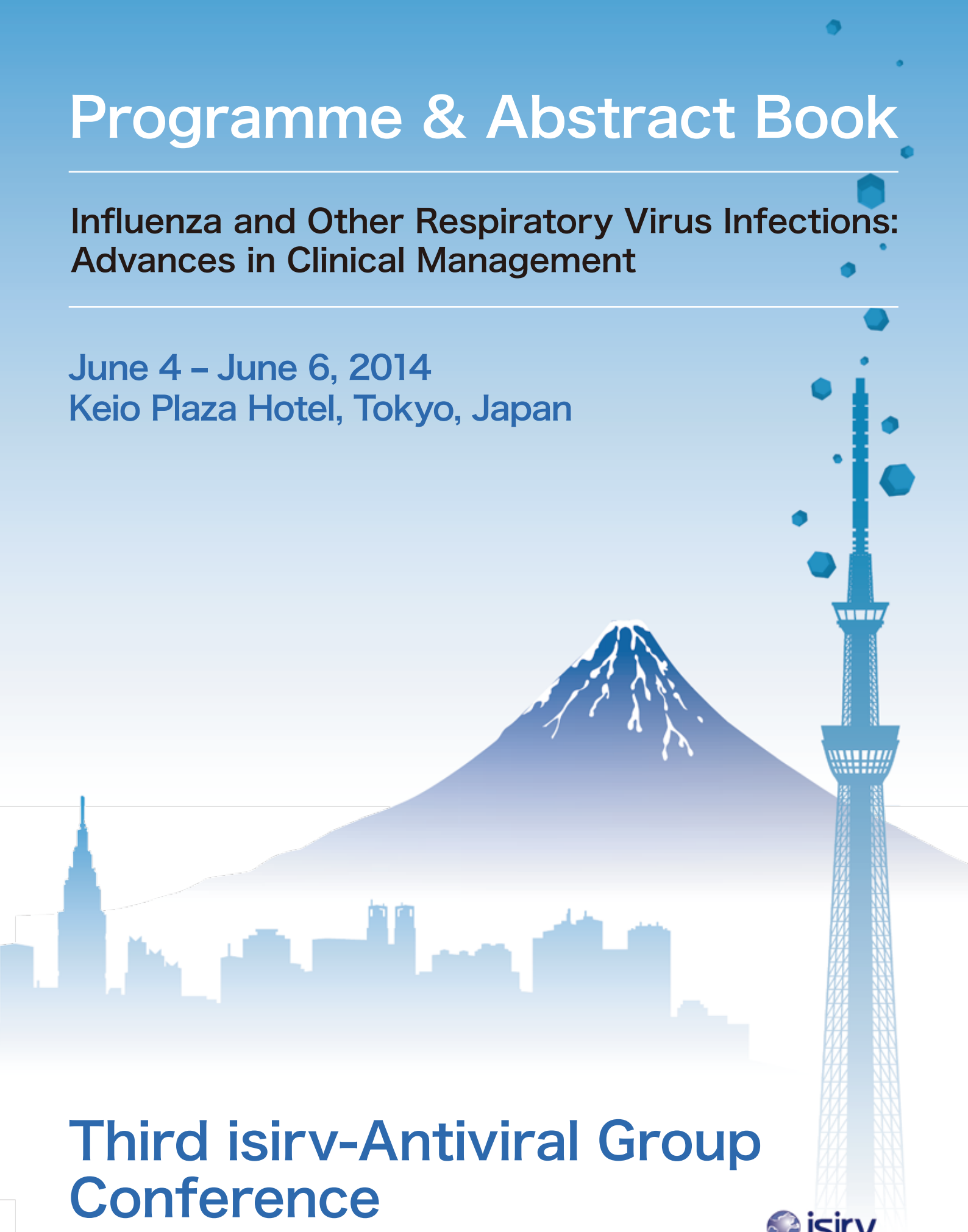
Programme & Abstract Book

Influenza and Other Respiratory Virus Infections: Advances in Clinical Management

June 4 – June 6, 2014
Keio Plaza Hotel, Tokyo, Japan



Third isirv-Antiviral Group Conference

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Lunch Seminar 1 Wednesday, June 4, 2014 13:00-14:00 Room 1
Co-sponsored by DAIICHI SANKYO CO., LTD.
Lunch Seminar 3 Thursday, June 5, 2014 13:00-14:00 Room 1
Co-sponsored by DENKA SEIKEN CO., LTD.
Lunch Seminar 4 Thursday, June 5, 2014 13:00-14:00 Room 2
Co-sponsored by Shionogi & Co., Ltd.

Third isirv-AVG Conference
Influenza and Other Respiratory Virus Infections: Advances in Clinical Management

Wednesday 4 - Friday 6 June 2014

Organizing Committee

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Japan

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World Health Organization
Geneva
Switzerland

Maria C. Zambon
Public Health England
London
UK

Welcome

Dear Colleagues,

I would like to welcome you to the Third isirv Antiviral Group (AVG) Conference. The programs of the first two conferences, in Rio de Janeiro in 2011 and in Hanoi in 2012, mainly concerned the effectiveness of anti-influenza drugs and resistance to anti-influenza drugs. The program of the conference in Tokyo encompasses every aspect of influenza, including anti-influenza drugs, influenza diagnosis, influenza vaccines, and the epidemiology of influenza, especially H7N9, one of the most important topics. MERS coronavirus, RSV, and parainfluenza virus will also be important subjects for discussion.

This will be the first time that such internationally renowned physicians and researchers convene in Tokyo for wide-ranging talks on influenza and other respiratory viral diseases. It is the best possible opportunity for Japanese to learn of the latest advances in the field.

At the previous international conferences, I was often asked how universal early treatment with neuraminidase inhibitors had been achieved in Japan. Participants from abroad could learn the answer in their discussions with Japanese participants.

Finally, I sincerely thank all the members of the scientific committee and organizing committee for their outstanding efforts in organizing the Third AVG conference. I am confident that we will have a stimulating and productive meeting, and I also hope you will have time to enjoy Japan as well as participate in the conference.

I am looking forward to seeing you in Tokyo.

Norio Sugaya, M.D.
Conference Chair,
Professor, Department of Pediatrics
and Department of Infection Control
Keiyu Hospital, Yokohama, Japan

Third isirv-Antiviral Group Conference Programme

Wednesday, June 4

9:00～10:00 Opening and Welcome — Room 1

10:00～13:00 Plenary Session 1 — Room 1

PS1: Emerging Respiratory Viral Threats

Chairs: Norio Sugaya, *Japan*
Alan Hay, *UK*

PS1-1 **Global Influenza Threats and Risk Assessment**

Daniel B. Jernigan
Influenza Division, Centers for Disease Control and Prevention (CDC), USA

PS1-2 **H7N9 in China: Virologic and Epidemiologic Features**

YueLong Shu
WHO Collaborating Center for Reference and Research on Influenza, National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China

PS1-3 **Clinical Comparison of Recovered Cases and Fatal Cases of Human Infection with H7N9 Avian Influenza in Shanghai**

Hongzhou Lu
Shanghai Public Health Clinical Center, Shanghai, China

11:30～12:00 Refreshment Break — Foyer

Chairs: Nahoko Shindo, *Switzerland*
Frederick G. Hayden, *USA*

PS1-4 **MERS-CoV Situation Update**

Maria C. Zambon
Public Health England, London, UK

PS1-5 **Update on MERS Coronavirus: Epidemiology, Clinical Features, Prevention and Case Management**

Ziad A Memish
Saudi Minister of Health & Alfaisal University, Riyadh, Kingdom of Saudi Arabia

13:00～14:00 Lunch Seminar 1 — Room 1

Chair: Frederick G. Hayden, *USA*

LS1 Clinical Effectiveness of Neuraminidase Inhibitors (NAIs) in Japan

Hideyuki Ikematsu
Influenza Study Group of Japan Physicians Association, Japan

13:00～14:00 Lunch Seminar 2 — Room 2

Chair: Masato Tashiro, *Japan*

LS2 The Clinical and Anti-influenza Virus Effects of Favipiravir, a Novel Anti-RNA Virus, Anti-influenza Agent

Carol L. Epstein
Medivector, Inc., Boston, USA

14:00～15:30 Oral Presentations — Room 1

Chairs: Yoshihiro Kawaoka, *Japan/USA*
Larisa Gubareva, *USA*

O1 Inferring Intra- and Inter-Host MERS-CoV Evolutionary Dynamics in a Transmission Chain by Deep Whole Genome Sequencing

Monica Galiano [1], Richard Myers [1], Nuno Rodrigues Faria [2], Kieren Lythgow [1], Juan Ledesma [1], Alison Bermingham [1], Oliver Pybus [2], Maria Zambon [1]
[1] Microbiology Services, Public Health England, Colindale, London, United Kingdom, [2] Department of Zoology, University of Oxford, Oxford, United Kingdom

O2 Molecular Epidemiology of Influenza A/H5N1 Clade 1 Circulation in Cambodia

Sareth Rith [1], Srey Viseth Horm [1], Touch Sok [2], San Sorn [3], Davun Holl [3], Sowath Ly [2], Lotfi Allal [7], Borann Sar [6], Savuth Chin [4], Paul Horwood [1], Reiko Tsuyuoka [5], Arnaud Tarantola [1], Sovann Ly [2], Philippe Buchy [1]
[1] Institute Pasteur in Cambodia, Phnom Penh, Cambodia, [2] Communicable Disease Department, Ministry of Health, Cambodia, [3] National Veterinary Research Institute, Ministry of Agriculture, Phnom Penh, Cambodia, [4] National Institute of Public Health, Phnom Penh, Cambodia, [5] World Health Organization, Cambodia, [6] Centers for Disease Control and Prevention, Cambodia Office, Phnom Penh, Cambodia, [7] Food and Agriculture Organization, Phnom Penh, Cambodia

O3 Hijacking the Host Factor of Type I Interferon Response into Proteosomal Degradation as Molecular Basis of Pathogenesis of 1918 PB1-F2

Eun-Sook Park [1,2], Yong Kwang Park [1], Young Ho Byun [4], Yo Han Jang [4,5], Yoon Jae Lee [4,5], Woo-Ry Han [1], Keo-Heun Lim [1], Kyun-Hwan Kim [1,2,3], **Baik L. Seong** [4,5]
[1] Department of Pharmacology and Center for Cancer Research and Diagnostic Medicine, IBST, School of Medicine, [2] Institute of Functional Genomics, [3] Research Institute of Medical Science, Konkuk University, Seoul, Republic of Korea, [4] Department of Biotechnology, College of Life Science and Biotechnology, [5] Vaccine Translational Research Center, Yonsei University, Seoul, Republic of Korea

O4 **Epidemiology of Influenza Infection Among Pregnant Women and Children Under 6 Months in Mongolia, 2013/14 Season: A Prospective Cohort Study**

L Chaw [1], A Burmaa [2], T Kamigaki [1], C Urtnasan [2], I Od [3], G Nyamaa [2], P Nymadawa [2,4], H Oshitani [1]
[1] Department of Virology, Tohoku University Graduate School of Medicine, Sendai, Japan, [2] National Influenza Center, National Center of Communicable Diseases, Ulaanbaatar, Mongolia, [3] Baganuur District, Ulaanbaatar, Mongolia, [4] Mongolian Academy of Medical Sciences, Ulaanbaatar, Mongolia

O5 **The ChILD-Score: A Standardized Clinical Outcome Parameter for Infants and Children with Influenza-Like Illness (ILI)**

Barbara Rath, Tim Conrad, Katharina Karsch, Franziska Tief, Patrick Obermeier, Xi Chen, Lea Seeber, Eleni Adamou, Janine Reiche, Brunhilde Schweiger
Department of Pediatrics, Division of Pneumonology-Immunology, Charité University Medical Center, Berlin, Germany

O6 **Sustained Viremia and High Viral Load in Sputum are Associated with Death in Adults with Adenovirus Pneumonia**

Li Gu, Jiu-Xin Qu, Bing Sun, Xiao-Min Yu, Hui Li, Bin Cao
Department of Infectious Diseases and Clinical Microbiology, Beijing Chao-Yang Hospital, Beijing Institute of Respiratory Medicine, Capital Medical University, Beijing, China

15:30～16:00 Refreshment Break Foyer

16:00～18:00 Plenary Session 2 Room 1

PS2: Influenza Impact and Management

Chairs: Nelson Lee, *China*
Akihiko Kawana, *Japan*

PS2-1 **Responding to Emerging RVI Threats: WHO Perspectives**

Nahoko Shindo
World Health Organization, Geneva, Swizerland

PS2-2 **Avian Influenza A(H5N1): Success and Challenges**

Tawee Chotpitayasunondh
Queen Sirikit National Institute of Child Health, Thailand

PS2-3 **Influenza and Other RVIs in Pregnancy**

Shigeru Saito
Department of Obstetrics and Gynecology, Graduate School of Medicine and Pharmaceutical Science for Research, University of Toyama, Toyama, Japan

PS2-4 **Did Neuraminidase Inhibitors Reduce Mortality During the 2009-10 Influenza Pandemic?**

Jonathan S. Nguyen-Van-Tam
The University of Nottingham, United Kingdom

18:30～ Conference Dinner Room 2

Thursday, June 5

9:00～11:00 Plenary Session 3 — Room 1

PS3: Advances in Respiratory Virus Diagnosis and Treatment

Chairs: Bin Cao, *China*
Jiro Fujita, *Japan*

PS3-1 **Advances in Diagnostics for Influenza and RVI's**

Lance C. Jennings
Microbiology Department, Canterbury Health Laboratories, Pathology Department, University of Otago, Christchurch, New Zealand

PS3-2 **Corticosteroids for Respiratory Viral Infections: When Are They Useful, When Are They Harmful?**

David Shu-Cheong Hui
Stanley Ho Center for Emerging Infectious Diseases, The Chinese University of Hong Kong, Shatin, Hong Kong, China

PS3-3 **Management of Adult CAP During Epidemic Influenza**

Bin Cao
Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

PS3-4 **Widespread Use of Neuraminidase Inhibitors in Japan and Several Issues to be Resolved**

Norio Sugaya
Department of Pediatrics and Department of Infection Control, Keiyu Hospital, Yokohama, Japan

11:00～11:30 Refreshment Break — Foyer

11:30～13:00 Plenary Session 4 — Room 1

PS4: Antiviral Resistance and New Agents

Chairs: Alan Hay, *UK*
Reiko Saito, *Japan*

PS4-1 **Antiviral Resistance: Detection and Assessment of Biologic Consequences**

Jennifer L. McKimm-Breschkin
CSIRO Materials Science and Engineering, Parkville, Australia

PS4-2 **Antiviral Resistance in A(H7N9) Viruses**

Hui-Ling Yen
School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

PS4-3

Advances in Therapeutics for Non-influenza Respiratory Virus Infections

Frederick G. Hayden
University of Virginia School of Medicine, Charlottesville, USA

13:00～14:00 Lunch Seminar 3 — Room 1

Chair: Nahoko Shindo, *Switzerland*

LS3

Universal Influenza Vaccination Program for Schoolchildren was Effective for Protection of Elderly and Young Children

Norio Sugaya
Department of Pediatrics and Department of Infection Control, Keiyu Hospital, Yokohama, Japan

13:00～14:00 Lunch Seminar 4 — Room 2

Chair: Jennifer L. McKimm-Breschkin, *Australia*

LS4

Neuraminidase Inhibitors Therapeutic Effect, Drug Resistance, Viral Transmission and Shedding Studies Conducted by Niigata University

Reiko Saito
Division of International Health, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

14:00～16:00 Oral Presentations — Room 1

Chairs: W. Abdullah Brooks, *Bangladesh/ USA*
Yasushi Itoh, *Japan*

O7

Middle East Respiratory Syndrome Coronavirus and the Effectiveness of Convalescent Plasma for the Treatment of Severe Acute Respiratory Infections of Viral Aetiology: A Systematic Review and Exploratory Meta-Analysis

J Mair-Jenkins[1], M Saavedra-Campos[2,3], K Baillie[4], P Cleary[3], FM Khaw[1], WS Lim[5], S Makki[1], KD Rooney[6], Convalescent Plasma Study Group, JS Nguyen-Van-Tam[7], **CR Beck**[7]
[1] Public Health England, Nottingham, UK, [2] Field Epidemiology Training Programme, Public Health England, UK, [3] Public Health England, Liverpool, UK, [4] University of Edinburgh, Midlothian, UK, [5] Nottingham University Hospitals NHS Trust, Nottingham, UK, [6] University of the West of Scotland, Hamilton, UK, [7] University of Nottingham, Nottingham, UK

O8

Safety and Efficacy of MHAA4549A, a Novel Monoclonal Antibody for Treatment of Severe Influenza A

José Trevejo, Rong Deng, Tracy Burgess, Jacqueline McBride, Michael Derby, Ning Chai, Summer Park, Min Xu, Lee Swem
Genentech Inc., South San Francisco, CA 94080

O9

Glycan Masking of Hemagglutinin Elicits Broadly Neutralizing Antibodies Against H5N1 Avian Influenza Viruses

Wen-Chun Liu [1] ,Shih-Chang Lin [1], Jia-Tsrong Jan [2], Suh-Chin Wu [1,3]
[1] Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan, [2] Genomics Research Center, Academia Sinica, Taipei, Taiwan, [3] Department of Medical Science, National Tsing Hua University, Hsinchu, Taiwan.

O10

Efficacy of a Vaccine and Neuraminidase Inhibitors Against H7N9 Influenza Virus in Cynomolgus Macaques

Yasushi Itoh [1], Misako Nakayama [1], Hideaki Ishida [1], Shintaro Shichinohe [2], Hirohito Ishigaki [1], Naoko Kitagawa [1], Takako Sasamura [1], Mayumi Sasada [2], Mayumi Endo [2], Masatoshi Okamatsu [2], Yoshihiro Sakoda [2], Hiroshi Kida [2], Kazumasa Ogasawara [1]
[1] Shiga University of Medical Science, Otsu, Japan, [2] Hokkaido University, Sapporo, Japan

O11

Evaluation of Oseltamivir Prophylaxis Regimens for Reducing Influenza Virus Infection, Transmission and Disease Severity in a Ferret Model of Household Contact

Ding Yuan OH [1], Sue LOWTHER [2], James MCCAW [3,4], Sheena G. SULLIVAN [1], Sook-Kwan LEANG [1], Jessica HAINING [2], Rachel ARKINSTALL [2], Anne KELSO [1], Jodie MCVERNON [3,4], Ian G. BARR [1,5], Deborah MIDDLETON [2], Aeron C. HURT [1,5]
[1] WHO Collaborating Centre for Reference and Research on Influenza, Australia, [2] Australian Animal Health Laboratory, Australia, [3] Vaccine and Immunisation Research Group, Murdoch Childrens Research Institute, Royal Childrens Hospital, Australia, [4] The University of Melbourne, Melbourne School of Population and Global Health, Melbourne, Australia, [5] Monash University, School of Applied Sciences and Engineering, Australia

O12

A Community Cluster of Influenza A(H1N1)pdm09 Virus Exhibiting Cross-Resistance to Oseltamivir and Peramivir in Japan

E Takashita, M Ejima, S Fujisaki, K Nakamura, A Sato, H Sugawara, M Tashiro, T Odagiri
National Institute of Infectious Diseases, Tokyo, Japan

O13

Novel Host-Targeted Approach for Control of Influenza Virus Infection

H Connaris [1], EA Govorkova [2], Y Ligertwood [3], BM Dutia [3], L Yang [1], S Tauber [1], MA Taylor [1], N Alias [1], R Hagan [1], AA Nash [3], RW Webster [2], GL Taylor [1]
[1] University of St. Andrews, St. Andrews, Scotland, [2] St. Jude Children's Research Hospital, Memphis, TN, USA, [3] The Roslin Institute, Edinburgh, Scotland

O14

Prevention of Lethal H7N9 Influenza Virus Infection by a Novel Receptor-Binding Protein

EA Govorkova [1], H Connaris [2], MA Taylor [2], BM Marathe [1], T Baranovich [1], L Yang [2], RG Webster [1], GLTaylor [2]
[1] St. Jude Children's Research Hospital, Memphis, TN, USA, [2] University of St. Andrews, St. Andrews, UK

16:00～16:30 Refreshment Break Foyer

16:30～18:00 Plenary Session 5 — Room 1

PS5: Influenza Vaccines

Chairs: Maria C. Zambon, *UK*
Makoto Yamashita, *Japan*

PS5-1 **Measuring Influenza Vaccine Effectiveness, Challenges and Needs**

Alain Moren
Department of Epidemiology, EpiConcept, Paris, France

PS5-2 **Influenza Vaccines for the Elderly**

Ann R. Falsey
University of Rochester School of Medicine, Rochester, USA

PS5-3 **Induction of Neutralizing Antibodies by Inactivated Intranasal Influenza Vaccine and Characteristic of Induced Secretory-IgA Antibodies in Human**

Hideki Hasegawa
Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

18:00～19:00 Special Evening Seminar — Room 1

Chair: Satoshi Iwata, *Japan*

ES **Current Progress in Influenza Research**

Yoshihiro Kawaoka
University of Tokyo, Tokyo, Japan/ University of Wisconsin, Madison, USA

19:00～21:00 Poster Session Reception — Room 2

P1 **Prognosis of 18 H7N9 Avian Influenza Patients in Shanghai**
Jie Liu

P2 **Clinical Presentation, Management and Outcomes of Influenza in Africa: Systematic Review, 2009-2013**
Martin Herbas Ekat

P3 **Elevated Pancreatic Enzymes in Patients with H7N9 Influenza: A Cross-Sectional Analysis of 18 Patients**
Tangkai Qi

P4 **Genetic and Antigenic Characteristics of Influenza Viruses Isolated in Egypt in Seasons 2010:2012**
Nagwa Elkholy

P5 **Interleukins Profile in Patients with Acute Respiratory Viral Infections**
Iaryna Iosyk

P6 **Effect of Human Rhinovirus Infection in Pediatric Patients with Influenza-Like Illness on the 2009 Pandemic Influenza A (H1N1) Virus**
Lin-qing Zhao

P7 **Tropism of Avian Influenza A (H5N1) Virus to Mesenchymal Stem Cells and $CD34^+$ Hematopoietic Stem Cells**
Maytawan Thanunchai

P8 **Different Immunity Elicited by Recombinant H5N1 Hemagglutinin Glycoproteins Containing Pauci-mannose, High-mannose, or Complex type N-Glycans**
Suh-Chin Wu

P9 **A Monoclonal Antibody Recognizes a Highly Conserved Neutralizing Epitope on Hemagglutinin of H6N1 Avian Influenza Virus**
Ching-Ho Wang

P10 **Estimated Effectiveness of Influenza Vaccine Using Influenza Rapid Diagnostic Tests - Test-Negative Case-Control Study Among Japanese Children**
Masayoshi Shinjoh

P11 **Phenotypic and Genotypic Significance of Influenza Viruses Identified in the Republic of Moldova**
C. Spinu

P12 **Antiviral Drug Profile of Human Influenza A and B Viruses Circulating in India: 2009-2014**
VA Potdar

P13 **Innate $CD8^{+}CD44^{hi}$ T Cells and IFN-γ Mediate Thymic Atrophy in Influenza A(H1N1)pdm09 Severely Infected Mice**
LIU Bo

P14 **Near Real Time Surveillance for Influenza in Primary Care Settings**
John Tamerius

P15 **An Immunoinformatics Approach for Designing Epitope Based Vaccine Strategy Against S Protein of Mysterious New Middle East Respiratory Syndrome Coronavirus (MERS-CoV)**
Kutub Uddin Muhammad Ashraf

P16 **Virological Estimation of Peramivir Administration in Children with Influenza**
Masatoki Sato

P17 **Genetic Variability of Group A Human Respiratory Syncytial Virus from a Large Referral Hospital in Alappuzha, Kerala State, India.**
B Anukumar

P18 **New Adamantane Derivatives Can Overcome Resistance of Influenza A(H1N1)pdm2009 and A(H3N2) Viruses to Rimantadine**
E.S. Kirillova

P19 **Identification of Resistance Mutations as Minority Species in Clinical Specimens from Hospitalised Adults with Influenza and Treated with Intravenous Zanamivir**
Phillip J Yates

P20 **Epidemiological, Biological, and Genetic Properties of A(H1N1)pdm09 Virus Strains Caused of Lethal Cases of Influenza Infection**
EI Burtseva

P21 **Susceptibility of Influenza A and B Viruses, Isolated in Russia During 2011-2014, to Oseltamivir (Tamiflu™) and Zanamivir (Relenza™)**
NV Breslav

P22 **A Community-Acquired Case of Oseltamivir and Peramivir Resistant Influenza A(H1N1)pdm09 Virus in Nagasaki, Japan in 2013-2014 Season**
Hiroki Kondo

P23 **NK Cells Aggravate Acute Lung Injury Via Up-regulation of NKG2D During Early Stage of H1N1 Influenza Infection**
Xulong Zhang

P24 **Predictors for In-hospital Mortality Among Adults with Influenza A (H7N9) Virus Infection with Emphasis on the Effect of Adjuvant Corticosteroid Treatment**
LI Hui

P25 **Oseltamivir Resistance Among Influenza Viruses: Surveillance in Northern Viet Nam, 2009–2012**
Pham Thi Hien

P26 **Oseltamivir Use in a Cohort of Young Children in Bangkok, Thailand**
Suntarattiwong P

P27 **Contribution of Respiratory Viral Infections Among Hospitalized Children Below 5 Years of Age in Pune, India: A Retrospective Study**
MS Chadha

P28 **Influenza B Viruses with Neuraminidase Inhibitor-Resistant Substitutions E119A and H274Y Possess Undiminished Fitness**
AJ Burnham

P29 **Emergence of G186D in the Presence of R292K in an Immunocompromised Child Infected with Influenza A/H3N2 Treated with Oseltamivir**
HK Lee

P30 **Molecular Surveillance of Antiviral Drug Resistance of Influenza A/H3N2 Virus in Singapore, 2009-2013**
HK Lee

P31 **Molecular Evidence of Transmission of Influenza on a University Campus in Singapore**
RK Virk

P32 **Impact of Live Poultry Market Closures on Reducing Risk of Human Infections with Influenza A(H7N9) Virus, China, Winter 2013-14**
Peng Wu

P33 **Respiratory Virus Infection and Clinical Characteristics of Hospitalized Children Younger than 5 Years with Severe Acute Respiratory Infection in Suzhou, China**
Qian Geng

P34 **Safety and Efficacy of Intravenous Zanamivir in the Treatment of Hospitalized Japanese Patients with Influenza Infection**
H Furukawa

P35 **Clinical Features of Severe Acute Respiratory Infections in Suzhou, China**
Yuejia Cheng

P36 **Vitamin D and Risk of Influenza in Children and Adults in Hong Kong**
Cuiling Xu

P37 **Viral Respiratory Infections in Okinawa**
Jiro Fujita

P38 **The Kinetics of Viral Load of Human Adenovirus Genotype 55 in Sequential Sputum and Blood Serum from an Immunocompetent Patient with Lethal Pneumonia**
JX Qu

P39 **2009 Pandemic H1N1 Influenza Can Rapidly Develop Escape Mutations in the Presence of a Host-Directed Vacuolar ATPase Inhibiting Drug**
Anika Singanayagam

P40 **No Middle East Respiratory Syndrome Coronavirus Detected in Pilgrims at Hajj 2013 with Laboratory-Confirmed Influenza-Like Illness**
J Kok

P41 **A Clinical Prediction Rule for Diagnosing Human Infections with Avian Influenza A(H7N9) in a Hospital Emergency Department Setting**
DKM Ip

P42 **Cost-effective Approaches for the Surveillance of Swine Influenza in Developing Countries**
BJ Cowling

P43 **Age Specific Epidemic Curves of Four Common Respiratory Viruses in Subtropical City Hong Kong, 2004-2013**
Lin Yang

P44 **Characteristics of Approved Influenza Rapid Diagnostic Testing in Japan, China and United States**
Takashi Miyazawa

P45 **Dynamics of Epidemics of Influenza A and B Viruses in Okinawa**
Satoko Sunagawa

P46 **Analysis of Influenza Virus Responsible for Persistent Infection After Drug Administration in an Immunosuppressed Patient**
C Kawakami

P47 **Comparison of Clinical Features Between Severe Cases Infected with H7N9 and H1N1pdm Influenza A in Jiangsu Province, China**
Xiang Huo

P48 **Peculiarities of Influenza Virus Infection in Hospitalized Patients in 2012-2014, Moscow, Russia**
S Trushakova

P49 **Optimization of Inoculum Dose of Inactivated Swine Influenza Vaccine for Immune Protection of Pigs**
SG Remyga

P50 **Monitoring Influenza Antiviral Resistance in Algeria: Establishment of a Local Surveillance of Influenza Viruses Susceptibility to Neuraminidase Inhibitors**
A. Ait Aissa

P51 **Wheezing Following Acute Respiratory Infections Versus Immunization in Young Children**
Barbara A. Rath

P52 **Novel *In Silico* Analysis of Pleiotropic Molecular Mechanism of Multitargeted Inhibitors for Influenza Virus Using the First-Principles Calculations**
Erika Ishitsubo

P53 **Phylogenetic and Clinical Virology Analyses of Influenza Virus Sequence Data from the First Four Years of the IRIS Study**
Martin Schutten

P54 **Activity of Thiazolides Against Other Respiratory Viruses than Influenza**
Jean-François Rossignol

P55 **Clinical Evaluation of Highly Sensitive Silver Amplification Immunochromatography Systems for Rapid Diagnosis of Influenza**
Mitamura K

P56 **Influenza Viruses in Children from a Rural Indian Community: A Post-Pandemic Surveillance Study**
Lalit Dar

P57 **Susceptibility of Avian Influenza A(H7N9) Viruses to FDA-Approved and Investigational Antiviral Drugs**
Gubareva LV

P58 **Flupep: a Novel Peptide for Treatment of Influenza Virus Infections**
Bernadette M. Dutia

P59 **Salt Bridge Modifications Resulted to Structural Differences Between the Avian and Human H7N9 Hemagglutinin Proteins: Implications in Viral Evolution**
ME Cueno

P60 **Tertiary-Care Hospital-Based Influenza Surveillance in India in 2010-2014**
R Kumar

P61 **Assays for Influenza Vaccines Evaluation and Correlates of Protection**
Emanuele Montomoli

Friday, June 6

8:30～12:30 Plenary Session 6 — Room 1

PS6: Respiratory Virus Infections

Chairs: Michael G. Ison, *USA*
Masayoshi Shinjo, *Japan*

PS6-1 **Paramyxovirus Impact (RSV, HMPV, PIV) in Children; New Studies of RVIs**

W. Abdullah Brooks
ICDDR,B, Bangladesh/ Bloomberg School of Public Health Johns Hopkins University, USA

PS6-2 **Molecular Epidemiology of Human Rhinovirus (HRV) in Patients with Asthma**

Hirokazu Kimura
Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

PS6-3 **Lower Respiratory Tract Viral Infections in Children, Asian Perspectives**

Yuan Qian
Laboratory of Virology, Capital Institute of Pediatrics, Beijing, China

PS6-4 **Non-Pharmaceutical Interventions for RVIs- Effectiveness and Consequences**

Benjamin John Cowling
School of Public Health, The University of Hong Kong, Hong Kong SAR, China

10:30～11:00 Refreshment Break — Foyer

Chairs: Frederick G. Hayden, *USA*
Isao Miyairi, *Japan*

PS6-5 **Unrecognized Burden of Adult RSV Infections in Asia**

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

PS6-6 **Prevention and Treatment Strategies for Respiratory Syncytial Virus Infections**

John DeVincenzo
The University of Tennessee School of Medicine, Knoxville, USA

PS6-7 **Challenge of RVIs in Immunocompromised Hosts**

Michael G. Ison
Divisions of Infectious Diseases and Orgen Transplantation, Northwestern University, Evanston, USA

12:30～13:30 Clinicians Panel: Open Q&A on Clinical Management Issues — Room 1

Chair: Tawee Chotpitayasunondh, *Thailand*

W. Abdullah Brooks
ICDDR,B, Bangladesh/ Bloomberg School of Public Health Johns Hopkins University, USA

Bin Cao
Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

John DeVincenzo
The University of Tennessee School of Medicine, Knoxville, USA

Frederick G. Hayden
University of Virginia School of Medicine, Charlottesville, USA

Michael G. Ison
Divisions of Infectious Diseases and Orgen Transplantation, Northwestern University Comprehensive Transplant Center, Evanston, USA

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

Kazunori Oishi
Infectious Disease Surveillance Center, National Institute of Infectious Disease, Tokyo, Japan

Nahoko Shindo
World Health Organization, Geneva, Swizerland

13:30 Closing and Final Remarks — Room 1

Norio Sugaya
Keiyu Hospital, Yokohama, Japan

Notes

Wednesday, June 4

Plenary Session 1: Emerging Respiratory Viral Threats

PS1-1

Global Influenza Threats and Risk Assessment

Daniel B. Jernigan

Influenza Division, Centers for Disease Control and Prevention (CDC), USA

In the last year, avian influenza A(H7N9) has emerged causing hundreds of human cases and deaths. In addition, human illness due to novel avian influenza A(H6N1) and A(H10N8) were reported in Asia. Increasing detection and recognition of novel influenza viruses are due to numerous causes, including improved awareness, surveillance, diagnosis, and reporting. These recent cases underscore several factors associated with emergence of novel influenza viruses such as increasing population density, travel and trade, and the convergence of animals and humans. Given the potential for an increasing number of human cases due to emerging novel influenza viruses from birds and swine, efforts are needed for improving risk assessment to inform clinical management, public health decision-making, and resource planning for interventions such as vaccines and antivirals. Following the global response to pandemic H1N1, a number of international and national activities were initiated to improve risk assessment, for use both before a novel influenza virus has gained sustained and efficient transmission, as well as after a pandemic has begun. New tools are being developed and evaluated which use available laboratory and epidemiologic data to 1) estimate the risk that a novel influenza virus will emerge to transmit from person to person, 2) estimate the severity of a newly circulating pandemic virus, and 3) more accurately estimate the actual number of cases occurring based on the detected number of cases. As recognition of novel influenza cases improves, new tools for better risk assessment may also improve readiness and response to emerging pandemic threats.

