II studies of antiviral agents.

- The investigator controlled timing of exposure to the virus;
- Most subjects became infected and shed predictable amounts of virus;
- Daily or more frequent monitoring of viral shedding, clinical symptoms, immune response, pharmacodynamics, etc. were possible;
- Many features of the response were very similar to typical influenza infection in a healthy adult;
- The timing of studies was not wholly dependent on seasonality.

He noted that there were also a number of disadvantages to the human challenge model.

- Some features of the model may not precisely mimic natural influenza infection, since the virus is extensively passaged in eggs which may alter virulence compared to wild-type virus.
- Viral pathogenesis may be altered due to the route of administration. Experimental infection was typically delivered via the nasal route rather than inhaled as droplets as in the case of natural influenza infection.
- Symptoms were generally not as severe as naturally occurring virus.
- Sampling for virus replication was primarily limited to the upper respiratory tract and peripheral blood.
- If studies with the challenge model gave a positive result, studies in naturally infected humans would still probably be required.

Dr. Treanor suggested that a critical feature of the model was that subjects have uniform susceptibility. All adults have some baseline level of immunity to influenza because of prior exposures. However, the hemagglutination-inhibition (HAI) antibody was a good, but not perfect, indicator of susceptibility. He also stated that other critical components included the availability of a wild type virus produced to GMP (Good Manufacturing Practice) standards. He specified that for unclear reasons, not all such GMP pools were equal in virulence. Finally, he stated that the studies were somewhat unique in that there was some risk to the subject and no direct benefit of participation and to prevent transmission to others, subjects were usually maintained in isolation, often in a hotel, during the period of virus shedding.

Outcome measures typically included virus shedding, assessment of symptom scores, nasal mucus weights, tympanometry, and generation of inflammatory mediators.

Dr. Treanor highlighted that studies using a human influenza challenge model have provided important data about the protective effect of vaccines and antivirals. H1N1, H3N2, and influenza B viruses have been well studied using this model. The model predicted efficacy of amantadine, rimantadine, zanamivir, and oseltamivir and demonstrated that nasal administration of zanamivir was not needed. The model predicted that peramivir had insufficient oral bioavailability to be effective and that LY217896 would not be an effective antiviral in humans, eliminating the need for further study of the drug.

Choosing, Qualifying, and Reviewing Viral Strains for Human Challenge Studies

Ms. Jean Hu-Primmer from the US Food and Drug Administration (FDA) discussed regulatory aspects of the human influenza challenge model. She defined the influenza challenge model as an experimental infection of a consented healthy participant with live influenza virus. She also noted that the influenza challenge strain was commonly referred to as the "challenge pool," and was a live influenza virus monovalent product that is used for experimental infection. Ms. Hu-Primmer cited that the legal basis for defining a challenge strain as a biological product is codified in the Federal Food, Drug and Cosmetic Act and the Code of Federal Regulations, as it "alters the structure and function of the body of man."

Ms. Hu-Primmer specified that if the chosen challenge strain was a wild-type influenza virus that was cultured from a clinical specimen, the source of the influenza challenge strain must be known and there must be information on how it was made, including details on the initial isolation, passage history, and qualification of raw materials and reagents used during isolation. However, she communicated that if the chosen challenge strain was a vaccine reference virus, generated by a WHO Collaborating Center, the documentation of quality, including source and passage history, must be available. She noted that regardless of which strain was chosen, GMP manufacture beginning with the source and quality control must be thoroughly monitored.

Ms. Hu-Primmer mentioned that several factors would need to be taken into account when performing trials with challenge material. She highlighted the safety of study participants and the need for full disclosure of risks involved via a consent form and an information brochure. Subjects must undergo health screening and safety follow-up is required. The extent of the health screening and safety follow-up depends on how much clinical safety information was available with the challenge strain. If the challenge strain was studied in a Phase I "first in humans," the screening and safety follow-up would be more extensive.

