

Ministry of Healthcare of the Russian Federation
Research Institute of Influenza
WHO National Influenza Centre of Russia

International conference “Trends in Influenza Research”

Saint-Petersburg,
September 18-20, 2017

Abstract book

Saint Petersburg
2017

UDK
BBK
F

International conference "Trends in Influenza Research":
Abstract book.
Saint-Petersburg, September 18-20, 2017

ISBN

The abstract book contains materials of the international conference "Trends in Influenza Research", held in St. Petersburg on September 18-20, 2017.

It contains reports in accordance with the thematic sections of the conference: "Influenza surveillance in humans and animals", "Influenza vaccines: from seasonal to universal", "Vaccines and diagnostic systems", "Influenza Virology: from Molecule to Virus", "Influenza Antivirals: Mechanisms of Actions and Delivery".

The book is intended for scientists, specialists and students interested in modern problems and achievements of virology.

UDK
BBK

ISBN

©Authors, 2017
©Research Institute of Influenza, 2017



Contents

Letter of Welcome	5
Local organizing committee	6
Scientific committee	7
Conference Supporters	8
Research Institute of Influenza – General Information	9
About Saint-Petersburg	10
Conference Program	11
Plenary Lectures	20
Oral Abstracts	30
Poster Abstracts	96
Author Index	156



Letter of Welcome

Dear colleagues,

I am glad to welcome each of you to the Trends in Influenza Research international conference which coincides with the 50th anniversary celebration of the founding of the Research Institute of Influenza. This special event will hopefully become the first in a series of international conferences, organized in Russia, which bring together leading virology experts to share their insights into influenza. Our conference is intended to highlight key developments and prospects in such areas as human and animal influenza surveillance, influenza diagnostics, universal and seasonal influenza vaccine development, influenza molecular virology, virus-host interaction research, and antiviral drug design.

It is a special pleasure for me to note that the program includes additional sessions. The first one is a separate session organized by the World Health Organization European Regional Office. This session represents a vote of confidence by the world's medical community in our Institute. Another highlight is a business session which will include a roundtable discussion where representatives of the pharmaceutical industry will discuss the prospects regarding influenza prevention, diagnostics, and treatment. The Conference will be held in the center of Saint Petersburg at the famous Hotel Astoria which has played an integral role in city's history and has been the setting for many key events in Russia's political and cultural life.

I would like to thank each of you for attending the Conference and for bringing your expertise to our gathering. You, as leading specialists in influenza research, have the vision, the knowledge, the wherewithal, and the experience to help us pave our way into the future. My personal respect and thanks goes out to all of you.

Sincerely,

Dr. Andrey Vasin



Local organizing committee

Andrey Komissarov,

Research Organizing department,

Laboratory of Molecular Virology, Research Institute of Influenza

Anna Shtro, PhD,

Research Organizing department, Research Institute of Influenza

Marina Dmitrieva, PhD,

Director`s office, Research Institute of Influenza

Lyudmila Tsybalova, PhD, Dr.Sc.,

Deputy Director for Science, Research Institute of Influenza

Tamara Lobova, PhD,

Scientific Secretary, Research Institute of Influenza

Nataliya Gyulikhhandanova,

Technology transfer and intellectual property department,

Research Institute of Influenza

Olga Konshina,

International Department, Research Institute of Influenza

Alina Narkevich,

Administration, Research Institute of Influenza

Dmitriy Petrov,

IT-specialist, Research Institute of Influenza

Scientific committee

Sergey Kraevoy, MD, PhD, Dr.Sc.,

Deputy Minister of Healthcare of the Russian Federation

Sergey Rumyantsev, MD, PhD, Dr.Sc.,

Corresponding member of Russian Academy of Sciences,
Pirogov Russian National Research Medical University

Andrey Rudskoy, PhD, Dr. Sc., Prof.,

Rector of Saint Petersburg State Polytechnic University,
corresponding member of the Russian Academy of Sciences

Andrej Egorov, PhD, Dr.Sc. Prof.,

Leading researcher of the Research Institute of Influenza,
CEO of Vacthera BioTech GmbH (Vienna, Austria)

Konstantin Skryabin, PhD, Dr.Sc., Prof.,

Head of Scientific Advisory Board of the
Federal Research Center “Fundamentals of Biotechnology” RAS

Alexandr Shneider, PhD,

Founder and leader of the consortium CureLab,
CEO / CSO CureLab Oncogy, Inc.

Larisa Rudenko, MD, PhD, Dr.Sc.,

Head of the Virology Department in the Institute of Experimental
Medicine (Saint Petersburg, Russia).

