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Meeting Report

# Prevention and treatment of respiratory viral infections: Presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV Antiviral Group conference

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ABSTRACT

The International Society for Influenza and other Respiratory Virus Diseases held its 5th Antiviral Group (isirv-AVG) Conference in Shanghai, China, in conjunction with the Shanghai Public Health Center and Fudan University from 14-16 June 2017. The three-day programme encompassed presentations on some of the clinical features, management, immune responses and virology of respiratory infections, including influenza A(H1N1) pdm09 and A(H7N9) viruses, MERS-CoV, SARS-CoV, adenovirus Type 80, enterovirus D68, metapneumovirus and respiratory syncytial virus (RSV). Updates were presented on several therapeutics currently in clinical trials, including influenza polymerase inhibitors pimodivir/JNJ6362387, S033188, favipiravir, monoclonal antibodies MHAA45449A and VIS410, and host directed strategies for influenza including nitazoxanide, and polymerase ALS-008112 and fusion inhibitors AK0529, GS-5806 for RSV. Updates were also given on the use of the currently licensed neuraminidase inhibitors. Given the location in China, there were also presentations on the use of Traditional Chinese Medicines. Following on from the previous conference, there were ongoing discussions on appropriate endpoints for severe influenza in clinical trials from regulators and clinicians, an issue which remains unresolved. The aim of this conference summary is to provide information for not only conference participants, but a detailed referenced review of the current status of clinical trials, and pre-clinical development of therapeutics and vaccines for influenza and other respiratory diseases for a broader audience.

## Background

Influenza and other acute respiratory virus diseases are of major global public health importance. The emergence of the pandemic influenza in 2009, the increasing numbers of human cases of avian influenza A(H7N9) virus infections in China, the emergence of Middle East Respiratory Syndrome coronavirus (MERS-CoV), continued outbreaks of highly pathogenic influenza A(H5N1) viruses in several countries, and novel strains of influenza now infecting humans, highlights the importance of international collaboration on respiratory virus research and development of new strategies for their prevention and control. The International Society for Influenza and other Respiratory Viruses Diseases (isirv) is an independent and international scientific professional society promoting the prevention, detection, treatment, and control of influenza and other respiratory virus diseases. The Antiviral Group is a special interest group of isirv (isirv-AVG) with specific objectives to promote understanding of the clinical use of antivirals against respiratory viruses, and to collate and provide up to date information on the emergence of antiviral resistance to the established therapeutics. It also aims to provide information on the evaluation of resistance to new therapies under development. To communicate advances in preclinical and clinical development of potent novel antivirals four previous conferences have been organized by the isirv-AVG.

The 5th isirv-AVG Conference was held in Shanghai, China, in conjunction with the Shanghai Public Health Center and Fudan University from 14-16 June 2017. The three-day programme encompassed presentations on some of the clinical features, management, immune responses and virology of respiratory infections, including influenza A(H1N1)pdm09 and A(H7N9), MERS-CoV, severe acute respiratory syndrome coronavirus (SARS-CoV), adenovirus Type 80, enterovirus D68, metapneumovirus and respiratory syncytial virus (RSV). Updates were presented on several therapeutics currently in clinical trials, including polymerase inhibitors, monoclonal antibodies and host directed strategies for influenza, and polymerase and fusion inhibitors for RSV. Updates were also given on the use of the currently licensed neuraminidase inhibitors (NAIs).

Given the location in China, there were also presentations on the use of Traditional Chinese Medicines (TCM). While TCMs have been used for thousands of years, and some may show benefits for treatments of respiratory virus infections, their development is complicated due to their formulation and combinations of compounds. The potency of herbal medications may also vary from manufacturer to manufacturer and from lot to lot. For the safe and effective use of herbal medicines, standardizing herbal formulations is essential for consistency in composition and comparable clinical effectiveness. Additionally, meta-analysis of efficacy of TCM in respiratory virus infections has emphasized the need for evidence based medicine, especially placebo controlled trials (Chen et al., 2011). *In vitro* and some clinical data are presented on the commonly used TCM.

*Abbreviations:* ARDS, Acute respiratory distress syndrome; AUC, Area under the curve; CAP, Community acquired pneumonia; HA, hemagglutinin; HPAI, Highly pathogenic avian influenza; LPM, Live poultry markets; MERS-CoV, Middle East respiratory syndrome corona virus; MN, microneutralization; NA, neuraminidase; NAI, neuraminidase inhibitor; PCT, Placebo controlled trial; PRNT, plaque reduction neutralization titer; RDB, randomized double blind; S/ARI, Severe/Acute respiratory infection; SARS-CoV, Severe acute respiratory syndrome corona virus; $TCID_{50}$, 50% tissue culture infectivity dose

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Following on from the previous conference, there were ongoing discussions on appropriate endpoints for severe influenza in clinical trials from regulators and clinicians, an issue which remains unresolved. The aim of this conference summary is to provide information for not only conference participants, but a review of the current status of clinical information, and development of therapeutics and vaccines for influenza and other respiratory diseases.

## 1. Keynote: zoonotic respiratory viral threats at the animal-human interface

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Around 70% of emerging human infectious diseases have arisen from animal reservoirs. Several viruses with global pandemic potential include SARS-CoV, MERS-CoV, vector-borne viruses (Zika virus, but also Dengue and Chikungunya viruses), and Ebola virus. For influenza viruses, an increased number of human infections with avian A(H7N9) viruses, and appearance of a sub-lineage that has acquired features of highly pathogenic avian influenza (HPAI) are recent notable events (Ke et al., 2017; Su et al., 2017). Live poultry markets (LPM) remain the mostly likely source of A(H7N9) infection, with 67% of patients exposed to an LPM, versus 33% with no history of exposure. HPAI A(H5N6) (Zhou et al., 2016) and A(H5N1) viruses continue to pose zoonotic threats. Influenza A(H5N8) viruses, though currently not causing zoonotic disease, continue to disseminate via wild bird migration. Responses to control of emerging influenza infections include: 1) early detection; 2) control at source (i.e. interruption of virus transmission chain at retail and wholesale LPM); and 3) innovation in predicting emergence of influenza viruses with pandemic potential. New high-throughput technologies and improved diagnostic capacity are essential for better control. LPM remain hubs for virus amplification, persistence and dissemination within poultry as well a source of transmission to humans. A high level of contamination of chicken carcasses with infectious avian influenza viruses (39.8% of 1230 samples) was also detected either at the retail LPM or at the dressed poultry stalls (Mao et al., 2017). Viral RNA or infectious avian influenza viruses of H5, H7 and H9 subtypes were detected in air samples from LPM in Guangzhou and Hong Kong SAR, and the use of de-feathering devices increased the quantity of virus-laden airborne particles (Zhou et al., 2016). Thus, both direct contact with live poultry, and airborne transmission of avian influenza viruses can increase zoonotic risks. Interventions to reduce risk of novel virus emergence in LPM, such as market rest-days (or even closure), ban of live poultry stalls in urban areas with central slaughtering and sales of poultry carcasses, have been implemented in China (Peiris et al., 2016; Yuan et al., 2015).

Influenza A viruses of swine can also be zoonotic pathogens. Influenza viruses of swine in China now carry multiple internal gene segments derived from the A(H1N1)pdm09 virus (Baudon et al., 2017). Most of these are H1 or H3 subtype viruses to which there is human population cross-immunity, but the possibility that swine viruses with the A(H1N1)pdm09 internal gene cassette may acquire avian influenza hemagglutinin (HA) or neuraminidase (NA) genes through genetic reassortment is a concern. Although it is difficult to accurately predict the pandemic threat of a virus, systematic algorithms addressing the risk that an avian or animal virus will achieve sustained human-to-human transmission and emerge as a pandemic (IRAT, TIPRA) have been developed (Trock et al., 2015). Such risk-assessments will need to be made on a continuing basis for pre-pandemic vaccine strain selection.

MERS-CoV is endemic in dromedary camels throughout the Middle East, South Asia and in West, North and East Africa, although zoonotic disease has only been reported from the Arabian Peninsula (Hemida et al., 2017). It is estimated that more than 45,000 people in Saudi Arabia are seropositive, however as mild cases do not seem to induce high levels of neutralizing antibody, many more may have been infected. In contrast, although many dromedary camels in Africa are both virus and seropositive, the disease is not seen. Reasons for this epidemiological pattern may include viral genetic heterogeneity, cultural differences in interactions with camels or lack of diagnosis of human MERS-CoV. The continuous surveillance of MERS-CoV in animal reservoirs, rapid identification and characterization of novel pathogens and their host interactions is required as it will decrease the potential virus threat to human health.

## 2. Novel coronaviruses

### 2.1. Insights from pre-clinical models of MERS-CoV and SARS-CoV

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Coronaviruses (CoVs) are enveloped viruses with a positive-sense single-stranded RNA genome and can infect humans and animals. SARS-CoV and MERS-CoV are newly emerged CoVs causing severe epidemic respiratory disease in human populations with high mortality (Channappanavar and Perlman, 2017). Some SARS-like viruses, such as SHC014-CoV, which is currently circulating in Chinese horseshoe bat populations (Ge et al., 2013) may also evolve into a human CoV with potential to cause global pandemics in the future (Menachery et al., 2015).

Using CRISPR-Cas9 gene editing to modify the mouse genome for expressing human dipeptidyl peptidase 4 (DPP4) the MERS-CoV co-receptor, a pre-clinical mouse model susceptible to MERS-CoV infection and replication was developed. Infection with a mouse-adapted MERS-CoV resulted in symptoms indicative of severe acute respiratory distress syndrome (ARDS). Treatment of these engineered mice with MERS-CoV neutralizing antibodies or vaccination with a MERS-CoV spike protein could protect against MERS-CoV-induced ARDS (Cockrell et al., 2016).

To combat diseases caused by current and future human CoVs broad-spectrum therapies capable of inhibiting CoV infections are needed. A nucleotide prodrug, GS-5734, currently in clinical development for treatment of Ebola virus disease, inhibited replication of SARS-CoV and MERS-CoV in several *in vitro* assay systems, including primary human airway epithelial cell cultures with submicromolar $IC_{50}$ values. Interestingly, GS-5734 is also effective against bat CoVs, prepandemic bat CoVs, and circulating contemporary human CoV in primary human lung cells. In a mouse model of SARS-CoV pathogenesis, prophylactic and early therapeutic administration of GS-5734 significantly reduced lung viral load and improved clinical signs of disease as well as respiratory function. These findings suggest that GS-5734 possesses broad-spectrum anti-CoV activity and has potential to be developed as a novel therapy for treatment of infection by endemic MERS-CoV, circulating human CoVs, and possibly the emerging CoVs of the future (Sheahan et al., 2017).

### 2.2. Clinical features and the virologic course of MERS

Myoung-don Oh, Seoul National University College of Medicine, Seoul, South Korea

On May 4th, 2015, MERS-CoV was imported by a returning traveller from the Middle East to Seoul. Subsequently, the virus spread within the hospitals in Seoul, resulting in 186 cases of confirmed infection, including 38 fatalities (Kang et al., 2017). Age > 55 years old was the greatest risk

factor. During the epidemic lasting 2 months, 16,993 individuals were quarantined for two weeks with an estimated economic cost of 8.3 billion USD. A total of 17 patients were categorized into severe and mild groups, depending on whether oxygen supplementation was used during the hospital stay. Viral load in these patients analyzed by rRT-PCR found that MERS-CoV concentrations peaked during the 2nd week of illness. The severe group had higher viral loads than those in the mild group. The patients in the severe group also had more prolonged viral shedding in respiratory secretions than those in the mild group. Importantly, lower respiratory tract specimens had higher and more prolonged levels of MERS-CoV RNA than those from upper respiratory tract. 10% of patients were still PCR positive at day 28. Throat swabs may be an alternative source of diagnostic samples when sputum cannot be obtained (Oh et al., 2016). After sequencing full viral genomes of strains isolated from 4 patients early and late during infection, who represented at least 4 generations of transmission, it was found that there was no evidence of changes in the evolutionary rate and no adaptive changes in viral proteins (Seong et al., 2016).

Nosocomial transmission is an important characteristic of MERS-CoV infection. Risk factors for transmission of MERS-CoV in healthcare settings are not well defined. The 186 patients who had laboratory-confirmed MERS-CoV infection are suspected as a source of viral transmission. They could be categorized into three spreader groups, including 5 super-spreaders, 10 usual-spreaders, and the non-spreaders group (n = 171). The significant risk factors in the spreader group include: 1) high temperature (≥38.5 ˚C), 2) pulmonary infiltration (≥3 lung zones), and more non-isolated in-hospital days. The super-spreaders had more non-isolated in-hospital days than the usual-spreaders (Mean, 6.6 vs. 2.9 days), suggesting that early active quarantine might help reduce the size of an outbreak (Kang et al., 2017).

### 2.3. Advances in developing vaccines and therapeutics against MERS-CoV

Shibo Jiang, College of Basic Medical Sciences, Fudan University, Shanghai, China

To date there are no vaccines or therapeutics for prevention and treatment of MERS-CoV infection. Previous studies have shown that the receptor-binding domain (RBD) in the S1 subunits of SARS-CoV spike (S) protein plays an important role in mediating virus binding to its receptor, angiotensin I converting enzyme 2 (ACE2), and contains the critical neutralizing domain, thus serving as an important target for developing a SARS-CoV vaccine (Du et al., 2009; He et al., 2005). Similarly, the RBD in MERS-CoV S protein can also effectively bind to its receptor, DPP4, and induce potent neutralizing antibody responses in the RBD-immunized mice (Du et al., 2013b). A subunit vaccine was engineered by linking MERS-CoV S-RBD with the Fc of human IgG (RBD-Fc) (Du et al., 2013a). The transgenic mice that globally expressed human DPP4 (hDPP4-Tg) immunized with RBD-Fc were fully protected from lethal MERS-CoV challenge (Tao et al., 2015). This RBD-Fc subunit vaccine was further optimized using immune refocusing and “neutralizing immunogenicity index” (NII) strategies (Du et al., 2016) suggesting that this vaccine candidate has potential to be further developed as an effective and safe subunit vaccine for prevention of MERS-CoV infection.

Based on the previous experience in developing peptide fusion inhibitors against HIV (Jiang et al., 1993) and SARS-CoV (Liu et al., 2004) a peptide derived from the S2 subunit HR2 domains of MERS-CoV S protein, designated HR2P, was found to be very effective in inhibiting MERS-CoV S protein-mediated cell-cell fusion and infection by both pseudotyped and live MERS-CoV with an $IC_{50}$ at the nM level (Lu et al., 2014). HR2P and its analogous peptides, such as HR2P-M2, have shown excellent *in vitro* and *in vivo* efficacy against MERS-CoV infection (Channappanavar et al., 2015) and protected hDPP4-Tg mice from lethal MERS-CoV challenge (Tao et al., 2015).

Using MERS-CoV S-RBD to immunize mice, a neutralizing monoclonal antibody (mAb) designated Mersmab1, was identified. This mAb specifically binds to MERS-CoV S-RBD and competitively interferes with the binding of the RBD to its cellular receptor DPP4, thus effectively blocking MERS-CoV entry into the host cells and potently neutralizing MERS-CoV infection (Du et al., 2014).

Using MERS-CoV S-RBD to screen a very large naïve-antibody library (containing $\sim 10^{11}$ antibodies), a series of RBD-specific human mAbs, including m336, m337, and m338, were discovered. These mAbs bound MERS-CoV S-RBD with high affinity and avidity. They bound to epitopes that overlap the receptor binding site on the RBD. The highest-affinity mAb, m336, is exceptionally potent in neutralizing both pseudotyped and live MERS-CoV with 50% neutralization at pM level (Ying et al., 2014). The hDPP4-Tg mice treated with m336 prior to or post lethal MERS-CoV challenge were fully protected (Agrawal et al., 2016). X-ray crystallographic analysis of the structure of Fab m336 in complex with MERS-CoV S-RBD reveals that its epitope almost completely overlaps with the RBD. Most interestingly, this human mAb is almost germline with only one somatic mutation in the heavy chain (Ying et al., 2015), suggesting that it can be safely used in humans.