Ms. Hu-Primmer brought up the need for an infection control plan and clearly defined halting rules. An Investigational New Drug (IND) Application is required, and must be submitted to the Agency and declared in effect prior to execution of any infection or challenge procedures. She stated that from the perspective of the Center for Biologics and Evaluation (CBER) at the FDA, review of a challenge strain is the same as a review of a live virus vaccine.

Ms. Hu-Primmer encouraged participants to request a meeting with FDA and initiate a dialogue to obtain feedback on the proposed manufacturing process, a proposed testing scheme, the design of a clinical protocol, and supportive pre-clinical information. She highlighted that the challenge pool must be demonstrably stable and sterile and the human infection model must be characterized prior to use in a product evaluation study.

Summary of Discussion on Human Challenge Studies

Experimental human influenza infection studies have provided important data about the protective effect of vaccines and antivirals. Many strains of influenza viruses have been well studied in humans to test efficacy of influenza vaccines including H1N1, H3N2, and influenza B viruses; and in general, the symptoms/shedding profile of experimentally infected subjects is less severe than individuals who are naturally infected. The human challenge model has also predicted efficacy of currently licensed antivirals (amantadine, rimantadine, zanamivir, and oseltamivir) and more recently, human challenge studies have been used to demonstrate the effectiveness of an alternate route of administration of an antiviral drug. Oral administration of zanamivir was shown to be as effective as the currently licensed nasal administration. Despite these successes, there are many factors to consider prior to use of the human challenge model. First, there are numerous regulatory requirements including filing an IND with details on the initial isolation, passage history, and qualification of raw materials and reagents used during isolation and development of the challenge virus that will be used. In addition, the challenge virus must comply with Good Manufacturing Production standards, be sterile, stable, and be fully characterized. Second, once the IND has been filed with the FDA, there are numerous other factors that need to be addressed prior to use, namely the safety considerations of the participating human subjects. Infection control measures must be in place and subjects must be screened for susceptibility to infection with the challenge virus and their overall health status. As a result, when considering human challenge studies, the resources needed for conducting the study and the safety of study participants must be weighed against the impact the data are anticipated to provide.

ANIMAL MODELS for INFLUENZA

Small Animal Models of Influenza (Mice, Ferrets, Hamsters, Cotton Rats)

Mice

Dr. Jacqueline Katz from the CDC discussed the use of mouse models to study avian influenza pathogenesis and transmission. She stated that in any animal model for influenza the goal should be to get as close as possible to the human situation, where there would be replication in the respiratory tract, a host response that would lead to shedding of virus, and possible transmission to a new host. The extent to which the situation in an animal model mirrors the human situation would vary for different viruses.

She described that in humans, mice, ferrets, non-human primates, and cats, avian influenza viruses bound to lower respiratory tract (RT) type II alveolar cells through a sialic acid-α-2,3-galactose link; in mice, the virus also bound to tracheal epithelium. Mice did not have a receptor for human influenza viruses, which utilized a sialic acid-α-2,6-galactose link. She mentioned that the avian influenza subtypes that were most relevant to humans were the H5, H7, and H9 viruses. Although not all avian influenza viruses replicated efficiently in mice, recent avian subtypes that have infected humans replicate efficiently in mice without prior adaptation.

Dr. Katz described using the mouse model to study pathogenicity of H5N1, using intranasal (i.n.) inoculation of a relatively large volume of virus to anesthetized animals. The H5N1 virus has two general phenotypes; the highly pathogenic (HP) phenotype caused experimental animals to rapidly lose weight and succumb to infection in seven days. Lung tissue was damaged, viral replication and increased cytokines were observed in lung and brain, and leukocytes were depleted. This outcome is in many ways analogous to the human outcome. Dr. Katz pointed to Table III, which was a comparison of H1N1, H3N2, H5N1, N7N7, H7N2, and H9N2 with respect to human disease and morbidity and mortality in the mouse and ferret models.

Dr. Katz described a number of advantages to studying influenza in the mouse model.