Caroline S. Brown, PhD,

Team Leader High Threat Pathogens, Lead “Better Labs for Better
Health” initiative, WHO Regional Office for Europe

Conference Supporters



Research Institute of Influenza – General Information

Research Institute of Influenza (RII) was founded in 1967 as a lead institution under the Ministry of Public Health of the USSR to explore the «Influenza and Influenza-Like Illnesses (ILI) problem». The area of research included virology, immunology, and epidemiology of influenza and other acute respiratory infections (ARI), and designing means for their prevention and therapy. During 1992–2010 the Institute was incorporated into the Russian Academy of Medical Sciences (RAMS). Since the end of 2010, the activities of RII were continued under the Ministry of Health and Social Development of the Russian Federation (RF MPHSD).

Russia joined global influenza surveillance system 40 years ago. The Institute became part of this network since 1971, functioning as a WHO National Influenza Centre.

Since its foundation, RII in the framework of the Influenza and ILI Problem Committee coordinated influenza and ARI investigations, analyzed research works, determined current trends, defined influenza prevention strategies, and conducted scientific conferences and workshops.

Results of the Institute's research activity were presented in multitude of scientific publications and guidelines

About Saint-Petersburg

The Trends in Influenza Research international conference will be held in Saint-Petersburg, one of the most beautiful cities in Russia. St. Petersburg was once the capital of the Russian Empire and is sometimes called The Venice of The North, due to its beautiful waterways. The city is famous and attracts tourists from all over the world due to its unique beauty.

The Hotel Astoria, the venue of the Conference, is located in the historical center of St. Petersburg. In 1990, the city center and its related monuments and architecture were included in the list of UNESCO World Heritage Sites. The city's architectural heritage includes very diverse styles, including Baroque, pure neoclassical, and traditional Russian-Byzantine. Saint- Petersburg is home to more than two hundred museums, more than 400 bridges, and a ring of suburbs.



Many architectural masterpieces are located with walking distance of the Conference venue including St. Isaac's Cathedral, the Mariinsky Palace and the State Hermitage Museum.

Conference Program

September 18, 2017

8:00 – 9:00 **Registration**

Opening session

9:00 – 9:05 Welcome from Director of Research Institute of Influenza
Andrey Vasin

9:05 – 9:10 Welcome from the Ministry of Healthcare of Russian Federation

Sergey Kraevoy

Deputy Minister of Healthcare of Russian Federation

9:10 – 9:15 Welcome from the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor)

Albina Melnikova

Deputy director of Epidemiological surveillance department

Welcome from Scientific Committee

9:20-9:25 **Caroline Brown**

WHO Regional Office for Europe

9:25 - 9:30 **Alexander Shneider**

CureLab Oncology Inc., USA

9:30 – 9:35 **Andrej Egorov**

Research Institute of Influenza, Saint Petersburg, Russia

Plenary Session 1. Interdisciplinary Studies in Influenza Research

9:40 -10:10 Diversity in Circulating Influenza Viruses in the WHO European Region during Seven Post-2009 Pandemic Seasons (2010-2011 to 2016-2017 Seasons)

Caroline Brown

WHO Regional Office for Europe

10:10 – 10:40 Broad Neutralization of Influenza Virus and Implications for a Universal Vaccine and Therapy

Ian Wilson

Skaggs Institute of Chemical Biology, USA

10:40 – 11:10 Human Quarantine and Challenge Contribute to the Science of Universal Influenza Vaccines

John Oxford, PhD

University of London, UK

11:10 – 11:30 Coffee break

11:30 – 12:00 Biological Diversity of Avian Influenza Viruses in Wild Birds in the Asian part of Russia

Alexander Shestopalov

Research Centre of Clinical and Experimental Medicine, Novosibirsk, Russia

12:00 – 12:30 Vaccine Potential of Influenza Vectors with Modified NS1 gene

Andrej Egorov

Research Institute of Influenza, Saint-Petersburg, Russia

12:30 – 13:00 Progress and Problems in Implementing the Global Plan for Pandemic Preparedness

Larisa Rudenko

Institute of Experimental Medicine, Saint Petersburg Russia

13:00 - 14:00 Lunch

ORAL PRESENTATIONS

Session 1. Influenza Surveillance in Humans and Animals

14:00 – 14:20 Influenza Surveillance in Russia: Status, Challenges and Future Developments.