PS1-2

H7N9 in China: Virologic and Epidemiologic Features

YueLong Shu, Dayan Wang, Jianfang Zhou, Rongbao Gao, Tao Chen

National Institute for Viral Disease Control and Prevention, China CDC, Key Laboratory for Medical Virology, National Health and Family Planning Commission. WHO Collaborating Center for Reference and Research on Influenza, Beijing, China

The novel avian influenza H7N9 virus with human infection was discovered in Yangzi river delta region in March, 2013, since then the virus had caused 391 human cases with 139 deaths by March 16, 2014 in the world, mainly in mainland of China. The H7N9 human cases have been reported sporadically even limited human-to-human transmission documented. More than 80% cases had a history of exposure to live animals including live poultry market visiting, suggested that the predominant infection source was from poultry.

The novel H7N9 virus was an avian-origin reassortant containing heamagglutinin derived from avian H7N3-like, neuraminidase from avian N9-like and six internal gene segments from avian influenza H9N2 virus. Its unusual features indicated a pandemic potential risk with perpetual challenges. The special “dual-receptor” binding profile of avian H7N9 virus, which caused by G186V and Q226L mutations in receptor binding site of HA, enabled the H7N9 virus could infect human upper respiratory tract easier than H5N1 virus. In addition to the amino acid substitutions, the dynamic reassortment of H7N9 virus with H9N2 virus resulted multiple genotypes, indicating the genetic compatibility of internal genes among H7N9 and H9N2 viruses and the potential multiple introductions from H9N2 viruses. The case fatality rate of H7N9 virus infection was more than 30%, and the “cytokine storm” contributed to the clinical severity. The animal model studies demonstrated that H7N9 virus was efficiently transmitted via direct contact, but less efficiently by airborne exposure, which further highlight the pandemic potential of the novel H7N9 influenza virus.

PS1-3

Clinical Comparison of Recovered Cases and Fatal Cases of Human Infection with H7N9 Avian Influenza in Shanghai

Hongzhou Lu

Shanghai Public Health Clinical Center, Shanghai, China

We describe the epidemiologic and clinical features among 17 fatal cases of human infection with H7N9 virus in Shanghai from February through October 2013. The median age of the patients was 73 years; 82.4% had one or more underlying medical conditions. The most frequent symptoms were fever (100%), followed by productive cough (47.1%), dry cough (35.5%), fatigue (17.6%) and sore throat (11.8%). Thirteen (76.5%) patients had dyspnea or respiratory distress, five (29.4%) had shock, and four (23.5%) had acute kidney injury. Leukopenia was found in five (29.4%) patients, 17 (100.0%) had lymphopenia, and eight (47.1%) patients had thrombocytopenia. There were marked abnormalities on chest radiography; involvement of both lungs was found by radiography in 14 (82.4%) patients at presentation. Fifteen (88.2%) patients were hospitalized. The median times from illness onset to hospitalization and to diagnosis confirmation were both six days. Eleven (64.7%) patients were admitted to the intensive care unit (ICU). Sixteen (94.1%) patients were treated with oseltamivir. The median time from illness onset to oseltamivir treatment was six days. Among 15 hospitalized patients, 12 (80.0%) required oxygen therapy, and nine (60.0%) required mechanical ventilation on admission. Among six patients who the duration of viral shedding was available, the median duration of viral shedding after oseltamivir treatment was 17 days. The median time from illness onset to death was 11 days. Refractory hypoxemia accounted for most deaths. Avian influenza A (H7N9) virus infection, characterized by multiple organ dysfunction, carries a high risk of death. This investigation reflects a significant delay in the diagnosis and antiviral treatment of patients with avian influenza A (H7N9) in Shanghai. Late antiviral treatment and a long duration of viral shedding may be associated with a fatal outcome in these patients. Strategies to facilitate rapid identification of cases and early antiviral treatment are urgently required.

PS1-4

MERS-CoV Situation Update

Maria C. Zambon

Public Health England, London, UK

MERS-Coronavirus emerged in 2012 causing a syndrome of severe respiratory infection. The animal origin of the virus remains uncertain, although bats and camels do host related Coronaviruses. Progress in understanding the pathology of disease, serological responses and developing interventions for this infection will be reviewed, including the potential for immumotherapy.

PS1-5

Update on MERS Coronavirus: Epidemiology, Clinical Features, Prevention and Case Management

Ziad A Memish

Saudi Minister of Health & Alfaisal University, Riyadh, Kingdom of Saudi Arabia

The Middle East respiratory syndrome (MERS) is a new killer respiratory disease caused by the MERS coronavirus (CoV) first reported from the Kingdom of Saudi Arabia (KSA) in September 2012, after identification of a novel betacoronavirus from a Saudi Arabian patient who died from a severe respiratory illness. Retrospective study of stored samples later showed that, earlier in April 2012, a cluster of severe respiratory illness occurred in a public health hospital in Zarqa, Jordan, where eight healthcare workers were among the 11 people affected, with two deaths attributed to MERS-CoV. Since the first KSA case report in September 2012, the KSA Ministry of Health (MoH) has recommended mandatory testing for MERS-CoV in all cases of respiratory illness requiring intensive care admission.
As of March, 2014, worldwide, 24 months since the first discovery of MERS-CoV in Zarqa, Jordan, there have been a total of 180 cases of MERS-CoV infection with 77 deaths (46% mortality) reported to the WHO. The trend in case detection rate does not suggest that an impending epidemic is inevitable. The majority of cases (156 and 63 deaths (40%) have been reported from KSA. All cases are linked to only six countries in the Middle East: KSA, Jordan, Kuwait, Oman, Qatar and UAE. Five countries outside the Arabian Peninsula (France, Italy, Germany, Tunisia and the UK) have detected MERS cases that were either transferred for care or travellers returning from one of the Middle East countries and subsequently became ill. Zoonotic transfer from an animal reservoir to humans has been shown to occur with the SARS-CoV. The quest to find an animal source for MERSCoV continues. Serological evidence for a cross-reactive virus in camels has been reported and a small fragment of MERS-CoV sequence have been identified in a bat from KSA. Recent identification of MERS-CoV in two camels in Qatar lends support to the previous assumption that an animal source of the route of transmission could be either direct contact, consumption of a contaminated food product or even
contact with a contaminated fomites. The KSA MoH is pursuing a vigorous search for the source of MERSCoV in animal hosts and other potential reservoirs, and their transmission routes to humans.
There were many unknowns at that time regarding MERS-CoV. Several priority research questions required urgent answers: the source of the virus; the route of transmission; infectious potential; the epidemiological and clinical features; occurrence in the community; transmission patterns; and evolution of the virus. A lack of accurate, sensitive and specific rapid serological diagnostic tests for surveillance hindered the conduct of case–control studies. The epidemiology, mode of transmission, clinical spectrum of illness and incidence in the community remained largely unknown.

Lunch Seminar 1

LS1

Clinical Effectiveness of Neuraminidase Inhibitors (NAIs) in Japan

Hideyuki Ikematsu

Influenza Study Group of Japan Physicians Association, Japan

The neuraminidase inhibitors (NAIs) are commonly used for the treatment of influenza in Japan, and treatment is recommended within 48 hours from the onset. The Japan Physicians Association has studied influenza since 2001. In brief, patients who are positive by rapid diagnosis test kit and have a temperature ≧37.5°C are registered for study. Patients suspected of having other viral or secondary bacterial infections following influenza or for whom more than 48 hours have passed after the onset of illness are excluded.

The duration of fever ≧37.5°C were compared among four NAIs. The duration of fever of patients treated with oseltamivir was longer for influenza B than for influenza A. A higher IC50 value for influenza B than for influenza A for four the NAIs may be responsible for this result.

A(H1N1) virus with the H275Y mutation, which shows high IC50 value for oseltamivir, was isolated at a high rate in North Europe in the 2006-2007 season and spread worldwide in the next two years. We investigated the influence of H275Y mutated virus on the clinical effectiveness of oseltamivir. The duration of fever was elongated in patients infected with H275Y mutated A(H1N1) compared to that of patients infected with A(H3N2) in the same season or to that of patients infected with the unmutated A(H1N1) of the previous season.

Virus subtype was also related to the clinical effectiveness of NAIs. The duration of fever of patients infected with A(H1N1)pdm was significantly shorter than that of the patients infected with A(H3N2), which may account for the severity, and mortality of influenza in Japan having been low compared to other countries during the A(H1N1)pdm09 pandemic.

Factors that influence the clinical effectiveness of NAIs, including the virus titer, will be presented, that give new insight into the pathogenesis of Influenza Infection.

Lunch Seminar 2

LS2

The Clinical and Anti-influenza Virus Effects of Favipiravir, a Novel Anti-RNA Virus, Anti-influenza Agent

Carol L. Epstein, Dennis Giesing, Robert P. Lenk, Jeffrey Finman, Yang Yijun, Sarah Frech
Medivector, Inc., Boston, USA

Favipiravir, a novel pyrazine molecule that inhibits replication of RNA viruses, has been in development for the treatment of uncomplicated influenza. Discovered by Toyama Chemical, Ltd., it has been shown to be effective against all strains of influenza in preclinical testing including H5N1 and H7N9, as well as other pathogenic RNA viruses, and resistance has not yet been demonstrated. It is currently undergoing global US Phase 3 testing as a treatment for uncomplicated influenza under a US IND, funded by the Department of Defense. The results of a Phase 2 program are presented below.

FDA requires demonstration of clinical efficacy for drugs intended to treat uncomplicated influenza. This consists of decreasing the time to resolution of six symptoms plus resolution of fever, in a clinically meaningful fashion. The first US study, US204, demonstrated anti-viral effects, but no significant clinical effects. We postulated that the dosing regimen needed adjustment and efficacy in this setting, where patients recover spontaneously in a matter of days, would require rapidly reaching intracellular therapeutic levels of the active metabolite, T-705RTP, and maintaining it over the five day course of therapy. A second US study was designed to test the PK and safety profiles of two new TID regimens, and then bring one into the clinical setting comparing it and a new BID regimen to placebo.

Analyses of the results showed that both the BID and TID regimen had anti-viral effects (decreasing the time to cessation of shedding and the amount of virus shed), but only the BID regimen had clinical benefit. The only drug-related adverse effect appears to be transient, asymptomatic elevations of uric acid. This BID regimen is currently being studied in the global Phase 3 program intended to lead to approval.

Oral Presentations

O1

Inferring Intra- and Inter-Host MERS-CoV Evolutionary Dynamics in a Transmission Chain by Deep Whole Genome Sequencing

Monica Galiano [1], Richard Myers [1], Nuno Rodrigues Faria [2], Kieren Lythgow [1], Juan Ledesma [1], Alison Bermingham [1], Oliver Pybus [2], Maria Zambon [1]

[1] Microbiology Services, Public Health England, Colindale, London, United Kingdom, [2] Department of Zoology, University of Oxford, Oxford, United Kingdom

Introduction: The recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV) was detected first in March, 2012. As of April 2nd, 2014, it has infected 207 persons, with 87 fatal cases. Four cases have been identified in the UK, with 3 of them epidemiologically linked in a family cluster where only the index case had history of travel to the Middle East. The use of next generation sequencing tools allowed investigation of the putative chain of transmission and virus populations observed in each patient. Characterisation of viral populations that evolve within a host and also those that can signal transmissions events are key for understanding the mechanisms that shape the genetic diversity of MERS-CoV in humans.

Objective: To interpret intra-host evolutionary dynamics and patterns of transmission of MERS-CoV using longitudinal samples from 3 cases in a chain of transmission.

Methods: Primers designed against sequences of MERS-CoV EMC/2012 and bat coronaviruses HKU4 & HKU5 were used to amplify two sets of 35 amplicons, each covering the entire genome. Amplicon products were sequenced with Illumina MiSeq sequencer. Sequence data was processed using BWA-MEM, Samtools and in-house scripts.

Results: A total of 12 sequential samples from 3 cases (Case 2 (index)=7; Case 3=4; Case 4=1) from a chain of transmission within a family cluster that occurred in February, 2013 in the UK were sequenced. Four different variants were observed in frequencies of >20%, with one of them codifying for a non-synonymous change in the spike protein. This polymorphism was present as a mixture in several of the sequential samples from Case 2, showing transient increase in its frequency from 4% on day 1 to 36% on day 6 and down to 4% again on Day 10. This time period coincided with the timing of contact between Case 2 and case 4. Sequence from the only sample available from case 4 showed this variant present in 100% of the sample viral population. In addition, this mixture occurred at different frequencies in samples from Case 2 taken on the same day from upper and lower respiratory tract. Case 3 showed a single synonymous change which was not found in any of the index case sequences. Additional results are being evaluated.

Conclusion: Significance of the presence of transient variants, transmission of a minority variant between cases and differential frequencies of variants in different body compartments will be discussed.

O2

Molecular Epidemiology of Influenza A/H5N1 Clade 1 Circulation in Cambodia

Sareth Rith [1], Srey Viseth Horm [1], Touch Sok [2], San Sorn [3], Davun Holl [3], Sowath Ly [2], Lotfi Allal [7], Borann Sar [6], Savuth Chin [4], Paul Horwood [1], Reiko Tsuyuoka [5], Arnaud Tarantola [1], Sovann Ly [2], Philippe Buchy [1]

[1] Institute Pasteur in Cambodia, Phnom Penh, Cambodia, [2] Communicable Disease Department, Ministry of Health, Cambodia, [3] National Veterinary Research Institute, Ministry of Agriculture, Phnom Penh, Cambodia, [4] National Institute of Public Health, Phnom Penh, Cambodia, [5] World Health Organization, Cambodia, [6] Centers for Disease Control and Prevention, Cambodia Office, Phnom Penh, Cambodia, [7] Food and Agriculture Organization, Phnom Penh, Cambodia

Highly pathogenic avian influenza (HPAI) H5N1 virus was first detected in Cambodian poultry in January 2004 and the first human case was detected in January 2005. To date, 56 human cases (including 36 deaths) and 43 poultry outbreaks have been recorded in Cambodia. The Virology Unit at the Institut Pasteur in Cambodia has conducted detailed genetic analysis, antigenic characterisation and antiviral resistance analyses on these viruses.
Prior to 2013, molecular characterisation revealed that all of the H5N1 viruses isolated from humans and poultry belonged to clade 1, genotype Z. In January 2013, a new genotype of clade 1.1 emerged containing HA and NA genes from clade 1.1.2 and internal genes from clade 2.3.2.1. During 2013 Cambodia faced an explosive increase in the numbers of human H5N1 cases diagnosed, resulting in the highest number of cases and deaths per 100,000 inhabitants in the world. Antigenic analysis using sera raised against reference viruses demonstrated that some of the strains developed a very significant antigenic drift by comparison to the A/Vietnam/1204/2004 reference strain (clade 1). These results were confirmed by the WHOCC for influenza at US CDC Atlanta.
In order to monitor the risk of human-to-human transmission of the avian influenza virus, nasopharyngeal swabs from contacts of H5N1 cases were tested by PCR. Acute and convalescent sera were also tested in the BSL3 of IPC by hemagglutination-inhibition and microneutralization tests. All of the results were so far negative, demonstrating that none of these close-contact persons were infected with H5N1 virus.

O3

Hijacking the Host Factor of Type I Interferon Response into Proteosomal Degradation as Molecular Basis of Pathogenesis of 1918 PB1-F2

Eun-Sook Park [1,2], Yong Kwang Park [1], Young Ho Byun [4], Yo Han Jang [4,5], Yoon Jae Lee [4,5], Woo-Ry Han [1], Keo-Heun Lim [1], Kyun-Hwan Kim [1,2,3], **Baik L. Seong** [4,5*]

[1] Department of Pharmacology and Center for Cancer Research and Diagnostic Medicine, IBST, School of Medicine, [2] Institute of Functional Genomics, [3] Research Institute of Medical Science, Konkuk University, Seoul, Republic of Korea, [4] Department of Biotechnology, College of Life Science and Biotechnology, [5] Vaccine Translational Research Center, Yonsei University, Seoul, Republic of Korea

The high mortality of 1918 pandemic influenza was reported to be associated with the PB1-F2 protein, but the molecular mechanism of pathogenesis still remains elusive.
We investigated the molecular and functional characteristics of 1918 and PR8 PB1-F2 proteins. Interestingly, 1918 PB1-F2 showed the proteasome-dependent degradation with low stability and strongly inhibited IFN induction compared to PR8 PB1-F2. Moreover, the low stability of 1918 PB1-F2 protein was linked with potent inhibition of type I IFN induction and enhanced pathogenicity in mice. The structural prediction shows that 1918 PB1-F2 belongs to the intrinsically disordered proteins (IDPs) and the mutations identified in 1918 PB1-F2 crucial for the virulence maps within the internal intrinsically disordered region (IDR). The interactome analysis of 1918 PB1-F2 revealed that an essential protein in type I IFN signaling pathway is bound and co-degraded with 1918 PB1-F2, but not with PR8 PB1-F2. The results suggest that 1918 PB1-F2 hijacks the mediator for IFN signaling into proteosomal degradation resulting in the inhibition of antiviral responses. Our results can explain, in part, the molecular mechanism of severe pathogenicity of pandemic 1918 influenza virus. The identification of the molecular determinants of highly pathogenic 1918 PB1-F2 and the host target is useful for future design of novel antiviral strategies against influenza infections.

O4

Epidemiology of Influenza Infection Among Pregnant Women and Children Under 6 Months in Mongolia, 2013/14 Season: A Prospective Cohort Study

L Chaw [1], A Burmaa [2], T Kamigaki [1], C Urtnasan [2], I Od [3], G Nyamaa [2], P Nymadawa [2,4], H Oshitani [1]

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Background
Pregnant women and children under 6 months old are known to have high risk for influenza-related hospitalizations and developing severe complications. Maternal influenza vaccination could be the best preventive measure for both groups. Disease burden information for both groups is sparse, especially among pregnant women. Hence, we aim to assess the influenza disease burden in a community.

Methods
From Oct 1st 2013 to April 31st 2014, we are conducting a prospective cohort study with pregnant women and children under 6 month's old living in Baganuur district, Ulaanbaatar, Mongolia. Influenza-like illness (ILI) cases are identified via biweekly follow-up calls. For those identified, illness information is collected and cases confirmed by rapid test kits (QuickNavi™ - Flu+RSV). Demographic, household, antenatal and newborn information are obtained from the whole study population.

Results (as of March 21st)
In total, 620 pregnant women are enrolled where their median age is 26 (range: 16-43) and median household size is 4 (range: 1-12). Among them, 111 (17.9%) ILI cases were detected. Out of the 105 samples tested (99.5%), 9.9%, 1.8%, and 1.8% tested positive for influenza A, influenza B, and RSV respectively. Among those positive for influenza A, the median age at infection is 31 (range: 21-41) and 6/9 (66.7%) got infected during the second trimester.

Among the enrolled 606 children under 6 months, 187 (30.9%) ILI cases were detected. Out of the 148 samples tested (79%), 3.7%, 2.7%, and 3.2% tested positive for influenza A, influenza B, and RSV respectively. Among those positive for influenza A, the median age at infection is 4.8 months (range: 4.3-6.0). The youngest case got infected with influenza B at 28 days old.

As data collection is ongoing, any changes and additional data will be presented at the conference.

Conclusion:
Higher incidence of influenza A (1.8%) was observed for pregnant women, when compared to influenza B and RSV. For children under 6 months old, similar incidence was observed for influenza A (1.2%), influenza B (0.8%), and RSV (1.0%).

O5

The ChILD-Score: A Standardized Clinical Outcome Parameter for Infants and Children with Influenza-Like Illness (ILI)

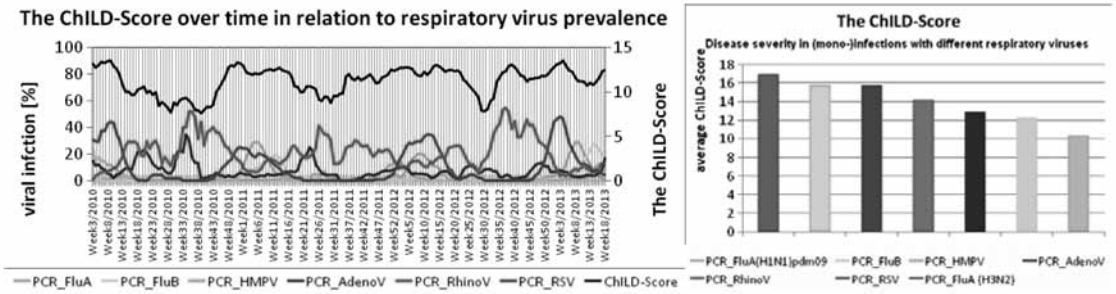
Barbara Rath, Tim Conrad, Katharina Karsch, Franziska Tief, Patrick Obermeier, Xi Chen, Lea Seeber, Eleni Adamou, Janine Reiche, Brunhilde Schweiger

Department of Pediatrics, Division of Pneumonology-Immunology, Charité University Medical Center, Berlin, Germany

Background:
The standardized assessment of disease severity in infants and children with influenza and other respiratory viruses provides a challenge. In adults, “pneumonia” and/or “hospitalization” have been used to discriminate mild from severe disease. Radiography however, is rarely performed in children, and pediatric hospitalizations depend on a number of factors, including parental support, social circumstances and the availability of age-appropriate resources. Well-differentiated universal scores are urgently needed as clinical outcome parameters in influenza-like illness (ILI) surveillance and antiviral research.

Methods:
The CHILD-Score was established in the context of the Charité-Influenza-Like-Disease (=ChILD) Cohort, a quality management program for children with ILI at the Charité Department of Pediatrics in collaboration with the National Reference Centre for Influenza at the Robert Koch Institute in Berlin, Germany. The ChILD Score, a 10-minute point-of-care assessment on tabloid computers, was performed on all patients fulfilling ILI case criteria, independent from routine care. The same quality management team also obtained nasopharyngeal swabs for blinded RT-PCR at the RKI. The ChILD Score is based on 22 detailed clinical criteria to reflect the full spectrum of disease presentations in infants and children aged 0-18 years. ChILD Score data are in full compliance with data standards and terminologies issued by the Clinical Data Interchange Standards Consortium (CDISC).

Results:
From January 2010-April 2013, a total number of 3542 infants and children were assessed prospectively. ChILD-Scores were normally distributed across all pediatric age strata, allowing cross-cohort comparison. Throughout >3 years of pediatric emergency room and inpatient surveillance, influenza, RSV, human metapneumovirus (HMPV), human rhinovirus (HRV), and adenovirus (AdenoV) affected disease severity differently. RSV caused significant disease severity, whereas influenza A(H1N1)pdm09 disease was the least severe.



Conclusions:
The ChILD-Score shows promise as an objective tool for the quantification of ILI disease burden at the point-of-care, with a focus on disease severity rather than disease incidence or prevalence. The ChILD-Score provides an important tool for standardized physician assessments of disease severity in multi-center clinical trials of antivirals, as well as for the monitoring of real-world vaccine effectiveness.

O6

Sustained Viremia and High Viral Load in Sputum are Associated with Death in Adults with Adenovirus Pneumonia

Li Gu, Jiu-Xin Qu, Bing Sun, Xiao-Min Yu, Hui Li, Bin Cao*

Department of Infectious Diseases and Clinical Microbiology, Beijing Chao-Yang Hospital, Beijing Institute of Respiratory Medicine, Capital Medical University, Beijing, China
Drs. Li Gu, Jiu-Xin Qu and Bing Sun contributed equally to the paper.
**Correspondence: Bin Cao (caobin1999@gmail.com)*

Objectives:
Severe adenoviral (AdV) pneumonia with high mortality has raised concerns among immunocompetent adults. But the mechanism of fatality is still unknown. This study investigated the relationship between viremia and viral load in respiratory tract secretions and clinical outcome.
Methods:
Laboratory confirmed hospitalized adenovirus pneumonia adults were prospectively enrolled in Beijing Chao-Yang hospital from March to June 2013. Clinical data and whole blood and sputum samples from both acute and convalescent period from such of patients were collected. Quantitative real-time polymerase chain reaction (PCR) was performed to quantify adenovirus load in whole blood and sputum.
Results:
A total of 14 Adv pneumonia cases were consecutively enrolled during the study period and four of them died in ICU. There are no difference of age, gender and underlying diseases between death and survival groups.Clinical symptoms and signs had no difference between two groups, except for higher PSI score (101.25 ± 8 vs 46.50± 26.67, p=0.002) and dyspnea ratio (100% vs 20%, p=0.015) in death group. The mean time from disease onset to initial PCR test for sputum and whole blood was 6.23 days and no difference between two groups. On admission (5-7 days after onset of illness), 100% of fetal cases presented with viremia, as compared with 85% of survival cases (p=0.64). However, fetal cases presented longer duration of viremia compared with survival cases as evaluated by positive ratio of viremia 12-14 day after onset of illness (100% vs 66.7%, p=0.017).Compared to survival patients, dead patients also had higher initial sputum viral load (8.578 ± 2.115 vs 6.263 ± 1.225, p=0.023)
Conclusions:
Sustained viremia and higher viral load (more than 10^8copy/ml) in sputum might be risk factors for death of immunocompetent adults with severe adenovirus pneumonia.

Plenary Session 2: Influenza Impact and Management

PS2-1

Responding to Emerging RVI Threats: WHO Perspectives

Nahoko Shindo

World Health Organization, Geneva, Swizerland

PS2-2

Avian Influenza A(H5N1): Success and Challenges

Tawee Chotpitayasunondh

Queen Sirikit National Institute of Child Health, Thailand

Avian influenza (AI) is an infectious viral disease of birds but in some instance these agents have also able to cause serious infection in human such as A(H5N1), A(H7N9). Human A(H5N1) was first recognized in 1997 in Hong Kong SAR and re-emergence in 2003 among poultry and human in part of Asia, northeast of Africa and recently in Canada. As of 4 April 2014, a total of 662 cases of laboratory confirmed A(H5N1) virus infection, resulting in 391 deaths (59.1%), had been reported in 16 countries.

The clinical disease usually aggressive, rapid deterioration especially severe pneumonia or multiorgan failure resulting high fatality rate. Many studies had reported the better outcome with early antiviral therapy but also be recommended in patient with late presentation. The new highly efficacious intravenous antiviral agents is challenging for these severely ill patient which drug absorption may be impaired. High-dose corticosteroid should be avoided in severe influenza including A(H5N1) virus infection.The appropriate and effective supportive cares such as high frequency ventilator, ECMO, fluid therapy,etc also very essential in clinical practice guidline.Strictly isolation of patient and appropriate PPE for health care workers are important to control the hospital transmission of A(H5N1) virus.

Studies on A(H5N1) vaccines as a pre-pandemic vaccine for human had shown safety and immunogenicity in all age groups but H5 vaccine for poultry is remaining a controversial issue of their benefit.

Conclusion: The biomedical and clinical research are essential for better understanding the new knowledges to provide the appropriate strategies for treatment and prevention of severe avian influenza disease. Scientists are concerning A(H5N1) virus have highly potential to mutate into more transmissible among human and pose potential of pandemic threats to global public health. The well prepared plan is urgently needed in increasing the ability to respond to a future pandemic.

PS2-3

Influenza and Other RVIs in Pregnancy

Shigeru Saito

Department of Obstetrics and Gynecology, Graduate School of Medicine and Pharmaceutical Science for Research, University of Toyama, Toyama, Japan

Pregnant women and infants are at high risk of influenza-related complication, and a considerable number of pregnant women died from the pandemic (H1N1) 2009 worldwide. However, no maternal mortality occurred in Japan during this pandemic. I would like to review the reasons why maternal mortality did not occur in Japan.
First of all, Japan Society of Obstetrics and Gynecology (JSOG) released these informations to the homepage and media. JSOG recommended the followings: (i) prompt use of antiviral drugs for treatment of pregnant women; (ii) active use of antiviral drugs for prophylaxis after close contact with an infected person; (iii) vaccination against the pandemic (H1N1). Our nationwide survey revealed approximately 30,000 to 40,000 pregnant women may have been infected with pandemic (H1N1). 40,000-50,000 pregnant women took antiviral medicines for prophylaxis. Around the half of the infected pregnant women took antiviral drugs for prophylaxis and 90% of hospitalized pregnant patients took antiviral medicine within 48 hours after symptom onset. And approximately 60% of pregnant women were vaccinated within 1.5 months after availability of a vaccine against pandemic H1N1 influenza. The JSOG surveyed infants born to mothers who took oseltamivir or zanamivir, and found that prognosis of infants exposed to oseltamivir or zanamivir in utero were not adversely affected.

PS2-4

Did Neuraminidase Inhibitors Reduce Mortality During the 2009-10 Influenza Pandemic?

Stella Muthuri*, Sudhir Venkatesan*, Puja R Myles, Jo Leonardi-Bee,
PRIDE Consortium Investigators ‡, **Jonathan S. Nguyen-Van-Tam**

*(*joint first authors; ‡ full membership of the PRIDE Consortium may be viewed at: http://www.nottingham.ac.uk/research/groups/healthprotection/documents/pride/ms-lancet-resp-online-version.pdf)*

Neuraminidase inhibitors were widely used during the 2009–10 influenza A(H1N1) pandemic, but evidence for their effectiveness in reducing mortality is uncertain. We performed a meta-analysis of individual patient data to investigate the association between use of neuraminidase inhibitors and mortality in patients hospitalised with pandemic influenza.

We assembled data on 29,234 patients (all ages) hospitalised worldwide with laboratory confirmed or clinically diagnosed pandemic influenza A(H1N1)pdm09 virus infection between March 1, 2009 (Mexico), or April 1, 2009 (rest of the world), until March 14, 2011. We adjusted for both treatment propensity and potential confounders, using generalized linear mixed modeling, and assessed the association with time to treatment using time-dependent Cox regression shared frailty modelling.

Compared with no treatment, neuraminidase inhibitor treatment (irrespective of timing) was associated with a reduction in mortality risk (adjusted odds ratio [OR] 0•81; 95% CI 0•70–0•93; p=0•0024). Compared with later treatment, early treatment (within 2 days of symptom onset) was associated with a reduction in mortality risk (adjusted OR 0•48; 95% CI 0•41–0•56; p<0•0001). Early treatment versus no treatment was also associated with a reduction in mortality (adjusted OR 0•50; 95% CI 0•37–0•67; p<0•0001). These associations with reduced mortality risk were less pronounced and not significant in children. In adult ICU patients, later treatment (started >48h after symptom onset) was also associated with a reduction in mortality (adjusted OR 0•65; 95% CI 0•46–0•93; p=0•0183). There was an increase in the mortality hazard rate with each day's delay in initiation of treatment up to day 5 as compared with treatment initiated within 2 days of symptom onset (adjusted HR 1•23 [95% CI 1•18–1•28]; p<0•0001 for the increasing HR with each day's delay).

We advocate early instigation of neuraminidase inhibitor treatment in adults admitted to hospital with suspected or proven influenza infection.

Thursday, June 5

Plenary Session 3: Advances in Respiratory Virus Diagnosis and Treatment

PS3-1

Advances in Diagnostics for Influenza and RVI's

Lance C. Jennings

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Acute respiratory tract infections are the most common infections in humans, and respiratory viruses cause the majority of these. New respiratory viruses and their subtypes are continuing to be discovered. Sensitive molecular amplification methods, largely PCR, have become standard practice for the laboratory diagnosis of all previously known respiratory virus infections and those recently discovered. Because most respiratory viruses cause similar symptoms, multiplex assays have proved popular, allowing the detection of multiple pathogens in a single test. However, the detection of multiple pathogens in the same sample along with high virus detection rates in asymptomatic individuals raises concerns on causality with some respiratory viruses. Quantitation of respiratory viruses may address some of these concerns and lead to more timely information becoming available for patient management.

There are an increasing range of commercial assays becoming available utilizing differing molecular methods and primer designs. These automated fully validated assays with quality controlled reagents and the auto-calling of results offer increased sensitivity and rapid turnaround times for respiratory virus diagnosis, however few head-to-head comparisons are available to allow assessment of their clinical usefulness. These will be needed as there is an increasing requirement by regulators for the certification of diagnostic laboratories and the use of in vitro diagnostic devices which are FDA-approved or CE-marked.

Next generation sequencing, microarrays, with their associated informatics, which cover all clinically viruses and many bacteria (~50,000 virus genomes) are contributing to our understanding of the molecular epidemiology of influenza and other respiratory viruses, while other technologies such as the Loop-mediated isothermal amplification (LAMP) system show promise for improving the real-time detection and quantitation of respiratory viruses. Digital PCR allows the absolute measure of target nucleic acids and other new technologies allow the counting of virus particles. These technologies will in time provide new assay systems capable of high throughput, speed and with high sensitivity and specificity and lead to improved patient management and public health.

PS3-2

Corticosteroids for Respiratory Viral Infections: When Are They Useful, When Are They Harmful?

David Shu-Cheong Hui

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Cytokine dysregulation has been reported in severe acute respiratory infections (SARI) such as Severe acute respiratory syndrome (SARS), influenza A(H1N1)pdm09, H5N1 or H7N9 influenza, and the Middle East Respiratory Syndrome (MERS). During the major outbreak of SARS in 2003, systemic corticosteroids were widely used in Asia based on CT scan and histo-pathological evidence of bronchiolitis obliterans organizing pneumonia in some patients, yet with conflicting results. Moreover, use of high dose of systemic corticosteroids was associated with increased risks of nosocomial infections (including fatal disseminated fungal infection), and avascular osteonecrosis in patients with SARS. A RCT approved and conducted in the late phase of SARS in HK showed that the use of systemic corticosteroid could lead to prolonged viraemia versus normal saline as control whereas patients with acute exacerbations of asthma/COPD due to seasonal influenza who received systemic corticosteroids were also observed to have delay in viral clearance.

Some of the patients with H7N9 or MERS were also given systemic corticosetroids for ARDS and yet with no benefits. The use of systemic corticosteroids was associated with increased risk of nosocomial infections and higher mortality in patients hospitalized with severe influenza A (H1N1)pdm09 esp among those who received late or no administration of neuraminidase inhibitors. Except for those who need systemic corticosteroids for underlying illness (eg Addision' disease, connective tissue diseases, etc) or in the setting of clinical trials, it is advisable to prescribe low dose hydrocortisone only for cases of SARI of viral aetiology complicated by refractory septic shock and for those with acute exacerbations of asthma/ COPD.