- The cost was relatively low, which allowed the investigator to use large numbers of animals and achieve statistically meaningful data on dosage, delivery, and timing strategies.
- Avian subtypes with pandemic potential (H5, H7, H9) replicated efficiently in the mouse upper and lower respiratory tract.
- The kinetics of mouse humoral and cellular immunity were well-studied and reagents are available.
- Immunocompromised mouse models and inbred and outbred strains have been developed.
- There were multiple markers for evaluation of efficacy, including survival from lethal challenge, weight loss (morbidity), viral titers and kinetics of viral clearance, and viral spread (for highly virulent strains).

She continued to describe some disadvantages to the mouse model system.

- The mouse sialic acid receptor specificity and distribution in airways was different from that in humans.
- Human virus subtypes generally required mouse adaptation to change the receptor binding preference of the viral HA.
- Pathogenesis in mice differed from humans.
- Delivery and pharmokinetics may differ from humans.

Table III. Comparison of Mouse/Ferret Models and Human Disease Outcomes for Human and Avian Influenza Virus Subtypes

Subtype	Avian Pathotype	Human Disease	BALB/c mouse (i.n. route)		Ferret (i.n. route)	
			Morbidity	Mortality	Morbidity	Mortality
H1N1 or H3N2	N.A.	Respiratory, Non-fatal	+	+/-	+	-
H5N1	HPAI[1]	Respiratory, Fatal	++	+	++	+
H5N7*	HPAI	Respiratory, Fatal	++	+	++	+
H7N7	HPAI	Conjunctival, Non-fatal	-	-	-	-
H7N2	LPAI[2]	Respiratory, Non-fatal	+	-	+	-
H9N2**	LPAI	Respiratory, Non-fatal	-	-	-	-

**De Wit et al., 2005 J Virol 79:12401; **Lu et al., 2001 J Virol 75:4896*
[1]High pathogenicity avian influenza virus
[2]Low pathogenicity avian influenza virus

Dr. Katz highlighted that there were differences in disease outcomes in mice infected with highly pathogenic (HP) and low pathogenic (LP) viruses. One could inoculate HP viruses in multiple sites in mice and virus could get in to the respiratory system and cause severe disease or death. In contrast, LP virus replicated in blood but did not cause disease. She emphasized that in some cases, viruses that were HP in mice was not in humans; virulence was different in different animals. Thus, she emphasized that the selection of the right virus to use in the model was critical.

Dr. Katz concluded by saying that overall, the mouse model was an excellent initial *in vivo* system to refine an experimental protocol for evaluation of antivirals.

Mice – Neurotropism of Influenza Infection

Dr. John Morrey from Utah State University discussed potential usefulness of the mouse model for studying influenza-associated encephalitis, which may be a serious problem in a pandemic situation. He highlighted that the extent to which HP avian influenza strains would be neurovirulent was not known but that in neurological diseases, early treatment was critical.

As background, Dr. Morrey briefly reviewed his studies on rodents infected subcutaneously or directly into the spinal column with West Nile virus (WNV), which affected the nervous system and caused paralysis. Intraperitoneal injection of a humanized monoclonal antibody as late as four days after injection of WNV into the thoracic spinal cord reduced paralysis and improved survival. Lesions for WNV-induced paralysis mapped to the thoracic and lumbar regions of the spinal cord.

He stated that mice were now being used to study the effect of influenza on the brain. Investigators have used stereotactic surgery to deliver the neurotropic influenza virus A/WSN/33 into the olfactory bulb. Future studies of this kind will provide an opportunity to evaluate agents that protect against influenza-induced neurological disease without the confounding effects of pulmonary disease.

Antiviral Studies in Mice

Dr. Robert Sidwell from Utah State University continued the discussion of antiviral studies in mice. He noted that the mouse model offered cost advantages, utilized compact housing space, and was readily biocontainable which is important when working with highly pathogenic viruses. In addition, mice offered low risk for personnel safety, could be obtained free of defined diseases, genetically well-defined and uniform in biological response, has many immunological reagents available, and was easier to get IACUC approval for use than it is for other larger animal models like the use of non-human primates.