Anna Sominina

Research Institute of Influenza, Saint Petersburg, Russia

14:20 – 14:40 Influenza in Pregnants: the Results of Monitoring During Epidemics 2012-2017, Moscow

14:40 - 15:00 Implementation of Next-Generation Sequencing in Influenza Surveillance: Experience of NIC Saint-Petersburg

Andrey Komissarov

Research Institute of Influenza, Saint Petersburg, Russia

15:00 – 15:20 Collection of Influenza and ARVI Viruses of the Research Institute of Influenza: Significance for Influenza Surveillance in Russia, Current State and Perspectives of Development

Mikhail Eropkin

Research Institute of Influenza, Saint Petersburg, Russia

15:20 – 15:40 Sentinel Surveillance of Influenza and Other Respiratory Viruses in Cuba. 2012-2016

Susana Borroto

Pedro Kourí Institute, Cuba

15:40 – 16:00 Viral Etiology of Influenza-like Illnesses in Hospitalized Children in Novosibirsk, Russia, in 2013 – 2017

Olga Kurskaya

Research Institute of Experimental and Clinical Medicine, Novosibirsk, Russia

16:00 - 16:20 Coffee break

Session 2. Influenza Vaccines: from Seasonal to Universal

14:00 – 14:20 Plants as Factories for the Production of Influenza Vaccines

Nicolai Ravin

Center of Bioengineering, Moscow, Russia

14:20 – 14:40 Efforts Made to Create a Universal Influenza Vaccine

Lyudmila Tsybalova

Research Institute of Influenza, Saint Petersburg, Russia

14:40 - 15:00 Novel Mucosal Influenza-Vectored Tuberculosis Vaccines: Preclinical and Clinical Evaluation

Marina Stukova

Research Institute of Influenza, Saint Petersburg, Russia

15:00 – 15:20 Parallel Testing the Subunit, Adjuvanted, Whole-Virion Inactivated and Live Influenza Vaccines Against Homologous and Heterosubtypical Challenge

Alexandra Gambaryan

Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products, Moscow, Russia

15:20 – 15:40 GRIPPOL Family Vaccines: Development History and Perspectives

Natalia Puchkova

“NPO Petrovax Pharm”, LLC, Moscow, Russia

15:40 – 16:00 Manufacturing Technology Development and Pre-clinical and Clinical Trials of Pandemic and Seasonal Influenza Vaccines

Ainur Nurpeisova

Research Institute for Biological Safety Problems, Gvardeiskiy, Kazakhstan

16:00 - 16:20 Coffee break

ORAL PRESENTATIONS

Session 1. Influenza Surveillance in Humans and Animals (continued)

16:20 – 16:40 Monitoring the Influenza Virus on the Human-Animal Interface and the Properties of Avian Influenza Viruses A(H5N8) Isolated in Russia in 2016-2017 Years

Vasiliy Marchenko

State Research Center of Virology and Biotechnology “Vector”, Rospotrebnadzor, Koltsovo, Russia

16:40 – 17:00 Isolation of Unusual Strains of H1N1 Swine Influenza Virus from a Pig in Russia, 2016-2017.

Ivan Sobolev

Research Institute of Experimental and Clinical Medicine, Novosibirsk, Russia

17:00 – 17:20 Swine Influenza Surveillance in Russia: Failures, Advances, Perspectives

Daria Danilenko

Research Institute of Influenza, Saint-Petersburg, Russia

17:20 – 17:40 The Study of Molecular-Biological Properties of H4N6 AIV Subtypes Isolated in Caspian Seal Population; the Assessment of Pathogenic Potential in Experimental Infection in Mice.

Marina Gulyaeva

Scientific Research Institute of Experimental and Clinical Medicine,
Novosibirsk State University, Novosibirsk, Russia.

17:40 – 18:00 Identification of Etiology of RVI Using PCR Tests in Order to Manage Etiotropic Treatment

Nicolai Lvov

Military Medical Academy, Saint Petersburg, Russia

18:00 – 18:20 Positive Association of Supersaturation Effects in the Human Airways with Influenza Seasons in Okinawa (2007-2012) – New Method for Analyzing and Forecasting in Subtropical Climate

Alexander Ishmatov

Scientific Research Institute of Experimental and Clinical Medicine,
Novosibirsk, Russia

Session 3. Vaccines and Diagnostic Systems

16:20 – 16:40 The Protective Effect of Associated Virus-Bacterial Vaccines Based on Live Attenuated Influenza Vaccine (LAIV) and Streptococcal Recombinant Peptides in Mouse Model of Post-Influenza *S. pneumoniae* Infection