References:

1) Hui DS, Lee N, Chan PK. Adjunctive therapies and immunomodulatory agents in the management of severe influenza. Antiviral Res. 2013;98(3):410-6.

2) Hui DS, Lee N. Adjunctive therapies and immunomodulating agents for severe influenza. Influenza Other Respir Viruses. 2013 Nov;7 Suppl 3:52-9.

PS3-3

Management of Adult CAP During Epidemic Influenza

Bin Cao

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Community-acquired pneumonia (CAP) refers to pneumonia acquired outside of hospital or long-term care facilities. Although CAP guidelines acknowledge respiratory viruses as a “cause” of adults with pneumonia, few recommendations are made regarding management, largely due to the short of data regarding prevalence and clinical severity, as most relevant data concern infants and children. In addition, the emergence of SARS, avian influenza A (H5N1), the 2009 pandemic influenza A (pH1N1) and the recent avian influenza A (H7N9) has emphasized the important role of respiratory viruses as causes of CAP.

Many microbial pathogens can cause CAP and the role of viruses may have been previously underestimated because of lack of appropriate diagnostic methods. In recent years, modern molecular techniques revealed that respiratory viruses accounted for about 22% of adult CAP cases. The most common viruses are influenza, parainfluenza, respiratory syncytial virus, metapneumovirus, and adenovirus in adults. Respiratory syncytial virus is more commonly encountered in elderly CAP patients. It has been reported that 29% to 55% of autopsied patients with A(H1N1) had evidence of bacterial coinfection. Though no consensus exists for patients with obvious viral community- acquired pneumonia need to be treated with antibiotics, the difficulties to clearly distinguish the cause of pneumonia make empiric use of antibiotics reasonable. The choice of antibiotics should follow the CAP guideline and narrow-spectrum antibiotics, not broad spectrum antobiotics are the appropriate choice as the main causative pathogens include S. pneumoniae, S. aureus, et al. Neuraminidase inhibitors for pneumonia caused by influenza viruses have been reported to reduce mortality, especially when used early. However, there is no clear role for use of specific antivirals to treat viral community-acquired pneumonia other than influenza virus. Further studies are needed to better understand the cause and pathogenesis of community-acquired pneumonia.

PS3-4

Widespread Use of Neuraminidase Inhibitors in Japan and Several Issues to be Resolved

Norio Sugaya

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Since the approval of the neuraminidase inhibitor (NAI) zanamivir as a drug for the treatment of influenza virus infection in Japan in 1999, and of oseltamivir in 2000, rapid diagnostic tests have been routinely performed by clinicians on patients who have an influenza-like illness, and patients whose test results have been positive, including otherwise healthy adults and children without any underlying illness, have usually been treated with NAIs. Using the rapid diagnostic test for influenza has enabled Japanese clinicians to accurately diagnose influenza and prescribe NAIs with confidence. Most of the cost of the rapid diagnostic test and NAI treatment is covered by the public health insurance systems. In addition to oseltamivir and zanamivir, the inhaled NAI laninamivir and intravenous NAI peramivir have been used in Japan since the 2010/11 season, bringing to four the total number of NAIs currently being used in hospitals and clinics nationwide.

Japan may have had the lowest case fatality rate for symptomatic illness (<0.001%, 198/20.7 million) in the H1N1/09 pandemic because of the universal implementation of early treatment with NAIs. A study of 1000 children hospitalized because of a H1N1/09 infection revealed that NAIs, primarily oseltamivir, had been used to treat 984 (98.4%) of them. Treatment of 88.9% of the children with NAIs was started within 48 hours after the onset of illness.

However, there have been some problems related to the routine use of NAIs to treat seasonal influenza. Neuropsychiatric disorders that were suspected of being adverse reactions to oseltamivir became a cause of concern in 2007, and the Ministry of Health, Labour and Welfare issued an emergency instruction to suspend the use of oseltamivir to treat patients between the ages of 10 and 19 years. Oseltamivir still cannot be prescribed for teenagers.

Japanese mass media sensationalized the emergence of the resistant viruses in the 2013/14 season. An outbreak in the northern city Sapporo caused by oseltamivir-resistant H1N1/09 virus was reported, and over 40 strains with H274Y mutations were detected.

The Japanese experience showed that medical expenditures decreased after the introduction of rapid diagnostic tests and NAIs, probably because of preventing hospital admissions for influenza.

Plenary Session 4: Antiviral Resistance and New Agents

PS4-1

Antiviral Resistance: Detection and Assessment of Biologic Consequences

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It is 15 years since the approval of the first of the neuraminidase inhibitors, Relenza (zanamivir), followed by Tamiflu (oseltamivir). Currently both zanamivir and oseltamivir are licensed globally for the treatment and prevention of influenza, with newer drugs peramivir and laninamivir licensed in parts of Asia only. While all the inhibitors are effective against all strains of influenza, since the drugs are chemically different and there are subtle differences in the neuraminidase active sites of different influenza viruses, there are mutations which only confer resistance to some of the drugs, and only confer resistance in certain subtypes. Some mutations are more common, such as the H275Y found in the N1 subtypes, and E119V and R292K in the N2 subtypes, which all confer oseltamivir resistance. Mutations can be detected by sequence analysis of the neuraminidase gene if it is a common mutation. However to confirm phenotypic resistance the virus needs to be amplified in either eggs or cells and tested in an enzyme inhibition assay. Structural studies can also provide valuable information on understanding why the mutations lead to decreased drug binding. Often clinical samples contain a mixed population of wild type and resistant viruses, hence amplification may not result in the same ratios as in the original sample. Resistance as defined by the fold difference compared to a wild type virus can also vary depending on whether a fluorescent or chemiluminescent enzyme assay is used. The impacts of the mutations on the virus fitness can be assessed by both in vitro and in vivo replication and drug sensitivity studies. Fitness can also be assessed by animal transmission studies. While many early mutants were compromised in their fitness, we have recently seen additional mutations which allow the neuraminidase to tolerate drug resistance mutations, without impacting on the fitness of the enzyme.

PS4-2

Antiviral Resistance in A(H7N9) Viruses

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Human infections with H7N9 avian influenza virus have raised concerns for an emerging pandemic influenza. Clinically, antiviral treatment with neuraminidase (NA) inhibitors reduced viral load in the throat swabs and improved clinical outcome in many H7N9 patients, even when treatment started 48 hours after illness onset. Persistent high viral load in spite of antiviral therapy was associated with adverse clinical outcome including the dependence on extracorporeal membrane oxygenation. An R292K mutation that confers significant resistance (>1,000 fold reduced sensitivity) to oseltamivir and peramivir and moderate resistance to zanamivir (>100 fold) has been reported in four out of more than 370 human H7N9 infections to date. The transmission potential of a human influenza A H7N9 isolate with a NA-R292K mutation was evaluated in the ferret model followed by genotyping assay to monitor the stability of the mutation in vivo. Both the H7N9 wild-type and the R292K variants transmitted at comparable efficiency to the direct or respiratory droplet contact ferrets. Genotyping assay identified the wild-type genotype gained dominance over the R292K mutant in inoculated or infected ferrets, suggesting H7N9 virus with the R292K mutation may transmit among ferrets but is not stably maintained in vivo. The emergence of the R292K NA inhibitor resistant variants should be closely monitored in H7N9 patients and would render the treatment with oseltamivir and peramivir ineffective, but i.v. zanamivir may remain a therapeutic option.

PS4-3

Advances in Therapeutics for Non-influenza Respiratory Virus Infections

Frederick G. Hayden

University of Virginia School of Medicine, Charlottesville, USA

Lunch Seminar 3

LS3

Universal Influenza Vaccination Program for Schoolchildren was Effective for Protection of Elderly and Young Children

Norio Sugaya

Department of Pediatrics and Department of Infection Control, Keiyu Hospital, Yokohama, Japan

In the past, Japan's influenza control strategy was to vaccinate schoolchildren based on the theory that so doing would prevent influenza epidemics in the community. A nationwide universal influenza vaccination program for schoolchildren was started in 1962, and during an approximately 20-year period from the early 1970s to the late 1980s, the vaccine coverage rate among schoolchildren ranged from 50% to 85% in Japan. During this period, schoolchildren were compulsorily vaccinated with influenza vaccine together at their schools. On the other hand, influenza vaccination was not recommended for elderly and high-risk patients. In 1987, however, new legislation allowed parents to refuse to have their children vaccinated against influenza. The Japanese government abandoned the universal vaccination program for schoolchildren in 1994, mainly because of lack of evidence that it was effective in preventing influenza epidemics in the community.

It recently emerged that excess deaths decreased in Japan during the period when the universal vaccination program for schoolchildren was in effect. Because over 90% of the excess deaths occurred in the elderly population, the most probable cause to be protection of the elderly by the herd immunity generated by the universal vaccination of schoolchildren.

Until the 1980s, the reports of influenza-associated encephalopathy were rare in Japan. However, after 1994, the year universal vaccination was stopped, the incidence of influenza encephalopathy increased sharply, and more than 100 encephalopathy deaths were reported annually from 1995 to 1999. The increased mortality in young children was attributable to the reduction in vaccine coverage rates among schoolchildren. Therefore, the Japanese universal vaccination program for schoolchildren not only protected elderly persons against influenza-associated mortality, but protected younger siblings of schoolchildren as well. Moreover, it was recently demonstrated that the universal vaccination for schoolchildren was effective in reducing the number of class cancellation days and absenteeism in the school.

In conclusion, the indirect protection of the elderly and young siblings against influenza and the direct protection of schoolchildren by the universal vaccination program for schoolchildren have been demonstrated through Japanese experience.

Lunch Seminar 4

LS4

Neuraminidase Inhibitors Therapeutic Effect, Drug Resistance, Viral Transmission and Shedding Studies Conducted by Niigata University

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Four kinds of neuraminidase inhibitors (NAIs) are used for influenza infections in Japan; oseltamivir, zanamivir, peramivir and laninamivir. In Niigata University we collect influenza specimens and clinical information directly from clinicians in various prefectures in Japan. We evaluate therapeutic effect of NAIs mainly by fever duration as observational studies. So far, fever duration is shorter in influenza A compared to B after the therapy and there are no differences among four drugs. Younger children took longer time until fever alleviation than older children and adults. In Japan, the period of school absenteeism for children infected with influenza virus was designated by the School Health and Safety Act. Current suspension period is after 5 days of fever onset. Our results of viral shedding after the therapy and evaluation of suspension period for children will be discussed.

We characterize type and subtype of our isolates, and screen all of A/H1N1pdm09 strains by cycling probe Real Time PCR whether to possess H275Y mutation in NA protein that confer resistance to oseltamivir and peramivir. In 2013-2014 season, we collected 414 nasopharyngeal samples from 6 prefectures in Japan, and 83 (41.7%) were A/H1N1pdm09, 25 (12.6%) were A/H3N2, 24 (12.1%) were B/Victoria lineage, and 67 (33.7%) were B/Yamagata lineage (as of 28 Feb 2014). One (1.2%) A/H1N1pdm09 had H275Y mutation in the NA. The samples showed 150 and 250 times reduction of susceptibility to oseltamivir and peramivir respectively, but it remained sensitive to zanamivir and laninamivir. The patient did not have history of travel or antiviral medication prior to his illness onset. Apart from our study, National Infectious Diseases Surveillance showed that the frequency of community acquired H275Y in Japan accounted for 62 (5%) of 1145 A/H1N1pdm09 isolates (as of 10 March 2014) and the spread of resistant influenza strains are a matter of concern.

Oral Presentations

O7

Middle East Respiratory Syndrome Coronavirus and the Effectiveness of Convalescent Plasma for the Treatment of Severe Acute Respiratory Infections of Viral Aetiology: A Systematic Review and Exploratory Meta-Analysis

J Mair-Jenkins [1], M Saavedra-Campos [2,3], K Baillie [4], P Cleary [3], FM Khaw [1], WS Lim [5], S Makki [1], KD Rooney [6], Convalescent Plasma Study Group, JS Nguyen-Van-Tam [7], **CR Beck** [7]

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Objectives
The objective of this study was to conduct a systematic review and exploratory meta-analysis to evaluate the clinical effectiveness of convalescent plasma, serum or hyperimmune immunoglobulin for the treatment of severe acute respiratory infections (SARIs) of viral aetiology, to help inform clinical management of MERS-CoV.

Methods
The study protocol was registered on PROSPERO (CRD42013005091). Healthcare databases and sources of grey literature were searched by two reviewers using a predefined search strategy. All records were screened against the protocol eligibility criteria by two researchers. Data extraction and risk of bias assessments were performed by paired independent reviewers using a piloted form. Results were narratively synthesized by viral aetiology for each research question. Exploratory *post hoc* random effects model meta-analyses were undertaken to pool studies irrespective of SARI viral aetiology. Statistical heterogeneity was quantified using I^2. Publication bias was examined through construction of funnel plots and assessed statistically using Egger's regression test.

Results
We identified 32 low or very low quality studies of SARS-CoV, Spanish influenza A(H1N1), avian influenza A(H5N1) and influenza A(H1N1)pdm09. We found consistent evidence for a reduction in mortality associated with convalescent plasma therapy, especially if given early after symptom onset. The evidence was strongest for SARS-CoV and influenza A(H1N1)pdm09 infections. The reported magnitude of benefit varied between studies and we encountered sources of clinical heterogeneity and potential confounding. Exploratory *post hoc* meta-analysis showed a statistically significant reduction in the odds of mortality following convalescent plasma use in SARI of viral aetiology compared with placebo or no therapy (n = 8 studies; pooled odds ratio 0.25; 95% confidence interval 0.14 to 0.45; $p < 0.001$; $I^2 = 0\%$). We found no evidence of serious adverse events or complications due to therapy. Evidence for other outcomes investigated was very limited.

Conclusion
Convalescent plasma therapy may reduce mortality in SARI of viral aetiology and appears safe. However, this is based on predominately low quality, uncontrolled studies. The precise magnitude of benefit for different viral aetiologies is unclear and likely depends on disease severity and timing of administration. Convalescent plasma therapy should be recommended for early use in patients hospitalised with MERS-CoV infection within the context of a clinical trial or other formal assessment of its effectiveness.

O8

Safety and Efficacy of MHAA4549A, a Novel Monoclonal Antibody for Treatment of Severe Influenza A

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Severe influenza in hospitalized patients represents a high unmet need with approximately 200,000 to 278,000 patients hospitalized with severe influenza infections annually in the United States alone (Zhou et al. 2012). Novel parenteral therapies that can be used in hospitalized patients are critical to addressing the threat of severe influenza. MHAA4549A is a human monoclonal IgG1 antibody to influenza A virus, cloned from a single human plasmablast cell isolated from an influenza vaccinated donor (Nakamura et al. 2013). This antibody binds to a highly conserved epitope on the influenza A hemagglutinin (HA) stalk region, which allows broad neutralization of the influenza A virus by blocking the HA mediated membrane-fusion event in the late endosome. Here we present preclinical and clinical data outlining the safety and efficacy of MHAA4549A.

Preclinical data demonstrates a significant response to lethal challenge in mice and ferrets in both group 1 (H1N1, H5N1) and group 2 (H3N2) strains of influenza A. The antibody demonstrated strong efficacy when dosed as late as 72h post-viral challenge. In combination with oseltamivir, a synergistic response was demonstrated when sub-efficacious doses of either drug were administered.

Phase 1 data in healthy volunteers was conducted at weight-based dose levels of 1.5mg/kg, 5mg/kg, 15mg/kg and 45mg/kg. MHAA4549A was safe and well tolerated at all tested doses and demonstrated a mean half-life of ~20 days (range 19.3−22.2). None of the subjects had anti-therapeutic antibodies when assessed up to 120 days post-administration. Preliminary data on a fixed dose of MHAA4549A (3600mg) was assessed in a human challenge model of influenza A based on the qPCR and $TCID_{50}$ of viral shedding. The subjects were also monitored for safety and tolerability in combination with oseltamivir.

In summary, MHAA4549A is a human monoclonal antibody that is being developed for treatment of severe influenza A. Pre-clinical and clinical data suggest that it is generally safe and well tolerated with a favorable PK profile. Clinical efficacy has been evaluated in an influenza challenge model to inform the potential of MHAA4549A to treat hospitalized patients with severe influenza A infection.

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O9

Glycan Masking of Hemagglutinin Elicits Broadly Neutralizing Antibodies Against H5N1 Avian Influenza Viruses

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The highly pathogenic avian influenza (HPAI) H5N1 virus, a known trigger of diseases in poultry and humans, is perceived as a serious threat to public health. There is a clear need for a broadly protective H5N1 vaccine or vaccines for inducing neutralizing antibodies against multiple clades/subclades. We constructed single, double, and triple mutants of glycan-masked hemagglutiinin (HA) antigens at residues 83, 127 and 138 (i.e., g83, g127, g138, g83+g127, g127+g138, g83+g138 and g83+g127+g138), and then obtained their corresponding HA-expressing adenovirus vectors and recombinant HA proteins using a prime-boost immunization strategy. Our results indicate that the glycan-masked g127+g138 double mutant induced more potent HA-inhibition, virus neutralization antibodies, cross-clade protection against heterologous H5N1 clades, correlated with the enhanced bindings to the receptor binding sites and the highly conserved stem region of HA. The immune refocusing stem-specific antibodies elicited by the glycan-masked H5HA g127+g138 and g83+g127+g138 mutants overlapped with broadly neutralizing epitopes of the CR6261 monoclonal antibody that neutralizes most group 1 subtypes. These findings may provide useful information in the development of a broadly protective H5N1 influenza vaccine.

O10

Efficacy of a Vaccine and Neuraminidase Inhibitors Against H7N9 Influenza Virus in Cynomolgus Macaques

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Since most humans do not have immunity against H7N9 avian influenza virus which outbreak occurs in China, development of vaccines and anti-viral drugs against the H7N9 virus is an urgent issue. We examined the efficacy of a vaccine candidate and NA inhibitors against the H7N9 virus using cynomolgus macaques. Firstly, we examined immunogenicity of an inactivated whole particle vaccine prepared from A/duck/Mongolia/119/2008 (H7N9), which was selected from our virus library. Subcutaneous inoculation with the vaccine induced neutralizing antibody against A/Anhui/1/2013 (H7N9) in sera. In the challenge infection with A/Anhui/1/2013 (H7N9), virus was detected for 2 days in the nasal swabs of vaccinated macaques, whereas virus was detected 7 days after inoculation in unvaccinated macaques. Secondly, oseltamivir phosphate or peramivir hydrate (30 mg/kg/day) was administered to unvaccinated macaques for 5 days after virus infection. No significant reduction in virus titers during treatment with either NA inhibitors were seen compared with those in untreated macaques. However, virus titers in the nasal samples of macaques treated with peramivir were significantly lower than those in the nasal swab samples of untreated macaques on day 6 post-infection. However, virus was detected on day 7 post-infection in the nasal samples of two of three macaques treated with either NA inhibitor. Furthermore, the virus with R294K mutation in NA was dominant in samples in one of three treated macaques. Altogether, although vaccination is effective to prohibit propagation of H7N9 influenza viruses, we need to monitor emergence of the resistant virus when NA inhibitors are used for treatment.

O11

Evaluation of Oseltamivir Prophylaxis Regimens for Reducing Influenza Virus Infection, Transmission and Disease Severity in a Ferret Model of Household Contact

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Objectives: The emergence of the pandemic influenza A(H1N1)pdm09 virus in 2009 saw a significant increase in the therapeutic and prophylactic use of neuraminidase inhibitors (NAIs) to mitigate the impact of this highly transmissible virus. Prior to the pandemic, many countries stockpiled NAIs and developed pandemic plans for the use of antiviral drugs, based on either treatment of high risk individuals and/or prophylaxis of contacts. However, to date there has been a lack of *in vivo* animal models to test the efficacy of NAI prophylaxis following exposure to an infected contact, in a household setting.

Methods: A ferret model of household contact was developed to study the efficacy of different prophylaxis regimens in preventing infection in contact ferrets exposed to influenza A/California/7/2009 A(H1N1) pdm09-infected 'index' ferrets. Index ferrets were naturally infected by 48 hours exposure to infected donor ferrets, prior to co-housing with contact ferrets. Prophylaxis regimens of contact ferrets consisted of once or twice daily dosing of oseltamivir phosphate (5 mg/kg) at 12- or 24-hour pre-exposure, or 24-hour post-exposure to infected index ferrets.

Results: Among the different prophylactic regimens, contact ferrets receiving oseltamivir prophylaxis twice daily from 24 hours pre-exposure or 12 hours post-exposure showed better outcomes than those receiving oseltamivir once daily. Benefits included a significant delay in the time to secondary infection, lower weight loss and higher activity levels. None of the prophylaxis regimens prevented infection or reduced the duration of viral shedding, although clinical symptoms did improve in ferrets receiving prophylaxis.

Conclusions: Different oseltamivir prophylaxis regimens did not prevent infections nor significantly reduce viral titres. However, oseltamivir prophylaxis did consistently result in a reduction of symptoms in infected ferrets. The combination of improved morbidity but little effect on viral shedding warrants further investigations in human.

O12

A Community Cluster of Influenza A(H1N1)pdm09 Virus Exhibiting Cross-Resistance to Oseltamivir and Peramivir in Japan

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Objectives

Between November and December 2013, all six A(H1N1)pdm09 viruses detected in Sapporo, the capital city of Hokkaido, Japan possessed an H275Y substitution in the neuraminidase (NA) protein, which confers resistance to oseltamivir and peramivir. No epidemiological link among the six cases could be identified and none of them had received NA inhibitors before specimen collection. We strengthen nationwide monitoring of A(H1N1)pdm09 viruses with the H275Y substitution since then.

Methods

A(H1N1)pdm09 viruses were subjected to NA gene sequencing and allelic discrimination assay to detect the H275Y substitution. The susceptibilities of viruses to four NA inhibitors (oseltamivir, peramivir, zanamivir and laninamivir) were determined by NA inhibitor susceptibility assay.

Results

As of March 2014, we found that 38 (39%) of 97 A(H1N1)pdm09 viruses tested possessed the H275Y substitution in Hokkaido. None of the 38 cases had received NA inhibitors before specimen collection. In another part of the country, 40 (2.8%) of 1,451 A(H1N1)pdm09 viruses tested possessed the H275Y substitution. Most of the cases were associated with the therapeutic use of oseltamivir or peramivir. The hemagglutinin and NA genes of the H275Y mutant viruses in Hokkaido were closely related to one another, suggesting clonal spread of a single H275Y mutant virus. All H275Y mutant viruses exhibited cross-resistance to oseltamivir and peramivir, but are sensitive to zanamivir and laninamivir.

The largest cluster of A(H1N1)pdm09 viruses with the H275Y substitution occurred in Newcastle, Australia, in 2011: 29 (15%) of 191 A(H1N1)pdm09 viruses possessed the H275Y substitution. The H275Y mutant viruses detected in the cluster in Australia possessed three additional substitutions (V241I, N369K and N386S) in the NA protein, which may offset the destabilizing effect of the H275Y mutation. All H275Y mutant viruses detected in Hokkaido possessed V241I, N369K and N386K substitutions.

Conclusions

A community cluster of influenza A(H1N1)pdm09 virus exhibiting cross-resistance to oseltamivir and peramivir occurred in Hokkaido, Japan. Surveillance of antiviral-resistant A(H1N1)pdm09 viruses should be continued for public health planning and clinical recommendations for antiviral use.

This study was carried out by collaboration with the influenza virus surveillance group of Japan.

O13

Novel Host-Targeted Approach for Control of Influenza Virus Infection

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OBJECTIVES: A novel approach in combating influenza infection is to target host cellular receptors using protein-based biologics that recognize sialic acid (SA). This approach can prevent viral attachment during initial stages of infection and avoids drug resistance issues associated with virus-targeted therapies. Here we report the engineering and characterization of multivalent proteins based on SA-recognizing carbohydrate-binding modules (mCBMs) and their potential in preventing influenza virus infection.

METHODS: mCBMs were generated as tandem-repeats or as oligomers using PCR-based cloning techniques using genes encoding the SA-binding domain from bacterial sialidases, *V. cholerae* nanH or *S. pneumoniae* nanA and the *P. aeruginosa* pseudaminidase trimerisation domain. To characterise binding of mCBMs to SA, SPR and fluorescence imaging were used. To examine antiviral activity, mCBMs were tested in MDCK and A549 cell lines against different influenza A and B strains. *In vivo* efficacy of mCBMs was examined in BALB/c mice where mCBMs were intranasally administered as single or repeat doses (0.1-500µg/mouse) up to 7d before or 24h after lethal challenge with either mouse-adapted influenza A/WSN/1933 (H1N1) or A/California/2009 (H1N1). Mouse weight loss, survival rates, viral titres and immune response levels were assessed.

RESULTS: Multivalent CBMs exhibited nanomolar binding for SA compared to monomeric forms, with interaction being promiscuous and linkage-independent. Cell protection by mCBMs was observed against influenza A/WSN/1933, A/Udorn/1972 (H3N2), A/PR/8/1934 (H1N1) and B/Hong Kong/1973 strains with EC_{50} values between 0.39µM–4µM. Fluorescence imaging of mammalian cells demonstrated mCBMs targeting cell surface SA receptors with binding abrogated following sialidase treatment. mCBMs conferred protection *in vivo* against lethal doses of both influenza A/WSN/1933 and A/California/2009 viruses, with a hexameric mCBM (*Sp*2CBMTD) demonstrating 100% survival when given as a single 1µg dose 7d before challenge, with repeat dosing being the most beneficial regimen for complete protection and no weight loss. Moreover, anti-viral antibodies were detected in all treated mice. Furthermore, stimulation of inflammatory mediators by mCBMs may contribute towards their protective ability.

CONCLUSIONS: Targeting host cell SA receptors using engineered mCBMs can prevent influenza infection when administered repeatedly in advance of viral challenge. mCBMs may also have a broader application against other SA-binding respiratory pathogens.

O14

Prevention of Lethal H7N9 Influenza Virus Infection by a Novel Receptor-Binding Protein

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OBJECTIVES: Compounds targeting cellular factors that are essential for influenza virus replication represent a novel, attractive approach to antiviral therapy. Such targets are less susceptible to mutation; therefore, the likelihood of drug-resistant influenza variants emerging is low. Here we examined the efficacy of a novel host-targeted biologic that was designed to mask specific sialic acid–containing cellular receptors against lethal infection of mice with the A/Anhui/1/2013 (H7N9) influenza virus.
METHODS: A multivalent sialic acid–binding protein, *Sp*2CBMTD, was genetically engineered using sialic acid–binding domains derived from bacterial sialidases. To explore the antiviral activity of this biologic in vivo, we administered the biologic intranasally as a single dose (0.1, 1, 10, or 100 µg) to BALB/c mice (6-8 weeks old) at 7, 3, or 1 day before H7N9 virus infection or at 6 or 24 hours post-infection. Administration of 2 or 3 doses of the biologic were also evaluated. Mice were lightly anesthetized with isoflurane, infected intranasally with 10 MLD_{50} of A/Anhui/1/2013 (H7N9) virus, and monitored daily for survival and weight loss. Viral lung titers, histopathologic changes, and pulmonary expression of cytokines and chemokines were assessed. Animals that survived the initial H7N9 infection were subsequently re-infected with a higher dose of homologous H7N9 virus or heterologous H5N1 virus.
RESULTS: A single administration of *Sp*2CBMTD (10 or 100 µg) protected 80%-100% of animals when administered 7 days before the lethal challenge. Repeated dosing with *Sp*2CBMTD before H7N9 virus infection provided the most beneficial protection and resulted in 100% survival of mice, even at the lowest dose (0.1 µg). *Sp*2CBMTD administration improved H7N9 virus clearance from lungs at later times post-infection, decreased the number of influenza-positive cells, and reduced influenza-induced lung pathology. Moreover, *Sp*2CBMTD stimulated the expression of pro-inflammatory mediators. Administration of the biologic did not interfere with the development of serum anti-HA antibodies, and the level of immune response was sufficient to protect against viral re-infections.
CONCLUSIONS: *Sp*2CBMTD, a novel host-targeted compound, showed high potency for preventing newly emerged H7N9 influenza virus infections. This host-targeted approach holds promise for preventing infection by either emerging or seasonal influenza strains.

Plenary Session 5: Influenza Vaccines

PS5-1

Measuring Influenza Vaccine Effectiveness, Challenges and Needs

Alain Moren, Marta Valenciano, Esther Kissling, on behalf of the I-MOVE network
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The content of influenza vaccines is reformulated every year and study designs used to measure their post marketing direct effect (influenza vaccine effectiveness-IVE) face major operational and methodological challenges. The test negative design (TND) is frequently used including in multicentre studies. It compares vaccine coverage between influenza like illness cases laboratory confirmed (RT-PCR) as influenza and ILI cases testing negative. The study design enables estimates, each season and during pandemics, of early and late IVE for influenza sub types, by age groups, target groups for vaccination, and control for positive and negative confounders. Results suggest that IVE is moderate, mostly ranging between 40 and 60%, with departure from those ranges as for example during the 2009 pandemic (IVE against A(H1N1)pdm09 > 70%) and in 2012 with IVE < 20% against A(H3N2) among elderly. Critical questions remain and require international collaboration and funding for their response. Among them: to study the potential decrease of IVE in the season suggesting either waning immunity or viral changes; to explore the role of former influenza vaccinations and the effect of natural immunity; to obtain larger sample sizes and provide IVE early in the season for each sub type and by type and brand of vaccines as requested by regulatory agencies; to develop large studies investigating simultaneously correlates for protection and effectiveness; to evaluate the validity of TND to measure VE against severe influenza at hospital level. Since 2013 the Global Influenza Vaccine Effectiveness (GIVE) network gathers scientists from the European Union, US, Canada, New Zealand and Australia and provides the WHO committee on vaccine content with IVE estimates that complement the laboratory components of the decision.

PS5-2

Influenza Vaccines for the Elderly

Ann R. Falsey
University of Rochester School of Medicine, Rochester, USA

Influenza is a major cause of morbidity and mortality in older adults. It is well recognized that the effectiveness of inactivated influenza vaccines (IIV) is consistently lower in adults over age 65 years compared with younger adults. Changes in the immune response associated with aging result in diminished antibody and cellular immune responses to vaccination. A number of strategies have been explored to improve vaccine efficacy in older age groups including the use of adjuvants, intradermal injection and the use of higher doses of antigen. Vaccines adjuvanted with ASO3 and MF59 and vaccines containing 60ug of hemagglutinin have been shown to be well tolerated and to produce a significantly greater serum antibody response compared to standard IIV. Two large efficacy trials have recently been conducted to assess whether higher serum antibody titers translate into improved vaccine efficacy in older adults. In global trial of 43,000 elderly adults comparing ASO3 adjuvanted IIV to non-adjuvanted vaccine, the relative efficacy was 12.1% and superiority was not established. Influenza infection rates during the population were low due to emergence of influenza 2009 H1N1 and may have impacted study results. However, in a subsequent trial of 32,000 older adults in the US and Canada, Fluzone® high-dose was compared to standard dose Fluzone® and a relative efficacy of 24.2% (9.7;36.5) was shown against any laboratory confirmed strain of influenza meeting predefined superiority criteria. Relative efficacy for high-dose vaccine was 32.4% (8.1; 50.6) in persons over age 75 years. Local reactions were more common in the high-dose group but were generally mild and transient. Serious adverse event rates were not significantly different and were low in both vaccine groups. Progress is being made in the quest for more efficacious influenza vaccines for older adults; however, continued efforts are needed to protect this vulnerable population from influenza infection.

PS5-3

Induction of Neutralizing Antibodies by Inactivated Intranasal Influenza Vaccine and Characteristic of Induced Secretory-IgA Antibodies in Human

Hideki Hasegawa

Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

The 2009 pandemic of influenza virus highlighted the difficulty in predicting the subtype and strain of influenza viruses which cause a coming pandemic. This fear of an emerging pandemic of new influenza underscores the urgency of preparing effective vaccines to meet the pandemic. One mean to mitigate current concerns is to develop a flu vaccine that is functional against drift influenza viruses. In our current situation, in which we can't predict which strain will cause a pandemic, cross-protective immunity plays a particularly important role in preventing the spread of highly pathogenic influenza viruses.

Intranasal administration of a vaccine induces cross-protective secretory IgA (S-IgA) antibodies on the surface of nasal mucosa which is not induced by parenteral injection of the vaccine. S-IgA antibody in nasal mucus can, unlike serum IgG, prevent homologous and heterologous virus infection. However little is known about the quaternary structures and neutralizing potencies of the polymeric S-IgA antibodies in human. In this study, antibody responses induced by intranasal vaccination with a seasonal influenza viruses and Highly Pathogenic Avian Influenza virus (HPAIV) A(H5N1) Whole Inactibvated Virion (WIV) were measured in serum and nasal wash samples of healthy adult volunteers. Moreover the neutralizing ability of S-IgA antibodies and those structures were analyzed. The result showed that the intranasal vaccination of WIV can induce neutralizing antibodies both in serum and nasal mucus in human. Moreover, the polymeric S-IgA antibodies induced by intranasal vaccination play a pivotal role in cross-protection and neutralization of the virus.