He specified that unlike humans, mice lack expression of Mx protein and that the absence of Mx protein resulted in prolonged viral replication in lungs which may be helpful in certain types of experiments. As mentioned earlier, mice have predominantly sialic acid-α-2,3-galactose receptors which in some cases required that strains of influenza virus be adapted to mice. Absence of sialic acid-α-2,6-galactose receptors resulted in H5N1 virus infection in the upper RT rather than in the lower RT, unlike what occurred in humans.

He continued to mention that the mouse offered a variety of parameters that could be used for evaluation of potential antiviral activity. These included weight loss, pneumonia (lung score, increased lung weight), arterial oxygen decline, rales, histopathological changes, serum α-1-acid glycoprotein increase, lung water content, virus replication in lung or other tissues, and death. In contrast, mice did not get fever or nasal discharge and so these parameters could not be measured in the mouse model.

Dr. Sidwell stated that multiple studies in mice using clinically active drugs appeared to correlate well with results from human clinical studies including studies involving amantadine, rimantadine, oseltamivir, zanamivir, and peramivir.

Dr. Sidwell summarized the results of these studies in Table IV.

Table IV. Comparison of Mouse Testing Data with Clinical Results of Selected Influenza Inhibitors

Compound	Mouse Results	Clinical Results
Amantadine, Rimantadine	Highly active given p.o. vs. influenza A/H3N2, some strains of A/H1N1. Inactive vs. influenza B.	Active orally vs. influenza A/H1N1, H3N2, not B.
Amantadine, Rimantadine	Significant resistance of influenza A/H3N2 virus after single passage through treated mice. Virus remained virulent	Resistance reported to quickly develop, resistant virus readily transmitted to other patients
Oseltamivir	Strongly active vs. influenza A/H1N1, H3N2, B when given p.o. Resistance not readily developed after many passages	Active orally vs. influenza A/H1N1, H3N2, B viruses in patients. Scattered reports of occasional resistance
Zanamivir	Weakly active given p.o. vs. influenza A/H1N1, H3N2, highly active given i.n. or by s.p.a. (small particle aerosol) vs. influenza A/H3N2, B	Not used orally in humans. Active given by oral inhalation vs. influenza A/H1N1, H3N2, B infections in patients
Peramivir	Highly active when given p.o. twice daily vs. influenza A/H1N1, H3N2, B. Less active when given once daily vs. these infections	Weakly active vs. influenza A infections in humans when given once daily

Cotton Rats

Dr. Martin Ottolini from the Uniformed Services University of Health Sciences introduced the use of the cotton rat model to study influenza infection and pathogenesis. He described the clinical parameters of influenza-infected cotton rats which included significant initial weight loss, tachypnea, and initial hypothermia in opposition to fever, which was not unexpected in an animal (as seen in babies) that had a large surface area compared to its mass. Viral replication occurred initially in the lung but rapidly dropped off.

Dr. Jorge Blanco from Virion Systems, Inc. continued the discussion of the cotton rat model. He mentioned that cotton rat genes for cytokines, chemokines, cell surface molecules, and housekeeping and other functions have been cloned and were available as reagents for use in molecular analysis. Some of these probes have been used in influenza-infected cotton rats to elucidate cytokine expression, chemokine expression, subcellular localization of Mx proteins, tissue localization of Mx RNA and protein, and tracheal influenza receptor localization. Based on his molecular studies, Dr. Blanco concluded that the cotton rat is permissive to unadapted strains of human influenza virus, did not have age dependent restrictions for infection, expressed functional Mx genes, and was permissive to avian strains of influenza.

A question was asked from the floor on what the advantage of the cotton rat model was over the mouse model, given that the cotton rat genome is not fully sequenced and multiple reagents exist for the mouse model. Dr. Blanco answered that the cotton rat could be infected with virus strains that did not infect mice. Because a large number of animals could be infected, statistically significant results could be obtained. He continued to mention that two inbred strains of cotton rat were available and several cotton rat reagents have been developed, many of which were available through R & D Systems Inc. In addition, he highlighted the fact that his group had several DNA libraries from which additional genes could be cloned. He also mentioned that the cotton rat had pulmonary clinical determinants one could follow and was cheaper than other models used (e.g., monkeys) and it did not need pre-screening for influenza antibodies before experimental use (like in the case of ferrets). Some disadvantages were that the viruses tested so far were not lethal in the cotton rat and because the cotton rat did not have a big sneeze reflex, it was not a useful model for studying transmission.