## 3. Emerging threats

### 3.1. Lessons from nosocomial outbreaks of MERS

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The Korean outbreak of MERS-CoV in 2015 resulting in 186 cases with a case fatality rate of 19.4% is the largest outbreak outside the Middle East (Oh et al., 2016). The major factors contributing for this nosocomial outbreak include: 1) overcrowded emergency departments, 2) delays in diagnosis, 3) substandard practices for infection control, 4) the neglect of emerging and re-emerging global infectious diseases, and 5) the lack of infrastructure for highly contagious infectious diseases and hospital infection control. Therefore, this outbreak should serve as momentum for government reform of the healthcare system in infectious diseases.

The kinetics of serologic responses to MERS-CoV infection were assessed by testing 95 sera from 17 MERS patients collected 2–46 days after symptom onset by using plaque reduction neutralization tests, microneutralization (MN), MERS-spike pseudoparticle neutralization and MERS S1-enzyme-linked immunosorbent assay (ELISA). In most patients, robust antibody responses developed by the third week of illness. Neutralization tests correlated well with each other and moderately well with S1 ELISA. Delayed neutralizing antibody responses were associated with more severe disease (Choe et al., 2017; Park et al., 2015a, 2015b).

### 3.2. Human adenovirus type 80: a novel adenovirus causing ARDS and disseminated infection with fatal outcome

Barbara Rath, Vienna Vaccine Safety Initiative, Berlin, Germany

Adenoviruses, which are nonenveloped viruses with a double stranded DNA genome, were first isolated from human adenoids in 1953. More than 50 distinct adenoviral serotypes can infect humans, causing a variety of diseases ranging from mild respiratory infection to life-threatening multi-organ disease (Smith et al., 2010). Neonatal adenovirus infection is a rare and fatal disease (Elnifro et al., 2005). Particularly, disseminated

adenovirus infection may cause ARDS with fatal outcomes in infants and neonates in intensive care units. From October 2009 to April 2015, 6073 patients (infants and children with mean age 3.1 years, range 0–18.8 years) in intensive care units, who presented with fever ≥38 °C and ≥1 respiratory symptom(s) and/or physician diagnoses of influenza-like illness, were tested for adenovirus infection. It was found that 584 of these patients tested adenovirus-positive (mean age 2.2 years, range 0–17.5, 92% < 5 years), while only 4 adenovirus infections occurred in neonates aged ≤28 days. One of these four neonates developed disseminated adenoviral disease with ARDS and fatal outcome. Whole genome sequencing revealed that this infant was infected by a novel human adenovirus type 80, although the pathogenesis of this novel virus and the host factors contributing to disease severity have not been defined yet. Therefore, timely detection of human adenovirus infection and development of effective antiviral treatment options for this highly vulnerable age group are urgently needed.

### 3.3. Epidemiological and clinical characteristics of enterovirus D68 (EV-D68) infection among children aged less than five years in the Philippines

Mariko Saito-Obata, Tohoku University Graduate School of Medicine, Miyagi, Japan

Human enterovirus D68 (EV-D68), a member of the *Picornaviridae* family, was first isolated from pediatric patients hospitalized with lower respiratory infection in California in 1962 (Schieble et al., 1967). Before 2000, EV-D68 was a rarely reported virus linked with respiratory disease. However, it has spread worldwide in the 21st century (Oberste et al., 2004; Tokarz et al., 2012). Most recently, the reported number of EV-D68 cases in acute respiratory infections (ARI) significantly increased (Biggs et al., 2017). To explore possible reasons for the recent increase, the epidemiological and clinical characteristics of EV-D68 in children (< 5-year-old) were analyzed. A hospital-based study on children with clinical diagnosis of severe pneumonia based on Integrated Management of Childhood Illness (IMCI) criteria was performed in several sites in the Philippines from 2008 to 2015. A prospective cohort study on children with symptoms of ARI was carried out in Biliran Island from 2014 to 2016. Nasopharyngeal swabs (NPS) were taken from children and respiratory viruses in the samples, including influenza virus, rhinovirus, respiratory syncytial virus, adenovirus and EV-D68 were detected by RT-PCR. To confirm detection of each virus and to determine genogroup of EV-D68, DNA sequencing was conducted.

Among the total of 5438 NPS samples collected from the hospitalized cases, 59 samples (1.1%) were confirmed EV-D68 positive, while among the 6608 NPS samples collected for the cohort study, 60 (0.9%) were EV-D68 positive. Case fatality rate was 8.5% in the hospitalized EV-D68 positive cases, whereas that in EV-D68 the hospitalized negative cases was 3.6% ($P = 0.06$). Phylogenetic analysis, showed these Philippine strains belonged to lineage 2 (L2) and 3 (L3). About 57.6%, 20.3%, and 22.0% of the hospitalized EV-D68 positive cases were L2, L3 and undetermined, respectivelty. There is no significant difference in the case fatality rate between L2 (5.9%) and L3 (16.7%)($P = 0.2$), and the proportions of very severe pneumonia (61.3% vs 50%, $P = 0.3$). Similarly, about 78.3%, 16.7%, and 5.0% of the EV-D68 positive cohort cases were L2, L3 and undetermined, respectivelty. Interestingly, the proportion of cases diagnosed as pneumonia/severe pneumonia/very severe pneumonia in L2 (57%) was significantly higher than that in L3 (20%) ($P = 0.048$). The results from this study suggest that though the positive rates of EV-D68 are low in both hospitalized children with pneumonia and cohort children cases with ARI in the Philippines, EV-D68 is associated with more severe pneumonia. The Philippine strains of EV-D68 belonged to L2 and L3, while L2 is the dominant genogroup and is associated with more severe disease in cohort children, confirming that EV-D68 is an important pathogen for ARI and its L2 and L3 possess different epidemiological and clinical characteristics.

### 3.4. Detection of influenza A(H1N1)pdm09 viruses exhibiting enhanced cross-resistance to oseltamivir and peramivir in Japan

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Continuous evolution in the influenza HA and NA glycoproteins can result in reduced efficacy of antiviral drugs and vaccines. For example, a single H275Y substitution in the NA of the A(H1N1)pdm09 virus confers resistance to the NAIs, oseltamivir and peramivir. The first widespread community cluster of the H275Y mutant A(H1N1)pdm09 virus was identified in Newcastle, Australia in 2011 (Hurt et al., 2012). During the 2013–2014 influenza season in Japan, a large community cluster of influenza A(H1N1)pdm09 virus exhibited cross-resistance to the NAIs, oseltamivir and peramivir (Takashita et al., 2014, 2015). In the 2015–2016 influenza season, surveillance of NAI-resistant viruses throughout Japan was conducted. A fluorescent NA inhibition assay was used to determine the susceptibilities of viruses to oseltamivir, peramivir, and other NAIs, zanamivir and laninamivir, according to the WHO criteria, which are based on the fold change of $IC_{50}$ values compared to the median $IC_{50}$ values of the same subtype/lineage (WHO, 2012).

After screening 2584 A(H1N1)pdm09 viruses, 50 (1.9%) viruses were found to possess an H275Y substitution in the NA protein. Two H275Y mutant viruses had an additional G147R or I223K substitution in the NA protein. Compared with the single H275Y mutant viruses, the dual H275Y/G147R and H275Y/I223K mutant viruses exhibited enhanced cross-resistance to oseltamivir and peramivir and reduced susceptibility to zanamivir and laninamivir. The dual H275Y/I223K mutant virus exhibited reduced *in vitro* replication capacity, while the dual H275Y/G147R mutant virus retained the ability to grow. These results suggest that the dual H275Y/G147R and H275Y/I223K substitutions exhibited synergistic effects compared with those for the single H275Y change. Because the dual H275Y/G147R mutant virus retained viral fitness, it has the potential to spread among humans.

### 3.5. Clinical and virological outcomes upon emergence of oseltamivir-resistant influenza A viruses in treated individuals: the IRIS study

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To assist in the early detection of IAVs resistant to antivirals and to monitor the clinical and virological outcome of adults and children infected with IAVs according to subtype and susceptibility, the Influenza Resistance Information Study (IRIS; NCT00884117) has been conducted in the United States, Australia, France, Germany, Hong Kong, Norway, and Netherlands between 2009 and 2016 (Roche, 2016). Over a 7-year period, 2213 oseltamivir-treated, IAV-infected patients with follow-up were analyzed for treatment-associated emergentce of resistance. Quantitative IAV RT-PCR was performed on nasal and throat swabs taken at day 1 (for baseline) and on days 3, 6 and 10, respectively. For genotyping and phenotyping of the virus, mutation specific RT-PCR (275Y, 119V, 292K) and next generation sequencing on original swabs were performed. Sanger sequencing and NA inhibition analysis were conducted by NA-STAR assay on cultured material. Resistance mutations were detected in 57 (2.6%) of oseltamivir-treated patients, including 39 infected by A(H1N1)pdm09 with the H275Y substitution and 18 infected by A(H3N2) strains with the R292K substitution. The resistance mutations were first detected on day 3, 6 and 10 in 27%, 68% and 5% of the oseltamivir-treated patients, respectively. At the next visit, the resistant mutant was cleared in 73% of these patients. The resistant viruses isolated from 27 patients were less than 10% of the virus population, as

detected by mutation-specific RT-PCR, but they were not detected by the NA-STAR assay. However, for 74% of the patients in whom resistant mutant strains were the majority species, an oseltamivir resistant phenotype was confirmed by the NA-STAR assay. For A(H1N1)pdm09 strains, median viral RNA clearance was 11.9 and 8.9 days for resistant and wild-type cases, respectively ($P < 0.0001$), while there is no signficant difference in clearance of A(H3N2) strains. In summary, oseltamivir-resistant mutants of IAVs were detected in 2.6% of oseltamivir-treated patients. Although these mutations are not associated with other major substitutions in HA and NA proteins that potentially aid viral fitness, they are associated with delayed clearance of A(H1N1)pdm09, but not with that of A(H3N2) virus.

In another IRIS substudy, viral shedding and susceptibility to oseltamivir in hospitalized immunocompromised patients infected with IAVs was monitored. By day 9 most patients had not cleared the virus. Four of 24 patients (17%) shed resistant virus (Fraaij et al., 2015). Correlation between symptom severity and level of compromise could not be determined.

### 3.6. Update and consolidation of WHO standard guideline for the clinical management of severe influenza infections

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Influenza viruses, particularly the zoonotic viruses, such as avian influenza viruses of A(H5N1), A(H7N9), and A(H9N2) subtypes and swine influenza viruses of A(H1N1) and A(H3N2) subtypes, pose an enormous threat to global public health. In March 2013, the first case of human infection with avian-origin influenza A(H7N9) virus was reported in China (Gao et al., 2013). So far, there have been five A(H7N9) influenza epidemic waves in China. The fifth wave beginning in October 2016 revealed the steepest increase in the number of human cases. Additionally the emergence of highly pathogenic A(H7N9) strains (Su et al., 2017) has now been reported.

In December 2009, the WHO issued updated guidelines for the prevention of A(H1N1)pdm09 infection in healthcare settings (Chor et al., 2012). In 2010, the current WHO guidelines for the clinical management of A(H1N1)pdm09 and other influenza viruses were published, which have contributed to the Chinese National guidance for influenza A(H7N9). The WHO standard guideline for the clinical management of severe influenza will be updated and consolidated to include the new evidence that has been published in recent years. This updated and consolidated WHO standard guideline will be developed by the Guideline Steering Committee (GSC) and the Guideline Development Group (GDG), a globally representative group with broad expertise including infectious disease, virology, paediatrics and public health. Important areas with new evidence for consideration include: 1) diagnostics (including point of care), 2) treatment with NAIs, including the newer licenced NAIs, and 3) the supportive care of severely or critically ill patients with hypoxia and/or shock. Systematic reviews will be commissioned to inform the guideline. The GDG will formulate recommendations based on the evidence quality as assessed using the GRADE approach, while the GSC will ensure that the guideline must meet the breadth of the WHO public health agenda, including subpopulations vulnerable to severe diseases. The Update and Consolidation of WHO Standard Guideline for the Clinical Management of Severe Influenza Infections is expected to be published early in 2018.

## 4. Avian A(H7N9) influenza

### 4.1. Infection prevention and control for patients with avian influenza A(H7N9) virus infection

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Influenza A(H7N9) virus has been detected and isolated in birds, their secretions and in LPM environments. Closure of LPMs has been effective in reducing the human risks of A(H7N9) infection (Yu et al., 2014b). Most of the human cases of A(H7N9) infection are sporadic, but there have been several family clusters in which limited, non-sustained human-to-human A(H7N9) virus transmission cannot be ruled out (Li et al., 2014). Limited human to human transmission in the hospital settings, together with risk factors such as an overcrowded ward environment and performance of aerosol-generating procedures, has been reported (Chen et al., 2016; Fang et al., 2015). When caring for patients with ARI, the WHO infection prevention and control (IPC) guidelines for ARI patient care that are applicable to A(H7N9) patients include early recognition and isolation of patients, application of routine IPC precautions (Standard Precautions) for all patients, additional precautions in selected patients (e.g. airborne precautions for high risk aerosol-generating procedures), and other IPC strategies in health-care facilities such as early recognition and source control, administrative controls, environmental and engineering controls, and personal protective equipment (WHO, 2014).

### 4.2. Epidemiology of human infections with A(H7N9) virus and control measures in mainland China

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Between 2013 and June 2017, 1503 cases of human infection with influenza A(H7N9) virus have been reported, with more than 500 fatalities. An earlier start and a steep increase in the number of human cases of A(H7N9) virus infection was observed during the fifth epidemic in China in 2016–2017 (Zhou et al., 2017a). Most human cases occurred in Eastern and Southern China in the earlier epidemics. However, six provinces in Central and Western China without previous cases, reported human infections in the fifth epidemic, indicating geographic expansion of A(H7N9) cases. In comparison with the earlier epidemics, the epidemiology characteristics such as sex and age of patients infected with A(H7N9) virus remained unchanged, but the proportion of rural residents among confirmed cases increased in the fifth wave. Severity remained high in the fifth epidemic: 90% of cases were diagnosed as severe, and 79% of cases were admitted into an intensive care unit. Most (90%) cases reported exposures to poultry, including 75% that had visited an LPM before illness onset or had contact with poultry purchased from an LPM. Since 2013, 37 clusters (comprising 78 cases), of at least 2 epidemiologically-linked confirmed cases have been identified, including 11 clusters reported in the fifth epidemic. There were no changes in the size or number of clusters per epidemic and there was no evidence of sustained human-to-human transmission. While most human cases confirmed since 2013 were caused by low pathogenic avian influenza (LPAI) A(H7N9) virus infection, 25 cases of HPAI A(H7N9) virus infection have been identified in three provinces in southern China during the fifth epidemic. Preliminary analysis indicated that HPAI A(H7N9) virus-infected patients were more likely to live in rural areas, have exposure to sick or dead poultry, and admitted to hospitals earlier than LPAI A(H7N9) cases. Extensive efforts are needed to prevent and control the spread of LPAI and HPAI A(H7N9) viruses among poultry, including in rural areas (Zhou et al., 2017b).

### 4.3. Virological update

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The Yangtze River Delta region, located in eastern China, is well-recognized as the original source for A(H7N9) influenza outbreaks. Based on the evolutionary analysis of A(H7N9) viruses from the first 3 epidemic waves, the Pearl River Delta region has been identified as another A(H7N9) outbreak source. A(H7N9) viruses are repeatedly introduced from these two sources to the other areas and the persistent circulation of A(H7N9) viruses occurs in poultry, causing continuous epidemic waves. The internal genes continue to reassort with the A(H9N2) viruses, and both the HA and NA proteins are evolving. More than 93 genotypes have been identified, with more than 80 of these being transient. The AnH1 genotype, which was predominant during the first epidemic wave, was replaced by JS537, JS18828, and AnH1887 genotypes during the second and third epidemic waves (Wang et al., 2016a)). In late 2016 and beginning of 2017, three patients with severe clinical symptoms were confirmed to be infected with HPAI A(H7N9) viruses. The viruses belonged to the Yangtze River Delta lineage. Four amino acids inserted (Lys-Arg-Thr-Ala) at the HA cleavage site enabled the A(H7N9) HPAI virus to display trypsin-independent infectivity (Zhu et al., 2017). Additionally, the HPAI viruses have acquired the L226Q substitution in the HA, although maintaining dual receptor-binding preference. The HPAI HA antigenicity is distinct from the LPAI A(H7N9) viruses. All viruses have the S31N substitution in their M2 protein, conferring amantadine resistance. Among 314 LPAI viruses tested for NAI susceptibility, 18/314 had decreased sensitivity, one with I222R, two with A246T and 15 with the R292K substitutions, the latter conferring a multidrug resistance phenotype. Among HPAI viruses tested, 4/21 also had reduced sensitivity, with three of those with the R292K substitution (Ke et al., 2017). All 292K isolates arose post-treatment. Therefore, surveillance of the virus, especially antiviral surveillance, is an essential component of pandemic preparedness.