Special Evening Seminar

ES

Current Progress in Influenza Research

Yoshihiro Kawaoka

University of Tokyo, Tokyo, Japan/ University of Wisconsin, Madison, USA

Friday, June 6

Plenary Session 6: Respiratory Virus Infections

PS6-1

Paramyxovirus Impact (RSV, HMPV, PIV) in Children; New Studies of RVIs

W. Abdullah Brooks

ICDDR,B, Bangladesh/ Bloomberg School of Public Health Johns Hopkins University, USA

PS6-2

Molecular Epidemiology of Human Rhinovirus (HRV) in Patients with Asthma

Hirokazu Kimura [1], Hiroyuki Tsukagoshi [2], Akihide Ryo [3], Haruyuki Ishii [4], Naoki Shimojo [5], Kazunori Oishi [1]

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Recent studies strongly suggest that various respiratory viruses may be associated with the initiation and/or exacerbation of asthma. A phenotype of the viral infection-associated asthma is now recognized as “virus-induced asthma”. Up to the present, many types of respiratory viruses have been confirmed. Human rhinovirus (HRV) belongs to the genus Enterovirus and family Picornaviridae. HRV is typically causative agents of mild acute respiratory infection (ARI), such as the common cold. In addition, HRV has been implicated as an agent of more severe ARI such as bronchiolitis, pneumonia, and virus-induced asthma. HRV are genetically classified into three species, HRV-ABCs. HRV-A and -C have many genotypes (maybe over 100 genotypes) and these viruses may be associated with ARI and virus-induced asthma. However, molecular epidemiology of HRV in patients with asthma are not exactly known. From the background, we have studied regarding epidemiology and genetic analysis of various respiratory viruses in the patients with ARI and/or asthma. As a result, we found that various genotypes of HRV-A and -C may be associated with virus-induced asthma. In this lecture, we summarize the molecular epidemiology of HRV in the patients with asthma.

PS6-3

Lower Respiratory Tract Viral Infections in Children, Asian Perspectives

Yuan Qian, Runan Zhu, Linging Zhao, Jie Deng, Fang Wang, Yu Sun
Laboratory of Virology, Capital Institute of Pediatrics, Beijing, China

Acute lower respiratory tract infection (LRTI) is one of the most important causes for morbidity and mortality in children, especially those younger than 5 years old. Viral infection is the most common cause for LRTI world wide and Asia as well. The viruses causing LRTI are RSV, Adenovirus, influenza virus (A and B), parainfluenza virus (1, 2, 3, 4). In recent years, metapneumovirus, bocavirus, coronaviruses have been more and more reported. Rhinovirus which was thought to cause common cold was found to be one of the viruses causing severe LRTI. Most of the studies about viral pathogens for LRTI were prospective and nasopharyngeal aspirates were used as clinical specimens. Virus isolation and antigen detection by immunofluorescence test are still the most reliable methods for etiological studies for LRTI in many laboratories and PCR/RT-PCR, real time PCR are becoming popular techniques in some of the laboratories in Asian countries. Because the multiplex PCR/RT-PCR were employed, it has been recognized that co-infection by more than one virus in one single child are common.

PS6-4

Non-Pharmaceutical Interventions for RVIs- Effectiveness and Consequences

Benjamin John Cowling
School of Public Health, The University of Hong Kong, Hong Kong SAR, China

A variety of “non-pharmaceutical” interventions are available to control epidemics of respiratory virus infections. At the individual level, hand hygiene is one of the most commonly recommended interventions and is known to reduce the risk of acute respiratory illnesses and gastrointestinal illnesses, although this intervention has only modest efficacy against influenza virus infections in randomized controlled trials. Surgical face masks can be used both by infected persons, for source control, and by uninfected persons to reduce susceptibility. Again, randomized trials have identified only modest efficacy of surgical type face masks. The hypothesis that influenza is an anisotropic infection (Milton et al. 2013) was used in re-analysis of results of controlled trials of face masks and hand hygiene for influenza to estimate that fine particles below 5 microns have an important role in influenza virus transmission in households in Hong Kong and Bangkok, and that the non-pharmaceutical interventions may not have benefited participants. Apart from individual-level measures, non-pharmaceutical interventions are also available at the community level including school closures and other measures to increase social distancing. The latest evidence on face masks and hand hygiene for respiratory viruses, and the potential consequences, will be discussed.

PS6-5

Unrecognized Burden of Adult RSV Infections in Asia

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Background. Complications and outcomes of adults hospitalized with Respiratory Syncytial Virus (RSV) infection is unclear, particularly among the Asian population.

Methods. We performed a large retrospective cohort study on adults hospitalized for virologically confirmed RSV infections during 2009–2011 (N = 607) in Hong Kong. Adults hospitalized for seasonal influenza during the period were used for comparison (N = 547). Main outcome measures were all-cause death, respiratory failure requiring ventilatory support, and hospitalization duration.

Results. The mean age of RSV patients was 75 (+/-16) years; 87% had underlying conditions. Lower respiratory and cardiovascular complications were diagnosed in 71.9% (pneumonia, 42.3%; acute bronchitis, 21.9%; chronic obstructive pulmonary disease/asthma exacerbation, 27.3%) and 14.3% of patients, respectively; 12.5% had bacterial superinfections. Supplemental oxygen and ventilatory support were required in 67.9% and 11.1%, respectively.

Crude all-cause mortality was 9.1% and 11.9% within 30 days and 60 days, respectively; mean length of stay of survivors was 12 (+/-13) days. Advanced age, radiographic pneumonia, requirement for ventilation, bacterial superinfection, and elevated urea level and white blood cell count were independently associated with poorer survival. Systemic corticosteroid use was associated with longer hospitalization and secondary infections. The overall outcomes of survival and length of stay were not significantly different from those in seasonal influenza.

Conclusions. RSV can cause severe lower respiratory complications and high mortality in older adults, similar to seasonal influenza. The unmet need for antiviral therapy and vaccination against RSV in adults should be promptly addressed.

PS6-6

Prevention and Treatment Strategies for Respiratory Syncytial Virus Infections

John DeVincenzo

The University of Tennessee School of Medicine, Knoxville, USA

PS6-7

Challenge of RVIs in Immunocompromised Hosts

Michael G. Ison

Divisions of Infectious Diseases and Orgen Transplantation, Northwestern University, Evanston, USA

Respiratory viral infections are associated with significantly increased morbidity and mortality among immunocompromised individuals. The epidemiology, risk factors, and outcomes of RVI in immunocompromised adults and children depend on the specific immune defect. With the wider availability of molecular diagnostics, the importance of RVI in immunocompromised. In this talk, I will discuss emerging data on the epidemiology, pathogenicity, and outcomes of RVI and review emerging data on noval antiviral therapies for influenza, RSV, PIV and adenovirus in immunocompromised patients.

Notes

Poster Abstracts

P1

Prognosis of 18 H7N9 Avian Influenza Patients in Shanghai

Shuihua Lu [1*,#], Tao Li [1*], Xiuhong Xi [1*], Qingguo Chen [1*], Xuhui Liu [1*], Binxing Zhang [1*], Jiaxian Ou [2*], **Jie Liu** [2*], Qin Wang [2*], Biao Zhu [3], Xinian Liu [1], Chunxue Bai [2], Jieming Qu [4], Hongzhou Lu [1], Zhiyong Zhang [1], Yuanlin Song [1,2,5#]

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Purpose: To provide prognosis of an 18 patient cohort who were confirmed to have H7N9 lung infection in Shanghai.
Methods: Patients' history, clinical manifestation, laboratory test, treatment strategy and mortality were followed and recorded for data analysis.
Results: A total of 18 patients had been admitted into Shanghai Public Health Clinical Center from April 8th to July 29, 2013. 22.2% of the patients were found to have live poultry contact history and 80% were aged male patients with multiple co-morbidities including diabetes, hypertension and/or chronic obstructive pulmonary disease (COPD). This group of patients was admitted to the clinical center around 10 days after disease onset. According to laboratory examinations, increased C reactive protein (CRP), Procalcitonin (PCT), Plasma thromboplastin antecedent (PTA) and virus positive time (days) were indicative of patients' mortality. After multivariate analysis, only CRP level showed significant prediction of mortality (P=0.013) while results of prothrombin time (PT) analysis almost reached statistical significance (P=0.056).
Conclusions: H7N9 infection induced pneumonia of different severity ranging from mild to severe pneumonia or acute lung injury/acute respiratory distress syndrome to multiple organ failure. Certain laboratory parameters such as plasma CRP, PCT, PTA and virus positive days predicted mortality of H7N9 infection and plasma CRP is an independent predictor of mortality in these patients.

P2

Clinical Presentation, Management and Outcomes of Influenza in Africa: Systematic Review, 2009-2013

Martin Herbas Ekat

Ministry of Health, Brazzaville, Republic of Congo

BACKGROUND:
This paper aims to review the effectiveness of diagnostic and management of influenza in Africa, specifically mortality, treatment and outcomes.
METHODS:
In two times, we searched the online databases PubMed™ and Scopus™ for articles and abstracts published in English and French between January 2003 and December 2013, with the following terms :(influenza OR flu) AND (clinical) AND (management OR outcomes) AND (Africa) at the first time, and online databases of internationals conferences for the abstract who do not meet the consent of the editor of scientific journals at the second time. Cross- sectional, longitudinal studies and randomized clinical trial on influenza were selected when clinical, management and outcomes were reported.
RESULTS:
Patients with influenza were more likely to present with fever as initial and main symptom, followed by shortness of breathing, cough, muscle & joint pain, sore throat, hemoptysis, dyspnea, and gastrointestinal complaints (vomiting and diarrhea), pneumonic infiltrations in the chest. For the diagnostic nasal secretions were collected in patients presenting with flu syndrome and follow-up by laboratory identification of viruses was performed by the ELISA technique using anti-A and anti-B monoclonal antibodies (immunocapture) and by isolation on MDCK cells, quantitative real-time polymerase chain reaction (qRT-PCR) assay of the upper respiratory tract is used increasingly to diagnose lower respiratory tract infections. Amongst samples analyzed for influenza; 1–45% had laboratory-confirmed influenza infections; including influenza virus A (H3N2) type, A (H1N1) type, A (H5N1) type and influenza virus B. All confirmed cases received oseltamivir in any setting. Among patient with influenza hospitalized in intensive care unit 90% had respiratory failure, 50% of the patients required invasive ventilation. Respiratory dysfunction can remain isolated but may also be associated with other dysfunctions or complications, such as, septic shock, seizures, myasthenia gravis exacerbation, Guillan-Barre syndrome, acute renal failure, nosocomial infections and biological disturbances. Among groups known to be at high risk of influenza-associated complications, Included age<5 years, asthma, cardiac disease, pregnancy, diabetes mellitus, active pulmonary tuberculosis and chronic malnutrition. Mortality rate was 28 – 68.4%. Female sex, age >15 years, and receiving the first dose of oseltamivir >2 days after illness onset, non vaccination against the virus the circulating influenza, cardiovascular complications and ventilatory associated pneumonia were identified as mortality predicting factors.
DISCUSSION:
The classic presentation of influenza in Africa is most often confused with malaria, low technical platforms limit the detection of virus in the samples. The progressive creation of influenza sentinel surveillance system will improve care in Africa

P3

Elevated Pancreatic Enzymes in Patients with H7N9 Influenza: A Cross-Sectional Analysis of 18 Patients

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Objective: To describe the prevalence of elevated pancreatic enzymes and its clinical significance, as well as its association with certain factors.
Methods: This is a retrospective cross-sectional clinical research. Diagnostic Criteria of H7N9: flu-like symptom and a positive result of H7N9 nuclear acid test by real-time RT-PCR according to WHO recommended protocol.
Results: 44.4% (8/18) of patients had evidence of elevated pancreatic enzymes. We did not find association between elevated pancreatic enzymes and factors including age, sex, alcohol abuse, biliary disease, ARDS, corticosteroid usage ,detectable virus in fecus or clinical outcome. One out of 8 patients had both amylaze and lipase over 5 times of upper limit of normal range, indicating probable pancreatitis.
Conclusions: Elevated serum pancreatic enzymes are prevalent in H7N9 influenza patients. H7N9 virus might have a potential of causing acute pancreatitis. Monitoring pancreatic enzymes should perhaps be included in the routine clinical management for human H7N9 influenza cases.

P4

Genetic and Antigenic Characteristics of Influenza Viruses Isolated in Egypt in Seasons 2010:2012

Nagwa Elkholy, Eman Fawzy, Marwa A. Hamid

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Influenza (flu) is a respiratory infection caused by a variety of flu viruses. Influenza A and B viruses are important human respiratory pathogens which transmitted mainly by droplets and aerosols originating from the respiratory secretions of infected people. Respiratory viral infections are a leading cause of morbidity, hospitalization, and mortality throughout the world. On April 15, 2009, novel swine-origin influenza A (H1N1) virus (S-OIV) was identified in specimens in the United States. In response to the spread of the new influenza A (H1N1) virus, the World Health Organization (WHO) declared pandemic influenza Phase 6 on 11 June 2009. All viruses are antigenically similar to the A/California/7/2009vaccine virus. Global Influenza Surveillance and Response System (GISRS) is actively monitoring the susceptibility of emerging influenza viruses to antiviral drugs. We need to study and provide information regarding circulating influenza subtypes and strains circulating. Such information is needed to guide decisions regarding influenza treatment and chemoprophylaxis and to formulate vaccine for the coming year. Samples from influenza-like illness (ILI) patients are collected through three successive seasons (2010-2012). Influenza viruses were isolated. It has been found that influenza viruses circulating and predominating in Egypt through season 2010 are A(H1N1)pdm09 which are genetically similar to California/7/2009 vaccine strain. Through season 2011 predominating strains are A(H1N1)pdm09 and B which are genetically similar to California/7/2009 vaccine strain & B/Brisbane/60/2008 vaccine strain respectively. Viruses predominated through season 2012 are H3N2 which are genetically similar to A/Victoria/361/2011 and the genetic group 3C. All isolates are resistant to M2 inhibitors (amantadine and rimantadine) but sensitive to neuraminidase inhibitors (oseltamivir and zanamivir).

Key words: Influenza, H1N1 pdm2009, isolation, RT-PCR, vaccine, antiviral agents.

P5

Interleukins Profile in Patients with Acute Respiratory Viral Infections

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Influenza and other acute respiratory viral infections (ARVI) the most common among human diseases, in the structure of infectious diseases they account for almost 70%.

Materials and methods

Where was studied cytokine profile in patients with ARVI. We were observed 30 patients in 18 to 58 years old who were treated in 2010-2011. Were determined different classes of cytokines in serum by enzyme-linked immunosorbent assay (ELISA).

Results

The level of cytokines was depended on the number of pathogens. The highest levels of pro-inflammatory interleukins (IL-2, IL-6, TNF-α) and the lowest – anti-inflammatory IL-4 is defined in patient with a combination of viruses in quantities of 5 or more compared to monoinfection.

Analysis of the data showed that in the acute phase was increased levels of all the studied proinflammatory cytokines – IL-2, IL-6, TNF-α ($p<0.001$) – in 8, 39, 9 times respectively relative performance of healthy individuals. Content of anti-inflammatory IL-4, by contrast, declined by 1.6 times ($p<0.001$).

In all cases the concentration of pro-inflammatory interleukins during early reconvalescence decreased ($p<0.001$), but remained at a high level without reaching the performance of healthy individuals. At the same time the level of anti-inflammatory IL-4 increased significantly – about 2.6 times the original level; renovation was well in excess of 1.5 times the indicator of healthy individuals ($p<0.001$).

In the acute phase of ARVI increased levels of pro-inflammatory cytokines was significantly higher, and depended on the severity of the disease. Level of IL-4 on the severity of the disease were independent. The concentration of pro-inflammatory IL- 2, IL-6, TNF-α and anti-inflammatory IL-4 in patients with ARVI, complicated by pneumonia, did not differ from that in patients with uncomplicated course.

Conclusions

In the acute period of ARVI were found raising of proinflammatory cytokines in serum which depended on the severity of the disease. Indicators of IL-4 were suppressed, but in early reconvalescence was increased concentrations of cytokines was observed in 2.6 times with respect to the initial level and even in excess of 1.5 times the indicator of healthy individuals.

P6

Effect of Human Rhinovirus Infection in Pediatric Patients with Influenza-Like Illness on the 2009 Pandemic Influenza A (H1N1) Virus

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Background: Some research groups have hypothesized that human rhinoviruses (HRVs) delayed the circulation of the 2009 pandemic influenza A (H1N1) virus [A(H1N1) pdm09] at the beginning of Autumn 2009 in France. This study aimed to evaluate the relationship between HRV and A(H1N1)pdm09 in pediatric patients with influenza-like illness in Beijing, China.

Methods: A systematic analysis to detect A(H1N1) pdm09andseasonal influenza A virus (FLU A) was performed on 4,349clinical samples from pediatric patients with influenza-like illness during the period June 1, 2009 to February 28, 2010, while a one-step real-time RT-PCR (rRT-PCR) assay was used to detect HRVin 1,146 clinical specimens selected from those 4,349 specimens.

Results: During the survey period, only one wave of A(H1N1)pdm09 was observed. The percentage of positive cases for A(H1N1)pdm09 increased sharply in September with a peak in November 2009 and then declined in February 2010. Data on the monthly distribution of HRVs indicated that more HRV-positive samples were detected in September (2.2%) and October (3.3%), revealing that the peak of HRV infection in 2009 was similar to that of other years. Among the1,146 specimens examined for HRVs, 21 (1.8%) were HRV-positive, which was significantly lower than that reported previously in Beijing (15.4% to 19.2%) ($P<0.01$). Overall, 6 samples were positive for both A(H1N1)pdm09 and HRV, which represented a positive relative frequency of 1.60% and 2.08% HRV, considering the A(H1N1)pdm09-positive and –negative specimens, respectively. The odds ratio was estimated to be 0.87(0.32; 2.44, CI95), $P=0.80>0.05$.

Conclusions: The results therefore indicate that HRVs and A(H1N1)pdm09 co-circulated in this Chinese population during September and October 2009, and that the HRV epidemic in 2009 did not affect A(H1N1)pdm09 infection rates in Beijing, China as suggested by other studies. However the presence of A(H1N1)pdm09 might explain the unexpected reduction in the percentage of HRV positive cases during the period studied.

Keywords: real-time PCR assay, human rhinovirus, A(H1N1)pdm09, pediatric patients, influenza-like illness

P7

Tropism of Avian Influenza A (H5N1) Virus to Mesenchymal Stem Cells and CD34+ Hematopoietic Stem Cells

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The presence of abnormal hematologic findings such as lymphopenia, thrombocytopenia, and pancytopenia were diagnosed in severe cases of avian influenza A H5N1. Whether direct viral dissemination to bone marrow (BM) cells causes this phenomenon remains elusive. We explore the susceptibility of the two stem cell types; hematopoietic stem cells (HSCs) and mesenchymal stromal cells (MSCs) isolated from human BM cells or cord blood, to infection with avian H5N1 viruses. For the first time, we demonstrated that the H5N1 virus could productively infect and induce cell death in both human stem cell types. In contrast, these activities were not observed upon human influenza virus infection. We also determined whether infection affects the immunomodulatory function of MSCs. We noted a consequent dysregulation of MSC-mediated immune modulation as observed by high cytokine and chemokine production in H5N1 infected MSCs and monocytes cocultures. These findings provide a better understanding of H5N1 pathogenesis in terms of broad tissue tropism and systemic spread.

P8

Different Immunity Elicited by Recombinant H5N1 Hemagglutinin Glycoproteins Containing Pauci-mannose, High-mannose, or Complex type N-Glycans

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Highly pathogenic avian influenza H5N1 viruses can result in poultry and occasionally in human mortality. A safe and effective H5N1 vaccine is urgently needed to reduce the pandemic potential. Hemagglutinin (HA), a major envelope protein accounting for approximately 80% of spikes in influenza virus, is often used as a major antigen for subunit vaccine development. In this study, we conducted a systematic study of the immune response against influenza virus infection following immunization with recombinant HA proteins expressed in insect (Sf9) cells, insect cells that contain exogenous genes for elaborating N-linked glycans (Mimic) and mammalian cells (CHO). While the antibody titers are higher with the insect cell derived HA proteins, the neutralization and HA inhibition titers are much higher with the mammalian cell produced HA proteins. Recombinant HA proteins containing tri- or tetra-antennary complex, terminally sialylated and asialyated-galactose type N-glycans induced better protective immunity in mice to lethal challenge. The results are highly relevant to issues that should be considered in the production of fragment vaccines.

Key words: H5N1, hemagglutinin, subunit vaccine, N-glycans, CHO cells

P9

A Monoclonal Antibody Recognizes a Highly Conserved Neutralizing Epitope on Hemagglutinin of H6N1 Avian Influenza Virus

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Objective: This report describes the identification of a hemagglutinin epitope at the globular head near the receptor binding site.

Methods: A monoclonal antibody, EB2 was prepared against the H6N1 avian influenza virus hemagglutinin. Flow cytometry of AIV-infected DF-1 cells and specific-pathogen-free embryonated eggs were used for testing the neutralizing activity of this mAb. To narrow down the binding region, partially overlapping HA fragments and synthetic peptides were used to map the epitope by immune-blotting.

Results: The linear motif RYVRMGTESMN, located at the middle of the HA protein, was identified as the epitope bound by EB2. Alignment of the EB2-defined epitope with other H6 AIVs showed that this epitope was conserved, and specific to H6. We propose that this motif is a linear B-cell epitope of HA protein, and is near the receptor binding site.

Conclusions: The identified epitope might be useful for clinical applications and as a tool for further study of the diagnosis, structure and function of the H6 AIV HA protein.

P10

Estimated Effectiveness of Influenza Vaccine Using Influenza Rapid Diagnostic Tests - Test-Negative Case-Control Study Among Japanese Children

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Objectives: In Japan, the influenza rapid diagnostic test (IRDT) has been used in daily clinical practice. Its sensitivity is more than 80%, and the specificity is 95 to 100%. In this study, we investigated the estimated influenza vaccine effectiveness (eVE) for medically attended children using the IRDT.

Methods: A prospective test-negative case-control study was performed in 24 hospitals during the 2013/2014 influenza season. The main epidemic viruses were A/H1N1pdm09 and B/Yamagata. All vaccinated outpatients aged 0 to 15 who had undergone IRDT and had a fever of 38°C and over were enrolled. All IRDTs used in this study can discriminate between influenza A and B. Patients with positive IRDT results were defined as cases and those with negative results were controls. Patient information was collected using questionnaires and medical records. This study was approved by the Keio University Ethics Committee (No. 2013216).

Results: All vaccine strains in this season (A/H1N1pdm09, A/H3N2, and B/Yamagata) matched the main epidemic viruses. Preliminarily, between November 2013 and March 2014, 2897 pediatric patients from 16 hospitals were analyzed. Among them, 674 were positive for influenza A, 841 were positive for influenza B, and 1382 were negative. The overall eVE was 47% (95% CI, 39-54%). The eVE for influenza A was 60% (95% CI, 52-67%). The influenza vaccine was effective against influenza A among the age groups of 1 to 5 years (72%, 95% CI, 64-79%) and 6 to 12 years (48%, 95% CI, 31-61%). However, the vaccine was not effective against influenza B in any age group (-1%, 95% CI, -19-14%). Ninety-one percent (1349/1476) of patients were diagnosed with influenza within 48 hours after the first episode of fever.

Conclusions: The test-negative case-control study using the IRDT among children in clinical settings was useful and showed similar VE to other reports based on the PCR test. As almost all patients with influenza-like illnesses are tested with IRDTs in Japan during influenza seasons, VE could be reported promptly in a large-scale study in Japan during each season.

P11

Phenotypic and Genotypic Significance of Influenza Viruses Identified in the Republic of Moldova

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An important segment of the clinical-epidemiological and virological surveillance system of influenza (ILI – influenza like infections), acute respiratory infections (ARI) and severe acute respiratory infection (SARI) morbidity is the results of the development and study of influenza viruses' phenotypic and genotypic properties. During the 2012-2014 period in the National Influenza Center recognized by WHO, in collaboration with Influenza Center of Institute "Cantacuzino", Bucharest and the WHO CC for Reference and Research on Influenza, National Institute for Medical Research, London were isolated in MDCK cells and MDCK - SIAT1 cultures and identified by haemagglutination inhibition test (HAI) 27 strains of influenza viruses: 10 strains of influenza A(H1N1)pdm, 6 – A(H3N2), and 11 – influenza B virus from positive in rRT-PCR samples collected from patients with presumptive clinical diagnosis "ILI", "ARI" , and "Pneumonia". Dominant influenza viruses during 2012-2013 influenza season were A(H1N1) pdm (48 %), and during 2013-2014 season - influenza A(H3N2) (95 %) viruses. Strains isolated and studied by HAI test were not significantly different from antigenic variants of reference influenza viruses and could be considered similar to A/California/7/2009 H1N1pdm and A/Texas/50/2012 H3N2 influenza viruses respectively. The phylogenetic trees (Northern Hemisphere) demonstrated that influenza viruses A(H1N1)pdm fall into genetic group 6C (common substitution D97N in gene HA1), influenza viruses A(H3N2) – genetic group 3C.3 (common substitutions N145S, V223I in gene HA1, and D158N in gene HA2 – resulting in the loss of a potential glycosylation site) and influenza B viruses – genetic groups 2 (common substitutions T121S, T75N, T181A, D196N in HA gene), and 3 (common substitutions S150I, N165Y, G229D, D196N).

It is important to note that all isolates of influenza viruses in neuraminidase inhibition assay were sensitive to neuraminidase inhibitors: Oseltamivir and Zanamivir and were similar to those included in the influenza vaccine formula recommended by WHO for 2012-2013 and 2013-2014 influenza seasons respectively.

Obtained results demonstrate that the segment regarding the highlight and evaluation of the phenotypic and genotypic properties of influenza viruses as component of the unique clinical-epidemiological and virological surveillance system of ILI, ARI and SARI morbidity has an important significance for the Republic of Moldova in the context of policy on influenza vaccine use, optimization of the treatment management and prophylaxis of mentioned infections, prognosis of the epidemic process and significant reduce of the negative impact on the health system.

Keywords: influenza virus, phenotypic, genotypic

P12

Antiviral Drug Profile of Human Influenza A and B Viruses Circulating in India: 2009-2014

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Background and Objective:
Antiviral drugs continue to be an important option for the treatment of influenza and probably the only option during the early phases of a pandemic. Limited number of drug classes licensed for treatment of influenza, and drug resistance to these raises concern. Two classes of drugs are presently licensed for treatment of influenza, M2 and neuraminidase inhibitors. M2 inhibitors are currently not recommended for treatment of influenza because of widespread resistance and resistance to neuraminidase inhibitors has been observed in seasonal and rarely to pandemic influenza. The study was undertaken to evaluate susceptibility of influenza viruses isolated from various parts of India to antiviral drugs.
Methods:
Influenza viruses isolated from 2009 to 2014 were analyzed genetically for known resistance markers by M2 and NA gene sequencing. Allelic discrimination rRT-PCR assay was used to assess H274Y mutation in clinical samples positive for A/H1N1pdm09 (n= 1245). Influenza A/H3N2 (340) viruses were tested for amantadine resistance (M2 gene; amino acid positions 26 to 34). 289 of the above A/H3N2, A/H1N1 pdm09 (237), and Type B (325) viruses were tested for Neuraminidase Inhibitor (oseltamivir) resistance (NA gene site H274Y (N1 subtype) , amino acid positions R118K, E119Q, D151E, R152K, I222V, R224K, E227D, H274Y, E276D, R292K, N294S and a Δ 244-247 (N2 subtype) and for Type B E119A, R152K, D198N/E, I222T, H274Y and R371K). Randomly selected influenza A(H3N2)(n=50) & A/H1N1 pdm09 (n=90) viruses and influenza B(n= 55) viruses were confirmed by phenotypic NAI assay.
Results:
In the study period, all A/H3N2 viruses were resistant to amantadine with S 3I N mutation in M2 gene. Two A/H1N1pdm09 viruses of 2013 showed resistant to oseltamivir with H 275Y mutation and increased IC 50 values in NAI assay which were also confirmed by allelic rRT-PCR using original clinical sample. All A/H3N2 and Type B isolates were sensitive to oseltamivir.
Conclusions:
In spite of extensive use of oseltamivir during pandemic, influenza viruses by and large remained susceptible to the drug. Sporadic emergence of pandemic influenza resistant strains emphasizes the need for continuous monitoring of drug susceptibility as a part of any National Influenza surveillance program in India.

P13

Innate CD8$^+$CD44^{hi} T Cells and IFN-γ Mediate Thymic Atrophy in Influenza A(H1N1)pdm09 Severely Infected Mice

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Thymic atrophy has been described as a consequence of infection by several pathogens including highly pathogenic avian influenza virus and is induced through diverse mechanisms. However, whether influenza A(H1N1)pdm09 infection induces thymic atrophy and the mechanisms underlying this process have not been completely elucidated. To further understand the mechanisms of thymic atrophy induced by influenza A(H1N1)pdm09 infection, BALB/c mice were intranasally inoculated with influenza A virus strain A/California/07/2009. Our results show that severe influenza A(H1N1)pdm09 infection induced a progressive thymic atrophy in the BALB/c mice model, especially the cortex region. Double positive (DP) T cells, which exist in cortex region, had a significant depletion due to apoptosis. The virus could be transported into thymus via dendritic cells from lungs, and then activated thymic innate CD8$^+$CD44^{hi} single positive (SP) thymocytes, which subsequently led to DP thymocytes apoptosis through secreting a large amount of IFN-γ. Milder thymic atrophy was observed in innate CD8$^+$ T cell deficient mice (C57BL/6J). Neutralization of IFN-γ could significantly rescue the atrophy, but Peramivir treatment did not significantly alleviate thymic atrophy. In this study, we demonstrated that thymic innate CD8$^+$CD44^{hi} SP T cells play critical roles in influenza A(H1N1)pdm09 infection-induced thymic atrophy through secreting IFN-γ. This exceptional mechanism might serve as a target for the prevention and treatment of thymic atrophy induced by influenza A(H1N1)pdm09.

P14

Near Real Time Surveillance for Influenza in Primary Care Settings

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Background: Delays in reporting are inherent in syndromic, mechanistic and laboratory surveillance of influenza. Consequently, public health acquisition of information and response to influenza outbreaks is typically offset by two-to-three weeks in the United States. The feasibility of creating and operating a statewide array of rapid influenza detection test (RIDT) analyzers that report results in real time was assessed. The analyzers use a wireless connectivity system to communicate results to a cloud-based server.
Methods: The primary outcome was the feasibility of a real-time primary care influenza surveillance network, including the time to recruit, engage, and train practices and for installation and implementation the analyzers. Secondary objectives included the assessment of performance for detection of influenza as compared to that observed with existing surveillance programs. Quidel's Sofia™—an immunofluorescence-based instrument with wireless capability—was used for this study. The RIDT was Quidel's Sofia Influenza A+B FIA. The cellular/wireless network employed was Quidel's Virena™ system. Both Sofia and the rapid immunofluorescence assay (FIA) are FDA-cleared in the United States. At least two Sofias were deployed in each of five public health regions of Wisconsin, starting in October 2013. Twenty primary care practices located in urban, suburban and rural locations were recruited and participated.
Results: After obtaining IRB exemptions and the university's administrative authorizations, healthcare practices were contacted and recruited. A full complement of participants was obtained by mid-November, 2013. Installations of Sofias were completed by mid-December 2013. Data were accumulated as soon as sites were activated and reporting from 16 sites (80%) was fully operationalized by 12/31/2013. This time course allowed for any local site IRB applications and for inserting and applying new technology into practices. Data were aggregated and analyzed on a daily and weekly basis by site, public health region, and for the Wisconsin composite. The system identified the onset of the 2013-2014 seasonal influenza outbreak extremely early.
Conclusions: Effortless, real time reporting of RIDT results was achievable over a very short time frame in practices using RIDT coupled with immediate wireless transmission of results. Such reporting eliminated the need for clinicians or laboratorians to identify time to aggregate and transmit information. This approach is a reasonable model for public health surveillance for any pathogen identifiable by clinic-based technology.

P15

An Immunoinformatics Approach for Designing Epitope Based Vaccine Strategy Against S Protein of Mysterious New Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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Objectives: In 2012, a deadly virus namely Middle East Respiratory Syndrome Coronavirus (MERS-CoV) has emerged from the Arabian Peninsula and it is striking fear in the hearts of public health officials throughout the world. Recent studies find that, similar to SARS-CoV, the spike (S) protein of MERS-CoV also plays important roles in receptor binding and viral entry that affects viral host range. As the major protein causing virus infection, S protein can be an ideal target for both vaccines and MERS-CoV entry inhibitors. Hence, analyzing the properties of MERS-CoV S protein is a high research priority.

Method: As there is no effective drug available, novel approaches regarding epitope prediction for vaccine development were performed in this study. In this study, we identified several immunodominant sites on the S protein by by immunoinformatics tools. Epitopes or peptide fragments as nonamers of these antigenic S proteins were analyzed by according to their proteasomal cleavage sites, TAP scores and IC50<250 nM, the predictions were scrutinized. Furthermore, the epitope sequences were examined by in silico docking simulation with different specific HLA receptors.

Results: This study suggests that the S protein is highly immunogenic and induces protection against MERS -CoV challenge and that neutralizing antibodies alone may be able to suppress virus proliferation, further advocating the rationale that vaccines against MERS-CoV can be evolved based on the S protein, which can provide high population coverage.

Conclusion: Although the computational approaches executed here are based on concrete confidence which demands more validation and *in vivo* experiments to validate such *in silico* approach.

P16

Virological Estimation of Peramivir Administration in Children with Influenza

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Objectives
To estimate peramivir administration in children.

Methods
Sequential serum samples and nasal swabs were collected before and after a single peramivir administration at 10 mg/kg/dose from 15 hospitalized patients diagnosed with influenza A or B using a rapid antigen test. We measured the serum concentration of peramivir, and determined the sequential virus copy number and the population of E119E/V and R292R/K at the N2 gene by quantitative real time RT-PCR. The isolated viruses were analyzed by a chemiluminescence-based neuraminidase inhibition assay to determine the IC50 and IC90 values.