Ferrets – Clinical Pathology

Thomas Rowe from the Southern Research Institute in Birmingham, AL described the ferret model of influenza pathogenesis. He noted an important feature of the ferret model stating that influenza viruses that infect humans did not need to be adapted in the ferret model due to its similarity in its receptor specificity to humans. Also, ferrets exhibited similar symptoms as humans including fever, weight loss, appetite loss, runny nose, and sneezing.

Mr. Rowe indicated that replication of seasonal influenza viruses was restricted to the respiratory tract (RT) whereas replication of H5N1 occurred in the respiratory tract as well as systemically. He also mentioned that ferrets infected with the H5N1 showed enhanced morbidity and mortality as compared to seasonal influenza. Specifically, one week after ferrets were infected intranasally with a highly pathogenic virus, the infected ferrets lost more than 25 percent of their body weight. They were lethargic and gross lung pathology showed deterioration. In addition, replicating virus was found in the lung and lung histology showed pneumonia and infiltration of inflammatory cells. Mr. Rowe continued to mention that replication of H5N1 also occurred in the brain and induced significant inflammatory cell infiltration. There was extensive brain pathology in the form of glial nodules, neuronophagia and meningitis. In addition to lung and brain, he discussed that replicating virus was also found in spleen and nasal passages. Furthermore, he noted that aerosol transmission of H3N2 between ferrets was efficient, compared to

transmission of H5N1, which was inefficient. Overall, ferrets were highly susceptible to influenza infection and selecting the right vendor was very important.

Ferrets – Immune Markers

Dr. David Kelvin from the University Health Network/University of Toronto continued the discussion on the ferret model, in particular he discussed immune markers in the ferret model. He presented data on gene expression patterns using microarray data. He showed that the pattern of immune markers generated by the ferret after H5N1 infection was similar to the pattern generated after SARS infection. Like SARS, H5N1 elicited waves of host immune responses and there were similar patterns in the early expression of interferon response genes and the early expression of the chemokine CXCL10. He highlighted that the continued expression of CXCL10 in severe SARS cases and H5N1 infected animals seem to contribute to pathological outcome through the continued infiltration of inflammatory cells to lung and other tissues. He indicated that in H5N1 infected ferrets there was a correlation between apoptosis and inflammatory cytokine gene expression in the brain. In addition, the microarray data in ferrets indicated differences between ferret responses to H5N1 and H3N2, which likely reflect host contributions to pathology. At the conclusion of his presentation, Dr. Kelvin mentioned that he is currently looking at immunomodulators that can block separate arms of the immune system.

Ferrets – Antiviral Resistance and Combination Therapy

More discussions occurred around the use of the ferret as a model for influenza infection. Dr. Elena Gorvokova from St. Jude Children's Research Hospital spoke about using the ferret model to study antiviral resistance. She noted that the choice of method to assess the emergence of drug-resistant variants was important and a preferred method of analysis was to sequence virus samples obtained from individual plaques in cell culture. This allowed for detection of both NA and HA mutations in the same sample. She further mentioned that minor populations of oseltamivir-resistant variants of H5N1 could be detected by this method and could be further evaluated to identify DNA sequence changes and amino acid variations in HA or NA that potentially might be the source of resistance. However, she indicated that the significance of a minor population of drug-resistant clones was unclear and required further study.