### 4.4. Antiviral treatment and resistance

Xiaonan Zhang on behalf of Junwen Hu, Shanghai Fudan University, China

The emergence of NAI-resistant variants of A(H7N9) viruses with an R292K NA substitution poses a therapeutic challenge. In a study of 14 patients with A(H7N9) infection complicated by pneumonia, NAI treatment was associated with a reduction of viral load in throat swab specimens in 11 surviving patients. However, 3 patients with persistently high viral loads in the throat, despite antiviral therapy, became dependent on extra-corporeal membrane oxygenation. An R292K NA substitution was identified in two of these patients, who had also received systemic corticosteroid treatment. In one of them, wild-type sequence R292 was noted 2 days after commencement of NAI treatment, and the resistant mutant K292 dominated 9 days after commencement of treatment (Hu et al., 2013). Using limited serial passage in the presence of oseltamivir, and plaque purification, an R292K variant of the Anhui1 lineage was isolated from a patient with clinical evidence of resistance to oseltamivir. *In vitro* and cell-based assays confirmed a high level of resistance of A(H7N9) virus carrying the R292K substitution to oseltamivir carboxylate and a moderate level of resistance to zanamivir and peramivir. Non-NAI antivirals, such as T-705, ribavirin and NT-300, efficiently inhibited both the variant and the wild-type viruses in cell-based assays. A combination of NAIs and non-NAIs did not exhibit a marked synergistic effect against the R292K variant in cell culture. However, the combination of two non-NAIs (T-705 and ribavirin) exhibited synergism against the mutant virus. In experimentally infected mice, the R292K variant showed delayed onset of symptoms, a reduced viral load and attenuated lethality compared with the wild-type. The study suggested non-NAIs should be tested clinically for A(H7N9) patients with a sustained high viral load. Possible drug combination regimens, such as T-705 plus ribavirin, should be further tested in animal models. A real-time RT-PCR and single nucleotide polymorphism probes have been developed to differentiate this mutant strain in mixed virus populations in human specimens (Wang et al., 2014b). Screening of 18 patients undergoing oseltamivir therapy identified viruses with mixed R/K sequence, not previously detected.

## 5. RVI diagnosis and treatment

### 5.1. Advances in respiratory virus infection molecular diagnostics

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Clinical manifestations of respiratory virus infections are usually non-specific, with significant overlap between viral, bacterial, and non-infectious causes. New technologies, with improved multiplexing capabilities, has allowed detection and differentiation of multiple viruses from a single respiratory sample. In order to be clinically useful, clinicians want rapid turn around with multiple pathogens detected. For a clinical microbiology laboratory handling low to medium specimen volumes, these platforms are preferred as they allow a simple workflow that can be used with minimal training, and afford a rapid turnaround time. However, these platforms are not capable of large volumes of testing. Batched testing high throughput platforms are useful for unexpected increases in testing volume such as nosocomial outbreaks, peak influenza seasons, and during pandemics. One system will not work for all situations. Laboratorians and clinicians should know the limitations of all test platforms available.

### 5.2. A simple tool-CLAP to assess the severity of influenza A(H7N9) viral pneumonia

Yeming Wang, Xuan Wu Hospital Capital Medical University, Beijing, China

Influenza A(H7N9) virus has mortality rates of up to 50%. A clinical severity assessment tool is needed to help triage patients with A(H7N9) infections. Current severity assessment tools have not been tested in this population. The goal of this study was to develop a new clinical severity tool, and compare to currently available tools such as PSI (Pneumonia Severity Index) (Fine et al., 1995), SMARTCOP (Charles et al., 2008) (systolic blood pressure, multilobar chest radiography involvement, albumin level, respiratory rate, tachycardia, confusion, oxygenation, and arterial pH), and CURB-65 (Lim et al., 2003) (confusion, urea nitrogen, respiratory rate, blood pressure, 65 years of age and older). Data was collected from patients who were hospitalized with influenza A(H7N9) infection in China. A derivation cohort was constructed from the clinical data of 613 patients hospitalized between April 2013 and March 2015. Of these, 285 patients had complete data. The 30-day mortality of the derivation cohort was 26.3%. A multivariate logistic regression was performed to identify clinical features at admission to hospital that were associated with mortality within 30 days. These results were converted into a simple point-based severity tool which was based on creatinine level, lymphocyte count, PaO2/FiO2, and age. The 30-day mortality of the validation cohort was 47.4%. Risk factors associated with 30-day mortality were creatinine $>$ 133 μmol/L (1 point), lymphocyte count $<$ 0.3 $\times$ 109/L (1 point), age $>$ 60 (1 point) or 80 years (2 points), and PaO2/FiO2 $<$ 100 (2 points) or 200 mmHg

(1 point). The scoring system is called CLAP. After derivation of the scoring system, a validation cohort was compiled with clinical data from 171 patients hospitalized between October 2016 and March 2017. Sensitivities and specificity of CLAP on admission identifying the 30-day mortality were 90.79% and 45.45%, respectively. The highest AUROCs were for the CLAP score (0.77; 95% CI, 0.72–0.82) and the PSI score (0.73; 95% CI, 0.68–0.78) compared with 0.62 (95% CI, 0.56–0.68) for SMART-COP ($P < 0.001$, compared with CLAP) and 0.59 (95% CI, 0.55–0.65) for CURB-65 ($P < 0.001$, compared with CLAP). CLAP is a simple scoring tool that my have utility in predicting the outcome of patients infected with influenza A(H7N9) viral pneumonia.

### *5.3. Investigation of antibody dynamics in the randomized study with Japanese pediatric patients with influenza A virus infection after treatment of four neuraminidase inhibitors*

Nobuo Hirotsu, Hirostu Clinic, Kawasaki, Japan

It is not known if antiviral treatment may affect the development of the adaptive immune response after influenza infections. In a study previously reported at Options IX for the Control of Influenza, 133 participants between 4 and 12 years of age that presented within 48 hours of illness onset were randomly allocated to be administered one of four NAIs: oral oseltamivir, inhaled zanamivir, intravenous peramivir, or inhaled laninamivir. The primary endpoint was time to undetectable virus titer. Peramivir showed a shorter time to viral clearance when compared to oseltamivir (adjusted $P = 0.035$). This analysis examined if the adaptive immune response, as measured by hemagglutinin-inhibition assay (HAI), was different among treatment groups. Approximately 80% of the enrolled patients were infected with influenza A(H3N2) virus, and 20% with influenza A(H1N1)pdm09. Antibody levels were evaluated at three timepoints: baseline, day 3–4, and day 14 ± 3. There were no significant differences in HAI titer increase (ratio of the baseline to the day 14 visit) between treatments. Prior vaccination, prior influenza A(H3N2) infection, and prior family history of influenza illness all were associated with higher pre-treatment antibody levels. Higher antibody increases from day 0 to day 14 correlated with faster resolution of viral shedding.

### *5.4. A randomized controlled trial on adjunctive macrolide treatment in adults hospitalized with influenza*

Nelson Lee, The Chinese University of Hong Kong, Hong Kong SAR, China

Hypercytokinemia is seen in severe influenza and associated with poor outcomes (Liu et al., 2016). Macrolides have anti-inflammatory properties, and may attenuate cytokine levels in acute influenza (Kanoh and Rubin, 2010). This was a randomized, open-label, multicenter trial among adults hospitalized for laboratory-confirmed influenza. Adults ≥18 years of age with lower respiratory tract infection that presented within 4 days onset of illness were eligible for the study. Subjects were not eligible if they were taking corticosteroids, or had baseline QTc prolongation on EKG. Participants were randomized in a 1:1 ratio to receive oseltamivir and azithromycin (500 mg/day), versus oseltamivir alone for 5 days. The primary outcome was the change in plasma cytokine/chemokine concentration over time (days 0–10). Fifty patients were randomized to the study. The treatment arms had comparable baseline characteristics: age was 57 ± 18 years; 70% had influenza A(H3N2), and viral loads were similar. Macrolide treatment was associated with lower pro-inflammatory cytokines IL-6 (reduction from baseline −83.4% vs −59.5%, $P = 0.016$), CXCL8/IL-8 (80.5% vs −58.0%, $P = 0.056$), IL-17 (−74.0% vs −34.3%, $P = 0.015$), CXCL9/MIG (−71.3% vs −56.0%, $P = 0.043$). However, the duration of fever, duration of symptoms, and viral shedding were not different between the two treatment arms.

### *5.5. Broad spectrum in vitro and in vivo activity of RO-7, a novel inhibitor of the influenza virus PA endonuclease*

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The influenza virus acidic polymerase (PA) is a promising antiviral target because of its essential role in virus transcription. RO-7 is a new PA protein endonuclease inhibitor (Jones et al., 2017). RO-7 was tested against 36 influenza A and B viruses in MDCK and differentiated human bronchial epithelial cells. RO-7 inhibited replication of multiple strains of influenza A (H1N1, H3N2, H5N1, H7N9, H9N2) and B viruses as well as NAI-resistant variants (mean $EC_{50}$ 2.9–105.3 nM).

A lethal BALB/c mouse model was established using 5$MLD_{50}$ of A/California/04/2009 A(H1N1)pdm09 ($1.1 \times 10^3$ 50% tissue culture infectious doses [$TCID_{50}$]) or B/Brisbane/60/2008 ($2.5 \times 10^5$ $TCID_{50}$) virus. The mice were treated with RO-7 at 6, 15 or 30 mg/kg/day twice daily for 5 days starting 4 hours before or 24 or 48 hours after virus inoculation. All dose levels of RO-7 exhibited reduced morbidity, increased survival (60–100%), reduced lung virus load, and decreased lung pathology compared to untreated controls. No RO-7 resistance was observed in viruses isolated from drug-treated mouse lungs. Virus with 60-fold reduced sensitivity was selected after 16 passages in MDCKs under increasing RO-7 concentrations, with an I38T substitution in the PA endonuclease domain. However, 99% of circulating viruses do not have this change.

### *5.6. The topaz trial: a phase IIB study of pimodivir (JNJ-63623872 or JNJ-872; formerly VX-787) as monotherapy or in combination with oseltamivir in the treatment of acute uncomplicated seasonal influenza*

Lorant Leopold, Janssen, Titusville, New Jersey, USA

Pimodivir is a PB2 subunit inhibitor of the influenza A polymerase (Fu et al., 2016). This was a Phase II study evaluating the safety and potential efficacy of pimodivir. Adults 18–65 years of age with acute uncomplicated influenza A virus infection that presented with less than 48 hours of symptoms were eligible for the study. Participants were randomized 1:1:1:1 to receive placebo, pimodivir 300 mg, pimodivir 600 mg, or the combination pimodivir 600 mg/oseltamivir 75 mg for 5 days. 967 participants were screened, 293 randomized, and 223 used for the primary analysis. The demographics were balanced across treatment arms, with 84% caucasians, 51% females and a median age of 41 years. When compared to placebo, 600 mg of pimodivir resulted in a greater decrease in qRT-PCR area under the curve (AUC) for viral load from day 1–8, compared to 300 mg [-4.5 (−8.0; −1.0); 0.012 vs −3.6 (−7.1; −0.1); 0.044 $\log_{10}$ copies/ml]. The combination of pimodivir and oseltamivir demonstrated further reduction when compared to pimodivir 600 mg (−4.1 $\log_{10}$ copies/mL, −7.4 to −0.7, $P = 0.017$). There was no change in median time to resolution of symptoms: pimodivir 300 mg, 99.0 hours (71.5–150.6), pimodivir 600 mg, 85.7 hours (55.3–114.9), pimodivir + oseltamivir, 70.4 hours (61.8–82.5), and placebo alone, 86.4 hours (68.7–117.3). The most common adverse event was diarrhea, typically mild, and similar across treatment arms. Although pimodivir showed a statistically significant decrease in viral area under the curve, this difference was not associated with improved clinical benefit. The dosage of 600 mg of pimodivir has been selected for Phase III studies.

### 5.7. IRC003: a randomized study of combination antivirals for the treatment of influenza

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Preclinical data suggests that a combination of anti-influenza antivirals could be more effective than oseltamivir alone in the treatment of influenza. This was a randomized, blinded, multicenter Phase II trial in the United States, Thailand, Mexico, Argentina and Australia. Participants that were either 65 years of age or older, had a chronic medical condition, and/or were obese with confirmed influenza A or B were eligible for the study. Enrolled subjects were randomly assigned to receive either the combination of oseltamivir, amantadine, and ribavirin or oseltamivir alone for 5 days, and were followed for 28 days. The primary endpoint was the percentage of participants with virus detectable by PCR in a nasopharyngeal swab at day 3. 881 participants were enrolled, and 633 were randomized. Seven participants were excluded from the ITT population: 3 were not randomized appropriately, and 4 withdrew before taking any study medication. The primary analysis included 394 participants, excluding 47 in the pilot phase, 172 without influenza confirmed in the central laboratory, and 13 without an endpoint sample. 80 of 200 (40.0%) participants in the combination arm had virus detectable at day 3 compared to 97 of 194 (50.0%) (95% C.I. 0.2–19.8%, $P = 0.046$) in the control arm.

There was no benefit in clinical outcomes across multiple parameters: the duration of clinical symptoms (4.5 days in the combination arm vs 4.0 days in oseltamivir monotherapy, $P = 0.44$), duration of fever (0 vs 1 day, $P = 0.69$), time to feeling as good as before the influenza illness (7.5 vs 6.5 days, $P = 0.0033$), nor time to return of pre-illness physical function using the physical domain of the SF-36 (7.0 vs 6 days, $P = 0.06$). The most common adverse events were nausea [65 (12%) vs 63 (11%)], vomiting [56 (10%) vs 64 (11%)] and diarrhea [39 (7%) vs 23 (4%)], and occurred in similar proportions in both arms. Although oseltamivir, amantadine, and ribavirin showed a statistically significant decrease in viral shedding at day 3 relative to oseltamivir monotherapy, this difference was not associated with clinical benefit.

## 6. Keynote: burden of respiratory virus diseases in China

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China is a geographically, economically, and climatologically diverse country with a population of approximately 1.37 billion. These factors, in part, contribute towards its substantial influenza mortality burden, estimated at 11–18 excess deaths per 100,000 in the southern and northern cities in the inter-pandemic seasons (Feng et al., 2012). Although seasonal influenza vaccination was introduced in China in 1998, it is not included in the national immunization program. The influenza vaccine coverage is still relative low: 2009/2010–8.5%, 2010/2011–9.5%, 2011/2012–4.3%, mainly in the elderly in wealthier cities. Additionally, substantial vaccination rate diversity is observed among low- and high-income cities and provinces. On the basis of multiyear laboratory-confirmed influenza surveillance data representative of a large majority of the Chinese population (2005–2011), three epidemiological regions were identified for influenza A virus infection: northern provinces (latitude $\geq 33^{\circ}$N) experience winter epidemics, southern provinces (latitude $< 27^{\circ}$N) experience peak activity in spring, while provinces at intermediate latitudes experience semi-annual epidemic cycles (Yu et al., 2013). Therefore, it is optimal for Northern China to follow the timing of vaccination typically recommended for the Northern Hemisphere, with annual campaigns starting in October. In contrast, most of southern Chinese provinces have to accommodate influenza activity peaking in April–June, and hence vaccination would be best initiated in February–March of each year, broadly coinciding with the recommended timing of vaccination for the Southern Hemisphere.