Results
Among the 15 children, 10 and 3 children were diagnosed with influenza A (H3) and B, respectively, based on the positive result of the real time RT-PCR. The serum peramivir concentrations measured at 0.5, 1, and 1.6 h after the initial doses were 97,746.7; 79,628.5; and 58,587.1 nM, respectively. The average serum peramivir concentrations measured on days 1, 2, 3, and 5 were 97.0, 27.4, 12.0, and 4.4 nM, respectively. Although the viral load of influenza A decreased to 10−3 of those before peramivir administration by day 2, the viral load in five children increased after day 3. The IC50 values for each isolated virus on every hospitalization day were within 0.15–0.2 nM. No E119V substitution was detected, and the percentage of R292K did not increase during the observation period. For influenza B, the ratio of the viral load decline was low compared with that of influenza A. The IC50 and IC90 values of type B influenza were within 1.27–1.47 nM and 11.16–20.62 nM, respectively

Conclusion
Regarding influenza A, the results that the viral load increased after day 3 following the peramivir administration, although the susceptibility of the isolated virus after peramivir administration was stable and no E119V strains or no increase in the percentage of R292K strains were observed, indicated that the peramivir concentration in the respiratory airways is below the concentration required to inhibit neuraminidase activity, and that peramivir readministration should be considered at 48 h after the first administration in severe cases. Regarding influenza B, it was found that the effectiveness of peramivir is low within the early days after peramivir administration, and that readministration should be considered at 24 h after the first administration in severe cases.

P17

Genetic Variability of Group A Human Respiratory Syncytial Virus from a Large Referral Hospital in Alappuzha, Kerala State, India.

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Human Respiratory Syncytial virus (HRSV) is one of the most important causes of acute lower respiratory tract infection (ALRI) in infants and young children globally. HRSV presents two antigenic groups (A and B) with a high genetic and antigenic variability between them. Genetic variability is also very high within each group. A dearth of information is available about the circulating group and genotype of RSV in the southern part of India. The present study was carried out in Alappuzha district in Kerala state, India. This region of India is surrounded by the coast of Arabian Sea on the west and is bounded by the Western Ghats in the east and has over 3% population of the country. The aim of the study is to identify the groups and genotypes of circulating HRSV in children below 3.0 years old age with acute lower respiratory tract infection. Conventional multiplex Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify the Nucleocapsid (N) gene to identify the RSV groups. Genetic characterization of the circulating group of HRSV was done by nucleotide sequencing of the C-terminal region of the Glycoprotein (G) gene. The nasopharyngeal swabs were collected from the children with ALRI and immediately transported to the laboratory for testing. During the period of January, 2012 to September, 2012, a total of 93 samples were collected. All the samples were typed by RT- PCR based assay. Of the 93 samples typed, 30 belongs to group A and one belong to group B virus. Genetic variability within RSV A was studied by sequencing the G gene of 12 representative samples. Phylogenetic analysis revealed that all sequences belonged to the NA1 genotype. Of these, two sequences showed the novel 72 nucleotide duplication in the C-terminal region of the G gene. The duplication in the sequences was first observed from Ontario, Canada and clustered in the newly designated ON1 genotype. From this study, we conclude that HRSV is a major pathogen in children with ALRI in Kerala and it was detected in 33.33% of children by RT-PCR. Both the groups (A and B) of virus were co-circulated and group A was a predominant type during the study period. Within group A HRSV, both the strain with 72 nucleotide duplication and without duplication also observed.

P18

New Adamantane Derivatives Can Overcome Resistance of Influenza A(H1N1)pdm2009 and A(H3N2) Viruses to Rimantadine

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One of the well-known method for influenza treatment and prevention is using of antivirals. Among them, rimantadine and amantadine are most available. Both are adamantane compounds suppressing the function of influenza A protein M2. The drugs are ineffective against influenza B virus. But the most epidemic strains of A(H1N1)pdm09 and A(H3N2) influenza viruses (90%) are completely resistant to amantadine and rimantadine now. It connects with spontaneous mutations in the virus genome, including position 31 of M2 protein transmembrane domain with substitution of serine residue for asparagine (Ser31Asn). This necessitates expanded research on the reasons for the development of resistance and ways of overcoming it by creating new antivirals or to improve the current ones.

Adamantane amino acid derivatives were obtained via the formation of amide bond in the reaction between amino acid carboxyl group and adamantine carbocycle amino group or between adamantane-carbonic acid carboxyl group and amino acid amino group using classical methods of peptide chemistry.

Antiviral activity of the new compounds was evaluated on rimantadine-resistant influenza A/IIV-Moscow/01/2009(H1N1)pdm09 and A/Moscow/26/2009(H3N2) strains by cell-ELISA. Rimantadine and the tested compounds (adamantane derivatives) at concentrations of 5.0 μg/mL were added at the same time that the cell MDCK monolayer was infected.

The percent inhibition of virus activity by the compounds was determined.

Analysis of the results showed that the compounds with the amino group protected by tert-butylhydroxycarbonyl(Boc) group inhibited influenza A(H1N1)pdm09 and A(H3N2) viruses. These compounds were amino acids ornithine (compound 1(Boc-Orn(Boc)-Rim) and sarcosine (compound 2(Boc-Sar-Rim). The percent inhibition of A(H1N1) pdm09 virus activity by the compounds was 71% and the percent inhibition of A(H3N2) virus activity was 66%;64%, respectively.

Rimantadine derivative with lipoic acid (compound 3(TOA-Rim) including cyclic disulfide exhibited significant (78%;89%) suppression of both influenza A strains.

The 1,6-diaminohexane derivative with 1-adamantane-carboxylic acid (compound 4(Ad-HDA) suppressed replication of both influenza A virus strains by 74%;63%, respectively.

The compound containing histidine residue (compound 5(H-His-Rim) exhibited stable suppression of influenza A viruses replication by 91%;94%.
Compound 6 (1,3-adamantane-diacetic acid (Ad*-$(CH_2Ser\text{-}OMe)_2$) proved to be one of the most active (98%;90%) amino acid derivative of adamantane acids.
These compounds have a practical interest as perspective antivirals for influenza A (H1N1)pdm09 and A(H3N2) viruses, which population is absolute resistant to rimantadine and amantadine now.

P19

Identification of Resistance Mutations as Minority Species in Clinical Specimens from Hospitalised Adults with Influenza and Treated with Intravenous Zanamivir

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Intravenous zanamivir (IVZ) is a neuraminidase inhibitor (NI) suitable for treatment of patients hospitalised with influenza. One of the main factors that can affect the efficacy of NIs is resistance development. Resistance mutations can be detected by population sequencing (PS) but can only be detected as minority species to a minimum of 25%. In this study clinical specimens were analysed for the presence of resistance mutations using Next Generation Sequencing (NGS).
A phase II study (NCT01014988) was initiated at the onset of the 2009 influenza A/H1N1 pandemic and included 130 symptomatic, hospitalised adults with influenza. Patients received IVZ for 5-10 days. Throat swabs were taken at various timepoints up to 23 days post-treatment (PT). The last visit sample from each subject with detectable virus ≥Day 4 was amplified using neuraminidase specific primers and analysed by NGS using Illumina MiSeq sequencing platform. Sequences were trimmed and aligned, and a variant calling workflow applied. A minimum sequencing depth coverage of 1000x and 1.5% minimum variance was used for detection.
Ninety six samples from 50 subjects (87 A/H1N1, 8 A/H3N2, 1 influenza B) were amplified by RT/PCR. Of these, 74 samples were RT/PCR positive (70 A/H1N1, 4 A/H3N2), which included 17 Day 1 samples, 35 during-treatment samples and 22 PT samples, and were analysed by NGS.
Many variants were identified, so to try and identify clinically relevant mutations a threshold value was selected. Mutations identified by PS and NGS were correlated, the NGS detection was in the range 20-100%, and a threshold of 15%, 5% below the level detected by PS, was selected.
Five resistance mutations were detected in viruses from five subjects: E119K (PT+5), E119D (two subjects, PT+16 and PT+5), L134S (Day 7), D199N (Day 3) and E277K (Day 5). None of the mutations were present at Day 1 (in 4/5 subjects, one Day 1 sample was not tested) and the first 4 mutations were not detected by NGS at later time points. Only one mutation, E119D, was detected by both NGS and PS. NGS is a suitable technique for minority species analysis. Five resistance mutations were detected in five subjects as minority species, the clinical relevance of which is not known.

Sponsored by GlaxoSmithKline.

P20

Epidemiological, Biological, and Genetic Properties of A(H1N1)pdm09 Virus Strains Caused of Lethal Cases of Influenza Infection

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Background
Clinical materials (nasal swabs, lavage, autopsy materials) from patients who was infected (450) and/or succumbed to lethal pneumonia (110) caused by influenza A(H1N1)pdm09 virus during 2009-2014 were examined in D.I. Ivanovsky Institute of Virology, Moscow, Russia.
Materials and methods
RT-PCR, virus isolation on MDCK and embrionated eggs (EE), Hemagglutiniation Inhibition, sequencing were used.
Results
Since 2009 in Russia influenza A(H1N1)pdm09 was the main etiological agent of epidemics during 2009-2010, 2010-2011, 2012-2013 and co-circulated with A(H3N2) during 2013-2014, but its activity was differed in different seasons. The mean age of patient with lethal pneumonia was 41.2 years (from 3 to 77 years); 52% of them were women; 8% - pregnant. The most of patients had chronic diseases, mainly metabolic (48,7%), cardiac (46,1%), diabetes (19,7%), asthma (14,5%), chronic alcoholism (13,2%). The mean duration of the illness was 10.5 days (from 2 to 26 days); duration of the illness before hospitalization was 5,5 days (from 2 to 15 days) and duration of the illness before lethal outcomes was 10.6 days (from 2 to 26 days).

We detected mutations in HA1 receptor-binding site in 110 samples [39% of total]. D222G and Q223R were the most common mutations found in autopsy materials. Meantime the results of observation of clinical materials from 450 patients with non-lethal influenza infection didn't find no one case with such mutations.

36 strains of A(H1N1)pdm09 were isolated from autopsy materials: 19 strains – on EE (53%), 12 strains – on MDCK (33%) and 5 strains – on both of EE and MDCK (14%). Most isolates were characterized as A/California/7/2009-like, the virus used as the influenza A(H1N1)pdm09 component of the 2009-14 influenza vaccines for the Northern hemisphere. Only two from 36 strains of them (6,0%) showed reduced titers with antiserum produced against the reference virus.
Conclusions
These results demonstrate that future monitoring of influenza viruses from patients with lethal pneumonia will provide the most important trends in A(H1N1)pdm09 virus evolution and the effectiveness of control/treatment/prevention measures.

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P21

Susceptibility of Influenza A and B Viruses, Isolated in Russia During 2011-2014, to Oseltamivir (Tamiflu™) and Zanamivir (Relenza™)

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The objective of the research work was the study of susceptibility to neuram-inidase inhibitors (NAIs) of influenza A and B viruses strains, which had epi-demic activity during 2011-2014 in Russia.

During 2011-2014 150 pandemic influenza A(H1N1) pdm09, 21 epidemic influenza A(H3N2) and 24 influenza B viruses strains were studied and the following methods were applied: fluorescent method with MUNANA substrate (determining of minimal inhibitory concentration of preparations - IC_{50}), RT-PCR and partial sequencing (identifying of marker mutations in neuraminidase gene, responsible for influenza viruses' resistance to preparations).

During 2011-2012 epidemic season all of the studied strains (13 A(H3N2) and 3 A(H1N1)pdm09) were susceptible to NAIs. During 2012-2013 season pandemic A/IIV-Moscow/34/2013(H1N1)pdm09 strain contained a marker mutation H275Y in neuraminidase surface protein, showing its resistance to oseltamivir, but not to zanamivir. This strain was isolated from a non-vaccinated 22 years old patient, with symptoms similar to uncomplicated in-fluenza (4-5 days of fever, malaise, shivering, cough, tracheitis, coryza, phar-yngitis). During the antiviral therapy course the patient was treated with 90 mg/day dose of Ingavirin™ for 5 days; the Tamiflu™ was not used. All the other pandemic strains (121) and 8 of epidemic A(H3N2) strains have pos-sessed susceptibility to NAIs. Were defined ranges of IC_{50} values for 24 influ-enza B virus strains, as follows: 34,1-248,5 nM for oseltamivir carboxylate and 8,3-42,4 nM for zanamivir. In comparison with reference values of IC_{50}, defined for susceptible to preparations influenza B virus, these concentration ranges showed normal activity of NAIs towards the studied strains. Prelimi-nary data received for 25 influenza A(H1N1)pdm09 virus strains, isolated in 2013-2014, showed that all of them were sensitive to NAIs, including one of them which had mutation in 222 position of hemagglutinin (D222G). This strain was isolated from broncho-alveolar lavage, which was taken from patient with severe influenza infection on 8-th day of illness.

In spite of worldwide sporadic cases of new resistant to NAIs strains emer-gence (less than 2%), it should be noted that resistance appearance usually correlates with the use of them. That is why neuraminidase inhibitors of influ-enza viruses remain the preparations of choice for treatment and prophylaxis of influenza infection.

P22

A Community-Acquired Case of Oseltamivir and Peramivir Resistant Influenza A(H1N1)pdm09 Virus in Nagasaki, Japan in 2013-2014 Season

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Objective: Prevalence of neuraminidase inhibitor (NAI) resistance of A(H1N1)pdm09 virus remained low at around 1-3%. However, community clusters of A(H1N1)pdm09 viruses with H275Y mutation in the neuraminidase (NA) were first reported in Australia in 2011, and recently from the US and Japan in 2013. In this study, we report a new case of influenza A(H1N1) pdm09 virus with H275Y mutation in Nagasaki, Japan.
Method: A nasopharyngeal swab was taken at first visit from a patient with no prior history of NAI treatment and no known contact with previously reported cases in Japan. One-hundred ul of the sample was inoculated onto MDCK cells and the isolate was confirmed to be A(H1N1)pdm09 virus possessing H275Y mutation by real-time PCR cycling probe method. This H275Y virus underwent fluorescent-based IC_{50} susceptibility assay for NAIs; oseltamivir, peramivir, zanamivir and laninamivir. We analyzed additional 8 isolates without H275Y mutation that were collected in Nagasaki and Kyoto in 2013-2014. Sequencing was performed on the hemagglutinin (HA) and NA genes for the 9 isolates. We constructed phylogenetic trees from viruses that were collected in 2013 in Japan, China, the US, and 2011 isolates in Australia.
Result: The Nagasaki strain with H275Y mutation showed a 157-fold and a 250-fold increase in IC_{50} value against oseltamivir and peramivir respectively. The H275Y virus remained sensitive to zanamivir and laninamivir. All other 8 strains were susceptible to the four drugs. The HA gene of all 9 strains belonged to clade 6B by WHO classification and were closely related to H275Y viruses from the US in 2013. Likewise, NA gene analysis showed that all 9 strains belonged to clade 6B, but 6 of the 9 viruses including our H275Y virus formed an independent clade. This clade is an intermediate clade between the US and Japan-China groups.
Conclusion: We identified an influenza A(H1N1) pdm09 virus with H275Y mutation in NA, which conferred resistance to oseltamivir and peramivir from Nagasaki, Japan. Surprisingly, this H275Y virus is closely related with the American strains than the recently reported Japanese strains. It was suggested that community acquired H275Y viruses were derived by various transmission routes from different nations.

P23

NK Cells Aggravate Acute Lung Injury Via Up-regulation of NKG2D During Early Stage of H1N1 Influenza Infection

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Acute lung injury was considered as the major pathological contribution of 2009 pandemic H1N1 influenza virus infection. NK cells were the first line to defend against virus infection, but their roles in the lung pathogenesis and virus elimination were not fully elucidated. Severe BALB/c mice infection model was intranasally inoculated with influenza A virus strain A/California/07/2009. Following virus challenge, the body weight was loss and survival rate was decreased. The infected lung showed severe lung injury, including pulmonary edema and capillary leak, and a large number of infiltrating lymphocytes were recruited to perivascular and parenchyma areas in mice model and patients. Total lymphocytes in lung and bronchoalveolar lavage fluid (BALF) were increased as the infection process, and the ratio and number of NK cells was significantly increased. But the ratio of T cells in lung had no change. Furthermore, NK cells were rapidly activated, and secreted a large amount of IFN-γ and increased high level of perforin and granzyme B. H1N1 infection induced significant high expression of NKG2D, but not NKG2A, on NK cells. NKp46, which can recognize virus HA, was also improved. NK cells CD11b(high)CD27(low) subset were decreased and CD11b(high)CD27(high) were increase after infection. Meanwhile, H1N1 infection induced significantly high expression of NKG2D ligands (RAE-1) and low expression of NKG2A ligands (Qa-1α). Depletion of NK cells with AsGM1 show lighter lung damage and weight loss, but higher virus titer compared with PBS control at early stage, accompanying with reduced secretion of IFN-γ. Our data demonstrated NK cells played dual roles in lung injury and virus elimination during the early stage of H1N1 virus infection.

P24

Predictors for In-hospital Mortality Among Adults with Influenza A (H7N9) Virus Infection with Emphasis on the Effect of Adjuvant Corticosteroid Treatment

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Objectives: To access the effect of adjuvant corticosteroid treatment on the outcome of adults with H7N9 infection.
Methods: Adult patients with laboratory confirmed severe H7N9 influenza in China from March to July 2013 were enrolled. Multi-variable logistic-regression analysis was used to assess independent predictors for death. The association between corticosteroid administration and clinical outcome was evaluated using multivariable logistic regression models and propensity score based analysis.
Results: In-hospital mortality was 31.8% among 110 patients included. 84 patients (76.4%) received adjuvant steroid treatment. Multi-variable analysis revealed that CRP≥80mg/L (adjusted odds ratio (aOR), 4.76; 95% CI, 1.32 to 17.13;) and shock before or within 48 hours on admission (aOR, 9.86; 95% CI, 1.33 to 72.87) were the independent risks factors of death. There was a trend that use of corticosteroids was associated with higher risk of death (aOR, 4.58), but it did not reach pre-determined statistical significance (95% CI, 0.85 to 24.64; P = 0.077). In propensity score analysis, again there was a trend that use of corticosteroid was associated with higher in-hospital mortality (aOR=4.3, 95% CI: 0.7-26.3).
Conclusions: Adjuvant corticosteroids appeared to be associated with higher in hospital mortality in patients with H7N9 viral pneumonia and should not be used unless for other compelling indications.

P25

Oseltamivir Resistance Among Influenza Viruses: Surveillance in Northern Viet Nam,2009–2012

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Introduction: Influenza infection causes annual epidemics throughout the world. Influenza A viruses caused several influenza pandemics in the 20th century, the recent pandemic in 2009 caused by the influenza A(H1N1)pdm09 virus. National influenza surveillance was initiated in Viet Nam in 2006, and the data collected so far have shown that influenza viruses circulate year-round with similar peaks and subtypes observed across all surveillance regions.
The neuraminidase inhibitors oseltamivir and zanamivir are the primary antiviral agents recommended for the treatment of influenza infections, yet antiviral resistance to influenza A viruses is increasingly being reported. Oseltamivir is currently recommended as the first-line option by the Viet Nam Ministry of Health for treating suspected infections of A(H5N1) and A(H1N1) pdm09. The limitations of other antiviral drugs, as well as the risk of oseltamivir resistance, have raised concerns about the efficacy of oseltamivir for influenza infection treatment.
We report here on a pilot study for the establishment of a routine antiviral resistance surveillance programme in northern Viet Nam.
Methods: We analysed specimens from two sources during the period 2009–2012: influenza-positive samples from influenza-like illness patients at sentinel clinics in northern Viet Nam and isolates from patients with confirmed A(H5N1)
infections. Pyrosequencing was used to detect mutations: H275Y [for A(H1N1) and A(H5N1)], E119V [for A(H3N2)] and I117V [for A(H5N1)]. A neuraminidase inhibition assay was used to determine the Inhibitory Concentration $_{50}$ (IC_{50})
values for all influenza A and B isolates.
Results: There were 341 influenza A positive samples identified; influenza A(H1N1)pdm09 was identified most frequently (*n* = 215). In 2009, oseltamivir resistance was observed in 100% (19 of 19) of seasonal A(H1N1) isolates and 1.4% (3/215) of A(H1N1)pdm09 isolates. This H275Y mutation was not found in influenza subtypes A(H5N1) or A(H3N2) isolates.
Discussion: In Viet Nam, seasonal and A(H5N1) influenza vaccines are not currently available; thus, effective treatment is required. The presence of oseltamivir-resistant viruses is therefore a concern. Active surveillance for oseltamivir resistance among influenza viruses circulating in Viet Nam should be continued.

P26

Oseltamivir Use in a Cohort of Young Children in Bangkok, Thailand

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Objective: Thai clinical guidelines recommend oseltamivir for patients with severe influenza and a high risk of severe disease, including children aged <2 years. We examined predictors of oseltamivir use in children with acute respiratory illness (ARI).
Methods: From August 2011-November 2013, we prospectively enrolled an equal number of healthy and chronically ill aged 0-36 months at the Queen Sirikit National Institute of Child Health. Study nurses contacted caretakers weekly for two years to identify children with ARI (presence of ≥2 of fever/ feverishness, cough, sore throat, and runny nose) and encouraged them to visit the hospital. Children with ARI underwent physical examination with nasal and throat swabs tested for influenza by rapid test. The rapid test results were shared with physicians who made treatment and management decisions. We analyzed predictors of oseltamivir use using Cox proportional hazards models.
Results: A total of 2,377 ARIs occurred in 784 children out of the 1,149 enrolled. Two hundred and twenty-nine (10%) ARIs were treated with oseltamivir, of which 147 (64%) were rapid test positive. Of those meeting treatment criteria, 10% were treated, whereas 10% of those not meeting treatment criteria were treated (p-value 0.84). Treatment was initiated within two days of illness onset in 133 (58%) episodes, and a 5-day course of treatment was completed in 212 (93%) episodes. Of all ARIs, 175 (7%) required hospitalization and 950 (40%) occurred in chronically ill children; 60 (34%) and 102 (11%) were treated with oseltamivir, respectively. Children were significantly more likely to receive oseltamivir if they were <2 years of age [adjusted hazard ratio (aHR) 2.4, 95% confidence interval (95% CI) 1.7-3.3], hospitalized (aHR 5.4, 95% CI 3.5-8.6), chronically ill (aHR 1.3, 95% CI 1.0-1.8), rapid test positive (aHR 24.7, 95% CI 17.2-35.7), or had high tympanic temperature (aHR 1.2, 95% CI 1.0-1.3).
Conclusions: Although overall use of oseltamivir was low, physicians were more likely to prescribe oseltamivir to severely ill children and those with high fever. Continued education among physicians should stress prompt empirical treatment with oseltamivir to maximize the benefits of treatment.

P27

Contribution of Respiratory Viral Infections Among Hospitalized Children Below 5 Years of Age in Pune, India: A Retrospective Study

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Background
Viral respiratory tract infections (RTI) are relatively understudied in India. In temperate countries, seasonal activity of respiratory viruses has been reported, particularly in association with temperature, while inconsistent correlation of respiratory viral activity with humidity and rain has been reported in tropical countries. A retrospective analyzes was carried out (2009–2013) to investigate the association of viruses with RTI among of children (≤5 years old) admitted in a various hospitals in Pune, Western India.
Methods
A total of 4761 respiratory samples from children ≤5 years of age were received at the National Institute of virology during 2009–2013. 1523 were tested by duplex real time RT- PCR, developed using previously published primer–probe sequences. The detected viruses included influenza virus A and B, Influenza A(H3N2), A(H1N1), and pandemic influenza (H1N1), parainfluenza 1-4 (PIV), human metapneumovirus (hMPV1 &2), respiratory syncytial virus (RSV A and B), rhinovirus (RV), human coronaviruses 229E, OC43 and NL63 along with internal control Rnase P.
Results
Out of 1523 individuals, 879 (57.7%) had at least one respiratory virus detected. The most commonly detected being RSV (241, 15.67 %), Rhinovirus (199, 13.06%), Influenza (180, 11.82%), PIV (56, 3.58%), hMPV (65, 4.27%), Adenovirus (49, 3.2%), Corona and Boca viruses were found in (24, 1.58%) individuals. Co- infections were noted in 64 (4.2%) individuals. Children infected with respiratory viruses were significantly younger (<1yr) except in case of hRSV B, Rhinovirus and Influenza where children of all age group showed comparable positivity. The five main viruses (RSV, Rhino, Influenza, hMPV and PIV) caused disease throughout the year, with enhanced activity observed for RSV in winter (November-January) and in rainy season (July–September).
Conclusion
Viral RTIs, particularly due to RSV, Rhino and Influenza were commonly detected in respiratory samples from hospitalized children. Quick etiological diagnosis using rapid and sensitive assays may be useful in patient management and for limiting usage of antibiotics.

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P28

Influenza B Viruses with Neuraminidase Inhibitor-Resistant Substitutions E119A and H274Y Possess Undiminished Fitness

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OBJECTIVES: Neuraminidase (NA) inhibitors (NAIs) are the only available therapy for patients infected with influenza B viruses and the potential emergence of NAI-resistant viruses is a public health concern. NA substitutions located within enzyme active site could not only reduce NAI susceptibility of influenza B virus but also affect virus fitness. In this study we investigated the effect of single NA substitutions on fitness of B/Yamanashi/166/1998 influenza viruses.
METHODS: Using reverse genetics we generated recombinant viruses containing either wild-type (WT) NA or NA with a substitution in the catalytic (R371K) or framework residues (E119A, D198E, D198Y, I222T, H274Y, N294S, N2 numbering). We assessed NAI susceptibility using a fluorescence-based assay, NA biochemical properties, cell surface NA protein expression, and virus replication in differentiated normal human bronchial epithelial (NHBE) cells with and without drug pressure.
RESULTS: We determined that four NA substitutions (D198E, I222T, H274Y, and N294S) reduced inhibition of influenza B virus by oseltamivir, and three (E119A, D198Y, and R371K) conferred highly reduced inhibition and cross-resistance by oseltamivir, zanamivir, and peramivir. All NA substitutions except for D198Y and R371K were genetically stable after 7 passages in MDCK cells. Cell surface NA protein expression was significantly increased by H274Y and N294S substitutions. Viruses with E119A, I222T, H274Y, or N294S substitutions were not attenuated in replication efficiency *in vitro* or in NHBE cells in the absence of drug. Addition of oseltamivir (10 μM) inhibited replication of the susceptible rg-WT virus but failed to completely suppress replication of any NAI-resistant viruses in NHBE cells. Zanamivir (10 μM) inhibited replication of rg-WT, rg-I222T, and rg-H274Y viruses, impaired replication of three other viruses (rg-D198E, rg-N294S, and rg-R371K), and importantly, failed to significantly reduce replication of the rg-E119A virus.
CONCLUSIONS: Our studies identified two NA substitutions of potential public health importance: 1) virus with the H274Y mutation had increased cell surface protein expression and sustained replicative fitness and 2) virus with the E119A mutation lead to highly reduced inhibition by multiple NAIs and productive replication in the presence of NAIs. Overall, viruses with E119A or H274Y NA substitutions possess fitness comparable to NAI-susceptible virus and their acquisition by circulating influenza B viruses should be closely monitored.

P29

Emergence of G186D in the Presence of R292K in an Immunocompromised Child Infected with Influenza A/H3N2 Treated with Oseltamivir

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An 11-year old, immunocompromised girl presenting with neutropenic fever, was treated with oseltamivir on her first day of illness, after early laboratory confirmation of an influenza A/H3N2 infection. Serial nasal swabs were taken on days 1, 4, 7 and 10 for quantitative influenza viral load testing to monitor response to therapy. Surprisingly, the viral loads started to rise on day 10 after initially falling in response to treatment: $3.43x10^9$, $6.59x10^6$, $6.20x10^6$, $3.47x10^7$ copies viral RNA/mL, on each of these days, respectively. Capillary genomic sequencing and pyrosequencing showed the emerging presence of the drug resistance-associated mutation, R292K, in the neuraminidase (NA) gene on days 4, 7 and 10. Another mutation, G186D, in the hemagglutinin (HA) gene, emerged on days 7 and 10, possibly contributing to the day 10 viral rebound by acting as a compensatory mutation for R292K. Several underlying related mechanisms may explain this observation: 1) the G186D mutation occurring near the receptor binding site (RBS) may have weakened the receptor binding ability of the HA protein, allowing the progeny virus to leave the host cell in the presence of NAI (i.e. without the need for the NA enzyme to release them). 2) this residue 186, located at the epitope B of the HA1 globular head, may be a specific target for immune selection by humoral B- and cell-mediated T-cell responses. As such, selection for viral variants leading to an immune escape viral phenotype, such as G186D that involve the RBS, may rapidly evolve in the presence of oseltamivir, enhancing the survival of this drug-resistant mutant in this patient. 3) hence, a reduced viral fitness due to R292K may therefore be potentially overcome by a secondary substitution in the HA gene, near the RBS. Thus, as demonstrated in this case report, for specific patients, viral load quantification followed by HA and NA sequencing to detect the presence of such drug-resistant/ compensatory mutations may be useful in the management of treatment-resistant cases. Further phenotypic characterization of this novel G186D-HA mutation in the presence/ absence of the accompanying R292K-NA mutation is warranted.

P30

Molecular Surveillance of Antiviral Drug Resistance of Influenza A/H3N2 Virus in Singapore, 2009-2013

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Adamantanes (M2-blocker) and neuraminidase inhibitors (NAIs) are the two classes of drugs available for chemoprophylaxis and treatment of influenza infections. A survey of antiviral testing and sequencing conducted in 2010 by the WHO GISRS cited an inadequate antiviral surveillance capability in the South-East Asia. To improve the understanding of the epidemiology of drug resistance in influenza A/H3N2 viruses in this region, we performed large-scale sequencing of NA and matrix protein (MP) genes directly (i.e. without initial culture amplification) from 241 clinical samples submitted to National University Hospital and Singapore General Hospital between May 2009 and November 2013. Of the 241 A/H3N2 samples, 229 NA (95%) and 241 MP (100%) complete sequences were obtained. Drug resistance mutations in the NA and MP genes were identified according to published studies and recent WHO guidelines. For the NAIs, a visual inspection of the aligned NA sequences of these H3N2 viruses revealed no known drug resistant genotypes (DRGs). For the adamantanes, the well-recognised S31N DRG was identified in all the 241 MP genes. In addition, there was a noticeable drift towards an increased number of viruses carrying D93G+Y155F+D251V (since May 2013) or D93G (since March 2011) in the NA gene. This D251V mutation results in an acidic-to-non-polar change of the amino acid, which is similar to D251G that causes 'low levels' of zanamivir resistance. However, neuraminidase inhibitor (NI) testing indicated that neither D93G+Y155F+D251V nor D93G alone conferred significant drug resistance against any of the 4 NAIs (oseltamivir, zanamivir, peramivir, and laninamivir). Lastly, an NA-I222T mutation that has previously been reported to cause oseltamivir-resistance in influenza A/H1N1/2009, B, and A/H5N1, was detected from a treatment-naïve patient. Further NI testing is required to confirm the true drug-resistance effect of this mutation specifically in A/H3N2 viruses. A reference influenza DRG surveillance programme should combine both sequencing and functional assays to identify and characterise novel, emerging DRGs (directly from clinical samples) in this continuously evolving virus.

P31

Molecular Evidence of Transmission of Influenza on a University Campus in Singapore

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Background/Objectives: University campuses and other closed settings are believed to be the foci of influenza transmission especially in tropical countries. We conducted a prospective surveillance study in a cohort of university students and staff to understand the epidemiology of influenza transmission on campus.

Methods: Nasopharyngeal swabs and basic demographic data were collected from all consenting students and staff with influenza-like illness visiting our university health centre from May 2007 through September 2009. A multiplex-PCR was employed to detect influenza types and subtypes. The criteria for "shared strains" was 100% amino acid identity in two or more strains.

Results: Overall, 153/500 (30.6%) of subjects had influenza A virus detected. Sanger sequencing of hemagglutinin (HA) gene of 11 seasonal (sH1N1 and sH3N2) and 41 pandemic (H1N1 2009) influenza viruses was successfully performed. There were 32 shared strains and 20 non-shared strains suggesting at least some degree of transmission on campus with a smaller number of introductions. All the 32 in the shared group were pandemic H1N1 2009 strain sequences which could be further sub-divided into 4 clusters with 100% identical amino acid patterns. Cluster A had 24 strains, B and C had three strains each and D had two strains. The non-shared group had 9 distinct pandemic H1N1 2009 strains and 11 distinct seasonal influenza strains (sH1N1 and sH3N2). Residence at a student hostel was identified as a risk factor for having a shared strain [OR (odds ratio)=4.2, 95% CI (confidence interval) 1.2-14.9; p=0.02]. Other factors such as, age <25 [OR=2.3 compared with age >25, 95% CI 0.6-8.9; p=0.22), gender [OR=0.8, 95% CI 0.2-2.7; p= 0.74], Engineering course of study [OR=1.7, 95% CI 0.5-5.9 compared with other courses of study; p=0.41], Singapore citizenship [OR=1.7, 95% CI 0.5-5.2 compared with overseas students; p=0.38] and smoking [OR=1.8, 95% CI 0.4-8.0; p=0.47], were not different between the subjects with shared and non-shared strains.

Conclusions: The clustering of identical strains in students staying on campus in hostels suggests that influenza transmission occurred on campus. Surveillance of influenza for seasonal or emerging influenza should correlate molecular and epidemiological data to identify foci of transmission for public health intervention.

P32

Impact of Live Poultry Market Closures on Reducing Risk of Human Infections with Influenza A(H7N9) Virus, China, Winter 2013-14

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BACKGROUND
The novel avian influenza A(H7N9) virus caused two epidemic waves in mainland China in 2013-14 with 390 laboratory-confirmed human cases reported by 25 March 2014. Temporary closure of live poultry markets in the 2013 spring wave was suggested to be highly effective in reducing in the incidence of H7N9 infections. The study aims to estimate the impact of market closure in reducing risk of human infections during the 2013-14 winter wave.

METHODS
Nine urban areas in the two most affected provinces during the 2013-14 winter wave were selected for analysis. Estimates of the ratio of incidence rates of H7N9 during versus prior to LPM closure from the model indicated the effectiveness of intervention by assuming a constant incidence rate of infection before and during the market closure and a lognormal distribution of the incubation period. An overall model was also fitted assuming the same incidence rate ratio across all areas.

RESULTS
Point estimates of the effectiveness of LPM closure varied from 61-89% in the nine areas with generally wide credibility intervals while the effectiveness was estimated to be 97% (95%CI: 89%, 100%) in the overall model. The incubation period distribution has a mean 3.4 days (95% CI: 2.2-5.0).