Dr. Gorvokova also described a mouse study that compared single-drug treatment to combination therapy for H5N1 infection. The H5N1 virus had been engineered to be amantadine sensitive. For all doses studied, the result of giving mice oseltamivir and rimantadine in combination, rather than individually, was superior in terms of survival and inhibition of virus replication in internal organs. She mentioned that combination therapy provided an advantage over administration of single drugs in preventing the spread of the H5N1 virus beyond the respiratory tract, including the CNS, and could be an option for control of infection with neurotropic influenza virus. In addition, she mentioned that direct sequencing did not reveal any amino acid substitutions in NA, HA, and M2 proteins that might decrease virus sensitivity to antiviral drugs during treatment. She indicated that this was encouraging and suggested that combination therapy might reduce the likelihood that drug-resistant influenza variants will emerge.

Cats

Dr. Albert Osterhaus from Erasmus Medical Centre in the Netherlands discussed the role of cats in the epidemiology of influenza and its use as a potential animal model for studying influenza infection. He described anecdotal evidence on 149 tigers in Thai zoos that strongly suggest H5N1 was transmitted from poultry to big cats and was lethal in those animals. The anecdotal evidence was consistent with histology performed on some of the dead felines.

He indicated that domestic cats in Asia and the Middle East also succumbed to H5N1 infection. He mentioned that in Germany there was transmission of virus from wild birds to cats. He stated previously, influenza A was known to replicate in domestic cats but not to cause disease or mortality; however, recent controlled studies performed by Dr. Osterhaus and colleagues revealed that avian H5N1 infection in cats caused systemic disease. These studies showed that the virus attached in the lower RT with the digestive tract as a possibility for a portal of entry.

Dr. Osterhaus discussed what is currently known about cats: they could become infected with H5N1 by contact with wild or domestic birds (or their excreta); develop severe to fatal disease; excrete virus from respiratory and digestive tracts; and could transmit infection to other cats.

He emphasized that there are still very important areas where more information was needed including the minimal infectious dose for cats; the duration of excretion; whether there were subclinical shedders; and whether cats can transmit virus to or other species including humans.

Dr. Osterhaus pointed out that the possible role of cats in the epidemiology of H5N1 has been largely overlooked, despite the fact that more cats than humans have died from the virus; unusual mortality in cats could be an early warning sign of pandemic avian influenza. Cats were not included in WHO /OIE (World Animal Health Organization) /FAO (United Nations Food and Agriculture Organization) guidelines for controlling the spread of H5N1 infection. He recommended that cats be kept indoors (or outdoors in case of already infected); that cats be quarantined and tested if clinical signs of disease were suspected or if contact with infected birds was suspected; and that cats showing unusual morbidity or mortality be tested for H5N1.

Non-Human Primates as a Model for Influenza

Dr. Michael Katze from the University of Washington described an integrated approach to studying viral infection that combines traditional histopathological, virological, and biochemical technologies with functional genomics and proteomics; the objective was to obtain signatures of virulence as well as insights into mechanisms of pathogenesis and the host defense response. Dr. Katze explained that genomics and proteomics provided a means to study the impact of virus infection on host gene expression, discover cellular regulatory pathways targeted by viruses, and identify new cellular targets for antiviral therapy. He pointed out that mining of genomics and proteomics data was generally part of discovery-based science, as opposed to hypothesis-driven science.

Dr. Katze discussed mouse (Balb/c) and macaque (pigtailed and Cynomolgus) models of influenza, focusing on studies on the host response to a mildly pathogenic human virus and later to the reconstructed 1918 Spanish flu (r1918) and HP H5 influenza virus and later presented data on biomarkers of infection and vaccine development in macaques.

- Histopathology on mice infected with r1918 revealed severe lung damage at 24 hours, the earliest time point tested; genomic microarrays revealed distinct molecular signatures that were unique to infection with the 1918 H1 virus.

- Highly pathogenic H5 infections in mice exhibited some signature responses that were shared with the 1918 influenza virus as well as others that were unique to the avian virus. Macaque models exhibited signatures of infection in lungs and blood.

- Macaque studies supported the use of functional genomics in vaccine studies to predict outcomes of vaccination and disease progression.

- Macaque studies suggested that a probable major contributor to the lethality of the 1918 influenza virus was the uncontrolled host inflammatory response and activation of cell death pathways. Viral pathogenesis was probably determined within hours, if not minutes, of infection.