The National Influenza Surveillance Network was launched in China in 2000. Before the 2009 influenza pandemic, only southern Chinese provinces and three northern provinces (including Gansu, Liaoning and Tianjin) conducted full-year influenza surveillance, although other northern provinces conducted half-year surveillance. Now this network includes 411 provincial- and prefecture-level Centers for Disease Control and Prevention (CDCs) and 556 sentinel hospitals situated in 31 provinces. Currently, all provinces are doing year-round influenza surveillance. Sentinel hospitals report the numbers of total outpatient visits and the numbers of visits by outpatients with ILI and age group. A retrospective telephone survey estimated the cost of treatment for out-patients for influenza was $155 USD and > $1500 for in-patients (Yang et al., 2015). The national sentinel hospital-based influenza surveillance network and surveillance for SARI in central China showed that outpatients, hospitalization, mortality and economic burdens associated with influenza are substantial in China. Significant variations were observed in influenza-associated ILI burden and SARI hospitalizations by provinces, age, and influenza virus type. In one survey of approximately 80 million patients presenting at sentinel hospitals 3.1% had ILI and of those tested for influenza, 12.3% were positive. Surveillance among 4 hospitals in Jingzhou from 2010-2012 showed that of 16,208 SARI cases tested, 2057 (13%) had confirmed influenza, including 1427 (69%) aged < 5 years (Yu et al., 2014a). Marked differences in seasonality of influenza A and B viruses were determined, with most of China experiencing annual influenza B activity in winter. Active surveillance for hospitalized patients with acute lower respiratory infections revealed the spectrum of viral agents in 24 provinces of China during 2009–2013: influenza in 1869 patients (6.6%), RSV in 2795 patients (9.9%), PIV in 1366 patients (4.8%), adenovirus in 957 patients (3.4%), bocavirus in 551 patients (1.9%), human metapneumovirus in 424 patients (1.5%) and hCoV in 393 patients (1.4%) (Feng et al., 2014).

## 7. RVI impact and community acquired pneumonia (CAP)

### 7.1. New clinical guidelines for CAP in China

Bin Cao for Jieming Qu, Shanghai Ruijin Hospital, Shanghai, China

The Assembly of Infectious Diseases of the Chinese Thoracic Society has revised the Clinical Practice Guideline for CAP and published an updated Chinese version in April 2016 (Qu and Cao, 2016). This version is based on new evidence that has emerged in the recent decade on the etiological profile of CAP in China, advances in laboratory diagnosis, newer anti-infective therapies, and accessibility to effective vaccines (Torres et al., 2016). The main contents of the Guideline include: definition and diagnosis of CAP, assessment of CAP severity, criteria for hospital admission, etiological diagnosis, anti-infective therapies, adjunctive therapies, assessment after initial therapy and the criteria for discharge, unusual types of CAP, and prophylaxis. The recommendations were graded based on the strength of the evidence according to international standards. *Streptococcus pneumoniae* and *Mycoplasma pneumonia* are the commonest bacterial pathogens, which have distinctive resistance patterns (penicillins, macrolides); gram-negative pathogens (e.g. *Klebsiella pneumonia*, *E. coli*) are more frequently found in the special populations such as the elderly; respiratory viruses constitute 15–35% of cases, with influenza being the most important pathogen. Community-acquired MRSA infections remain rare. The CURB-65 score is recommended as a tool to assist decision on hospitalization of Chinese patients. The use of culture-based, antigen-based and molecular assays, as well as biochemical markers (e.g. procalcitonin) for diagnosis is discussed; empirical antibacterials and antivirals are recommended based

on the epidemiological data, clinical scenario and resistance profiles. NAIs are recommended for influenza pneumonia. Influenza vaccines and pneumococcal vaccines (polysaccharide, conjugated) are recommended for prevention. An English version of this practice guideline is under preparation.

### 7.2. Contact patterns of healthcare workers and transmission of infectious diseases

Lili Jiang, National University of Singapore, Singapore

Healthcare workers (HCWs) may play a significant role in transmitting pathogens within the hospital setting, and even from the hospital to the community (and vice versa). A prospective 'contact-diary' survey of hospital-based HCWs (at 3 public hospitals in Singapore) investigated their contact patterns, and compared these with working adults in the general population. Participants recorded their contact patterns (2-way conversation with $\geq 3$ words or skin-to-skin contact) for 24-hour periods, demographics of the contact person, location of contact, and the contact duration. Altogether, 211 HCWs and 1028 working adults reported 4066 and 15932 contacts respectively. HCWs reported more work-related contacts (median of 13 vs 4), and less household contacts (2 vs 3). HCWs also had more work-related skin-to-skin contacts (6 vs 1). Among different HCW subgroups, doctors reported the highest while ward-based nurses reported the lowest total work-related contacts (17 vs 11). However, skin-to skin work-related contacts were highest for ward-based nurses compared with other HCWs (7 vs 5). Multivariate linear regression showed significant differences in work-related contacts among various HCW subgroups, adjusted for gender and institutional effects. It was concluded that HCWs experienced more physical contacts than the general population and may place them at higher risk of acquiring and spreading infections.

### 7.3. Human metapneumovirus and pediatric ARI hospitalizations in Vietnam

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Human metapneumovirus (hMPV) is a known cause for ARI, and is especially common among children (Edwards et al., 2013). However, detailed clinical and molecular epidemiological information of hMPV remain limited in the developing countries. The seasonal pattern and molecular characterization of hMPV in pediatric patients hospitalized for ARI in central Vietnam, was monitored as part of a population-based ARI surveillance program (2007–2015). A total of 6167 cases were enrolled; of these, 206 (3.3%) tested positive for hMPV by a multiplex PCR assay. Yearly hMPV incidence was 127 (per 100,000) among children $< 5$ years prior to 2009, which increased to 216 (per 100,000) in the period 2010–2015. There was no seasonal trend. The presenting feature included wheezing and pneumonia (58.5% and 34.1% respectively). hMPV Group-A and B were co-circulating, with Group-A being predominant in most of the seasons. Time-scaled phylogenetic trees (F gene) showed that the recently dominant A2c lineage diverged from A2b around 2004 (95%HPD: 2002–2006) and became dominant after 2009. Multivariate analysis results indicated that cases infected with the A2b lineage were clinically more severe than A2c (adjusted OR 6.30, 95% CI 1.65–24.09 and 6.93, 95% CI 2.34–20.50 for wheezing and pneumonia respectively). Group B virus infections appeared milder. These findings indicate the importance of hMPV in pediatric ARI hospitalizations, and provide useful information for future surveillance and vaccine development.

### 7.4. Genomic and epidemiological dynamics of RSV in Australia

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RSV is one of the most important causes of severe respiratory infection in infants and young children, as well as in older adults and the immunocompromised (Lee et al., 2013; Shi et al., 2017). However, understanding of the dynamics driving the on-going evolution and epidemiology of RSV transmission in humans is rather limited. To study genetic diversity, as well as potential baseline resistance to novel therapeutics, the genomes of RSV strains collected from adult and pediatric patients in Australia from 2010 − 2016 (RSV-A, n = 70; RSV-B, n = 80) were amplified and sequenced using a 4x4 Kb overlapping RT-PCR strategy that targeted both RSV-A and RSV-B subtypes. Nextera XT libraries were prepared and sequenced using an Illumina MiSeq. Following de novo assembly, RSV sequences were aligned with global references and analyzed using a phylodynamic approach. A distinct seasonality was observed with most infections occurring during the late winter period (with subtle shifts in the seasonal relative predominance of RSV-A and RSV-B strains). Multiple co-circulating lineages were identified with the Australian strains dispersed throughout the global diversity. Spatial and temporal clustering was observed at local and regional scales highlighting the complex fine-scale dynamics (e.g. institutional outbreaks), as well as gene flow within the Asia-Oceania region. This gene flow with local endemic lineages was consistent with other respiratory pathogens suggesting viral traffic is driven by human movement and interactions. A strong-temporal structure was also observed with similar mean rates of evolutionary change at global scales for both RSV-A and RSV-B subtypes at $6.5 \times 10^{-4}$ nucleotide substitutions per site per year (subs/site/year) and $7.5 \times 10^{-4}$ subs/site/year, respectively. Like previous observations, most variation was found in the G protein and intergenic regions. However, fixed changes were observed within the fusion protein at potentially antigenic sites of recent RSV-A strains. Resistance associated mutations at the F protein and the polymerase genes seemed rare. Their data add to the understanding of RSV genetic diversity, transmission, and disease dynamics which may benefit future vaccine design.

### 7.5. Etiology and incidence of SARI among hospitalized patients in Madagascar

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The etiology and incidence of SARI among hospitalized patients, was part of an on-going surveillance program in Madagascar from 2014 − 2016. Upper respiratory samples were analyzed for influenza, RSV and rhinovirus using real-time RT-PCR assay. A total of 668 patients with SARI patients were enrolled. The median age was 7 years, with most cases (n = 318; 47.6%) being children $< 5$ years old. The detection rates for influenza, RSV and rhinoviruses were 21.7% (46/668), 21.4% (143/668) and 6.3% (42/668), respectively. The age group of 2–5 years was most affected by RSV (OR = 14.2; $P < 0.001$); whereas age groups of 5–15 years (OR = 2.5; $P = 0.026$) and 15–50 years (OR = 1.6; $P = 0.04$) were more frequently infected with influenza viruses. The overall incidence of SARI was 15,521.2 per 100,000 person-years, which varied significantly from year to year. The incidence of influenza-associated SARI was 3322.7 per 100,000 person-years, with the highest incidence in the age group of 15–50 years (6864.6 per 100,000 person-years) (OR = 2.9; $P < 0.001$). The overall incidence for RSV was 3254.4 per 100,000 person-years, with the highest incidence among children aged $< 2$ years (6103.8 per 100,000 person-years) (OR = 4.9; $P < 0.001$). Data indicated that disease burden caused by these respiratory viruses is high in African countries, particularly among children, and highlighted the importance of continuous surveillance to provide

data for planning of public health measures in the region (Lafond et al., 2016; McMorrow et al., 2015).

## 8. Pathogenesis of SARI

### 8.1. What part do bacteria play in severe influenza?

Peter Openshaw, Imperial College, London, United Kingdom

Severe influenza may in part result from an over-exuberant host response driven by a high viral load, but bacterial overgrowth (usually of resident pathogenic species such as *S. pneumoniae* and *H. influenzae*) can also contribute to morbidity and mortality (Lee et al., 2015). Due to overlapping clinical features, empirical antibacterial treatment is frequently prescribed, perhaps unnecessarily, whereas empirical antiviral treatment may be under-utilized (Myles et al., 2012). Therefore, it is important to distinguish these mechanisms to refine and direct specific antiviral, antibacterial, and (potentially) immunomodulatory therapy. In cases where it is not clear what therapy to use, the host response to infection might act as an indicator (for example, analysis of transcriptional signatures in the peripheral blood) (Suarez et al., 2015) but the dependence of such responses on severity, phase and complications of influenza infection remain unclear.

A comprehensive analysis of whole blood RNA signatures and local/systemic mediators was carried out in a cohort of 256 adults with influenza-like illness in 2009/10 and 2010/11 seasons (The Mechanisms of Severe Acute Influenza Consortium, MOSAIC.) (Cole et al., 2017). Interferon-related antiviral pathways genes were overexpressed during the early phase of illness (0–5 days), followed by activation of the inflammatory cell pathways including neutrophils, monocytes and NK cells (days 5–10). The molecular scores were related to severity and were affected by bacterial sepsis, but depended most critically on timing since onset of symptoms. Identification of specific patterns of immune activation that might be amenable to therapeutic manipulation may ultimately assist in management of influenza, but require careful interpretation.

### 8.2. Severity and phase linked virus specific CD4 and CD8T cell responses in human pH1N1 infection

Hui Li, Capital Medical University, Beijing, China

The role of CD4 and CD8 T cell responses in influenza has not been fully characterized (Zhao et al., 2012) The antigen-specific T cell responses and correlations with disease severity were examined in 79 patients with A(H1N1)pdm09 influenza infections in China. Samples were taken during the acute phase (7 ± 3 days) and recovery phase (21 ± 3 days) and were tested against live A(H1N1)pdm09 virus and peptide pools of influenza viruses. The longitudinal changes in influenza-specific $CD4^+$ and $CD8^+$ T cell responses with or without pneumonia were characterized and T cell activation and migration-related cytokines/chemokines in plasma were measured. Compared with milder infections, influenza-specific $CD4^+$ and $CD8^+$ T cell responses and virus shedding were elevated in patients with pneumonia. During the acute phase of illness, effector $CD8^+$ T cells (CD45RA + CCR7-) with highly-expressed inhibitory immune receptor CD200R dominated the response (IFN-γ secreting). In the recovery phase, effector memory $CD4^+$ T (CD45RA-CCR7-) cells with highly-expressed PD1, CTLA4 and LAG3 were higher in those with severe infections compared to mild infections. The magnitude of T cell responses correlated with pneumonia severity (as indicated by PaO2/FiO2, lung injury score, APACHE II score). In addition, T cell activation and migration-related cytokines/chemokines were associated with disease severity. Thus $CD4^+$ and $CD8^+$ T cell responses may play a role in pathogenesis and disease progression in A(H1N1)pdm09 pneumonia.

### 8.3. AIM2 inflammasome is critical for influenza induced lung injury and mortality

Jieru Wang, University of Pittsburgh, Pittsburgh, USA

The Absent-In-Melanoma 2 (AIM2) inflammasome is important in the host response against a range of bacterial and viral infections, but little is known about its role in IAV infection. It is activated by dsDNA upon infection of cells, leading to cleavage and activation of caspase1 and cleavage of IL-1β and IL-18 to their active forms. The role of AIM2 was studied using knock-out mice infected with lethal doses of IAV A/PR8/34 and A/California/07/09. In AIM2-deficient mice, induced dsDNA release, caspase-1 activation and release of cleaved IL-1β in the lungs were significantly reduced. In addition, AIM2 deficient mice exhibited attenuated lung injury (with fewer neutrophil infiltration) and improved survival against IAV challenge; virus burden in the lung was unaltered. Deficiency of AIM2 was not shown to affect the adaptive immune response against IAV infections. *In vitro* experiments with siAIM2 treated and AIM2 deficient human and mouse lung alveolar macrophages and type II cells indicated a macrophage-specific function of AIM2 in the regulation of IAV-stimulated proinflammatory responses (Zhang et al., 2017). These findings suggest that influenza infection activates the AIM2 inflammasome, which may play an important role in IAV-induced lung injury and mortality.

### 8.4. Identification of DDX19B as an essential host factor for uncoating step during influenza virus entry

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Using siRNA 18,000 host genes in human lung epithelial A549 cells were targeted, and screened for effects on multi-cycle infection of influenza A/WSN/33 (H1N1) virus. Of 209 siRNAs which decreased IAV infection, the 20 top-scored siRNAs were further validated. A DEAD-Box RNA helicases 19B, DDX19B, was identified as an essential factor for IAV replication. Depletion of DDX19B by siRNA resulted in up to a 2 $\log_{10}$ decrease in virus yields in single- and multi-cycle replication kinetics. The growth defect was also found in other influenza subtypes, including A(H1N1)pdm09, H3N2, H7N4, and type B. The effect was early since by 4 hours after infection (pi) levels of mRNA and vRNA were reduced. Normally the RNPs are imported into the nucleus by 3–4 hours pi, but there was little NP staining in the nucleus. Furthermore, there was no M1 staining in the cytoplasm, indicating that while DDX19B is known to function in mRNA nuclear export, the block was earlier at the uncoating step. This contrasts with others who showed the DDX19B enhanced the nuclear export of influenza mRNA (Diot et al., 2016).