CONCLUSIONS
Our analysis found that live poultry market closure was highly effective in reducing the risk of human infections with H7N9 virus during the 2013-14 winter wave.

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Respiratory Virus Infection and Clinical Characteristics of Hospitalized Children Younger than 5 Years with Severe Acute Respiratory Infection in Suzhou, China

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Objective: Severe acute respiratory infection (SARI) is an important cause of morbidity and mortality in children with a worldwide disease burden. This study was to learn the viral etiologies and the clinical characteristics of SARI in eastern Chinese children.
Methods: Since March 2011,a surveillance study on SARI children was conducted in Soochow University Affiliated Children's Hospital. Nasopharyngeal aspirate were collected for virus detection by real-time RT PCR reaction or direct immuno-fluorescent antigen test. Data on demographics, clinical characteristics, therapy, direct medical costs,etc. were collected by chart review, telephone survey and billing data requirement.
Results: From March 2011 to June 2013,a total of 2164 SARI children <5yrs were enrolled and 61.5% of them were boys. The median age was 1.2 years(IQR: 0.6-2.7). The median length of hospital stay was 7days (IQR: 6-8) in all SARI cases.The most common pathogens were respiratory syncytial virus(RSV,14.6%), and influenza virus(12.5%, Flu A: 7.2%, Flu B:5.2%), followed by bocavirus(6.4%) and parainfluenza virus type 3(5.6%). The median age of influenza positive cases was higher than that of RSV(1.5 years vs. 0.9 years, P<0.001).Wheezing was more easily developed in RSV infection cases than influenza infection cases(45.4% vs. 18.3%, P<0.001). However, high fever(>39.1℃) was more common in cases infected with influenza than those with RSV(46.5% vs. 30.1%, P<0.001). The children with RSV or influenza infection were more likely diagnosed with pneumonia than other cases (90.3% vs.83.5%, P=0.001).Compared with non-RSV and non-influenza infection children, RSV or influenza infection children had a higher usage rate of antibiotics(99.2% vs. 98.6%, P>0.05), anti-viral agents(89.2% vs. 84.7%, P=0.023) and glucocorticoids(85.4% vs. 78.4%, P=0.003). For all SARI cases, the median medical cost for hospitalization was US$753.0 (IQR: 603.2-935.4), and no significant difference between RSV or influenza infection children and non-infection children.
Conclusions: RSV and influenza were the most common etiologic viral agents of childhood SARI in Suzhou, China. Our finding indicated a high economic burden of SARI hospitalization to local families.

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Safety and Efficacy of Intravenous Zanamivir in the Treatment of Hospitalized Japanese Patients with Influenza Infection

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Zanamivir is a neuraminidase (NA) inhibitor with a favorable resistance profile approved as an oral inhaled formulation for influenza. Intravenous zanamivir (IVZ) is under investigation for the treatment of patients hospitalized with influenza. The primary objective of this study (NCT01527110) was to evaluate the safety of IVZ in hospitalized Japanese patients with influenza. Secondary objectives included clinical efficacy, virologic response, and viral resistance. Subjects (>16 years) with fever and onset of influenza symptoms within 6 days received IVZ 600 mg twice daily for 1-6 days. Twenty one subjects were enrolled; one subject withdrew due to a non-drug related fatal bacterial pneumonia during the follow-up period. The study population consisted of males 57%, median age 75 (range, 18-95) years, and baseline supplemental oxygen requirement 43%. Median duration of hospitalization was 7 (range, 4-23) days with no subjects requiring ICU stay. Seventeen subjects were infected with influenza A/H3N2, 3 subjects with influenza B, and one subject with strong suspicion of influenza infection. A drug-related adverse event during treatment occurred in one subject (injection site redness) and a drug-related serious adverse event during treatment occurred in one subject (decreased hemoglobin). The median time to clinical response was 3.5 (range, 0.5-22) days, return to pre-morbid level of activity 3.7 (range, 0.9-30) days, improved respiratory status 1.5 (range, 0.2-14) days, improved oxygen saturation 0.5 (range, 0.1-2.1) days, and absence of fever 1.0 (range, 0.4-3.4) days. The median time to virologic improvement was 3.0 (range, 2-5) days and to no detectable viral RNA by culture and qRT-PCR from nasopharyngeal samples 3.0 (range, 2-4) and 5.5 (range, 4-7) days, respectively. The influenza A median change from Baseline on Day 3 was -2.74 $\log_{10}$ copies/mL (range, -5.5-0.9). The mean IC_{50} (mean fold change) for influenza A/H3N2 was 2.04 nmol/L (1.78) and for influenza B 9.88 nmol/L (0.53). There was no identified resistance associated with NA mutations or mutations in the NA active site. Although a small study, there were no significant safety findings and efficacy of IVZ was observed. The data are valuable in understanding the profile of IVZ for treatment of hospitalized Japanese patients with influenza. Study sponsored by GlaxoSmithKline.

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Clinical Features of Severe Acute Respiratory Infections in Suzhou, China

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Severe Acute Respiratory Infections (SARI) remained the most important reason responsible for deaths of children under 5 years old. The study was aimed at describing the clinical features and complications of SARI cases, and exploring the risk factors that influence prognosis among Pediatric Intensive Care Unit (PICU) patients.
A retrospective study was conducted to all PICU SARI patients from 2007 to 2012 by chart review in Soochow University Affiliated Children's Hospital, Jiangsu Province, China.
In total, 977 SARI patients were enrolled. Among all patients, 73.8% were <1 year old. Median age was 4.1 months (*IQR:* 2.1-12.2 months). The most common symptoms were pharyngalgia (94.6%), cough (93.2%) and tachypnea (67.6%). Premature born, psychiatric disorders, expectoration and crackles happened more in children less than 4 months. While elder patients (>4 months) tended to develop rhonchus and fever (>38℃) more. Laboratory tests confirmed 37.7% cases infected by virus, 13.0% bacteria and 48.9% mycoplasma, respectively. RSV (30.5%) remained the most common detected virus, and 3.6% cases were influenza positive. A total of 99.3% cases had a complication of pneumonia, 39.5% respiratory failure and 32.5% heart failure. There were 31(3.2%) cases died. At the time of discharge, only 1.1% of the SARIs got cured, 93.5% improved and 2.3% uncured. Prognosis was slightly associated with complications (*R*=0.12, *p*<0.001). Cases with underlying medical conditions were more likely to have worse prognosis (*Z*=-2.26, *p*=0.024). Vomiting (*OR*=2.21, *95%CI*:1.31-3.70) and dyspnea (*OR*=2.77, *95%CI*:1.83-4.18) indicated a possible infection for severe pneumonia. About 89.3% patients were directly admitted into PICU. The median length of PICU stay was 4 days/case (*IQR*: 3-7 days) and it increased with age (*F*=278.34, *p*<0.001). After PICU treatment, the cases stayed in the wards for another 6 days (*IQR*: 4-9 days). In total, the length of hospitalization was 11 days (*IQR*: 8-15 days).
In conclusion, a worse prognosis of SARI patients was impacted by underlying conditions and complication developments. Patients less than 4 months had different clinical features compared with elder children.

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Vitamin D and Risk of Influenza in Children and Adults in Hong Kong

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Background: Some studies have hypothesized that Vitamin D may have a role to play in protection against influenza infection and illness. The objective of our study was to investigate whether serum vitamin D level is associated with the incidence of influenza infection and illness in school-age children and their family members in Hong Kong.
Methods: In a large trial in 2009-10, children and their household members were followed up for acute incidence of respiratory infections and illnesses, and paired sera are available from children, and also from their family members, to permit determination of infection by changes in humoral antibody titers, as well as determination of serum vitamin D levels. The sera from children and their household members were collected in 3-4 different study phases to allow us evaluate the seasonal pattern of vitamin D. We used the vitamin D level of the subjects during October to November 2010 as baseline vitamin D level to estimate the association of baseline vitamin D level and influenza infection.
Results: Vitamin D levels varied seasonally, peaking in September. We found the baseline serum vitamin D level was not statistically significantly associated with the incidence rates of influenza A(H1N1)pdm09, A(H3N2) and B virus infections, and ARI, by PCR or indicated by serology among children and adults, after adjusting for age, sex and vaccination. We did identify a higher risk of influenza-like illness among adults with lower serum vitamin D levels.
Conclusion: While lower levels of vitamin D possibly contribute to infection of influenza and other respiratory tract virus due to lower host immunity, our present finding provided evidence that seasonal variation in vitamin D levels does not determine the seasonality of influenza in subtropical regions.

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Viral Respiratory Infections in Okinawa

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About 200 million cases of viral community-acquired pneumonia occur every year-100 million in children and 100 million in adults. Molecular diagnostic tests have greatly increased our understanding of the role of viruses in pneumonia, and findings indicate that the incidence of viral pneumonia has been underestimated. In adults, viruses are the putative causative agents in a third of cases of community-acquired pneumonia, in particular influenza viruses, rhinoviruses, and human metapneumoviruses. Among them, influenza virus is a common cause of lower respiratory tract infections in adults. Infections can occur as a pandemic, epidemic, or sporadic outbreaks. Clinical pneumonia attributable to influenza virus infection is uncommon, but when it does occur, secondary bacterial pneumonia, as well as pure influenza viral pneumonia, needs to be considered as the possible cause.

In Okinawa, Japan, we have experienced two epidemics of influenza caused by the pandemic H1N1 2009 virus. Given this epidemic history, we compared the characteristics of critically-ill (intubated) patients observed during the first epidemics (2009-2010) and during the first post pandemic seasonal activity (2010-2011) caused by pandemic H1N1 2009 in Okinawa, Japan. For the first epidemics, the outbreak of influenza started in August 2009 and ended in February 2010 (seven months' duration). In contrast, in the first post pandemic seasonal activity, the outbreak started in December 2010 and ended in February 2011 (three months' duration). In the first epidemics, younger children were more likely to deteriorate and require intubation mainly because of influenza encephalopathy. In addition, 3 of 21 intubated patients died during the first epidemics. The age distribution of near-fatal influenza observed during the first epidemics had the highest rates in children under the age of 10 years, and intubation rates declined significantly for most age groups. In contrast, during the first post pandemic seasonal activity, the status of patients with increased age deteriorated, and 7 of 15 intubated patients died. The age distribution of near-fatal influenza infection during the first post pandemic seasonal activity was highest in adult patients aged 50-59 years, as well as adult patients aged 70-79. We also evaluated the clinicopathological findings of three autopsied cases and one survived case of influenza A virus pneumonia, and demonstrated that hyaline membrane formation and pulmonary hemorrhage play significant roles in pathogenesis of pure influenza A virus pneumonia. Our experiences concerning clinical features of nosocomial outbreaks caused by human metapneumoviruses as well as parainfluenza virus type 3 will also be presented.

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The Kinetics of Viral Load of Human Adenovirus Genotype 55 in Sequential Sputum and Blood Serum from an Immunocompetent Patient with Lethal Pneumonia

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Objective
Human adenovirus genotype 55 (HAdV-55) is a cause of severe and lethal pneumonia in immunocompetent adults. The main objective of this study is to describe the kinetics of viremia and viral shedding of HAdV-55 in sequential respiratory and blood samples.
Methods
Blood serum of an immunocompetent patient with lethal pneumonia was collected each day until death. Sputum, bronchoalveolar lavage fluid and pleural effusion samples were collected when available. Microbiological tests covering viral, bacterial and fungal pathogens had been performed. The hexon and fiber genes of adenovirus were both amplified to determine the genotype. Quantitative real-time polymerase chain reaction (qPCR) was conducted to quantify adenovirus load in all samples.
Results
The patient (male, 32 years old) was diagnosed as severe pneumonia and viremia caused by HAdV-55, without any clues of bacterial, fungal or other respiratory viral infections. Although a downtrend of the vriemia and viral load had been found after antiviral and support treatment in ICU over a period of 18 days, the patient died from respiratory failure and septic shock. The viral load in blood serum and sputum decreased from initial 2.1×10^8 copies/ml and 3.3×10^9 copies/ml to 4.5×10^3 copies/ml and 3.3×10^5 copies/ml until death. Surprisingly, the viral DNA concentration of pleural effusion decreased from 9.3×10^6 copies/ml to 3×10^4 copies/ml, then rose to 4.8×10^6 copies/ml again at the last day. The viral load increased with the order of blood serum, sputum, bronchoalveolar lavage fluid and pleural effusion samples collected at the eighth day after hospitalization (3.4×10^5, 5.2×10^5, 4.8×10^6 and 9.3×10^6 copies/ml).
Conclusions
Patient with lethal HAdV-55 pneumonia presented consecutive viral shedding in respiratory and blood samples. The further investigation on relationship between viremia, viral load in pleural effusion and clinical outcome is needed.

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2009 Pandemic H1N1 Influenza Can Rapidly Develop Escape Mutations in the Presence of a Host-Directed Vacuolar ATPase Inhibiting Drug

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Emergence of treatment-associated oseltamivir resistance in 2009 pandemic H1N1 (pH1N1), H5N1 and H7N9, and global circulation of seasonal oseltamivir-resistant A(H1N1) in 2007/8 has highlighted the limitations of our currently available antiviral armament. Anti-influenza drugs currently licensed for clinical use target the virus, but this strategy can lead to rapid drug resistance. Recently designed drugs targeting host factors critical for viral replication is one approach that may alleviate the problem of drug resistance, but so far the possibility of viral escape from a host-targeted strategy has not been well explored.

Across multiple genome-wide RNA interference screens, vacuolar (v)ATPases were identified as a critical host factor for influenza replication as they inhibit viral uncoating. Using the established vATPase inhibitor drug, bafilomycin A1 (BafA), we tested the hypothesis that influenza may escape vATPase inhibition by evolving to uncoat earlier in the endosomal pathway. As higher fusion pH has been associated with increased pathogenicity in influenza A viruses, we hypothesised that mutants may exhibit a more virulent phenotype.

BafA was effective at inhibiting replication of pH1N1, a reassortant H5N1 and a recent seasonal H1N1 strain in MDCK and CALU3 cells at nanomolar concentrations. Sensitivity of these viral strains to BafA varied, with reassortant H5N1 being least sensitive and the recent H1N1 most sensitive. Upon serial passage of pH1N1 in the presence of BafA, mutants were rapidly selected that were less inhibited by BafA than the parental virus. Plaque purified virus passaged with BafA selected for HA mutations A19T and S210N (H3 numbering) with no M gene mutations. In contrast, virus passaged in the absence of BafA had no HA or M gene mutations. BafA passaged mutant virus induced greater weight loss in BALB/c mice than control virus.

Our results show that exposure to a vATPase inhibiting drug leads to viral escape via mutations in HA which may result in a more virulent variant in mice, likely due to modulation of fusion pH enabling fusion earlier in the endosomal pathway. Whilst host-directed therapy presents exciting possibilities for developing highly efficient and broad-acting treatment for influenza, our results highlight the potential for rapid viral escape to host-targeting drugs and the importance of understanding the mechanism of viral resistance to these drugs.

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No Middle East Respiratory Syndrome Coronavirus Detected in Pilgrims at Hajj 2013 with Laboratory-Confirmed Influenza-Like Illness

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Background: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) may be transmitted amongst pilgrims attending Hajj. The mass dispersion of infected pilgrims can result in the rapid worldwide dissemination of MERS-CoV.

Methods: Nasal swabs (FLOQSwabs™; Copan) collected from Australian, Qatari and Saudi Arabian pilgrims attending Hajj from 13th – 18th October 2013 with an influenza-like illness (ILI; defined as subjective or measured temperature >38℃ and at least one respiratory symptom of cough, sore throat or rhinorrhoea) were placed into Universal Transport Medium (UTM™; Copan), frozen at -80℃ and transported from Saudi Arabia to Australia. Following nucleic acid extraction, respiratory viruses were detected using an in-house, real-time, multiplex RT-PCR assay targeting MERS-CoV, other human coronaviruses (OC43, 229E, NL63), influenza A, influenza B, respiratory syncytial virus, parainfluenza viruses 1 – 3, human metapneumovirus, rhinoviruses, enteroviruses and adenoviruses.

Results: ILI occurred in 112/1030 (10.9%) pilgrims; mean age was 35 (range 18 – 75) years, 49 (43.8%) were male, 35 (31.3%) received the trivalent influenza vaccine pre-Hajj. One or more respiratory virus was detected in 42/112 (37.5%) samples. No MERS-CoV was detected. The viruses detected were: rhinoviruses (30 [26.8%]), influenza A (5 [4.5%]; 4 A/H3N2 and 1 A(H1N1)pdm09), human coronaviruses OC43/229E (3 [2.7%]), adenoviruses (3 [2.7%]), parainfluenza virus 3 (2 [1.8%]) and parainfluenza virus 1 (1 [0.9%]). Viral co-infection was present in two samples (rhinovirus/adenovirus and rhinovirus/coronavirus OC43/229E).

Conclusions: No MERS-CoV was detected in pilgrims during the Hajj week of 2013. Rhinoviruses were the most common viruses detected. Infection control and vaccination measures should be enforced to prevent transmission of respiratory viruses at mass gathering events.

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A Clinical Prediction Rule for Diagnosing Human Infections with Avian Influenza A(H7N9) in a Hospital Emergency Department Setting

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Human infections with avian-origin influenza A(H7N9) virus were associated with a severe clinical picture and high mortality. Earlier identification of potential cases could inform triage decision of hospitalization and management in a timely and objective manner. We developed and evaluated a clinical prediction rule for predicting A(H7N9) infection in patient older than 12 years of age on their first presentation with acute respiratory infection to a hospital out-patient clinics and emergency room during an outbreak. We analyzed clinical details on presentation of 121 patients hospitalized with confirmed A(H7N9) in China from March to May 2013, and 2,777 patients hospitalized for other causes of acute respiratory infections captured by a hospital surveillance system in Hubei province of China from January 2010 through September 2012. We developed the clinical prediction rule using a 2-step coefficient-based multivariable logistic regression scoring method, and evaluated with internal validation by bootstrapping. In step 1, predictors for A(H7N9) included male sex, a history of poultry exposure, and the presence of fever, haemoptysis, or shortness of breath on history and physical examination. In step 2, haziness or pneumonic consolidation on chest radiographs and low lymphocyte count were also associated with a higher probability of A(H7N9). The observed risk of A(H7N9) was 0.2% for those assigned to the low-risk group by the model; and was 2.9%, 4.2%, and 43.8% for tertiles 1 through 3 respectively in the high-risk group. This prediction rule achieved good model performance, with an optimism-corrected sensitivity of 0.93, a specificity of 0.81, and an area under the receiver-operating characteristic curve of 0.96. This simple decision rule utilizing data readily obtainable in the setting of patients' first clinical presentation can be helpful in predicting their risk of A(H7N9) infection and the making of important clinical and public health decision in an timely and objective manner.

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Cost-effective Approaches for the Surveillance of Swine Influenza in Developing Countries

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In South-East Asian countries such as Vietnam, swine respiratory diseases are a major concern for both animal and human health. Diseases such as the Porcine Respiratory and Reproductive Syndrome are one of the most important burdens for the local pig farmers. Also since the H1N1 2009 pandemic, the critical need to monitor the circulation of swine influenza viruses (SIV) has become readily apparent, especially in countries with high human, pig and poultry populations. However, there is a lack of disease surveillance strategies suitable to the context of developing countries. This study aimed at testing and evaluating different surveillance protocols for the detection of swine respiratory diseases and more specifically influenza.

From May 2013, swine respiratory disease surveillance were implemented in collective and local slaughterhouses, sentinel farms and live pig markets in Northern Vietnam (Hung Yen province and Hanoi) for a one year period. A syndromic surveillance was also set up with the weekly collection of health-related indicators from local veterinarians, drug sellers and slaughterhouses.
From May to October 2013, 17 SIV (out of 850 swabs) were isolated from the collective slaughterhouse including A(H1N1)pdm09 and a novel H3N2 reassortant. From November 2013 to February 2014, 18 additional SIV (out of 600 swabs) were isolated but not yet subtyped.

No viruses were isolated from the other protocols. However pigs in the sentinel farms showed seroconversion at different period and ages. The syndromic surveillance had a good compliance and observance, analysis of the results is ongoing.

Our study demonstrated a high circulation of swine influenza in the region, and a potential seasonal trend with a higher circulation starting from August with a peak in the winter season. A(H1N1)pdm09 and multiple reassortant viruses were isolated from the industrial sector. The outcomes of this study will allow to further characterize SIV circulating in the study area and to improve the design of targeted surveillance protocols. The syndromic surveillance approach should allow for early detection of swine respiratory infection and targeted sampling.

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Age Specific Epidemic Curves of Four Common Respiratory Viruses in Subtropical City Hong Kong, 2004-2013

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Background Understanding age heterogeneity in seasonal transmission of respiratory viruses is critical for formulation of targeted control measures. However, age specific seasonal patterns remain poorly understood for most respiratory viruses in subtropical/ tropical regions.
Methods In this study we examined the age profile of laboratory confirmed cases with infections of influenza, respiratory syncytial virus (RSV), adenovirus and parainfluenza in subtropical city Hong Kong during 2004 to 2013. Wavelet analysis was adopted to characterize the temporal variations of virus activities in the age groups of 0-4, 5-17, 18-64 and 65+ years, respectively. Annual epidemic durations were also compared across age groups for each virus. To assess the effects of the 2009 H1N1 pandemic, age-specific epidemic curves of viruses other than the pandemic virus A(H1N1)pdm09 were compared between the pre-pandemic, pandemic and post-pandemic periods.
Results During the study period, there were a total of 9952, 1509, 5012, 1467 and 3391 cases confirmed with influenza, RSV, adenovirus and parainfluenza, respectively. For different types/subtypes of influenza, positive proportions of influenza A(H3N2) were similar in all age groups, whereas school children aged 5-17 years had higher positive proportions in A(H1N1), A(H1N1)pdm09 and B. Seasonal patterns of different types/subtypes of influenza markedly varied, but all the age groups showed highly synchronized virus activities during the study period. There were two waves of A(H1N1)pdm09 occurred in Hong Kong, the first in September 2009 with a higher attack rate in young and school children, and the second in February 2011that mainly attacked young children. Positive proportions of viruses other than A(H1N1) pdm09 significantly decreased during the pandemic period. Positive proportions of A(H3N2) in the post-pandemic period was slightly higher than those in the pre-pandemic period, but those of influenza B and RSV remained slightly lower. RSV, adenovirus and parainfluenza showed less clear seasonal patterns and their epidemic curves were not synchronized across age.
Conclusions Influenza virus activity was synchronized across different age groups in subtropical Hong Kong. Change in the age patterns of respiratory viruses other than A(H1N1)pdm09 after the 2009 pandemic suggests a possibility that the novel pandemic virus had interfered with the circulation of other respiratory viruses.

P44

Characteristics of Approved Influenza Rapid Diagnostic Testing in Japan, China and United States

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Influenza (flu) infection is acute respiratory tract infection caused by influenza viruses. Common symptoms include fever over 38 degrees Centigrade, joint pain and fatigue (tiredness). People 65 years and older, infants and people with chronic medical conditions such as heart disease, respiratory disease, kidney disease, or diabetes are at high risk of developing serious illness and at times can lead to death.
In 2009, there was a pandemic influenza season caused by influenza type A (H1N1) 2009pdm. A total of over 18000 influenza-related deaths were reported in 214 different countries and areas worldwide. According to a report from WHO, death rate in Japan was 0.16 per 10000 population and the number was the smallest among the world. One of the reasons to contribute Japan's small mortality rate is the early diagnosis by appropriate use of rapid diagnostic tests and proper use of flu antiviral drugs for treatments.
In this study, we focused on influenza rapid diagnostic tests approved and used by various countries and compared their performances. Using flu vaccine strain in 2013/2014 flu season, we evaluated sensitivity, reaction time and operation procedures of 3 commercial tests approved and used in Japan(made in Japan), 4 commercial tests approved and used in China(made in China and Korea) and 3 commercial tests approved and used in U.S. (made in U.S.).
Except certain products, commercially available tests including all the Japanese commercial tests proved to provide the same results when used according to their package insert. The results suggested the appropriate and active use of rapid diagnostic tests can contribute to global flu infection control and indicated the importance of outreach activity of proper use of rapid diagnostic tests in future.

P45

Dynamics of Epidemics of Influenza A and B Viruses in Okinawa

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Climatic conditions may have affected the incidence of influenza during the pandemic of 2009 as well as in previous instances. This study evaluated the effects of climatic conditions on influenza incidence in Okinawa, a subtropical region in Japan, during the 2009 pandemic using surveillance data from rapid antigen test (RAT) results. Weekly RAT results performed in four acute care hospitals in the Naha region of the Okinawa Islands from January 2007 to June 2014 were anonymously collected for surveillance of regional influenza prevalence. Lower ambient temperature, normally associated with higher influenza incidence during pre- and post-pandemic periods, did not maintain this trend during the pandemic of 2009. The association between climatic conditions and influenza incidence was less prominent during the pandemic of 2009 than during other pre- and post-pandemic periods. Interestingly, in Okinawa, we experience several episodes of influenza epidemics during summer months. Although frequently found in the literature, there are several reports that describe influenza epidemics throughout the summer, the pathogenesis driving summer epidemics of influenza has not been clarified. To evaluate the reason why influenza epidemics occur during summer in Okinawa, we evaluated full genome sequences of 48 representative human influenza A and influenza B viruses isolated in Okinawa between 2001 and 2013. Phylogenetic and antigenic data analysis revealed that different H1N1 and H3N2 viruses consistently co-circulate in Okinawa, despite being characterized by different temporal dynamics and degrees of genetic diversity. In addition, in Okinawa, influenza B viruses play a significant role for summer epidemics, almost every year. Ultimately, the analysis showed that new phylogenetic lineages and antigenic variants emerging in summer were likely to be the progenitors of the epidemic strains in the following traditional winter flu season. The synchronized seasonal patterns and high genetic diversity of influenza A viruses observed in Okinawa make it possible to capture the evolutionary dynamics and epidemiological rules governing antigenic drift and reassortment and may serve as an ''advanced warning'' system that recapitulates the global epidemic.

P46

Analysis of Influenza Virus Responsible for Persistent Infection After Drug Administration in an Immunosuppressed Patient

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The administration of influenza antiviral drugs such as neuraminidase (NA) inhibitors is essential in immunosuppressed patients because influenza infection not only interferes with treatment of the current disease, but also worsens their medical condition. However, neuraminidase inhibitors can readily lead to the development of drug-resistant variants in patients. In this study, we investigated the characteristics and drug-resistance properties of influenza virus isolated from a patient with a persistent infection after receiving a neuraminidase inhibitor.

In early February 2012, a girl aged 2 years undergoing treatment for neuroblastoma was infected with influenza A virus while receiving preventive oseltamivir. Nine days after treatment with the neuraminidase inhibitor peramivir, it was re-administered due to recurrent fever. Although her fever subsided, rapid diagnostic test findings showed that she was positive for influenza A virus until early April. We isolated the variants of influenza A virus from the patient on three separate occasions after drug administration, determined the sequence of the hemagglutinin (HA) gene, and reconstructed phylogenetic trees using the neighbor-joining method. The NA and M genes were screened for known markers of resistance. In addition, to reveal drug susceptibility, the 50% inhibitory concentration values (IC50 values) of four drugs were determined by fluorescence-based assays.

The HA gene in variant A/Yokohama/125/2012 had a mutation from adenine (A) to guanine (G) at nucleotide 721 with no substitution of the amino acid. The NA gene showed 100% homology in all three variants, with no known resistance mutations. Moreover, no changes in the IC50 values of the four neuraminidase inhibitors were observed, demonstrating that drug susceptibility was maintained in all variants.

Viral shedding was observed in the patient for 2 months after neuraminidase inhibitor administration, suggesting the possibility of persisting influenza infection under suppressed immune conditions. Although no variants resistant to neuraminidase inhibitors were isolated from the present patient, a drug-resistant virus in an immunosuppressed patient has been reported in the literature. It is important therefore to accumulate additional cases in order to establish an appropriate treatment strategy against influenza in immunosuppressed patients.

P47

Comparison of Clinical Features Between Severe Cases Infected with H7N9 and H1N1pdm Influenza A in Jiangsu Province, China

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Objective: To investigate the differences in clinical characteristics of severe cases infected with H7N9 and H1N1pdm Influenza A viruses.
Methods: We included all confirmed cases of severe H7N9 (n=37) as of April 4, 2014 and all confirmed cases of severe H1N1pdm (n=184) reported from June of 2009 to February 2010 in Jiangsu Province, China. Clinical information was obtained from the national system for reporting of notifiable infectious diseases. According to the Diagnosis and Treatment Scheme published by the National Health and Family Planning Commission of China, the criteria for severe infections of these 2 viruses were comparable. Mann-Whitney Test and Chi-square Test were used for statistical analyses.
Results: The median age (59.0 years) of severe H7N9 patients was significantly older than that of severe H1N1pdm (27.0). Male accounted for a significantly higher proportion in severe H7N9 patients than severe H1N1pdm. No significant difference in BMI was observed between those two groups of patients (median,23.9 vs. 22.9, ***P*** = 0.084). H7N9 patients were more likely to have at least one chronic medical disorder (55.6%) than H1N1pdm patients (34.5%) (***P*** = 0.018). 25.0% of H7N9 patients had cardiovascular disorders (excluding hypertension), which was significantly higher than that in H1N1pdm patients (7.30%, ***P*** = 0.004). Most of the patients of both H7N9 and H1N1pdm were treated with neuraminidase inhibitors, antibiotics and glucocorticoid with no significant differences. However, the clinical outcome was quite different. Nearly 85% of severe H7N9 patients were admitted to ICU compared with a proportion of 65.2% in severe H1N1pdm patients (***P*** = 0.026). Complications including ARDS, respiratory failure, liver and renal dysfunction occurred more frequently in severe H7N9 patients, resulting a significantly higher fatality rate than in severe H1N1pdm patients (45.7% vs. 15.3%, ***P*** < 0.001). The most common complications were ARDS and respiratory failure in both H7N9 and H1N1pdm patients. The time intervals from illness onset to first medical consultation, to hospitalization, to neuraminidase inhibitors administration and to death were all significantly longer in severe H7N9 patients.
Conclusion: Clinical features were quite different between severe H7N9 and H1N1pdm patients. Our study findings help the clinician for clinical management of those patients more efficiently

P48

Peculiarities of Influenza Virus Infection in Hospitalized Patients in 2012-2014, Moscow, Russia

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Introduction
The influenza A virus, especially A(H1N1)pdm09, is known to often cause severe diseases. This work presents the results of influenza monitoring in hospitalized patients in Moscow based on the Global Influenza Hospital Surveillance Network (GIHSN) protocol.

Methods
Patients with influenza like-illness (ILI) were admitted to Hospital #1 for infectious diseases in Moscow. Hospitalized patients of all ages presenting with ILI within 7 days between the onset of symptoms and admission were swabbed. The information on health conditions was obtained by face to face interview and review of clinical records. RT-PCR was applied to detect influenza A(H3N2), A(H1N1)pdm09 and B.

Results
During the 2012-2013 influenza season 1449 hospitalized patients were tested for influenza infection. Most patients (1088) were adults aged 15-64, 47 patients were the elderly and 311 – children aged 0-14. Influenza A(H1N1)pdm09 virus was identified in 18% of patients, A(H3N2) – 5,2% and influenza B – 9,2%. The highest percentage of positive influenza cases was found in patients with chronic obstructive pulmonary disease - 60%; cardiovascular diseases, asthma and renal impairment - 40%; cirrhoses - 30%. Almost half of pregnant women (36% of the admitted patients) were found to have influenza and 29% of them were positive for influenza A(H1N1) pdm09.
Preliminary data of the 2013-2014 season have shown that the dominant virus is influenza A(H3N2) – 16,8%, influenza A(H1N1)pdm09 - 3,2%, influenza B – 0,4%. Most pregnant women (37%) have been positive for influenza, mainly A(H3N2).

Conclusions
Influenza A(H1N1)pdm09 virus dominated in January-March 2012-2013, followed by A(H3N2). Influenza B virus was predominant at the end of the influenza season in Moscow. The 2013-2014 season is different compared to the previous one: the dominant influenza virus is A(H3N2), A(H1N1)pdm09 has been much more rarely found. Influenza B virus has low activity. Further gathering and analysis of the received data will determine particularities of the current season.

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P49

Optimization of Inoculum Dose of Inactivated Swine Influenza Vaccine for Immune Protection of Pigs

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The amount of vaccine immunizing dose can significantly affect the rate of active immunity formation, intensity and the number of positively react animals in the herd to the vaccination.
Objective of the study:
The objective of our research was to determine the vaccination dose of emulsified inactivated vaccine against swine influenza virus (SIV) for immunization of pigs.
Experimental procedure:
We used five experimental groups of 5 two months old pigs in each group. Pigs were immunized intramuscular in the upper third of the neck by experimental model emulsified inactivated vaccine against swine influenza type A, subtype H1N1. Pigs of group 1- in a volume of 0.5 cm^3; group 2 - 1.0 cm^3; group 3 - 2 cm^3; and group 4 - 3 cm^3. The fifth group remained intact. The SIV antigen with titer 6 log_2 was used as a specific immunogenic component in the preparation of the vaccine. In accordance to the volume of injected vaccines the final concentrations of antigen in experimental doses were 3.8, 4.8, 5.8 and 6.4 log_2. Revaccination of pigs was carried out on Day 21 in the same volume.
14 days after revaccination blood sera were collected from the piglets and tested in the hemagglutination inhibition assay (HI) for presence of specific antibodies to the SIV. The vaccine was considered effective if the antibody titers to the SIV in the sera of the 80% of vaccinated pigs after 14 days of revaccination were not less than 6.0 log_2.
Results:
According to the test results it was determined, that the immunization of pigs by experimental vaccine in doses containing 3.8 and 4.8 log_2 SIV antigen caused the formation of a weak immune response, resulting in low titers of antihemagglutination antibodies; 4.4 and 5.6 log_2, respectively. At the same time, the immune response for pigs, vaccinated with the drug, containing antigen SIV at doses of 5.8 and 6.4 log_2 was high and expressed high titers of antihemagglutination antibodies – 9.4 log_2.
Conclusions:
Thus, the optimal immunizing dose for 2 months old piglets is 5.8 log_2. The existing level of technology allows to contain this titer in a 2 cm^3 of the drug. Immunization of the animals with this dose provided the production of antibodies to the SIV in high titers.