- The rhesus macaque genome has been sequenced, which increased the usefulness of that and other closely-related species as models for human disease.

A question was asked from a participant regarding the use of genomics to monitor infection and vaccines. Dr. Katze replied that this would be a great idea and can even be expanded to include antivirals and genomics.

Summary of Discussion on Animal Models for Influenza

Animal models have been very useful in helping researchers better understand pathogenesis and virulence of influenza infections. However, there are multiple animal models each having advantages and disadvantages. No one model seems to provide data that can be used to answer all the questions about influenza disease. Some important information has come from studies done in mice; for example, tremendously important observations on MHC (major histocompatibility complex) and CTL (cytotoxic T lymphocytes) have translated to humans. The mouse model has been a good tool for identifying virulence factors, and immunological markers. They are relatively less expensive and easy to handle, statistical data can be generated and reagents are readily available. Yet the mouse is not the natural host and as a consequence, influenza viruses must be adapted to cause infection prior to their use in mice. The rabbit model (which was not discussed at this meeting) is a good model to study toxicity but not a useful model to study pharmacokinetics. The ferret model is considered more useful because it has receptor specificity similar to that in humans and viral resistance and transmission could be studied. However, viral pathogenesis in the ferret is systemic, whereas in humans the infection is primarily in the respiratory tract. This implies that each model seems to be uniquely positioned to define and understand very specific aspects of the disease including host immune response to infection, safety and efficacy studies of novel therapeutics, histopathology, symptomology, and transmission.

Despite the varying opinions of the group on the pros and cons of each animal model and individual preferences for their use, most agree that it was important to find good correlation between human responses and those observed in animals. Tools can be further developed to address the underlying problems to designing animal studies: developing standards (endpoints, outcome measures) and the continued standardization of animal models will be essential in building data sets for evaluating safety and effectiveness of novel therapeutics in human clinical trials; developing animal-specific reagents as tools to study influenza disease is crucial for advancement of the field; and because licensure of new control measures such as antiviral agents and vaccines for pandemic influenza may depend on animal, not human, data, obtaining pharmacokinetic data and data from challenge studies in animals will be very important.

SUMMARY OF OPEN DISCUSSION

The urgency for pandemic preparedness was highlighted during the meeting. Although there continues to be numerous accomplishments in influenza research, there remains a need for more data in basic science research, animal model development, and data from clinical trials to address key questions that might pose a concern during a pandemic. It is clear that as part of this effort, all invested parties (public health agencies, the private sector and Industry) will need to work together in accomplishing these goals including partnering with the private sector to develop novel therapeutics and prevention strategies, and improve existing strategies such as by using alternative formulations that can be evaluated in a clinical

research setting. In addition, there is also a need for a cost effective, rapid and sensitive diagnostic assay that can be commercially developed for use in hospital settings and in the field to better detect circulating influenza viruses. These steps will help identify and bridge the gap between basic science and clinical research. Lessons learned from the SARS outbreak underscore the need for preparedness at all levels and globally prior to the next influenza pandemic.

CONCLUSION

The meeting was convened to assess the current state of development and use of antivirals for treating seasonal and pandemic influenza. Participants included researchers and administrators from multiple federal agencies within and outside of HHS, including NIH, CDC, and FDA; scientists from academia; representatives of the World Health Organization's Global Influenza Program; and clinical practitioners specializing in infectious diseases.

Participants discussed current knowledge of influenza virology, treatments, animal models, and how data from clinical research and human challenge studies can be used to inform needed research to further the development of new antivirals. Details of the regulatory process for approval of new antivirals and how it would function in the event of a public health emergency were also covered.

In addition, open discussion sessions yielded ideas and action items that extend beyond NIAID's research activities to other partners involved in optimizing development and use of antivirals for pandemic influenza. Recommendations specific to clinical trials and protecting vulnerable populations were offered. NIAID appreciates the expertise provided by the meeting participants and will use insights gained to advance research planning and activities.