### 8.5. Studies to gain insights into drug resistance of influenza A(H7N9) viruses

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There are still no established correlates of clinically relevant resistance to the NAIs (Li et al., 2015). Some viruses may show reduced fitness *in vitro* or *in vivo*, while others retain their fitness. Influenza A(H7N9) wild-type (WT) and mutant viruses were propagated, plaque-purified, and

characterized by the NAI assay and genomic sequencing. In mice, the R292K virus still has a similar $MLD_{50}$, whereas viruses with E119V, I222K and I222R all showed higher pathogenicity, with lower $MLD_{50}$s. In mice infected with 5 $MLD_{50}$ of WT A(H7N9), oseltamivir treatment reduced the lung virus titer. In contrast, oseltamivir treated mice infected with the R292K virus, showed no decrease in virus titer, and still had significant weight loss. Sixteen recombinant N9 NAs (recN9) were also generated using a transient baculovirus expression system. They were tested against all 4 available NAIs. As seen with the N2 subtype, R292K caused reduced inhibition (RI) or highly reduced inhibition (HRI) depending on the NAI tested; E119V conferred HRI by oseltamivir. Conversely, recN9 harboring H274Y displayed HRI by oseltamivir, setting it apart from N2 that exhibited normal inhibition.

In the ferret model (Marjuki et al., 2015), the R292K virus showed reduced replication (2 $\log_{10}$ $TCID_{50}$ reduction in viral titers), while E119V, I222R and I222K viruses replicated similarly to the WT. Treatment with oseltamivir (5 and 25 mg/kg/dose; BID) for 5 days, showed 0.7–1.5 $\log_{10}$ reduction in nasal wash viral titers, while no reduction was detected in those infected with either R292K or E119V viruses. A small reduction in viral titers was seen in I222K-infected animals. In ferrets infected with WT virus, and treated with oseltamivir, resistant viruses with the R292K change emerged in 4/6 virus-infected ferrets on day 5 of treatment. The laboratory has tested the BD iART assay, designed for detection of resistant viruses in clinical samples (Gubareva et al., 2017). Pure populations with > 50-fold resistance were readily detected. However, with mixed populations, as the R292K NA has lower activity, it needed > 80% of the R292K population to be detected. However, modifying the buffer to a lower pH, enabled detection of 20% mutant. One R292K virus also had an HA mutation, D19G in the HA2. This virus appeared to have reduced sensitivity *in vitro* and *in vivo* to the Genentech Human mAb, 81.39a (Marjuki et al., 2016). This highlights the further challenges for developing phenotypic assays for resistance to the new mAbs under development.

## 9. RVI therapeutics

### *9.1. Understanding antiviral targets (compounds identified by using a systems biology approach regulate the pathogenicity of influenza A virus)*

Yoshi Kawaoka, University of Wisconsin, Madison Wisconsin USA and University of Tokyo, Tokyo, Japan

A systems biology approach has been used to investigate critical host regulators as potential antiviral targets. Using microarray analysis of A(H1N1)pdm09 and A(H5N1) virus-infected cells, transcriptional data sets from Calu-3 cells were compared. 51 potentially critical genes were targeted using siRNA knockdown, looking for where knockdown results in an increase in virus titer. 19 of these led to a 5-fold change in virus titer. Using a PR8 luciferase reporter system, 287 compounds were screened from an in house library, of which 50 had an $IC_{50}$ less than 1000 nM and $CC_{50}$ greater than 2500 nM. 36 of these were further prioritized based on existing human approval, no toxicity in mice, and information available on the delivery route. A A(H1N1)pdm09 mouse challenge model has identified 3 compounds which protected mice and 3 which increased pathogenicity. The aim is now to identify how these drugs affect host response networks and identifying the mechanisms through which they affect A(H1N1)pdm09 pathogenicity.

### *9.2. Prospects for antivirals for RSV disease*

Peter Openshaw Imperial College, London, United Kingdom

RSV infects the ciliated cells of the respiratory mucosa, causing respiratory disease of variable severity. hRSV infects about two-thirds of children in the first year of life and virtually all children by the age of 3. It repeatedly reinfects humans throughout life, apparently by inducing immunological amnesia in the host. In winter one in six pediatric beds is occupied by bronchiolitis patients, 80% of which are caused by RSV. RSV is also associated with high mortality in stem cell transplants and up to 10% mortality in the frail elderly. RSV is also important in the exacerbations of COPD and asthma. The standard method for diagnosis of RSV uses nasopharyngeal aspiration (NPA) for obtaining samples; however, this is both unpleasant and may be inaccurate. Nasosorption (Thwaites et al., 2017) uses an absorptive matrix and has allowed measurement of RSV load and the mucosal inflammatory response, showing correlation with disease severity, virus load, and length of hospital stay which was not seen with NPA sampling.

No antiviral is yet approved, but palivizumab, a humanized monoclonal antibody, is used for long term prophylaxis in high risk infants. GS-5806 (Perron et al., 2015), a potent small molecule inhibitor, targets the RSV F protein inhibiting F protein-mediated cell-to-cell fusion. A double-blind placebo controlled trial in healthy adults challenged with RSV (DeVincenzo et al., 2014), tested various doses, with the primary endpoint being the AUC for viral load, and the secondary endpoint was mucus weight and symptom scores. Treatment reduced the viral load and severity of clinical disease. ALS-008176 is an orally bioavailable prodrug of ALS-008112, a cytidine nucleoside analogue (DeVincenzo et al., 2015). ALS-008112 is phosphorylated to form a nucleoside triphosphate analogue, which inhibits the RSV polymerase L, limiting virus replication. In an RSV challenge study with various doses, there was more rapid RSV clearance, a greater reduction in virus load and decreased mucus weight compared to the placebo group.

### *9.3. Novel RSV inhibitor AK0529: update on clinical development*

Stephen Toovey, Ark Biosciences, Shanghai, China

AK0529 is a small molecule RSV fusion protein inhibitor. Passaging in the presence of AK0529 led to the emergence of resistant viruses. It has a $CC_{50}$ greater than 100 μM in HEp-2 cells, and in an immunocompromised mouse model, 50 mg/kg or 12.5 mg/kg resulted in a 2 or 1 $\log_{10}$ decrease in virus titer respectively. It is non-toxic in juvenile and adult rats and monkeys. Oral AK0529 was well tolerated in healthy adult volunteers in single (SAD) and multiple ascending doses (MAD) (Reynolds, 2015). No serious adverse events were reported and no safety concerns or signals emerged with maximum dosing: 1200 mg SAD, 600 mg daily MAD. An initial open label Phase I study in infants hospitalized with natural RSV infection was converted to a double-blind placebo controlled Phase IIa study with ongoing PK, clinical and virological safety reviews. AK0529 has shown better bioavailability than anticipated, which means a lower dose may be feasible. The study is ongoing and still blinded, but many patients have shown improvement in symptoms.

### 9.4. Usefulness of probiotics for RVI mitigation

Liisa Lehtoranta DuPont Nutrition & Health, Kantvik, Finland

Probiotics are defined as live micro-organisms which, when administrated in adequate amounts, confer a health benefit for the host. Most commercial probiotics belong to the genera Lactobacillus or Bifidobacterium. Probiotics may have favorable effects against viral ARI. Meta-analyses of 12 trials has suggested that probiotics may reduce the incidence and duration of viral ARIs, and antibiotics use and cold-related school absence when taken prophylactically (Hao et al., 2015). However, the quality of the evidence was very low. A randomized, double blind placebo control trial (PCT) in 464 healthy active men and women in Australia showed some delay in respiratory virus infections in those taking probiotics compared to the placebo (West et al., 2014). Another randomized, PCT determined the effect of administration of *Bifidobacterium animalis* subspecies lactis Bl-04 on innate and adaptive host responses to experimental rhinovirus challenge (Turner et al., 2017). Supplement was taken from −28 to 5 days post challenge, and nasal wash samples were taken for virus load and chemokine ligand CXCL8 levels. Probiotics resulted in higher levels of nasal CXCL8 on D0 prior to challenge, but lower levels post-infection compared to placebo. Probiotic use was associated with a reduction in nasal lavage virus titer, but there was no difference in symptom scores or infection rate.

### 9.5. Which traditional Chinese medicines are most beneficial?

Bin Cao China - Japan Friendship Hospital, Beijing, China

Traditional Chinese medicines (TCM) have been used for thousands of years in China. They may be composed of extracts from a single or multiple plant species or other materials, in various formulations. They may have antiviral activity or inhibit virus replication by modulating the immune response (Ma et al., 2015). Some of the following are reported to have some antiviral effects in preclinical studies.

a) Paeonia delavayi root extracts significantly inhibited NA activity, with two of seven identified constituents significantly inhibiting the NA (Li et al., 2016b). No cell or animal testing has been done.
b) Lianhuaqingwen Capsule inhibited the proliferation of various strains of influenza viruses in plaque reduction assays, and impaired the nuclear export of the viral RNP (Ding et al., 2017). It also suppressed virus-induced NF-kB activation and reduced levels of inflammatory cytokines.
c) Laggera pterodonta Benth extract was fractionated, and one component acted on the early stage of influenza virus replication and prevented the increased expression of cytokines and chemokines (Wang et al., 2017).
d) Puerana lobata Ohwi P. lobata showed antiviral activity against HRSV-induced plaque formation in HEp-2 and A549 cells. It acts at an early stage of infection, inhibiting viral attachment and internalization (Lin et al., 2013).
d) Scutellaria baicalensis root extracts were fractionated, and baicalin was found to be responsible for antiviral activity against RSV in a plaque assay. In a mouse model baicalin treatment resulted in reduction of T lymphocyte infiltration and gene expression of proinflammatory factors, while the treatment moderately reduced RSV titers recovered from the lung tissues (Shi et al., 2016).
e) Andrographolide is an essential active ingredient extracted from the plant Andrographis paniculata. However, its poor water solubility limits its bioavailability. Oral administration of an Andrographolide derivative reduced the death rate, inhibited lung consolidation, and reduced viral titers in the lung in A(H1N1), A(H9N2), and A(H5N1) infected mice (Chen et al., 2009). Other derivatives are reported to inhibit replication of an A(H3N2) virus in cells (Yuan et al., 2016).

A trial has been designed to test Andrographolide in patients hospitalized with CAP. Primary outcome will be time to clinical stability; secondary outcomes include duration of fever, hospital stay, duration of antibiotics. Administration will be 500 mg/day by injection. Approximately 460 patients will be needed. However, there are many unknowns in designing the trial, including dosage, PK/PD data, AEs etc. Additionally, the main component of TCM injection is the andrographolide total ester rather than a single chemical compound. Hence, the extraction and purification of active ingredients from the raw herbs may affect its safety and effectiveness compared to the extract.

Clinically evaluated:

f) Maxingshigan-yinqiaosan. A prospective, nonblinded, randomized, controlled trial with 410 persons with laboratory-confirmed H1N1 influenza were treated with oseltamivir, 75 mg twice daily, maxingshigan-yinqiaosan decoction (composed of 12 Chinese herbal medicines), 200 mL 4 times daily; oseltamivir plus maxingshigan-yinqiaosan, or no intervention (Wang et al., 2011). Interventions and control were given for 5 days. Oseltamivir and maxingshigan-yinqiaosan, alone and in combination, reduced time to fever resolution in patients with H1N1 influenza virus infection, with a greater reduction in the combination therapy. However, there were no differences in symptom scores as compared to the control.
g) Xuebijing injection is a formula composed of five medicinal herbs. In a multi-center blinded RPCT it was evaluated in ~700 patients with severe pneumonia to measure its efficacy in reducing the pneumonia severity risk rating (Wang et al., 2016b). Unpublished results suggest there was some improvement in clinical symptoms at days 4 and 8, and some decrease in 28-day mortality.

## 10. Development of influenza antivirals

### 10.1. Building the pre-clinical pipeline for influenza therapeutics

Amy Krafft NIAID, Bethesda, Maryland, USA

NIAID's preparedness priorities are focused on the development of universal flu vaccines and new classes of antivirals to prevent or treat seasonal and pandemic flu aiming to strengthen the pipeline of antivirals through Phase II. Grants fund translational research, drug discovery, new drug target identification and validation, lead candidate optimization and preclinical development of potential new antivirals with novel mechanisms of action and immunotherapeutics. Contracts fund the advanced development of a promising new therapeutic through early clinical trials (Phase II).

Inhibitors can be viral or host directed, but they must be effective against all strains of influenza, including highly pathogenic strains, have a low likelihood of resistance, a novel mechanism, long treatment window, and improve patient outcomes including preventing life threatening complications in high risk patients. Candidate drugs are initially tested *in vitro*, followed by efficacy testing in mouse models of influenza infection. If further testing is warranted, gap-filling preclinical services are available to advance a product towards regulatory approval. Clinical testing units for

Phase I studies are offered for the most promising therapeutic candidates. Similar services are available for evaluating new compounds against a panel of other respiratory viruses.

Some of the array of compounds tested against influenza include M2 blockers against the S31N amantadine resistant mutants, FluBITE-TCN-032, a bispecific reagent targeting the M2e and a T cell epitope, trying to improve some of the NAIs for wider applications, anti HA stem antibodies, VIS410, MHAA4549A, or peptides – Flufurtide, FF-3, Arbidol a small molecule, PA endonuclease inhibitor, S-033188, PB1 polymerase inhibitor T705, PB2 cap snatching inhibitor Pimodivir, VX-787/JNJ63623872, human hyperimmune plasma, and host targeting compounds Danrixin a CXCR2 antagonist and Verdinexor an XPO1 nuclear export antagonist.

Information on NIAID's services are available at https://www.niaid.nih.gov/research/microbiology-and-infectious-diseases-resources

### *10.2. Pimodivir (JNJ-63623872): a brief overview*

Lorant Leopold, Janssen Titusville, New Jersey, USA

Pimodivir (JNJ-63623872, formerly VX-787) is a novel non-nucleotide PB2 subunit inhibitor of the influenza A viral polymerase. It occupies the 7-methyl GTP ($m^7$ GTP) cap-binding site of PB2, selectively inhibiting the production of mRNA (Byrn et al., 2015). In a lethal mouse model of A(H5N1) infection, treatment with 10 mg/kg twice daily protected 100% of mice even when treatment was delayed up to 4 days (Byrn et al., 2015). Some protection was still seen after delaying treatment until 5 days post-infection. In a human challenge model 46% of drug is orally available, with 90% of the available metabolite unchanged. Half life is around 24 hours, with more than 75% fecal elimination. It is metabolized by CYP3A4, but has no effect on cytochrome P450 levels. In trials evaluating safety and dosing efficacy, among 200 patients administered a single dose up to 3200 mg, and 446 patients given multiple doses of 600 mg twice daily for 10 days, the most common adverse event was defined as “loose stools”. In a Phase IIb trial patients were given 600 mg twice daily for 7 days, with or without 75 mg of oseltamivir. Preliminary results show there was a significant decrease in nasal viral load AUC by qRT-PCR in the pimodivir treated group. Pimodivir plus 75 mg of oseltamivir showed a greater decrease in AUC than 600 mg of pimodivir alone. Ten percent of samples had resistant viruses, previously shown to occur by mutations in the PB2 (Byrn et al., 2015). No drug-drug interactions with oseltamivir, Pitavastatin, or oral contraceptives were seen, nor any cardiac safety concerns. A Phase III trial is planned for winter 2017–2018.

### *10.3. In vitro susceptibility of influenza virus isolates to cap-dependent endonuclease inhibitor S-033188: results from a randomized, placebo-controlled phase II study*

Takeki Uehara Shionogi, Osaka, Japan

S-033188 is small molecule inhibitor of the cap-dependent endonuclease PA inhibitor of influenza A and B viruses. It is hydrolyzed to the active form, S-033447 (Uehara et al., 2016), inhibiting initiation of virus mRNA synthesis, preventing virus protein production. *In vitro,* S-033447 inhibited virus replication with an $EC_{90}$ of around 1 nM, up to 100-fold more potent compared to the NAIs. In mice, a single dose of 1.5 mg/kg reduced titers by 1 $\log_{10}$, while 15 mg/kg reduced lung virus titers by more than 3 $\log_{10}$. A single dose has a long half-life of 49–90 hours, and in a Phase I trial administration of a 6 mg dose of S-033188 exceeded the exposure level of the target potency.