P50

Monitoring Influenza Antiviral Resistance in Algeria: Establishment of a Local Surveillance of Influenza Viruses Susceptibility to Neuraminidase Inhibitors

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Background and Objectives : Following the emergence in Algeria in 2007 of oseltamivir-resistant seasonal influenza A(H1N1) strains, the National Influenza Center (NIC), Institut Pasteur of Algeria, launched a project to establish a monitoring susceptibility of influenza viruses to neuraminidase (NA) inhibitors once oseltamivir has been introduced in clinical practice for the treatment and prevention of influenza. The objective of this study was to develop and validate laboratory testing methods to help the clinician to quickly adapt the treatment especially for populations considered at high risk of complications.
Methods : We conducted this study by testing the oseltamivir-sensitivity Algerian strains isolated during four seasons from the 2009 pandemic season through the 2012–2013 season, including reference strains sensitive and resistant to oseltamivir, provided by the National Institute for Medical Research, United Kingdom and the Influenza Division, Centers for Disease Control and Prevention, Atlanta, USA. Neuraminidase inhibition assays (determination of IC50 values) were performed using two substrates, the chemiluminogenic NA-Star (Applied Biosystems) and the fluorogenic MUNANA (Sigma). To detect the H275Y substitution in neuraminidase, an RT-PCR allelic discrimination assay was performed using LightMix Kit Influenza A Virus HxN1 Tamiflu resistance [H274Y] (Tibmolbiol – Roche).
Results : We found that all pandemic and post-pandemic A(H1N1) viruses were susceptible to oseltamivir, with IC50 values ranging from 0.1 nM to 1 nM. These results showed a good correlation with those obtained by allelic discrimination. Otherwise, our results showed that chemiluminescent assay provides higher sensitivity for detection of neuraminidase activity with low virus concentrations and higher Signal/Noise ratio than fluorescent assay. However, the IC50 assays comparison has shown good correlation between IC50 values obtained with both substrates fluorogenic MUNANA and chemiluminogenic NA-Star.
Conclusions : Determining IC50 values provide valuable information for the detection of resistant strains, but getting outliers indicates the need for a molecular test to monitor genetic changes in the NA. The aim of our study was the establishment in Algeria of a Local influenza antiviral surveillance for monitoring the emergence of NA inhibitor-resistant viruses and to inform public health structures for the control of influenza infections.

P51

Wheezing Following Acute Respiratory Infections Versus Immunization in Young Children

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Objectives: Wheezing is a common symptom in early childhood, often triggered by acute respiratory infections and influenza like illness (ILI). At the same time, i.e. during the first 5 years of life, the majority of childhood vaccines are administered. Recently, wheezing has also been reported as an adverse event following live-attenuated influenza vaccination. So far, syndromic surveillance data exploring the prevalence of wheezing following infection with different types of influenza and other respiratory viruses are missing, especially in the context of recent immunization.

Methods: This inception cohort study was conducted in the context of a quality management (QM) program for children with Influenza like illness (ILI) at the Charité Department of Pediatrics in collaboration with the National Reference Centre for Influenza at the Robert Koch Institute (Charité Influenza-Like Disease = ChILD Cohort). All pediatric patients' (aged 0-18) fulfilling pre-defined ILI case criteria and presenting to the emergency room or inpatient units were enrolled consecutively from 10/2009 through 11/2013. A specifically trained QM team performed highly standardized physical examinations and comprehensive medical assessments in real-time. Nasopharyngeal swabs were collected for rapid antigen testing at the point of care in addition to RT-PCR at the Robert Koch Institute. Multivariate association analysis correlating wheezing to multiple clinical and demographic variables was conducted using binary logistic regression.

Results: A total of 4158 cases were enrolled in the study. Less than 5% received antiviral therapy during the course of illness. Audible wheezing and/or bronchial obstruction (n=1272; 31%) was most prevalent in infants aged less than 2 years. Presence of any underlying pulmonary condition further increased the risk of wheezing. Amongst viral infections, RSV ($OR_{adjusted}$>3; p<0.0001), metapneumovirus ($OR_{adjusted}$>2; p<0.0001) and rhinovirus ($OR_{adjusted}$>1.5; p<0.0001) were most significantly associated with occurrence of wheezing after adjusting for age, gender, time since the latest childhood immunization (TSLCI), hospitalization, and underlying risk factors. With respect to TSLCI, children recently vaccinated with hexavalent Diphtheria-Tetanus-Pertussis-HBV-Polio-HIB vaccine, p<0.0001, and Meningococcal C immunization were more likely to show wheezing in the course of ILI (p<0.001).

Conclusions: RSV, metapneumovirus and rhinovirus infections are significantly more likely to be complicated by wheezing/obstruction, causing hospitalizations and considerable disease burden. Infants and young children with recent hexavalent or meningococcal immunization may be at increased risk. This study underlines the importance of timely diagnostics and accurate immunization histories in the pediatric acute care and inpatient settings. Antivirals are needed for the treatment of RSV, rhinovirus and metapneumovirus infections.

P52

Novel *In Silico* Analysis of Pleiotropic Molecular Mechanism of Multitargeted Inhibitors for Influenza Virus Using the First-Principles Calculations

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Several furan derivatives including Mumefural have find in Japanese apricot fruit-juice concentrate. The derivatives effectively inhibit influenza A virus. Note that Mumefural has inhibitory activities against not only the Neuraminidase (NA) but also the Hemagglutinin (HA) of influenza virus. The multifunctional inhibitory activities of Mumefural are also effective to influenza A/Narita/1/2009(H1N1) pandemic virus. Mumefural is expected as a pleiotropic multitarget lead compound of new anti-Influenza drug. But the inhibitory molecular mechanism and active point of derivatives to the influenza virus has not clear.

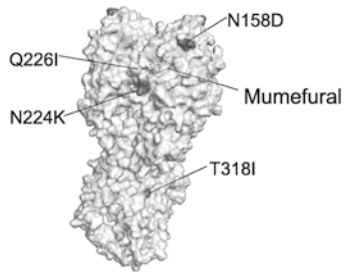

Fig.1 H5HA mutant complex with Mumefural

We, therefore, computationally investigated how the derivatives inhibit infection and budding of influenza virus by using novel *in silico* procedures based on the first-principles calculations and molecular dynamics (MD) simulations. And we theoretically analyzed inhibitory activities for several single/multiple point mutants of the HA/NA, and predicted the inhibitory activities for highly toxic mutants of the H5HA [1]. We, furthermore, analyzed which S- or R-Mumefural is a better inhibitor for the influenza virus by using the fragment molecular orbital (FMO) method at the correlated MP2/cc-pVDZ levels, because two different optical isomers for Mumefural due to a chiral center. With such a methodology, we can reliably estimate the electrostatic and van der Waals dispersion interactions between Mumefural and hydrophic/hydrophobic residues of the HA/NA influenza virus. Recently, flexible structure change of 150-loop in the N1NA was reported. [2] We traced dynamical structure change of the HA/NA with the derivatives by using MD simulations. We elucidated the origin of inhibitory difference between R- and S-Mumefurals to the HA/NA. S-Mumefural more effectively bound than R-Mumefural to both HA and NA, S-Mumefural could bound to the sialic acid pocket in the HA, and form a stable hydrogen bond with an amino acid residue in 150-loop in the N1NA. We tried to carry out optical resolution for racemic mixture of Mumefural, and experimentally determine the absolute structure for each optical isomer. We will compare experimental and theoretical structures of Mumefural and their HA/NA complexes.

Reference:
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P53

Phylogenetic and Clinical Virology Analyses of Influenza Virus Sequence Data from the First Four Years of the IRIS Study

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Objective: Extensive virological and clinical data has been collected from patients participating in a global observational trial, the Influenza Resistance Information Study (IRIS; NCT00884117). We investigated the phylogenetics and clinical virology of infecting viruses from baseline samples in Years 1–4 of IRIS.
Methods: Patients in Europe, North America, South-East Asia and Australia with influenza-like illness and/or a positive rapid test result for influenza had throat/nose swabs collected at baseline (day of study entry) for real-time reverse transcriptase polymerase chain reaction (RT-PCR) analyses of influenza type, subtype and neuraminidase inhibitor (NAI) resistance. Symptoms including fever were noted on diary cards. Positive samples were cultured, sequenced and phenotypically tested for NAI resistance. Full-length haemagglutinin (HA), neuraminidase (NA) and matrix protein 2 (M2) sequences obtained from Day 1 samples from individuals infected with A/2009H1N1, A/H3N2 and influenza B viruses recruited during Years 1–4 of IRIS were analysed by principal component analysis. Phylogenetic analysis was done by construction of maximum-likelihood trees. Clinical parameters were analysed by mixed effect models.
Results: Sequences were obtained from 2072 patients: 872 A/2009H1N1, 539 A/H3N2, 563 B/Victoria and 98 B/Yamagata sequences were obtained. Due to limited numbers B/Yamagata were not further analysed. Seeding from Asia into North America and Europe was observed for A/H3N2 but not for A/2009H1N1 and B/Victoria. Baseline viral loads were higher in febrile than in non-febrile patients for A/2009H1N1 ($p=0.04$), A/H3N2 ($p=0.0009$) and B/Victoria ($p=0.04$). Severity scores did not show significant differences between influenza subtypes; instead they were non-linearly associated with patient's age. In A/2009H1N1 and B/Victoria patients, a slight increasing trend in baseline viral load was observed over the course of the study ($p=0.008$ and 0.06 respectively). Mutations at the following positions were correlated with changes in phenotypic susceptibility to oseltamivir and zanamivir (multiple-testing adjusted $p<0.05$): for A/2009H1N1, 202 and 468 of HA, and 241 and 369 of NA; for A/H3N2, 174, 205 and 228 of HA, and 367, 369 and 464 of NA; and for B/Victoria, 73 of HA, and 27, 43, 65, 51, 199, 329 and 574 of NA.
Conclusions: Evolutionary history of influenza sequences collected in Years 1–4 of IRIS were reconstructed; temporal and geographical patterns in virus distribution and temporal change in baseline viral load were observed. Mutations correlated with change in NAI susceptibility were also identified. Additional sequences from Year 5 of IRIS are currently under analysis.

P54

Activity of Thiazolides Against Other Respiratory Viruses than Influenza

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Nitazoxanide and RM-5038 are broad-spectrum antiviral agents effective against 18 types of both RNA and DNA viruses with EC_{50s} for both drugs ranging from 0.03 to 1.5 µg/mL. Thiazolides are highly effective against influenza A and B viruses and nitazoxanide has completed its clinical development in the United States, Canada, Australia and New Zealand in approximately 2,500 adults and adolescents from 12 to 65 years of age in the treatment of uncomplicated influenza A and B and is now undergoing human clinical studies in the treatment of severe acute respiratory illness (SARI). RM-5038 is in phase 1 clinical trials in the United States.

Tizoxanide and RM-4848, the active circulating metabolites of nitazoxanide and RM-5038 are effective in cell culture assays against other respiratory viruses such as the two paramyxoviridae, parainfluenza virus and respiratory scyncytial virus and the coronaviridae such as the canine coronavirus and in clinical trials nitazoxanide is effective against four species of human coronavirus (229E, HKU1, NL63 and OC43) isolated in approximately 120 patients with influenza-like-illness (ILI). Thiazolides are not effective against rhinovirus.

Strain	Cell line	Assay type	Antiviral determination	EC50	SI50
Paramyxoviridae					
Parainfluenza Sendai virus (SeV)					
Tizoxanide	37RC	Single step (m.o.i. 5 HAU/10^5 cells)	HA	0.5 µg/ml	>100
RM-4848				0.7 µg/ml	>71.4
Respiratory Syncytial Virus (RSV)					
Tizoxanide	HeLa-ATCC	Single step (m.o.i. 1 $TCID_{50}$/cell)	IFA	0.5 µg/ml	10
RM-4848				0.4 µg/ml	13.3
Coronaviridae					
Canine Coronavirus S-378 (CCoV)					
Tizoxanide	A72	Single step (m.o.i. 5 PFU/cell)	$TCID_{50}$	1.0 µg/ml	>50
RM-4848				2.0 µg/ml	5
Picomaviridae					
Human Rhinovirus type 2					
Tizoxanide	HeLa R19	Single step (m.o.i. 3 $TCID_{50}$/cell)	$TCID_{50}$	>50	-
RM-4848				>50	-

P55

Clinical Evaluation of Highly Sensitive Silver Amplification Immunochromatography Systems for Rapid Diagnosis of Influenza

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Objectives:
Rapid diagnostic tests (RDTs) for influenza have been playing important role in early diagnosis of influenza. We evaluated the clinical usefulness of silver amplification immunochromatography (SAI) influenza virus detection kit, which employed new photographic development technology to increase the sensitivity of the conventional immunochromatographic assay.
Methods:
The detection limits of SAI system and other conventional immunochromatographic tests were calculated for comparison, using influenza virus strains. For clinical evaluation, four types of upper respiratory specimens were collected from the patients with influenza-like illness. The performance of SAI system was compared with viral culture and the conventional test (ESPLINE).
Results:
The detection limits of SAI system were smaller than other RDTs. A total of 1118 respiratory specimens were collected from patients between 2009 and 2012. The sensitivities of SAI system were 91.2% for type A and 94.4% for type B viruses and higher than those of the conventional RDT. The specificities of SAI system were 95.8% for type A and 98.0% for type B viruses.
Conclusion:
The SAI system was revealed to have high sensitivity and specificity, and it was easy to use. In conclusion, the SAI system is clinically useful for diagnosis of influenza from early stages of the illness.

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Influenza Viruses in Children from a Rural Indian Community: A Post-Pandemic Surveillance Study

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Background: Influenza-associated morbidity and mortality occurs every year, but the epidemiology and influenza disease burden is not well-defined among children living in rural India. As a part of ongoing active surveillance, we analyzed the post-pandemic trends of influenza incidence in a rural community in Ballabgarh, Haryana.

Methods: From March 2010 through February 2014, 2708 nasal and/or throat swabs were collected from children below 15 years of age presenting with influenza-like illness (ILI) to the pediatrics outpatient clinic at Ballabgarh. All samples were tested by real-time reverse transcription polymerase chain reaction (rRT-PCR) for seasonal and pandemic influenza viruses. All positive samples were processed for isolation in the MDCK cell line.

Results: Of 2708 children tested, 315 (11.6%) were influenza-positive by rRT-PCR; 67 (21.3%) for the influenza A/H1N1(pdm), 115 (36.51%) for A/H3N2, and 133 (42.2%) for influenza B. Over these four years, the highest positivity (13.8%) was observed during March 2013- February 2014. Age-wise analysis showed the highest incidence at 5-10 years of age (16.3%). Influenza peaked in the monsoon (rainy) season, between July and September each year. The highest influenza peaks occurred in September 2010 (38.5%) with predominantly A/H1N1(pdm), along with influenza B; in July 2011 (39.0%) with H3N2 alone; in September 2012 (41.4%) with predominantly influenza B, along with A/H1N1(pdm); and in July 2013 (52.4%) with A/H3N2 alone. Interestingly, a smaller peak (28.4%) was again seen in March 2013 due to A/H1N1(pdm). Influenza viruses were confirmed by isolation in the MDCK cell line in 73.7% of the real time PCR positive cases.

Conclusions: The influenza burden in children in this rural Indian community was substantial, with 11.6% positivity in ILI cases. The 2009 A/H1N1 pandemic was followed by predomination of influenza B and seasonal influenza A (H3N2) in alternate years, though pandemic influenza A continued to persist.

P57

Susceptibility of Avian Influenza A(H7N9) Viruses to FDA-Approved and Investigational Antiviral Drugs

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The ongoing outbreak of an avian influenza A(H7N9) virus represents a serious public health concern. Human infections with this recently emerged virus have been associated with substantial morbidity and a high rate of mortality. Infected patients have been treated with neuraminidase (NA) inhibitors, especially oseltamivir, because the H7N9 virus is resistant to M2 channel blockers. Treatment with oseltamivir has been reported to cause selection of H7N9 variants carrying changes in the NA, with R292K (R289K) most commonly detected. In the functional NA inhibition assay, R292K change was accompanied by >1000-fold rise in IC_{50} values for oseltamivir and peramivir, ~50-fold for zanamivir and ~25-fold for laninamivir. Other NA variants (e.g. E119V) showed a different drug resistance profile. We also assessed the effect of non-NA inhibitor antiviral drugs on replication of H7N9 viruses and their NA variants *in vitro* and *in vivo*. All antiviral compounds tested (favipiravir, fludase, nitazoxanide, and anti-HA mAb) showed substantial antiviral effect in cell culture. In a mouse model, fludase efficiently inhibited replication of the wild-type and R292K H7N9 viruses, even when treatment was delayed by 48 hours. Noteworthy, the same R292K virus exhibited a high degree of resistance to treatment with oseltamivir in mice. Furthermore, oseltamivir treatment failed to suppress replication of the R292K virus in the upper respiratory tract of ferrets. Experiments assessing efficacy of anti-HA mAb against wild-type and R292K H7N9 viruses are underway. Our data highlight the challenges associated with treatment of H7N9 infections and the need for new anti-influenza therapeutics.

P58

Flupep: a Novel Peptide for Treatment of Influenza Virus Infections

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Objectives: Development of effective antivirals for the treatment of influenza virus infections remains a major challenge. Current antiviral drugs which are active in control of disease can be divided into two major categories. These include neuraminidase inhibitors (oseltamavir and zanamivir) which block the exit of virus from the infected cell and adamantane derivatives such as amantadine and rimantadine which block the function of the M2 ion channel protein. Resistance to the neuraminidase inhibitors and the adamantanes is widespread and these drugs have limited usefulness for treatment of seasonal influenza. The potential rapid development of resistant viruses means that their efficacy may be short lived in pandemics. A third class of antivirals under development are novel peptides which act by preventing virus entry into cells. We have identified a family of peptides which restrict influenza virus replication *in vitro* and have now assessed their efficacy *in vivo*.

Methods: 5-6 week old BALB/c mice were infected intranasally with $5x10^3$ pfu influenza A virus (A/WSN/33) under isofluorane anaesthesia. Mice were treated with peptides by intranasal instillation at the time of infection or at various times post-infection. A range of peptides with modifications to improve solubility and half-life were used. Mice were monitored daily for clinical signs and weight loss and animals with severe clinical signs or 25-30% weight loss were humanely culled. At appropriate times lungs were removed for virus assay, histology or cytokine analysis.

Results: Prophylactic administration of Flupep was effective at preventing weight loss and clinical signs in infected mice. Therapeutic administration of pegylated peptide at 24 and 48h post-infection resulted in up to 100 fold lower lung virus titres 4 days post-infection. Treatment at 72, 96 and 120h post-infection resulted in over 10 fold lower lung virus titres at day 7. Mice treated with 50ug peptide at 72, 96 and 120h recovered from infection whereas untreated mice were euthanized at day 9. Treated mice had lower levels of inflammatory cytokines in the lungs at day 7 and histological examination showed less inflammatory infiltrate.

Conclusions: Flupep is effective in preventing lethal influenza virus disease in a mouse model of infection. The peptides result in lower lung virus titres, a lower inflammatory response and prevent lethal infection. Flupep has potential to be developed into a new therapeutic treatment for severe influenza virus infections.

P59

Salt Bridge Modifications Resulted to Structural Differences Between the Avian and Human H7N9 Hemagglutinin Proteins: Implications in Viral Evolution

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Background: Influenza A hemagglutinin (HA) is a homotrimeric glycoprotein composed of a fibrous globular stem supporting a globular head containing three sialic acid binding sites responsible for infection. The H7N9 strain has consistently infected an avian host, however, the novel 2013 strain is now capable of infecting a human host which would imply that the HA in both strains structurally differ. A better understanding of the structural differences between the avian and human H7N9 strains may shed light into viral evolution and transmissibility. In this study, we elucidated the structural differences between the avian and human H7N9 strains.

Methods: Throughout this study, we generated HA homology models from protein sequences available in the NCBI website, verified the quality of each model by determining the QMEAN scores, superimposed HA homology models to determine structural differences using SuperPose, and, likewise, elucidated the probable cause for any structural differences by determining changes in salt bridge formation. We used the Phyre 2 server to predict the protein conformation and J-mol software for structural analyses.

Results: We detected two different patterns of structural differences between the avian and human H7N9 strains, wherein, Pattern-1 showed three non-overlapping regions while Pattern-2 showed only one non-overlapping region. Moreover, we found that superimposed HA homology models exhibiting Pattern-1 contain three non-overlapping regions which we designated as: Region-1 ($S157_1$-$A160_1$); Region-3 ($R262_1$-$S265_1$); and Region-4 ($S270_1$-$D281_1$), whereas, superimposed HA homology models showing Pattern-2 only contain one non-overlapping region designated as Region-2 ($S137_1$-$S145_1$). We attributed the two patterns we observed to either the presence of salt bridges involving the $E114_1$ residue or absence of the $R141_1$:$D77_1$ salt bridge.

Conclusion: We propose that the putative absence of the $R141_1$:$D77_1$ salt bridge coupled with the putative presence of the $E114_1$:$R262_1$ and $E114_1$:$K264_1$ salt bridges found in the 2013 H7N9 HA homology model is associated to human-type receptor binding.

P60

Tertiary-Care Hospital-Based Influenza Surveillance in India in 2010-2014

R Kumar [1], Y Singh [1], R Guleria [1], AB Dey [1], A Choudekar [2], A Babu [1], D Namdeo [1], A Kumar [1], S Broor [2], DT Mourya [3], MS Chadha [3], L Dar [1]*

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Background: Seasonal influenza severity and predominating viral subtype vary annually. The epidemiology and influenza disease burden in India is not well characterized. We analyzed the post-pandemic trends of influenza incidence in patients attending clinics at All India Institute of Medical Sciences, New Delhi (AIIMS), a premier tertiary care hospital of India.

Methods: From March 2010 through February 2014, 2234 nasal and/or throat swabs were collected from patients presenting with influenza-like illness (ILI) to specific outpatient clinics at the AIIMS. All samples were tested by real-time reverse transcription polymerase chain reaction (rRT-PCR) for seasonal and pandemic influenza viruses. Positive samples were processed for isolation in the MDCK cell line.

Results: Of 2234 patients tested, 234 (10.5%) were influenza-positive by rRT-PCR; 102 (43.6%) for influenza A/H1N1(pdm), 75 (32.1%) for A/H3N2, and 57 (24.4%) for influenza B. Over these four years, the highest positivity (21.6%) was observed during 2010-2011. Age-wise analysis showed the highest incidence in patients below 10 years of age (16.9%) followed by those >10 to <20 years of age (14.5%). Influenza peaked twice a year, between July and September, and January to March. The two highest influenza peaks, during the monsoon of 2010 in August (36.5%), and during the winter of 2013 in February (28.2%), were both predominantly due to influenza A/H1N1(pdm). Distinct influenza peaks also occurred in July in 2011 (16.7%) and 2013 (20.7%) due to A/H3N2 alone; as well as in September 2012 (23.5%) predominantly due to influenza B. Influenza viruses could be isolated in MDCK cells in 71.4% of the real time PCR positive cases.

Conclusions: Amongst ILI cases presenting for tertiary care, children and young adults showed the highest incidence of influenza in the population studied. After the 2009 pandemic, the influenza A/H1N1 virus continued to circulate, and even showed some resurgence in early 2013, despite other strains predominating in 2011 and 2012.

P61

Assays for Influenza Vaccines Evaluation and Correlates of Protection

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Influenza is characterized by the occurrence of frequent unpredictable epidemics, and much less frequent worldwide pandemics. Epidemics arise because different strains of influenza are constantly generated through antigenic drift, which are able to evade the hosts pre-existing immunity. The potential risk of an H5N1 pandemic has increased research efforts in finding innovative substrates for viral production and improving immune system performance by accurately identifying correlates of protection. While correlates of protection against influenza viruses have not been fully defined, it is widely believed that protection against influenza can be conferred by serum hemagglutinin (HA) antibodies. European Medicines Agency (EMA) Guideline (*CPMP/BWP/214/96*) defines the single radial haemolysis (SRH) and haemagglutination inhibition (HI) as the main assays for FLU-seasonal vaccine. Considerable inter laboratory variation occurs between different laboratories and it is thus necessary to identify additional criteria in order to correlate the different methods of assessing surrogate correlates of protection. Serological assays used for FLU vaccine licencing were standardized several decades ago. A further updating of the EMA guideline (EMEA/CPMP/VEG/4717/03-Rev1) clearly identifies the need to introduce new assays for the evaluation of vaccine immunogenicity overall for pandemic strains, such as the Micro Neutralization (MN) test and Cell-mediated immunity (CMI) analysis. The standardisation and correlation between traditional serological assays (SRH, HI) and assays that allow the quantification of functional antibodies (MN test), could result in a more realistic evaluation of vaccine immunogenicity. One of the major drawbacks of the MN assay platform is the necessity to handle wild-type virus and the associated costs of high-level biocontainment facilities (i.e. Biosafety Level 3 laboratory) when studying the serology of highly pathogenic strains, such as H5 and H7. In this instance the use of influenza HA pseudotypes as surrogates for the wild-type virus is a safer alternative that may also have increased throughput capability and ease of standardization benefits. Pseudotype-based antibody assays have been shown to have broad utility for the detection of neutralizing antibody responses in avian and human sera, from natural infection and pre/post-vaccination against both avian and human influenza viruses.

Speaker's Biographies

Bin Cao

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Bin Cao is the Director of the Department of Infectious Diseases and Clinical Microbiology, Beijing Chaoyang Hospital, Beijing Institute of Respiratory Medicine, Capital Medical University.

Dr Cao's research interests include pneumonia and influenza. He has more than 80 papers published in international and national journals. His recent publication includes two original articles on H7N9 in an article in New England Journal of Medicine (May and June 2013), a paper in the Ann Intern Med (August 2011) entitled "Oseltamivir Compared With the Chinese Traditional Therapy Maxingshigan–Yinqiaosan in the Treatment of H1N1 Influenza: A Randomized Trial" and a paper in New England Journal of Medicine (December 2009) entitled "Clinical Features of the Initial Cases of 2009 Pandemic Influenza A (H1N1) Virus Infection in China."

Dr Cao acts as associate editor of ***Clinical Respiratory Journal*** and editorial board member of the ***Chinese Journal of Mycology, Chinese Journal for Clinicians and Chinese Journal of Respiratory & Critical Care Medicine***. He also serves as committee member of Chinese Medical Society, Beijing Infectious Disease Branch as well as member of Respiratory & Infectious Disease branch under the Chinese Society of Respiratory Disease. He had the honor of 14th Mao Yisheng Beijing Youth Science and Technology Award in 2011. He received grants from Chinese National Natural Science Foundation (2008, 2010 and 2012) and Ministry of Education of "New Century Excellent Talents Support Program" (2010).

Tawee Chotpitayasunondh

Queen Sirikit National Institute of Child Health, Thailand

Dr Tawee Chotpitayasunondh is currently Associate Professor (Honour), Senior Medical Officer and a paediatric infectious diseases specialist consultant at the Queen Sirikit National Institute of Child Health, Ministry of Public Health, Thailand. A/ Professor Chotpitayasunondh gained his medical degree from Siriraj Hospital, Mahidol University, Thailand, and has been a paediatric infectious diseases specialist for 38 years. His particular areas of interest include vaccines, tuberculosis, HIV/AIDS, antibiotics and influenza, Emerging infectious diseases and he has served as a WHO temporary consultant on paediatric HIV/AIDS, antivirals, avian/pandemic influenza.

A/Professor Chotpitayasunondh has been the recipient of several professional awards, including: the Best Senior Pediatrician Award 2011 from Royal College of Pediatricians, Thailand; the Best Academic Physician Award 2006 from Siriraj Medical School Alumni, Mahidol University, Thailand; the Best Research Award 2000 from MoPH, Thailand; and the Charles C Shephard Science Award 2000 from the US Centers for Disease Control and Prevention. He has over 68 scientific publications both in Thai and international medical journals to his name, and has published 35 chapters on paediatric infectious diseases in Thai textbooks.

Benjamin John Cowling

The University of Hong Kong, Hong Kong SAR, China

After graduating with a PhD in statistics from the University of Warwick, Dr COWLING spent a year at Imperial College London before moving to the University of Hong Kong in 2004. He is currently Associate Professor and Head of the Division of Epidemiology and Biostatistics in the School of Public Health at the University of Hong Kong, and a member of the Center for Communicable Disease Dynamics at the Harvard School of Public Health. He is an elected board member of the *International Society for Influenza and other Respiratory Virus Diseases* and serves on the editorial boards of *Influenza and Other Respiratory Viruses* and *PLoS ONE*.

Dr Cowling conducts research into the epidemiology of influenza and other respiratory viruses. His research team has characterized how easily seasonal and pandemic influenza viruses can spread in households, and the effectiveness of measures to reduce the risk of infection and transmission. His recent research has focused on the effectiveness of influenza vaccines in children, and the complex transmission dynamics of respiratory viruses. In 2013 he assisted China CDC with the public health research response to the avian influenza A(H7N9) virus outbreak, characterizing the clinical severity and epidemiologic characteristics of human infections. He has authored more than 160 peer-reviewed publications.

John DeVincenzo

The University of Tennessee School of Medicine, Knoxville, USA

Dr. DeVincenzo is a Professor of Pediatrics, Division of Infectious Diseases and Professor of Microbiology, Immunology and Biochemistry at the University of Tennessee School of Medicine. His research has focused on understanding the pathogenesis of Respiratory syncytial virus (RSV) directly in children and using this understanding to develop therapeutic and prevention strategies against this virus. He is the author of over 140 original published abstracts and papers on this subject.

He runs an active academic laboratory studying RSV and has received numerous honors for his research. For his groundbreaking proof of concept work applying RNA interference concepts to develop human therapeutics, Dr. DeVincenzo's study was listed as one of the most influential papers in medicine in 2010 (American Society for Microbiology). He is a practicing pediatric infectious disease specialist and also serves as the medical director of Le Bonheur Children's Hospital Molecular Diagnostics and Virology Laboratories. Dr DeVincenzo received his MD from Vanderbilt Medical School.

Carol L. Epstein

Medivector, Inc., Boston, USA

Carol Epstein MD is Executive Vice President and Chief Medical Officer of MediVector, Inc., a drug development company located in Boston, MA. She received her MD from Yale University School of Medicine, her SB from Massachusetts Institute of Technology, and did her internal medicine and rheumatology training at NYU-Bellevue in NYC. She has been in drug development for almost thirty years, with extensive experience in oncology, infectious disease, rheumatology and cardiology, among others. Dr. Epstein is one of the founders of MediVector, which has consulted to a range of large and small biopharmaceutical companies for both drugs and devices. MediVector began work on favipiravir for influenza for Toyama Chemical Ltd. and Fujifilm Holding in 2009. Dr. Epstein has led the clinical development of favipiravir since 2012, when MediVector obtained a US Department of Defense contract to take favipiravir from Phase 2 through FDA NDA approval. Favipiravir is currently undergoing global phase 3 studies.

Ann R. Falsey

University of Rochester School of Medicine, Rochester, USA

Dr Falsey is a Professor of Medicine at the University of Rochester School of Medicine.
The focus of her research has been clinical and translational research in the field of respiratory viral infections in adults. Dr Falsey received her Bachelor of Science degree in Biology at Providence College and Doctorate in Medicine at Vanderbilt University School of Medicine. She completed her residency in Internal Medicine at Strong Memorial Hospital at the University of Rochester and infectious disease fellowship at Yale University and the University of Rochester. Initially the focus of her research was defining the epidemiology and impact of respiratory syncytial virus in adult populations. More recently, Dr Falsey has broadened her research to include numerous viral respiratory pathogens including influenza, coronaviruses, parainfluenza viruses and human metapneumovirus. She has conducted numerous adult surveillance and vaccine studies in a variety of settings including ambulatory older adult clinics, nursing homes and senior daycare centers. She has extensive experience in the development and performance of diagnostic and serologic assays for influenza and other respiratory viruses including cell culture, RT-PCR, EIA and neutralization assays. Dr Falsey has been a standing member of the Clinical Studies and Field Research Study Section and has served as an ad hoc reviewer for numerous NIH study section reviews. She is a member of the steering committee for the Global Influenza Initiative, the Infectious Diseases Society of America and the American Virology Society. Dr Falsey has published over 200 peer reviewed articles, reviews, book chapters and abstracts. She currently serves as the Co-Director for the National Institute of Allergy and Infectious Diseases Respiratory Pathogen Research Center recently awarded to the University of Rochester. The role of the center is to provide NIH with the capability of conducting translational and clinical research focused on the development and optimization of control measures for viral and bacterial respiratory pathogens.

Hideki Hasegawa

National Institute of Infectious Diseases, Tokyo, Japan

Hideki Hasegawa graduated from the Hokkaido University school of medicine in 1993 with M.D. and finished graduate school at Hokkaido University in 1997 with PhD. He studied at the Rockefeller University from 1995 to 1996, and University College Dublin from 1996 to 1997 as a postdoctoral fellow. After finishing his studies he joined to National Institute of Infectious Diseases (NIID) where he is extending his research to development of mucosal vaccine against influenza viruses and human pathology of infectious diseases. In 2011 he promoted to Director of the Department of Pathology at NIID.

Frederick G. Hayden

University of Virginia School of Medicine, Charlottesville, USA

Dr Hayden is Stuart S. Richardson Professor of Clinical Virology and Professor of Medicine at the University of Virginia School of Medicine in Charlottesville, Virginia, USA. During 2006-2008 he served as a medical officer in the Global Influenza Programme at the World Health Organization, Geneva and during 2008-2012 as influenza research coordinator within International Activities at the Wellcome Trust, London.