In a Phase II trial, 400 influenza positive adult patients at $\leq$48 hours after symptom onset were randomized to receive a single dose of 10, 20 or 40 mg or placebo. Nasal/throat swabs were taken for virus titers and $EC_{50}$ determination in a plaque assay, and blood samples were taken at 24 hours post-treatment for plasma concentrations. There was a rapid decrease in virus load of 3–4 $\log_{10}$ by 24 hours, and all doses showed up to a 16 hour earlier resolution of fever and 28 hour earlier alleviation of all symptoms compared to the placebo group (mean ~50 vs s 77 hours). Adverse events were similar to placebo, and adverse drug interactions were higher in the placebo group. Levels of drug in plasma at 24 hours were mostly proportional to the dose. The median $EC_{50}$s of isolates at baseline were 40 nmol/L for A(H1N1)pdm09, 1.05 nmol/L for A(H3NX) and 6.90 nmol/L for type B viruses, comparable to earlier studies. There was a significant negative correlation between drug dose and a change in virus titer at days 2 and 3. Resistance studies were reported in poster P50 (Shishido et al.). Isolates from 4/112 patients infected with A(H1N1)pdm09 virus, and 1/56 infected with B virus had substitutions in the PA gene. Only the PA I38T/F in the A(H1N1)pdm09 PA reduced susceptiblity in a plaque reduction assay.

### *10.4. MHAA4549A monoclonal anti-HA*

Man Wah Tan Genentech, South San Francisco, California, USA

MHAA4549A is a human immunoglobulin G1 (IgG1) monoclonal antibody that binds to a highly conserved epitope on the stalk of influenza A HA. It blocks the HA-mediated membrane fusion in the endosome, promoting ADCC of the infected cell, and also leads to direct virus binding, inhibiting the release of the virus genome. It remains bound even at pH 5.0 preventing unfolding of the fusion peptide. It neutralizes all known human influenza A strains (Gupta et al., 2016). In a lethal mouse model there is dose dependent protection up to 72 hours post-infection, with 300 μg providing > 90% protection. It is being developed to treat patients hospitalized with influenza. In Phase I trials it demonstrated linear serum pharmacokinetics consistent with those of a human IgG1 antibody (Lim et al., 2016). In a Phase IIa study 60 subjects in three single-dose groups (400, 1200, or 3600 mg) received MHAA4549A intravenously 24–36 hours after inoculation with influenza A virus (Deng et al., 2017; McBride et al., 2017). All subjects started a 5-day course of oseltamivir (twice daily) from day 7. MHAA4549A exhibited dose-proportional serum PKs, but not dose-proportional nasal PKs. No PK drug–drug interaction between MHAA4549A and oseltamivir was observed (Deng et al., 2017). Only the 3600 treated group showed significantly reduced virus burden by RT-PCR and $TCID_{50}$ assay (McBride et al., 2017). Subjects with a maximum nasal concentration ($C_{max}$) greater than the median nasal $C_{max}$ value had shorter times to resolution of viral shedding compared with volunteers in the placebo group (median 75.8 vs 113.7 hours). Further trials are planned in hospitalized patients, comparing a high and low dose of MHAA4549A plus oseltamivir, compared to oseltamivir alone NCT02293863. However, the challenges remain with no clinical or virological endpoints yet clearly defined.

### *10.5. VIS410, a broadly neutralizing antibody in development for treatment of hospitalized patients with influenza A, demonstrates in vitro activity against the newly emergent 2016/2017 strains of A(H7N9)*

José Trevejo, Visterra Cambridge, Massachusetts, USA

VIS410 is a broadly neutralizing human IgG1 anti-HA antibody, which reacts with a region of the HA stalk conserved in Group 1 and 2 influenza viruses (Baranovich et al., 2016; Tharakaraman et al., 2015). It prevents maturation of the HA to the fusogenic form, thus preventing virus-endosome fusion. In a lethal BALB/c mouse model of A(H7N9) virus infection, VIS410 was administered via intraperitoneal injection of either a single 50 mg/kg dose 12 hours before or treated with 2, 10, or 50 mg/kg dose 24 hours after infection (Baranovich et al., 2016; McKimm-Breschkin and Fry, 2016). Prophylactic administration of the antibody resulted in 100% protection of the mice; a single administration of 50 mg/kg 24 hours after infection also provided 100% survival, whereas the 10 mg/kg dose protected 70% of the A/Anhui/1/2013 virus-infected (oseltamivir sensitive) and 90% of the A/Shanghai/1/2013 virus-infected mice (oseltamivir resistant). The A(H7N9) has undergone recent changes in pathogenicity (Su et al., 2017), with mutations in the HA, including an E57A substitution in the mAb binding region. However, this mutation did not lead to loss of VIS410 binding.

In a Phase I trial volunteers were administered a single intravenous dose of 2, 5, 15, 30, or 50 mg/kg. VIS410 exposure was approximately dose-proportional with a mean half-life of 12.9 days (Wollacott et al., 2016) suggesting a single dose is sufficient. No drug related serious AEs were recorded, and although some had mild diarrhea this was prevented by pre-treatment with 50 mg of Benadryl and 400 mg ibuprofen. In a Phase II A(H1N1)pdm09 challenge study (NCT02468115) there was a 91% decrease in the virus AUC by qRT-PCR, with a 2.2 $\log_{10}$ decrease in the median peak virus load. The median viral load was undetectable sooner, and the median time to resolution of symptoms was shorter by around 2 days.

### *10.6. Experience with favipiravir in phase III and implications for future studies*

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Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) a small molecule nucleoside analogue, selectively inhibits the RNA-dependent RNA polymerases of influenza and many other RNA viruses (Furuta et al., 2013). To date no resistance *in vitro* or from patient samples has been detected. It was reported at the previous isirv-AVG conference that in two Phase II dose finding trials there was no significant benefit in the first, and in the second there was a statistically significant decrease in the time to resolution of 6 symptoms, but not for fever (McKimm-Breschkin and Fry, 2016). There was also a more rapid decrease in virus titers compared to the placebo group. In two Phase III trials, for healthy adults with uncomplicated influenza, (NCT02026349, NCT02008344) patients were treated with two loading doses of 1800 mg on day 1, and 800 mg BID days 2–5. The adverse effect profile appears to be similar to untreated patients, with the exception of asymptomatic elevations of uric acid. Both trials showed a significant antiviral effect with time to undetectable virus by culture shortened by one day. In two of the Phase II and III studies there was an approximately 17% decrease in time to resolution of symptoms. In one trial, there was a much shorter time to resolution of symptoms in the placebo group, thus making it harder to show a full 24 hours decrease in the treated group. There was a 15 hours difference in one trial, and only a 6 hours difference in the other. However, there were different strains of virus predominating in the two trials. Analysis of the neutrophil-lymphocyte ratio (NLR) as a measure of systemic inflammation showed the difference in time to resolution of symptoms between placebo and favipiravir-treated subjects increased with higher NLRs. They are currently analyzing retained PK samples for cytokine levels, to further explore the concept of increasing benefit in patients with more inflammation.

### *10.7. Nitazoxanide: a first-in-class indirect-acting antiviral for treatment of respiratory virus infections*

Jean-Francois Rossignol Romark, Morristown, New Jersey, USA

Nitazoxanide, (NTZ) a small MW inhibitor used extensively for treatment of *Giardia* and *Cryptosporidium* infections is being repurposed for the treatment of influenza and other viral diseases, including PIV and RSV (Rossignol, 2014; Shakya et al., 2017). It blocks maturation of the influenza HA at a post-translational level (Rossignol, 2014). For paramyxoviruses it targets the F protein folding, by inhibiting, ERp57 a member of the protein disulfide isomerase family located in the endoplasmic reticulum, (Piacentini et al., 2016). Silencing of the ERp57 in cell culture decreased virus replication. NTZ also appears to decrease pro-inflammatory cytokines in PBMC. In an earlier Phase IIb/III trial in recipients with acute uncomplicated influenza 600 mg, but not 300 mg twice daily for 5 days was associated with a reduction in the duration of symptoms of up to 36 hours, and a 1 $\log_{10}$ decrease in virus titer (Haffizulla et al., 2014). In total, Phase II/III clinical trials in 2889 patients with uncomplicated influenza A and B, showed that the drug reduced the duration of the influenza illness when compared to placebo ($P < 0.05$) (Stachulski et al., 2017). In two recently completed RDBPCT Phase III trials (NCT01610245), NTZ given orally 600 mg twice daily as monotherapy for 5 days was compared with NTZ in combination with oseltamivir, and with oseltamivir alone. Overall, there was no difference in the time to resolution of symptoms between the NTZ, oseltamivir, and NTZ and oseltamivir combination vs the placebo groups. In one subset of these studies, among 324 patients aged 12–65, the mean time to resolution of symptoms was 32 hours compared to placebo, which was significant. Daily monitoring of these patients was thought to lead to the better outcome.

### *10.8. IV zanamivir for treatment of severe influenza: a program update and phenotypic/genotypic virologic analyses from phase II and phase III studies*

Sumita Roy-Ghanta GSK, Philadelphia, Pennsylvania, USA

Phase II and III clinical trials to evaluate the safety and efficacy of intravenous zanamivir for the treatment of patients hospitalized with severe influenza have been conducted between 2011–2015. Despite more than 3000 patients being supplied with intravenous zanamivir (IVZ) for compassionate use globally, it was a challenge to meet the 200 target for the Phase II study and the 600 for the Phase III study. For the Phase II study, subjects with confirmed influenza received 600 mg IVZ BID (adults) or an age-adjusted, weight-based dose BID (pediatrics) for 5–10 days. Safety, clinical, PK and virology were endpoints (Marty et al., 2014). The Phase III studies were carried out at 160 sites in 26 countries. Patients were enrolled within 6 days of symptom onset, and received 300 IVZ or 600 IVZ BID or oseltamivir (OS) 75 mg BID for 5–10 days (Marty et al., 2017). Time to clinical response, a composite of vital sign resolution and hospital discharge was the primary endpoint. Secondary endpoints included clinical, virology, PK and safety. Virological assessments included nasopharyngeal swabs, throat swabs and endotracheal samples, where available. There was an approximate 0.5 day improvement in time to clinical response in the 600 mg IVZ group compared to oseltamivir (not signficant or superior), but there was no difference in the median change in viral load between the three groups.

Drug sensitivity was evaluated by phenotypic analysis in the MUNANA based enzyme assay, after culture from upper and lower respiratory tract samples. In the Phase II study, of 130 adult and 71 pediatric subjects enrolled, 80% of adults and 70% of pediatrics had prior OS. The mean $IC_{50}$ values were within the expected range (Yates et al., 2016). Sequence analysis revealed three adult and 2 pediatric subjects had viruses with baseline resistance substitutions (Y155H, D199G, S247N, V149A, H275Y) and two subjects had viruses with treatment emergent NA resistance substitutions,

(E119D and E119G). However, these latter were not able to be cultured, demonstrating their lack of fitness.

In the Phase III study, of 626 subjects enrolled, 78% had confirmed influenza, 49% had prior OS. 514 samples from 265 subjects were culture positive. Mean OS and zanamivir $IC_{50}$s were in the expected range. 12 viruses from 10 subjects showed shifts in susceptibility to NAIs. 797 NA sequences from 379 Phase III subjects identified 21 resistance substitutions in 50 subjects (11 in H1N1pdm09; 36 in H3N2; 3 in B viruses), most were present at day 1, thus were not selected during treatment. Five had H275Y NA substitution, four of whom were in the oseltamivir arm, and the fifth although in the IVZ arm, had prior OS treatment (Marty et al., 2017). Four treatment-associated NAI-resistant substitutions were identified in viruses that could not be cultured: two in OS, R292R/K (day 7) and D198D/G (day 3) and two in 300IVZ, N294S/N (day 2) and T325I (day 2) (Marty et al., 2017). No novel zanamivir resistance substitutions were detected from the clinical studies evaluating IV zanamivir in severe influenza.

### *10.9. Umifenovir (Arbidol) – antiviral drug against influenza viruses*

Irina Leneva Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia

The HA targeted fusion inhibitor umifenovir (Arbidol) is licenced in Russia for treatment and prophylaxis of influenza A and B infection. Umifenovir binds in a hydrophobic cavity in the HA trimer stem at the interface between two protomers. By functioning as molecular glue, umifenovir stabilizes the prefusion conformation of HA that inhibits the large conformational rearrangements associated with membrane fusion in the low pH of the endosome (Kadam and Wilson, 2017). In cell culture, umifenovir inhibits replication of both influenza A and B viruses, including A(H1N1)pdm09, A(H3N2), A(H5N1), A(H7N9) and NAI-resistant viruses. Resistance arose after serial passaging of an A/Chicken/Germany/27 (H7N7) virus in culture, with amino acid substitutions in the HA2 subunit (Leneva et al., 2009). Mice challenged with A/California/04/2009 (H1N1) pdm09 virus were treated orally with 20, 30 or 60 mg/kg/day of umifenovir. Only 60 mg/kg enhanced survival, 50% compared to 0% in the placebo group (Leneva et al., 2016). Lung virus titers were decreased by $\geq$ 2 logs in the umifenovir-treated mice. Retrospective observational studies have been carried out on the 2010–2011 (Leneva et al., 2016) and 2014–2015 seasons with 5287 patients hospitalized with ILI who were treated with 4 × 200 mg umifenovir or 2 × 75 mg oseltamivir within 48 hours of symptom onset. Duration of fever in influenza-infected patients treated with umifenovir (< 2 years old) was 4.13 days and 2.45 days (> 65 years old), respectively as compared to 3.67 and 4.27 for age matched untreated groups. This characteristic was 3.55 in umifenovir-treated high risk patients compared to 4.55 for untreated high risk patients. In the 2010-11 studies, no significant differences were found in the duration of illness and main symptoms of influenza between the umifenovir and oseltamivir treated groups. Pneumonia as a complication of influenza was observed in 0.3% of the patients treated with umifenovir, in 23.7% of the patients who did not receive antiviral therapy ($P < 0.001$), and in none of the patients treated with oseltamivir. However, it was noted there was poor compliance with the four times daily dosing, and a long acting formulation is needed, as well as PK and PD studies.

### *10.10. A randomized, double-blind, placebo controlled study to evaluate the safety, tolerability and clinical effect of oral Danirixin (GSK1325756) in the treatment of adults with acute, uncomplicated influenza (201682)*

Sumita Roy-Ghanta GSK, Philadelphia, Pennsylvania, USA

Oral Danirixin (DNX) is a selective, competitive reversible inhibitor of CXC chemokine receptor 2 (CXCR2) (Busch-Petersen et al., 2017). CXCR2 is a key receptor in the chemotaxis of neutrophils to sites of inflammation. Oral DNX is currently in clinical development for uncomplicated influenza and for intravenous therapy for patients hospitalized with influenza. A RDB PCT in 45 outpatients with uncomplicated influenza evaluated the safety and tolerability of 75 mg DNX with and without 75 mg of oseltamivir (NCT02469298). Patients were treated twice daily for five days. 35 patients were influenza positive by qPCR. The highest incidence of AEs was in the placebo group (57%), followed by DNX + OS (44%), DNX (20%) and OS (0%) groups. In all treatment groups, mean peripheral neutrophils decreased from baseline to day 3, increased on day 5 while on therapy, and resolved by day 8. There were no confirmed bacterial AEs in patients with neutropenia (n = 7), all of whom were influenza positive. This agrees with COPD studies in which no bacterial AEs were observed in patients treated with 75 mg daily for 52 weeks. There was a trend toward shorter median times to resolution of all 10 influenza symptoms in the DNX + OS group (112 hours) than in the OS group (267 hours), although the latter was unexpectedly longer than the placebo. Decreases in PCR viral load were similar for all treatment groups including placebo. As there were no safety concerns they are now recruiting for the hospitalized study (NCT02927431).

### *10.11. Developing therapeutic human antibodies from transchromosomal (Tc) bovine for treating influenza infection*

Hua Wu SAb Biotherapeutics, Sioux Falls, SD, USA

The Transchromosomic (Tc) bovine platform uses a triple knockout of bovine immunoglobulin (Ig) genes including the Ig H chains and λ chains, and replacement with the full repertoire of human Ig genes. Tc-bovines produce fully-human immunoglobulin (hIgG) (Dye et al., 2016). Upon hyperimmunization with an antigen of choice, Tc-bovines can rapidly produce up to 600 grams of specific polyclonal hIgG per month per animal. Anti MERS hIgG in a Phase I trial has shown no serious AEs, normal PK for an hIgG, and no immunogenicity. Another trial for an hIgG against *Mycoplasma hominis* (SAB 136) has shown initial signs of efficacy without AEs. Tc-bovines hyperimmunized with a tri-valent seasonal influenza spilt virion have been used to produce SAB-100.

In a mouse model of lethal influenza A(H1N1)pdm09 virus infection the dosage of SAB-100 was evaluated prophylactically (−24 hours before infection) or therapeutically (+12 h post-infection (pi). A single prophylactic dose of SAB-100 at 1.33, 4, or 12 mg/kg provided 100% protection. A single treatment with 12, 24, or 48 mg/kg dose of SAB-100 provided 100% protection. In a second study, the therapeutic efficacy of SAB-100 was compared with human derived hyperimmune anti-influenza IVIG by treating infected mice with a single dose of 6, 12, 24, or 48 mg/kg SAB-100 or anti-influenza human IVIG at +12 h pi. While 100% protection was provided with all SAB-100 doses, only the 48 mg/kg dose of human anti-influenza IVIG provided significant protection (90%).

In a third mouse study, synergy between sub-therapeutic doses of SAB-100 (1.5 mg/kg) and oseltamivir (0.3 mg/kg) was evaluated compared to either agent alone when administered at 12 h pi. Mice that received the combination therapy had 90% survival, whereas only 60% survival was observed in mice that received 1.5 mg/kg of SAB-100 alone, and 40% survival in mice that received 0.3 mg/kg of oseltamivir alone. Phase I human trials are planned for 2018.

## 11. Keynote: advances in vaccines for respiratory virus infections

Barney S. Graham, Vaccine Research Center, NIAID, Bethesda, Maryland, USA

RSV is a pneumovirus in the family Paramyxoviridae and is the leading cause of severe respiratory disease and hospitalization in young children. To date, an RSV vaccine is unavailable and there are a number of challenges impeding its development: 1) young age status of patients with serious disease, 2) multiple viral mechanisms to interfere with induction and effector function of Type I interferon, 3) failure of natural immunity to protect against reinfections, 4) traditionally difficult to boost responses in adults, 5) legacy of vaccine-enhanced disease (Graham, 2017). RSV fusion (F) glycoprotein mediates viral entry into the cells and is a primary antigenic target for vaccine development. Recent advances towards defining RSV (F)-specific neutralization-sensitive epitopes and solving the structure of the prefusion (pre-F) and postfusion (post-F) conformations of the trimer of F glycoprotein (McLellan et al., 2013) have led to a better understanding of the mechanisms of neutralization, serological responses to natural RSV infection and vaccination, pathogenesis of disease, and mechanisms of viral inactivation (Liang et al., 2015). Elucidating the structure and function of F glycoprotein has led to the design of a vaccine antigen that recapitulates the surface of the functional pre-F molecule. Stabilizing the pre-F through mutagenesis has further improved its immunogenicity. In a primary infection in infants, there is more antibody against the post-F, which have poor neutralizing ability. In contrast, most antibodies in adults recognize the pre-F form. Clinical trials of a pre-F RSV vaccine started in February 2017.

Structure-guided vaccine antigen design has now been applied to beta-coronaviruses (beta-CoV), influenza and other paramyxoviruses that utilize class I fusion proteins for entry. The coronavirus spike (S) protein binds cellular receptors and mediates membrane fusion, and thus determines in part the cell tropism and host range of beta-CoV. The prefusion trimer structures for multiple S glycoproteins of beta-CoV have been solved alone and in complex with neutralizing monoclonal antibodies (Kirchdoerfer et al., 2016), suggesting that it may be possible to define a universal solution for stabilizing CoV prefusion S trimers by mutagenesis to produce highly stable and immunogenic CoV vaccine antigens. Immunogens based on full-length S plasmid DNA and S1 subunit protein elicit robust serum-neutralizing activity against several MERS-CoV strains in mice and non-human primates (Wang et al., 2015). Immunization of rhesus macaques confers protection against MERS-CoV-induced radiographic pneumonia, as assessed using computerized tomography, supporting this strategy as a promising approach for MERS-CoV vaccine development.

Major biological challenges for universal influenza vaccines include: 1) rapid antigenic and genetic variations, particularly within zoonotic reservoirs, 2) potential for increased fitness among reassortment and adaptive mutations, 3) pre-existing immunity that includes immunodominance of serotype-specific epitopes and antibody lineages with limited breadth, 4) influenza on B cell phenotypes, 5) most severe disease occurs at extremes of age and in vulnerable populations with compromised immunity. New technologies (particularly structural biology, rapid isolation of human monoclonal antibodies, high-through sequencing, protein engineering, single-cell analysis, and their derivatives) have provided new strategies for influenza vaccines. Influenza HA has discreet structurally-defined sites of vulnerability, and specific antibody lineages have been defined that can provide broad immunity. Antigen designs for influenza HA based on full-length HA, HA stem, or HA receptor-binding site have been produced to pursue distinct strategies for eliciting cross-protective antibody response (Yassine et al., 2015). Vaccine antigens displayed on self-assembling nanoparticles elicit high magnitude antibody responses (Kanekiyo et al., 2013).

The future of viral vaccine design will be characterized by atomic-level protein engineering to preserve critical structures on trimeric fusion machines targeted by potent, broadly cross-reactive neutralizing antibodies. The technologies of structural biology, rapid isolation of human monoclonal antibodies, high throughput sequencing, analysis of single sorted B-cells and T-cells, and new antigen display and delivery options have dramatically changed the process of vaccine development and have created new opportunities to prevent respiratory viral infections.

## 12. Vaccines and preventive strategies

### *12.1. Natural A(H7N9) infection in humans boosts cross-group stalk-specific antibody responses*

Lu Liu, Shanghai Public Health Clinical Center, Shanghai, China

After infection with influenza A(H7N9) virus, patients develop a relatively weak protective antibody response as measured by HAI and neutralizing antibody (nAb) titers (Guo et al., 2014). It is not known, however, if these antibodies protect against other A(H7N9) viruses or other influenza strains. The presence of cross-group immune responses were evaluated in a cohort of patients hospitalized in Shanghai with influenza A(H7N9) virus infections. Sera from 18 adult patients (age 47–88) hospitalized with influenza A(H7N9) were obtained early in the clinical course (up to 16 days after onset of symptoms) and late (13–36 days), and compared to 11 age matched asymptomatic non-infected controls (Liu et al., 2017). As expected, those infected with influenza A(H7N9) had higher HAI, nAb and binding antibodies (bAb) to the homologous influenza A/Shanghai/1/2013 A(H7N9) compared to controls. Those infected with influenza A(H7N9) also had higher HAI, nAb, and bAb to heterologous H7N7 and H7N2 viruses.

Those infected with influenza A(H7N9) virus also had higher antibody titers to other HAs using representative viruses from H1 - H15 subtypes as measured by ELISA. Using recombinant HA and NA proteins as substrates, antibody levels against the HA were 3- to 10-fold higher compared to controls against group 1 viruses, and 18- to 40-fold higher to group 2 viruses. These cross-reactive antibodies were shown to be neutralizing (2- to 11-fold higher in group 1 viruses and 4- to 43-fold higher in group 2 viruses) using pseudotyped viruses. Increases in NA antibody titers were 18-fold against the N9, and 2- to 4-fold against other group 2 viruses, N2, N3, N7 and only up to 3.6-fold against group 1 NAs. The antibody response to the head and stalk were evaluated separately by using chimeric HAs and HA head-only constructs in an ELISA. Cross-group responses were largely mediated by stalk-specific antibodies. These anti-stalk antibodies were induced early after infection, whereas antibodies against the head were induced later.

### *12.2. A single IM dose of a plant-made VLP vaccine bearing the H1 hemagglutinin elicits a balanced humoral and cellular response and protects young and aged mice from influenza H1N1 challenge*

Breanna Hodgins, McGill University, Montreal, Quebec, Canada

Seasonal influenza vaccine effectiveness may be lower in adults over the age of 65 years than younger adults (Beyer et al., 2011). This reduction in vaccine effectiveness may be partially explained by lower immune responses after vaccination in this population (Goodwin et al., 2006). Plant-made virus-like particles (VLPs) resembling influenza may be more immunogenic than split-virus formulations. VLPs lack genetic material, but

mimic viral structure displaying the targeted protein of interest. To develop the VLP for influenza, recombinant monomeric HA protein from influenza A/California/04/2009 (H1N1)pdm09 virus was expressed in Nicotiana benthamiana (a close relative of the tobacco plant) using transient expression vectors. The plants are then harvested, and plant-produced VLPs are recovered by fractionation. To evaluate immunogenicity, young (6–8 weeks) and aged (16–20 months) female BALB/c mice were given a single dose of HA (3 μg) by either the intranasal (IN) or intramuscular (IM) route. There were two controls: first were mice given influenza A/California/07/2009 (H1N1)pdm09 split vaccine by IM injection, and second were unvaccinated mice. At three weeks post-vaccination, HAI antibodies were only detected in the young H1-VLP IM group (geometric mean titer, 14.5). Mice were challenged IN with a sublethal dose or lethal dose of influenza A/California/07/2009 (H1N1)pdm09 virus. After viral challenge, the young H1-VLP IM group lost the least amount of weight and had 100% survival, as did the young H1N1 split IM group. Aged mice vaccinated by IM administration with the VLP also had minimal weight loss and had 80% survival compared to the split vaccine that had 60% survival. When standardized to the young naïve mice, the young VLP IM vaccine group had the greatest fold decrease in viral load (0.852) compared to other vaccine groups. Among the aged animals, the groups that received VLP by IM or IN routes had the greatest fold decrease (0.928 and 0.925, respectively) when compared to the naïve and split vaccine groups. Although IN administration of the VLP vaccine elicited no detectable systemic response, 75% of the young and 50% of the aged animals were protected from challenge.

### *12.3. An mRNA-based technology for the next generation of prophylactic influenza vaccines*

Susanne Rauch, CureVac AG, Tuebingen, Germany

mRNA-based vaccines allow rapid generation of sequence specific, clinical-grade material in a scalable cost-effective process. mRNA also has a higher safety profile as it doesn't cross the nuclear barrier. The encoded antigens can be rapidly changed, matching evolving viral strains, since each mRNA is produced from the same basic material in the same production site. RNActive® is one such mRNA based vaccine platform. Initial studies with an intradermal influenza RNActive® vaccine gave similar HAI titers in mice (1:320) when compared to quadrivalent split virus vaccine (1:160). A newer formulation of the RNActive® vaccine with lipid encapsulation enables IM delivery. RNActive® was able to induce potent immune responses when applied IM using low doses (μg) of mRNA, giving 64-fold higher HAI titers as compared to quadrivalent non-encapsulated RNActive® and quadrivalent split virus vaccine. Vaccination of mice with this RNActive® formulation encoding for influenza HA revealed an increase of both humoral and cellular immune responses, analyzed via functional antibody levels and ICS (intracellular cytokine staining) of T cells, respectively, compared to previous RNActive® formulations. In non-human primate studies, 10 μg of the lipid encapsulation formulation for the quadrivalent RNActive® vaccine gave good antibody titers that did not decline over 1 year, and better cellular immune responses as measured by the intracellular cytokine staining of T cells.

### *12.4. Development and validation of a non-egg-adapted wild-type influenza A H3N2 strain (A/Belgium/4217/2015 (H3N2)) as a challenge agent for human volunteer challenge studies*

Martin Schutten, Clinical Virology and Diagnostics, Noord-Holland, Netherlands

Current human challenge models for influenza use egg-grown viruses and have low attack rates (< 80%) (Memoli et al., 2015). Egg adaptation of human viruses increase their affinity for Sia (α2–3) Gal-containing receptors, and thus improve replication in eggs, but impair their ability to bind to Sia (α2–6) Gal-terminated receptors and likely decrease the fitness for replication in humans (Gambaryan et al., 1999). A wild-type non-egg-adapted virus may be better for a challenge model. An influenza A/Belgium/4217/2015(H3N2) virus was propagated 2 times in MDCK cells and a single passage in egg. Three cohorts of 12 healthy volunteers each were intranasally inoculated with increasing doses of influenza A/Belgium/4217/2015 A(H3N2) (cohort 1 = $10^5$; cohort 2 = $10^6$; cohort 3 = $6.76 \times 10^6$ $TCID_{50}$/mL). Inclusion criteria included a MN titer of less than or equal to 1:20 against the challenge strain. Overall attack rates (defined by at least two consecutive positive viral load by qRT-PCR) were 75% for cohort 1 and 2, and 100% for cohort 3. Viral shedding, as measured by AUC also increased with increased dose (cohort 1–495 and 154; cohort 2–774 and 227; cohort 3–888 and 250 by qRT-PCR ($log_{10}$ copies day/ml) and quantitative culture ($log_{10}$ $TCID_{50}$s day/ml) respectively. Participants with a baseline MN titer above or equal to 1:10 had an attack rate of 70% compared to 88% if the MN was less than 1:10. Viral AUC by qRT-PCR was 511 $log_{10}$ copies day/ml for those with a MN above or equal to 1:10 compared to 799 if the MN was less than 1:10. Similar results were found for viral AUC by quantitative culture (141 $log_{10}$ $TCID_{50}$s day/ml compared to 238). The mean composite influenza symptom score increased with the higher inoculum (cohort 1 = 8.9; cohort 2 = 14.8; cohort 3 = 21.8).

### *12.5. A Treg cell based novel RSV vaccine*

Bin Wang, Key Laboratory of Medical Molecular Virology of MOH and MOE, Fudan University, Shanghai, China

RSV infection is a major cause of respiratory tract disease in children under 5 years old. Prior RSV vaccine efforts, using formalin-inactivated RSV vaccine (FI-RSV), caused several cases of vaccine-enhanced disease (VED). VED may be due to lack of regulatory T cells (Treg), as these cells are important immunoregulatory cells to control inflammation and minimize tissue damage (Acosta et al., 2015). This can be demonstrated in FI-RSV vaccinated mouse and models (Cannon et al., 1988). The inflammation was due to the induction of a Th2-type response in lungs and overproduction of Th2 cytokines, that led to neutrophil infiltration, peribronchiolitis, and alveolitis (Castilow et al., 2007). Low dose cyclosporin A (CSA) has been shown to induce a Treg response (Brandt et al., 2009).

This study evaluated a strategy of immunizing animals with a recombinant RSV G protein together with low dose CSA (Li et al., 2016a). To evaluate the immunogenicity of recombinant G protein with CSA, female 6- to 10-wk-old BALB/c mice were immunized IM or SC on day 0, and on day 14 with 10 μg recombinant G protein, CSA alone, or G protein + CSA, or FI-RSV. The levels of anti-RSV antibodies and neutralizing antibodies were higher in the group immunized with G protein + CSA compared with the G protein alone or FI-RSV groups.

To evaluate protective efficacy of the recombinant G protein + CSA, animals were challenged IN with $5 \times 10^7$ PFU of RSV (A2 strain) on day 28. The viral loads in the lung were analyzed 4 days after the challenge. The recombinant G protein + CSA strategy reduced the viral load in lung significantly compared to G protein alone ($P = 0.0053$) or FI-RSV ($P = 0.0013$). When lung pathology was reviewed, the groups vaccinated with G protein or FI-RSV alone demonstrated significant perivasculitis, peribronchiolitis, and alveolitis, whereas the recombinant G protein + CSA group had only minor peribronchiolitis and nearly normal alveolar morphology. Animals that had received the recombinant G protein + CSA had higher levels of Treg cells in the spleen and lymph nodes compared to the other vaccination strategies. Stimulating the Treg response by adding CSA to the G

protein vaccine may potentiate the immune response while minimizing vaccine-enhanced disease.