Dr Hayden received his medical degree from Stanford University School of Medicine in 1973 and completed his clinical training in internal medicine and infectious diseases at Strong Memorial Hospital, University of Rochester, New York. He joined the faculty of the University of Virginia in 1978 and became Richardson Professor in 1990. His principle research interests have been on respiratory viral infections with a particular focus on the development and application of antiviral agents for influenza and rhinovirus infections. He has published over 350 peer-reviewed articles, chapters, and reviews, and co-edited the textbook *Clinical Virology*, the third edition of which was published in 2009 by the American Society for Microbiology.

Dr Hayden chaired the writing committees for two WHO clinical consultations on avian H5N1 and one on pandemic 2009 H1N1 influenza and continues to serve as a consultant to WHO on respiratory viral infections including avian H7N9 and MERS-CoV. In 2012-13 he worked with WHO colleagues to develop a new initiative, the Battle against Respiratory Viruses, to foster research on this important public health problem. During his work at the Wellcome Trust he also helped to establish a new federation of clinical research networks called the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) to improve the clinical research response to respiratory and other emerging infectious disease threats. Since October 2013 he has been serving as the interim chair of ISARIC. He is a member of multiple editorial boards and served as section editor of *Antiviral Therapy* for respiratory viruses for 18 years through 2013. He is a member of the board of the International Society of Influenza and Other Respiratory Viruses and a Fellow of the Infectious Diseases Society of America, American Academy of Microbiology, American Society for Clinical Investigation, and Association of American Physicians.

David Shu-Cheong Hui

The Chinese University of Hong Kong, Shatin, Hong Kong, China

Prof Hui is the Stanley Ho Professor of Respiratory Medicine and a Chair Professor at the Dept of Medicine & Therapeutics, The Chinese University of Hong Kong (CUHK). He is also the Director of the Stanley Ho Centre for Emerging Infections Diseases, CUHK.

He trained in Respiratory Medicine and Sleep Medicine in Sydney. During the major outbreak of SARS in 2003 and the H1N1 influenza pandemic in 2009, he was heavily involved in the clinical management of severe cases at the Prince of Wales Hospital in HK.

In Feb 2004, he attended the early human cases of H5N1 in Vietnam as a WHO advisor. Since then, he has frequently served as an advisor to the WHO on guidelines related to clinical management of severe influenza (including avian influenza) and emerging severe acute respiratory infections.

Prof Hui has published well over 220 peer-reviewed journal articles and 23 book chapters since joining the CUHK in 1998. His research interests include the clinical management of severe acute respiratory infections, safety of respiratory therapy and hospital infection control in the post SARS era.

Hideyuki Ikematsu

Influenza Study Group of Japan Physicians Association, Japan

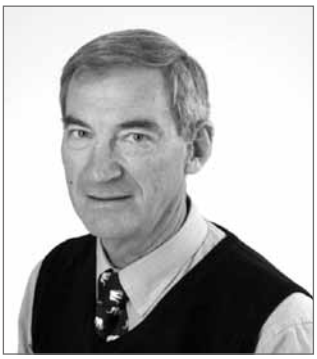
1982 Graduated, Faculty of Medicine, Kyushu University, Fukuoka, Japan
1982 Resident, the first department of internal medicine of Kyushu University Hospital.
1989 Visiting Fellow, National Institute of Health, Maryland, USA
1990 Research Associate, Department of Pathology, New York University Medical Center, New York, USA
1992 Stuff, Internal Medicine and Department of Clinical Research Hara-Doi Hospital, Fukuoka, Japan
1999 Visiting Scholar, Department of Molecular Immunology, Cornel University, New York, USA
1991 Chief of Internal Medicine and Department of Clinical Research Hara-Doi Hospital, Fukuoka, Japan
2011 Professor, Center of Advanced Medical Innovation, Kyushu University, Fukuoka, Japan
2014 Kurume Clinical Pharmacology Clinic
Specialties: Infectious diseases and internal medicine
Investigating the clinical feature of influenza and the clinical effectiveness of neuraminidase inhibitors though clinical research in Japan as a member of the Influenza Study Group of the Japan Physicians Association, since 2002.

Michael G. Ison

Northwestern University, Evanston, USA

Dr. Michael Ison completed his medical school training at University of South Florida College of Medicine and the obtained training in Internal Medicine at Oregon Health Sciences University in Portland, Oregon followed by Infectious Diseases at the University of Virginia. He joined the faculty of the Divisions of Infectious Diseases and Organ Transplantation at Northwestern University Feinberg School of Medicine in 2005. He is currently the Medical Director of the Transplant & Immunocompromised Host Infectious Diseases Service, Northwestern University Comprehensive Transplant Center.

He has continued to be a leader in the respiratory viruses research arena. He has two prospective interventional studies in hospitalized patients as well as a lead investigator for studies to determine how to prevent and treat influenza in immunocompromised patients. He has recently provided advice to the President's H1N1 Subcommittee, NIH, and BARDA on issues related to influenza in hospitalized and immunocompromised patients. Additionally, he is considered an expert in adenovirus infections in immunosuppressed patients.



Lance C. Jennings

Canterbury Health Laboratories & University of Otago, New Zealand

Associate Professor Lance Jennings is Clinical Virologist to the Canterbury District Health Board, Director of New Zealand's WHO National Measles Laboratory, Clinical Associate Professor in the Pathology Department, University of Otago, New Zealand, Fellow of the Royal College of Pathologists, London and a Founding Fellow of the Science Faculty, Royal Australasian College of Pathologists. He has been instrumental in the development of influenza control strategies for New Zealand, including the introduction of free influenza vaccine, establishment of influenza awareness education (NISG) and pandemic planning. He is co-founder in 2002 and current chairperson of the Asia Pacific Alliance for the Control of Influenza (APACI), a Charitable Trust, and initiated the First Asia Pacific Influenza Summit in the region in 2012. He is also the current chairperson if the International Society for Influenza & Other Respiratory Viruses (ISIRV).

Dr Jennings has been a member of WHO/WPRO Avian Influenza Outbreak Response and Expert Influenza teams in Asia and has held WHO short-term consultancies on measles and influenza in Asia and Europe. He was appointed as a Companion of the Queen's Service Order in 2006 in recognition of his service to virology in New Zealand and internationally.

Daniel B. Jernigan

Centers for Disease Control and Prevention (CDC), Atlanta, USA

Daniel B. Jernigan, MD MPH is the Deputy Director of the Influenza Division in the National Center for Immunization and Respiratory Diseases at CDC where he is responsible for oversight and direction of 320 staff members executing a broad scientific program to improve the detection, prevention, treatment, and response to seasonal, novel, and pandemic influenza. The Influenza Division is responsible for national surveillance of influenza and serves as a World Health Organization Collaborating Center for the Surveillance, Epidemiology and Control of Influenza.

Dr. Jernigan completed training at Duke University and Baylor College of Medicine, and is Board-Certified in Internal Medicine. He joined the CDC in 1994 as an Epidemic Intelligence Officer, and has been studying respiratory and emerging diseases since then. Dr. Jernigan has published numerous articles and book chapters on influenza and other emerging infections, and has led epidemiology and surveillance teams for national and international responses, including bioterrorism-related anthrax, West Nile virus, SARS in Taiwan, and others. He served as the Senior Science Officer and Lead for the Federal Epidemiology and Laboratory Task Force responding to the 2009 H1N1 influenza pandemic, and joined the WHO Consultation to the Kingdom of Saudi Arabia in June 2013 to assist in risk assessment for MERS-CoV.

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Yoshihiro Kawaoka

University of Tokyo, Tokyo, Japan/ University of Wisconsin, Madison, USA

Dr. Yoshihiro Kawaoka obtained his education in Japan, receiving his DVM in 1978 and his Ph.D. in 1983 from Hokkaido University. Early in his career, he identified the critical determinant for high pathogenicity of avian influenza viruses; this information is now used by the USDA and the Office International des Epizooties (World Organisation for Animal Health, OIE) as a criterion for identifying lethal and non-lethal avian influenza viruses. Dr. Kawaoka established reverse genetics, which allows the generation of 'designer' influenza viruses. This technology – coupled with his findings regarding the attenuation of deadly influenza viruses – has been used to develop candidate H5N1 influenza virus vaccines, which have proven efficacious in clinical trials. Through his studies of the 1918 Spanish influenza virus, Dr. Kawaoka revealed that the 1918 virus induced an abnormal immune response on infection. Dr. Kawaoka's research has been and continues to be invaluable to global influenza pandemic planning by public health agencies.

In 2006, Dr. Kawaoka was awarded the prestigious Robert Koch Award for innovative research in the field of influenza virology. In 2013, He was elected as a Foreign Associate of the United States National Academy of Sciences.

Hirokazu Kimura

National Institute of Infectious Diseases, Tokyo, Japan

Education
Mar.1999: Ph.D. in Graduate School of Bioscience, Gunma University.

Professional career
Apr. 2003-Mar. 2006:
Senior Researcher, Gunma Prefectural Institute of Public Health and Environmental Sciences
Apr.2006-Present:
Head, Viral Laboratory Training Division, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan
Apr. 2009-Present:
Guest Professor, Faculty of Medicine, Yokohama City University

Speciality
Virology (respiratory viruses) and host defense mechanisms

Professional memberships:
Guest Associate Editor, Frontiers in Virology
Editorial Board, Japanese Journal of Infectious Diseases
The member of The Japanese Society for Virology
The member of The Japanese Association for Infectious Diseases

Publications (as of 2013)
173 original articles (peer-reviewed)
Representative: J Allergy Clin Immunol, Blood, Sci Signal, Oncogene, Pediatrics, J Immunol, J Biol Chem, Infect Genet Evol, J Clin Microbiol

10 Review articles (peer-reviewed)
Representative: Front Microbiol, Curr Drug Targets

Nelson Lee

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

Professor Nelson Lee is the Head of Division of Infectious Diseases in Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong (CUHK); Consultant and Chief of the clinical infectious disease service at the Prince of Wales Hospital; and the Clinical Faculty Associate of the Stanley Ho Centre for Emerging Infectious Diseases (CEID), CUHK. Professor Lee is also serving as the Chairman of the specialty board of Infectious Diseases in The Hong Kong College of Physicians, and as an expert member in various advisory boards in the Hospital Authority, Center for Health Protection and Department of Health of Hong Kong. He is leading research, professional training, and inform clinical practices related to infectious diseases in the region.

Professor Lee's major research interests include emerging infectious diseases such as Influenza, Severe Acute Respiratory Syndrome, and severe pneumonia. His studies have contributed to the understanding of the clinical manifestations, diagnosis, treatment, viral kinetics, immunopathogenesis, transmission and control of these new infections, focusing on the severely ill hospital patients. He has published over 200 research articles, book chapters and conference papers in these areas; many of these have received high citations and referenced in clinical guidelines for health professionals. He has received multiple academic awards, both regionally and internationally. Professor Lee is also serving as an associate editor or referee for more than 20 regional and international medical journals.

Hongzhou Lu

Shanghai Public Health Clinical Center, Shanghai, China

Hongzhou Lu, M.D., is a physician- at Shanghai Public Health Clinic Center who will lead the clinical component of assembling a prospective cohort of HIV-infected persons with suspected TB in Shanghai. Dr. Lu has prior research experience and international publications in the realm of molecular diagnostics for central nervous system infections, pandemic H1N1 influenza, H7N9 influenza and HIV-TB co-infection. Dr. Lu is the PI for a current prospective TB immune reconstitution inflammatory syndrome (TB-IRIS) prospective cohort study in collaboration with NFSC and intra-mural NIH NIAID and a multi-center, prospective study on HIV/TB treatment in China. Dr. Lu is an Expert Consultant, Ministry of Health for HIV/AIDS. Dr. Lu is the team leader, AIDS sector of the Society of Chinese Medicine Infectious &Parasitic Disease Dr. Lu is the author of more than one hundred peer-reviewed publications and 27 reference books for medical education.

Jennifer L. McKimm-Breschkin

CSIRO Materials Science and Engineering, Parkville, Australia

Dr Jennifer McKimm-Breschkin, CSIRO Materials Science and Engineering Dr McKimm-Breschkin (BSc Hons, Monash), PhD (Pennsylvania State University, USA) joined CSIRO in 1987 after postdoctoral positions at Melbourne University, and the Walter and Eliza Hall Institute of Medical Research. She was part of the team involved in the development of the world's first influenza specific drug, Relenza, including carrying out preclinical studies on drug resistance. She continues to work in the evolving field of drug resistance, and has worked with both national and international pharmaceutical companies on the development of first and second generation influenza antivirals. She is a member of the Australian and American Societies for Microbiology, and is on the Antiviral Group committee of the International Society for Influenza and other Respiratory Viruses.

Ziad A. Memish

Saudi Minister of Health & Alfaisal University, Riyadh, Kingdom of Saudi Arabia

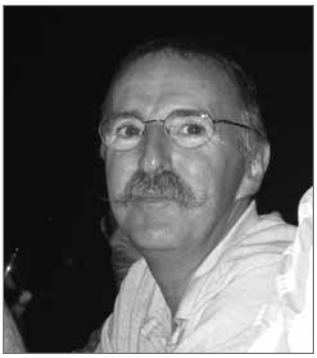
Ziad Memish is currently the Deputy Minister of Health for Public Health in Saudi Arabia. He is recently designated as Director, WHO Collaborating Centre for Mass Gathering Medicine. He is a senior Consultant Adult Infectious Diseases at King Fahad Medical City, Professor College of Medicine, Alfaisal University and Adjunct Professor in the Hubert Department of Public Health Rollins School of Public Health, Emory University.

Ziad Memish obtained his medical degree from the University of Ottawa in 1987. Additional qualifications include those from the American Board of Internal Medicine in 1990 and the American Board of Infectious Diseases in 1992, and the American Certification of Infection Control. He has received Fellowships of the Royal College of Physicians and Surgeons of Canada in Internal Medicine (1991) and Infectious Diseases (1992). He is a fellow of the American College of Physicians (1993), of the Infectious Diseases Society of America (1997), of the Society of Healthcare Epidemiology of America (2000), and Fellow of the Royal College of Edinburgh in 2011 and London 2012.

International committee memberships include the Council of the International Society of Infectious Diseases, the International Infection Control Informal Network at WHO and the Core WHO Hand Hygiene Guidelines Committee in Geneva. On a regional level he is a member of the WHO EMRO Research Advisory Group and Regional Expanded Program for Immunization Advisory Group. Professor Memish is also Ex-Chairman of the SHEA External Affairs Committee. Nationally he is the chair of the Hajj executive preventive medicine committee in addition to more than 14 National Committees including the National AIDS Committee, the National Committee for Communicable Diseases, the National Immunization Technical Advisory Committee and the National Committee for TB. He established and chaired the WHO collaborating center for Infection Control and the National Infectious Diseases training program and Infectious Diseases exam committee from 2006-2011 at the Saudi Council for Health Specialities.

In November 2007, he was awarded by the Custodian of the Two Holly Mosques King Abdullah Bin Abdulaziz Al Saud “The King Abdulaziz Medal from the First Degree” - the highest award on a National level in Saudi Arabia for achievements in the field of infectious diseases and infection control.

Professor Memish has presented more than 200 abstracts internationally and published more than 250 peer reviewed papers and chapters in books. A reviewer for 17 peer reviewed journals, he initiated 2 Elsevier journal in the MEA. The Editor-in-Chief of Journal of Infection and Public Health and Journal of Epidemiology and Global Health. Was the associate editor of the *American journal of Infection Control* and continues to be a ccorresponding editor of the *International Journal of Infectious Diseases*. Professor Memish sits on the editorial board of the *Joint Commission Journal on Quality and Patient Safety*, Current infectious diseases reports, the *Iranian Journal for Medical Sciences and the Journal of Chemotherapy*.



Alain Moren

Department of Epidemiology, EpiConcept, Paris, France

Dr Alain Moren is a medical epidemiologist currently Director of the epidemiology department of EpiConcept in Paris, France. Dr Moren holds a medical degree from the University of Caen France, an MPH from Johns Hopkins University, a PhD in epidemiology and an accreditation as a research director from Bordeaux University in France. Dr Moren was first trained as an internist (1976-1981) and then joined Médecins Sans Frontières (1981-1985) working mainly in African emergency settings among refugees and displaced populations. He was then selected as an EIs officer (1985-1987) by the US centre for disease control in Atlanta and was assigned for two years to the Pennsylvania department of Health. Upon returning to France Dr Moren developed Epicentre, the scientific department of MSF, carrying out research activities (infectious diseases and nutrition) in complex emergency situations. Later Dr Moren contributed to the development of the European programme for Intervention Epidemiology Training (EPIET: 1995-2006). The department of Epidemiology of EpiConcept, among other activities, coordinates for the European Centre for Disease Control and Prevention (ECDC) several vaccine effectiveness and impact studies.

Jonathan Nguyen-Van-Tam

The University of Nottingham, UK

Jonathan Van-Tam (JVT) graduated in Medicine from the University of Nottingham in 1987, trained in Public Health Medicine from 1991, and became a Senior Lecturer at the University of Nottingham in 1997, before joining the pharmaceutical and vaccines industries. He moved to the UK Health Protection Agency in 2004, where he was Head of the Pandemic Influenza Office, before returning to Nottingham in late 2007 as Professor of Health Protection. His special interest in influenza spans almost 25 years and focuses on: epidemiology; transmission; vaccinology; and pandemic preparedness. He is co-Editor of the textbook: Pandemic Influenza, which is now in its second edition. He has been a consultant to the World Health Organization since 2004. He sat on the UK Scientific Advisory Group for Emergencies (SAGE) during the 2009-10 pandemic crisis. His unit is an official WHO Collaborating Centre for pandemic influenza and research. He will shortly take up a role as Chair of UK NERVTAG (New and Emerging Respirator Virus Threat Advisory Group).

Yuan Qian

Laboratory of Virology, Capital Institute of Pediatrics, Beijing, China

Dr. Yuan Qian, Director of Laboratory of Virology, Capital Institute of Pediatrics, Beijing, China; Director of the National Network Laboratory for Surveillance of Influenza.
She graduated from the Department of Medicine, Jilin Medical University in 1977 and worked at the Department of Pediatrics, Liao Yuan Women and Children's Hospital as a Paediatrician for 3 years. She received her Master Degree of Medical Science in Medical Virology from Institute of Pediatrics, Peking Union Medical College in 1982 and has been working in this field since then. She worked as a Visiting Associate with Dr. Albert Kapikian at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, USA from 1988 to 1991 and with Dr. Linda Saif as a Visiting Scientist at Ohio State University, USA from Oct 1998 to Oct 1999. Her research interests: Viral Infections for children, especially respiratory viruses and the viruses causing diarrhea in children.
She has been a Full Member of American Society for Virology since 1991. She is now the Vice President of Chinese Society for Virology, Chinese Association for Microbiology.

Reiko Saito

Division of International Health, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

Dr. Reiko Saito is professor and head of Division of International Health, Graduate School of Medical and Dental Sciences, Niigata University, Japan. She received medical degree in 1991, completed medical training in internal medicine, and worked as a cardiologist for several years. Her interest changed to influenza and respiratory viruses after she moved to Public Health Department in Niigata University, where she obtained PhD in medicine in 2002. She became assistant professor in 2002, lecturer in 2006, and obtained current position in 2011 in Department of Public Health (International Health) in Niigata University. She worked in Zambia as a laboratory manager for Japan International Cooperation Agency (JICA) during 1997-1998. She worked for WHO Western Pacific Regional office as a short term consultant during 2003 - 2005 for control of SARS and avian influenza A/H5N1.
Her research interests are interface between clinical medicine and basic science on influenza virus; effectiveness of NAIs, antiviral resistance surveillance, genetic and antigenic characterization of influenza viruses, transmission and viral shedding, vaccine effectiveness, geographic spread of influenza infections in the community, and evaluation of school closures.

Shigeru Saito

Department of Obstetrics and Gynecology, Graduate School of Medicine and Pharmaceutical Science for Research, University of Toyama, Toyama, Japan

Professor S. Saito has received Bachelor of Medicine in 1980 from Nara Medical University, Japan, and Ph. D. from Nara Medical University in 1985. During a postdoctoral period at Kyoto University Virus Center, he studied molecular biology and immunology, especially cytokines. Since 1990 he has served as associate Professor in the Department of Obstetrics and Gynecology, Nara Medical University. Since April 1998, he has been Professor and Chairman in the Department of Obstetrics and Gynecology, Toyama Medical and Pharmaceutical University. He is one of the Editor-in-Chief of J. Reprod. Immunol.

His research interests have centered primarily at understanding the immunology at the maternal and fetal interface with particular emphasis on the roles of cytokines and chemokines and the immune cells that produce them in reproduction and perinatal medicine.

He is also interested in influenza infection. His team accomplished no maternal mortality occurred in Japan during pandemic (H1 N1) 2009.

Nahoko Shindo

Influenza and Respiratory Diseases, Department of Pandemic and Epidemic Diseases World Health Organization, Geneva, Switzerland

Professor Shindo is Medical Officer and leads Influenza and Respiratory Disease Team of Pandemic and Epidemic Disease Department at the World Health Organization in Geneva. Her background is in medicine, infectious disease and public health. She completed medical trainings at St Thomas' Hospital, Radcliffe Infirmary, Jikei University Hospital and her Ph D in Medical Science (microbiology and cell immunology) at Jikei University School of Medicine followed by specialist training in infectious disease/public health at Infectious Disease Surveillance Centre, National Institute of Infectious Diseases (NIID) in Tokyo, which is one of five WHO Influenza Collaborating Centres.

She has worked with WHO since 2002. During her time at WHO Professor Shindo has been involved in activities such as epidemic intelligence and verification, outbreak responses and influenza pandemic preparedness. She participated in major WHO responses including SARS, avian influenza, Indian Ocean Tsunami, the deadly viral hemorrhagic fever outbreaks in Africa, and more recently, 2009 influenza pandemic.

Since January 2012, she has been leading Influenza and Respiratory Diseases Team in the department. Her responsibility also extends to cover WHO Public Health Research Agenda for Influenza, assessment of influenza disease impact and influenza pandemic preparedness. Her other area of work is clinical aspects of influenza virus infection including use of antiviral medicine. During emergencies, she serves as one of the operational staffs of WHO's Strategic Health Operations Centre and deals with severe acute respiratory disease and avian influenza outbreaks of international public health concern. She and her team also combat against highly contagious and dangerous pathogens in the field to control the outbreak.

YueLong Shu

National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China

Dr Shu is the director of the WHO CC and CNIC, and deputy director of the NIVDC, at the China CDC. He finished his PhD studies in 1998, at the Institute of Virology, Chinese Academy of Preventive Medicine. He was a post-doctorate fellow at the Institute of Gene Therapy, Mount Sinai Medical School (1998–1999), and in Department of Molecular Pharmacology Medical School, University of California, Los Angeles (1999–2002). In 2002, he came back to China and worked as the director of Beijing JDK Bio-Tech Institute, National Engineering Research Center for Viral Biotechnology. Since 2004, he was designated as the director of CNIC, NIVDC of China CDC. The CNIC is a technical unit which is responsible for management of national influenza surveillance network in mainland of china, and is also a member of global influenza surveillance and response system (GISRS). His research area is mainly on influenza infection mechanisms and pathogenesis mechanisms, molecular virology research, new detection technique development and vaccine and drug-related research. He is currently undertaking several international research projects and national research projects. So far, he has published more than 50 papers in different journals, including Nature, Science and the New England journal of medicine.

Norio Sugaya

Keiyu Hospital, Yokohama, Japan

Norio Sugaya, M.D., is a professor and the director of the department of infection control and the department of pediatrics, Keiyu Hospital, Yokohama, Japan. He received his Ph.D. from the Keio University School of Medicine for a thesis on diagnostic testing for influenza. He has been active in the area of influenza diagnosis, treatment with neuraminidase inhibitors, influenza vaccines, and the epidemiology of influenza for over 30 years. His international experience includes participation as a panel member of Rapid Advice Guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus, held at World Health Organization in 2006 and as a panel member of Pharmacological Management of Pandemic Influenza A (H1N1), held in 2009-2010 at World Health Organization. He is also a member of Multinational Influenza Seasonal Mortality Study of NIH/FIC. In Japan, he is a central member of committees for influenza under the auspices of the Japanese Association for Infectious Diseases and the Japan Pediatric Society. He has done much to establish universal early treatment with neuraminidase inhibitors in Japan.

Hui-Ling Yen

The University of Hong Kong , Hong Kong, China

Dr. Hui-Ling Yen received her Ph.D. in Epidemiological Science from The University of Michigan, Ann Arbor and her postdoctoral training at St. Jude Children's Research Hospital, Memphis, TN. She is an Assistant Professor at the Center for Influenza Research, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong. The major research focuses have been on understanding the mechanism facilitating the transmission of influenza A virus among and between different reservoirs, exploring the potential virus-host interaction that affect viral pathogenicity and the host clinical outcome, and to examining the molecular determinants that confer antiviral resistance.

Maria C. Zambon

Public Health England, London, UK

Maria is Director of UK Public Health England (PHE) Reference Microbiology Services. The Centre's remit includes provision of UK national microbiology reference facilities and infectious disease surveillance.

Maria is medically and scientifically qualified and is a clinical virologist with R&D interests on vaccines, antivirals and surveillance. Main research interests are the diagnosis of viral infections in humans, especially RNA viruses, emerging infections and the development of new vaccines. Maria is Head of UK National Influenza Centre, and has a research group focused on influenza including antivirals and vaccines.

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Notes

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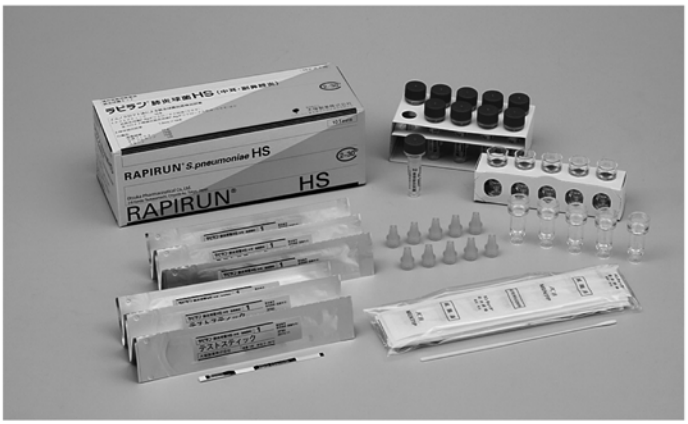

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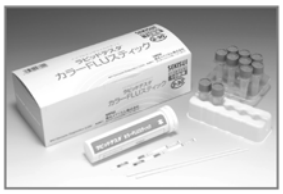

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【内　　容】

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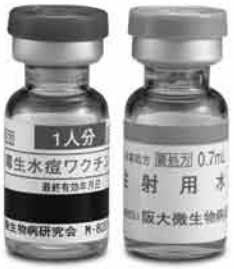

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生物由来製品・劇薬・処方せん医薬品(注)［健保等一部限定適用］

組織培養不活化狂犬病ワクチン

生物学的製剤基準　乾燥組織培養不活化A型肝炎ワクチン
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注）注意－医師等の処方せんにより使用すること

※効能・効果、用法・用量、接種上の注意等につきましては、製品添付文書をご参照下さい。

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Sofia shown with Virena router.

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※A型又はB型インフルエンザウイルス感染症

【警　告】
1. 本剤を治療に用いる場合は、本剤の必要性を慎重に検討すること。
2. インフルエンザウイルス感染症の予防の基本はワクチン療法であり、本剤の予防使用はワクチン療法に置き換わるものではない。

【禁　忌】（次の患者には投与しないこと）
本剤の成分に対して過敏症の既往歴のある患者

【効能・効果】
A 型又は B 型インフルエンザウイルス感染症の治療及びその予防

効能・効果に関連する使用上の注意
1. 本剤を治療に用いる場合には、抗ウイルス薬の投与が全てのA型又はB型インフルエンザウイルス感染症の治療には必須ではないことを踏まえ、本剤の使用の必要性を慎重に検討すること。
2. 本剤を治療に用いる場合、インフルエンザ様症状の発現から2日以内に投与を開始すること。
3. 本剤を予防に用いる場合には、原則として、インフルエンザウイルス感染症を発症している患者の同居家族又は共同生活者である下記の者を対象とする。
（1）高齢者（65歳以上）（2）慢性心疾患患者（3）代謝性疾患患者（糖尿病等）（4）腎機能障害患者
4. 本剤はC型インフルエンザウイルス感染症には効果がない。
5. 本剤は細菌感染症には効果がない（「1.重要な基本的注意（4）」参照）。

【用法・用量】
1. 治療に用いる場合
通常、成人及び小児には、ザナミビルとして1回10mg（5mgブリスターを2ブリスター）を、1日2回、5日間、専用の吸入器を用いて吸入する。
2. 予防に用いる場合
通常、成人及び小児には、ザナミビルとして1回10mg（5mgブリスターを2ブリスター）を、1日1回、10日間、専用の吸入器を用いて吸入する。

用法・用量に関連する使用上の注意
1. 本剤を治療に用いる場合、発症後、可能な限り速やかに投与を開始することが望ましい（症状発現から48時間経過後に投与を開始した患者における有効性を裏付けるデータは得られていない）。
2. 本剤を予防に用いる場合には、次の点に注意して使用すること。
（1）インフルエンザウイルス感染症患者に接触後1.5日以内に投与を開始すること（接触後36時間経過後に投与を開始した患者における有効性を裏付けるデータは得られていない）。
（2）インフルエンザウイルス感染症に対する予防効果は、本剤を連続して服用している期間のみ持続する。
3. 気管支喘息及び慢性閉塞性肺疾患等の慢性呼吸器疾患のある患者に対し、慢性呼吸器疾患の治療に用いる吸入薬（短時間作用発現型気管支拡張剤等）を併用する場合には、本剤を投与する前に使用するよう指導すること（「1.重要な基本的注意（3）」参照）。

【使用上の注意】
1. 重要な基本的注意
（1）因果関係は不明であるものの、本剤の使用後に異常行動等の精神神経症状を発現した例が報告されている。小児・未成年者については、異常行動による転落等の万が一の事故を防止するための予防的な対応として、本剤による治療が開始された後は、①異常行動の発現のおそれがあること、②自宅において療養を行う場合、少なくとも2日間、保護者等は小児・未成年者が一人にならないよう配慮することについて患者・家族に対し説明を行うこと。なお、インフルエンザ脳症等によっても、同様の症状が現れるとの報告があるので、上記と同様の説明を行うこと。
（2）免疫低下状態の患者に対する使用経験が少ない。免疫低下状態の患者に投与する場合には、患者の状態を十分に観察しながら投与すること。
（3）軽度又は中等度の喘息患者（ただし、急性のインフルエンザ症状を有さない症例）を対象とした海外の臨床薬理試験において、13例中1例に気管支攣縮が認められた。
インフルエンザウイルス感染症により気道過敏性が亢進することがあり、本剤投与後に気管支攣縮や呼吸機能の低下がみられたという報告がある（呼吸器疾患の既往歴がない患者においても同様な報告がある）。このような症状があらわれた場合、本剤の投与を中止し、適切な処置を行うこと。
また、気管支喘息及び慢性閉塞性肺疾患等の慢性呼吸器疾患のある患者に本剤を投与する場合には本剤投与後に気管支攣縮が起こる可能性があることを患者に説明することとし、必要時に使用できるよう短時間作用発現型気管支拡張剤を患者に所持させること。
なお、慢性呼吸器疾患の治療に用いる吸入薬（短時間作用発現型気管支拡張剤等）を併用する場合には、本剤を投与する前に使用するよう指導すること。
（4）細菌感染症がインフルエンザウイルス感染症に合併したり、インフルエンザ様症状と混同されることがある。細菌感染症の場合には、抗菌剤を投与するなど適切な処置を行うこと（「効能・効果に関連する使用上の注意」参照）。
（5）本剤投与後に失神やショック症状があらわれたとの報告がある。この失神やショック症状はインフルエンザウイルス感染症に伴う発熱、脱水等の全身状態の悪化に加え、本剤を強く吸入したこと、または長く息を止めたことが誘因となった可能性がある。患者には使用説明書に記載されている吸入法を十分に理解させ、くつろいだ状態（例えば座位等）で吸入するよう指導すること。また、このような症状があらわれた場合には、患者に仰臥位をとらせ安静に保つとともに、補液を行うなど適切な処置を行うこと。

2. 副作用
治療：
〈成人〉
国内臨床試験において、総症例291例（40mg/日　111例、吸入・鼻腔内噴霧40例を含む）中、50例（17.2%）に臨床検査値異常を含む副作用が報告された（承認時）。
使用成績調査及び特定使用成績調査5393例中、68例（1.3%）に副作用が報告された。その主なものは下痢13例（0.24%）、発疹7例（0.13%）、悪心・嘔吐7例（0.13%）、嗅覚障害6例（0.11%）であった（再審査終了時）。
〈小児〉
国内臨床試験において、総症例145例中、3例（2.1%）に臨床検査値異常を含む副作用が報告された（承認時）。特定使用成績調査784例中、13例（1.7%）に臨床検査値異常を含む副作用が報告された（再審査終了時）。
予防：
国内臨床試験において、総症例161例中、2例（1.2%）に臨床検査値異常を含む副作用が報告された（承認時）。特定使用成績調査289例中、副作用は報告されなかった（再審査申請時）。
（1）重大な副作用
1）**ショック、アナフィラキシー**：ショック、アナフィラキシー（血圧低下、呼吸困難、咽頭・喉頭浮腫等）（頻度不明[注1),2)]）が起こることがあるので、観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと。
2）**気管支攣縮、呼吸困難**：気管支攣縮、呼吸困難（いずれも頻度不明[注1),2)]）が起こることがあるので、観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと（「1.重要な基本的注意（3）」参照）。
3）**中毒性表皮壊死融解症（Toxic Epidermal Necrolysis：TEN）、皮膚粘膜眼症候群（Stevens-Johnson症候群）、多形紅斑**：中毒性表皮壊死融解症、皮膚粘膜眼症候群、多形紅斑（いずれも頻度不明[注1),2)]）等の重篤な皮膚障害があらわれることがあるので、観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと。
注1）自発報告又は海外のみで認められている副作用については頻度不明とした。
注2）海外での頻度：0.01%未満

●使用上の注意の詳細については、製品添付文書をご参照ください。

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