### 12.6. Evaluation of the effectiveness of oseltamivir against influenza variants with the H275Y neuraminidase mutation in ferrets

Ding Oh, WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia

In influenza viruses, amino acid substitutions in the NA glycoprotein can alter drug binding and cause reduced sensitivity of viruses to NAIs. The H275Y NA amino acid substitution has been seen in A(H1N1)pdm09 viruses in patients undergoing oseltamivir treatment. Based on *in vitro* NAI susceptibility assays, the H275Y variant viruses have a 600- to 1500-fold reduction in sensitivity to oseltamivir. Epidemiological data suggest the fitness and transmission potential of resistant viruses may vary between strains (Wong et al., 2012).

To evaluate the fitness and transmission potential of resistant viruses, ferrets were infected with wild-type (WT) or H275Y variants of seasonal influenza A(H1N1) and A(H1N1)pdm09 at $1 \times 10^6$ $TCID_{50}$. In a prophylaxis model, oseltamivir 10 mg/kg/day or placebo was administered to an uninfected ferret 2 hours prior to co-housing with an influenza virus-infected ferret, followed by twice daily dosing for 10 days. For seasonal influenza A(H1N1)-WT virus, oseltamivir fully prevented infection, with no detectable virus shedding or clinical signs. In animals infected with the H275Y virus, oseltamivir had no effect on viral shedding, activity, fever or body weight compared to controls. However, oseltamivir-treated ferrets did have decreased nasal inflammation compared to controls. For A(H1N1)pdm09 virus, oseltamivir prevented infection in 50% (2/4) of ferrets exposed to the WT viruses, but had no effect on infection and clinical symptoms with the H275Y variant.

To investigate the effectiveness of oseltamivir treatment, 10 mg/kg/day oseltamivir or placebo was given orally to ferrets 48 hours after co-housing with an influenza virus-infected ferret, followed by twice daily dosing for 5 days. Oseltamivir had no effect on viral shedding, activity, fever, body weight, or nasal inflammation in either WT or H275Y infected ferrets, thus making it an unsuitable model for evaluating drug susceptiblity.

## 13. Clinical research issues

### 13.1. Updating the WHO influenza research agenda

Jacky Chan, WHO, Geneva, Switzerland

In late 2009, after the first influenza pandemic of the century, the WHO Global Influenza Program developed a Research Agenda for Influenza together with global partners. The goal of the WHO Public Health Research Agenda for Influenza is to identify the evidence needed to better understand the disease impact, strengthen public health guidance and actions essential for limiting the impact of pandemic, zoonotic and seasonal influenza on individuals and populations. The agenda is a broad-based public health research strategy for influenza and is organized around a framework of five key research areas or 'streams' (WHO, 2017).

By 2012 there were more than 4000 peer-reviewed journal publications addressing the priority research questions of the agenda (WHO, 2013). An update in 2016–2017 retains three major substreams from the 2009 agenda. This includes factors associated with pathogenesis and clinical severity, improved clinical management of patients and health care capacity and response. Updates includes integration, revisions and a new recommendation. Specific research initiatives or projects have been identified that could contribute key evidence to improve patient management. One new recommendation is to optimize use of current antiviral treatments, including understanding barriers to availability and increased utilization for treatment of influenza. A higher research priority is recommended for optimizing the effectiveness of current and novel antiviral treatments. This comprises development of new formulations, delivery routes or systems, antiviral drug combinations, and strategies to address emergence and treatment of antiviral resistance.

### 13.2. What endpoints make sense in clinical trials of severe influenza and related illnesses?

Menno de Jong, University of Amsterdam, Amsterdam, Holland

Registration trials for antiviral agents against influenza historically targeted previously healthy patients with uncomplicated influenza in an outpatient setting. The focus of the current therapies under development is however on patients with underlying illnesses or those hospitalized with severe disease (Treanor et al., 2000) in whom there is a lack of formal evidence of efficacy of current licensed agents. This is further complicated by the lack of validated clinical endpoints for efficacy studies in severely ill patients (Ison et al., 2010). Clinical endpoints can include time to resolution of fever, or time to normalization of vital signs such as respiratory or heart rate, blood pressure, oxygen saturation, or mortality. Endpoints in hospitalized patients are problematic due to the heterogeneity of clinical backgrounds and disease severities at admission, (de Jong et al., 2014; Marty et al., 2017). Other endpoints can include patient reported outcomes, such as the FluiiQ™ questionnaire (Osborne et al., 2011), or the Flu-Pro© survey (Powers et al., 2016). Virological endpoints have shown good correlation with clinical measures (Carrat et al., 2008) and virus load at admission has been shown to correlate with the length of hospital stay (Clark et al., 2016). Virus load may thus minimize the issues around the heterogeneity of the population. Additionally it is not ethical to carry out placebo controlled trials, so the current requirement is to prove superiority to the standard of care, which is usually oseltamivir therapy, which has remained a challenge (de Jong et al., 2014). A combination of clinical and virological endpoints may be necessary to achieve consistency.

### 13.3. Developing influenza therapeutics - a European regulator's perspective

Regine Lehnert, BfArM, Germany

In 2011 the US FDA issued a guidance document on developing drugs for treatment and/or prophylaxis of influenza (FDA-CDER, 2011), but no such document is yet available for Europe. New antivirals are being dealt with individually within so-called scientific advice procedures. The Europeans Medicine Agency (EMA) has a concept paper on Guidelines for new influenza therapeutic under development. The therapy needs to show it is effective in severe influenza, safe and well tolerated, has high potency, is rapid acting and has a longer treatment window than the NAIs, low likelihood of resistance emerging, and is effective against known resistant strains. It may be more effective in combination with another therapy, a single treatment is preferable and results in a decrease in virus load. None of the currently approved drugs has demonstrated a clinical benefit in an RCT in patients with severe influenza disease. Challenge studies do not always reflect the natural infection, and results in previously healthy populations do not extrapolate to those with severe disease. In patients at risk of complicated influenza heterogeneity with respect to baseline status

and severity of disease as well as type and severity of complications may lead to difficulties in designing a valid study. Due to pre-existing immunity, data from adults may not always be extrapolated to children. Studies in severe influenza have used time to normalization of vital signs or respiratory function. Another endpoint is based on an ordinal scale at a given point in time after first administration of study drug with death > ICU with mechanical ventilation > ICU w/o mechanical ventilation > hospital floor > hospital discharge, suggested (King et al., 2016). A draft EU-guideline on the evaluation of medicinal products indicated for treatment of influenza is envisaged to be released in early 2018.

### *13.4. Feasible or infeasible? investigational drug monotherapy for patients hospitalized with laboratory-confirmed influenza*

Kimberley Armstrong, BARDA, Washington, DC, USA

The Biomedical Advanced Research and Development Authority (BARDA) is working with partners to develop therapeutics for the hospitalized influenza-infected population. To understand clinical and institutional attitudes towards clinical trial design for the hospitalized influenza population, BARDA conducted a feasibility study by sending a questionnaire to 339 clinical investigators who had worked on previous hospitalized infectious disease clinical trials. They were questioned about three potential arms of a trial, 1) oseltamivir, 2) oseltamivir + new drug, 3) new drug monotherapy. 104 unique responses from 26 countries were received. Topics covered the SOC for hospitalized influenza patients as well as the feasibility of using investigational drug monotherapy for hospitalized subjects. Most clinicians listed NAIs as SOC at their institution (80–90%) regardless of global region; however, the percentage of patients who receive a NAI varies by region and is lower than SOC would predict (50–60%). Most clinicians believed a clinical study protocol including an investigational drug only arm would be allowed by their institution (60–70%), but when asked about the likelihood of Institutional Review Board or Ethics Committee approval, only 60% of US participants versus > 80% of European participants thought approval would be given. In the United States, 60% of clinicians believed that including an investigational monotherapy arm would negatively impact their ability to recruit subjects, versus minimal impact in Western Europe. Given the other challenges already present for hospitalized influenza clinical trials, including an investigational drug monotherapy arm could restrict the clinical sites available for the study and hamper the ability to recruit subjects.

## 14. Issues in clinical management

### *14.1. Under-recognized and unusual presentations of influenza*

William Fischer, University of North Carolina, Chapel Hill, North Carolina, USA

Influenza associated myocarditis (IAM) has been reported to occur in 0.4–13% of adults hospitalized with influenza infection and may occur in the absence of severe respiratory complications. In 45 cases of fatal influenza B histopathological findings of IAM were observed in 69% (20/29) of patients with cardiac samples available (Paddock et al., 2012). Complications of IAM include congestive heart failure and in 44 adult patients with IAM, 50% of patients required advanced cardiac support, with a mortality of 23% (Sellers et al., 2017). Oseltamivir treatment for influenza is associated with significant decrease in the risk of recurrent cardiovascular events in subjects with a history of cardiovascular disease (Casscells et al., 2009). Neurological complications of influenza include febrile convulsion, encephalitis, encephalopathy and Guillain Barre Syndrome (Meijer et al., 2016; Tomas et al., 2015). Influenza associated encephalitis is more common in pediatric patients but has been reported in 4% of hospitalized adults, with a mortality rate of 21% while 25% of patients may have residual neurological deficits (Sellers et al., 2017). MRI appears to be more diagnostic than CT scans (Meijer et al., 2016).

### *14.2. Managing RVIs in transplant recipients*

Michael Ison, Northwestern University, Chicago, Illinois, USA

Respiratory viruses cause a range of infections in solid organ (SOT) and hematopoietic stem cell transplant (HSCT) recipients with early onset after transplant resulting in more severe and prolonged infections and possible rejection. Influenza, parainfluenza and RSV generally cause more severe infections than rhinoviruses. In SOT patients, diagnosis can be difficult as 20% may not have fever at presentation. Degree of lymphopenia at onset of disease (higher risk with CD4 ≤100 c/mL) and transplant type also affect severity (allo-HSCT > auto-HSCT; lung > other SOT). The respiratory viruses cause a range of acute direct effects (i.e. viral pneumonia) and long-term complications (bronchiolitis obliterans (BOS) and late onset airflow obstruction). Early antiviral therapy with oseltamivir resulted in lower progression to pneumonia, reduced need for ICU-level care, lower mortality and lower incidence of BOS for influenza (Kumar et al., 2010). In the event of resistance to oseltamivir, treatment with zanamivir is initiated. Two Phase II studies of ALN-RSV01 provided proof of principal for anti-RSV therapy reducing development of BOS in lung transplant recipients (Waghmare et al., 2016). Studies in hospitalized infants are underway of ALS-8176, a nucleoside analog targeting RSV polymerase and Phase II trials are underway in hospitalized and adults transplant recipients for GS-5806 an oral RSV entry inhibitor (Waghmare et al., 2016). The replacement of inhaled ribavirin with oral ribavirin is now also being investigated. DAS181, an inhaled sialidase, is undergoing clinical development for the treatment of PIV in adults and children (Russell and Ison, 2017; Waghmare et al., 2015) although there has been some failure reported, due to coinfection with bacteria or fungus.

### *14.3. Corticosteroids, statins, and other adjunctive therapies in SARI*

David Hui, Chinese University of Hong Kong, Hong Kong SAR, China

Systemic corticosteroid has been used frequently for treatment of influenza related ARDS. However, a meta-analysis of data predominantly related to treatment of severe influenza caused by A(H1N1)pdm09 virus has shown that systemic corticosteroids were associated with an increase in mortality (OR 3.06, 95% CI 1.58 to 5.92) (Rodrigo et al., 2016). In comparisons to controls, high-dose corticosteroids (> 150 mg/d methylprednisolone eqv) was associated with increased risks of 30 day mortality (38.5% vs 7.7%, $P = 0.021$) and 60 day mortality (50% vs 15.4%, $P = 0.022$) and longer viral shedding (15 vs 13 days, $P = 0.039$) in patients with A(H7N9) viral pneumonia, while there was no difference between low dose (25–150 mg/d methylprednisolone) and controls (Cao et al., 2016). In a study of critically ill patients infected with influenza A(H1N1) pdm09 requiring invasive mechanical ventilation (IMV), addition of a mammalian target of rapamycin (mTOR) inhibitor, Sirolimus 2 mg/d for 14 days to oseltamivir and prednisolone (n = 19) was associated with a higher frequency of liberation from IMV (84.2 vs 47.4%, $P = 0.04$), a shorter

duration of IMV (13.8 vs 33 days, $P = 0.03$) and a higher chance of achieving lower respiratory tract viral RNA negativity by day 7 (75% vs 33%, $P < 0.05$) than without addition of Sirolimus (n = 19) (Wang et al., 2014a). A study of adults hospitalized for A(H3N2) has shown that a triple combination of 2 days of clarithromycin 500 mg, naproxen 200 mg and oseltamivir 75 mg twice daily, followed by 3 days of oseltamivir reduced both 30- and 90-day mortality and length of hospital-stay versus oseltamivir 75 mg twice daily without placebo for 5 days as control (Hung et al., 2017). In contrast, another RCT comparing dual therapy of oseltamivir and azithromycin against oseltamivir alone has shown significant anti-inflammatory effects with adjunctive macrolide treatment in adults with severe influenza infections although virus control was unimpaired (Lee et al., 2017). Exploratory post-hoc meta-analysis of studies of SARS and severe influenza showed a significant reduction in the pooled odds of mortality following convalescent treatment vs placebo or no therapy (OR 0·25; 95% CI 0·14 to 0·45) (Mair-Jenkins et al., 2015).

### *14.4. Mutagenesis analysis to identify determinants for neuraminidase inhibitor resistance in influenza viruses of N8 and N9 subtypes*

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As human infections with A(H7N9) and A(H10N8) viruses have occurred, both random mutagenesis and site-directed mutagenesis were used to investigate potential NA substitutions in N8 and N9 proteins that may confer resistance to NAIs, which would be used for treatment of patients infected with these viruses. Pools of recombinant A(H1N8) and A(H1N9) viruses containing random mutations in the NA head domain were passaged *in vitro* under increasing concentrations of oseltamivir and zanamivir. NA mutations including A266V in the A(H1N8) virus and T87A, T247A in the A(H1N9) virus were identified after serial passages; however, none of these mutations directly confer resistance to NAIs when introduced into the respective recombinant A(H10N8) or A(H7N9) viruses. Introducing NA substitutions previously reported to confer resistance to NAIs in N1 (E119V, Q136K, I222R, H275Y, N294S) and N2 subtypes (E119V, Q136K, I222R, A246T, R292K, N294S) by site-directed mutagenesis into A(H10N8) or A(H7N9) recombinant viruses, respectively, conferred comparable, but not fully identical resistance profiles in the respective group 1 (N1 and N8) and group 2 (N2 and N9) NA proteins (AVWG, 2016). Both H275Y and E119V substitutions decreased replication compared to the WT virus.

## 15. Conclusions

As at previous conferences updates were provided on various antivirals and vaccines against influenza, RSV and other respiratory viruses in preclinical and clinical studies. The issues remain over determining appropriate clinical and virological endpoints for antiviral therapies. Baseline clinical parameters are clearly different for previously healthy patients, compared to high risk and hospitalized patients. In clinical trials, new influenza virus inhibitors showed potency in decreasing virus titers in the upper respiratory tract, like VX787/JNJ63623872 and triple combination therapy of oseltamivir, ribavirin and amantadine, however there was no improvement in the clinical benefit. In contrast, the broadly neutralizing VIS410 anti HA monoclonal antibody led to both an earlier decrease in virus levels as well as earlier resolution of symptoms. Both an RSV anti-F (GS-5806) and polymerase inhibitor (ALS-008112) showed some promise with greater reductions in virus load and decreased mucus weights compared to the placebo groups. The NIAID has been very supportive of trying to facilitate development of novel therapies and vaccines for respiratory viruses, but given the seasonal nature of these viruses, enrolling sufficient patients in clinical trials remains challenging, especially when multiple therapies are in the pipeline for trials.

## Declarations of interest

Nelson Lee has previously received honoraria for consultancy work and remunerated lecture from Shionogi Ltd., Janssen Pharmaceuticals Inc., AstraZeneca Hong Kong Ltd., Visterra Ltd., and Seqirus Inc.; and support for travel to meetings from Sanofi-Aventis Hong Kong Ltd, MSD (Asia) Ltd., Pfizer Corporation Hong Kong Ltd., Gilead Sciences Hong Kong Ltd., and Janssen Pharmaceuticals Inc. Other authors nothing to declare.

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