Yulia Desheva

Institute of Experimental Medicine, Saint Petersburg, Russia

16:40 – 17:00 Bioprocessing of Influenza Vaccines

Mats Lundgren

GE Healthcare Life Sciences, Sweden

17:00 – 17:20 Development of New Multiplex Diagnostic Methods for Sepsis

Sergey Klotchenko

Research Institute of Influenza, Saint-Petersburg, Russia

17:20 – 17:40 Microarray Based Approach for Multiparameter Analysis of Influenza Virus

Rustam Heydarov

Engelhardt Institute of Molecular Biology, Moscow, Russia

17:40 – 18:00 Automated mariPOC® Platform Enables Highly Sensitive and Rapid Antigen Detection and Subtyping of Influenza Viruses

Janne O. Koskinen

ArcDia International Oy Ltd, Finland

18:00 – 18:20 Etiological Laboratory Diagnostics of ILI in Surveillance and Clinical Practice.

Svetlana Yatsyshina

Central Research Institute of Epidemiology Rospotrebnadzor, Moscow, Russia

18:20 – 20:00 Welcome reception



September 19, 2017

8:00 – 9:00 Registration

Plenary Session 2. Interdisciplinary Studies in Influenza Research

9:00 – 9:30 Influenza A Drift: Reconsidering Influenza A Virus Evolution and Immunity

Jonathan Yewdell, MD, PhD

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, USA

9:30 – 10:00 Influenza Virus-Host Interactions

Adolfo Garcia-Sastre

Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, USA

10:00 – 10:30 Flu Host Jump: from H1-H16 to Bat-Derived H17/18

George Fu Gao

Chinese Center for Disease Control and Prevention, China

10:30 – 10:50 Coffee Break

10:50 – 11:20 Controlling Influenza and Other Respiratory Virus Infections Using Therapeutic Interfering Particles

Jeffrey Almond

Oxford University, UK

11:20 – 11:50 Molecular Mechanisms of Broad Antiviral Activity of Azolo-Azines Derivatives

Andrey Vasin

Research Institute of Influenza, Saint Petersburg, Russia

11:50– 12:20 Development of Oncolytic Influenza A Viruses

Michael Bergmann

Medical University of Vienna, Austria

12:20 – 12:50 “Live and Not Let the Virus Die” as Therapy Principle

Nicolai Bovin

Institute of Bioorganic Chemistry, Moscow, Russia

12:50 – 13:50 Lunch

ORAL PRESENTATIONS

Session 4. Influenza Virology: from Molecule to Virus

13:50– 14:10 Effects of PA-X Deficient Viruses in Chicken Embryos and Implications on Yield of Candidate Vaccine Viruses (CVVs)

Saira Hussain

Francis Crick Institute, UK

14:10 – 14:30 Human T-cell Immunity to Emerging Highly-Pathogenic Viruses

William J. Liu

National Institute for Viral Disease Control and Prevention, China CDC

14:30– 14:50 Defining RNA Structured Regions and Finding RNA Structured Regions in H1N1 Influenza Virus

Alexander Shneider

CureLab Inc., USA

14:50 – 15:10 Structural Bases of PB1 Derived Peptide Mechanism of Action

Yana Zabrodskaya

Research Institute of Influenza, Saint Petersburg, Russia

15:10 – 15:30 RNA Secondary Structure Analyses of the NS Segment of Influenza A Virus

Irina Baranovskaya

Research Institute of Influenza, Saint Petersburg, Russia

Session 5. Influenza Antivirals: Mechanisms of Actions and Delivery

13:50– 14:10 Immunochemical and Virus Neutralization Properties of a Recombinant Human Immunoglobulin A Specific to Influenza Virus A Hemagglutinin

Teimur Aliev

Moscow State University, Moscow, Russia

14:10 – 14:30 Multi-Contact Ligands As One of the Ways to Overcome Viral Resistance

Vladimir Tsvetkov

Research Institute of Influenza, Saint Petersburg, Russia

14:30– 14:50 New Strategies for Effective Delivery of Antiviral siRNA Against Influenza A Virus

Alexander Timin

RASA center in Tomsk, Tomsk Polytechnic University, Tomsk, Russia

14:50 – 15:10 The Efficacy of the Influenza Treatment with Antiviral Drugs with Different Mechanism of Action

Nicolai Malyshev

Federal Research Center of Epidemiology and Microbiology named after N. F. Gamaleya, Moscow, Russia

15:10 – 15:30 Some Prospects toward Poly-Inhibitors of Influenza and other Human Viruses

Alexander Serbin

A.V. Topchiev Institute of Petrochemical Synthesis, Moscow, Russia

15:30 – 15:50 Coffee break

RAPID ORAL PRESENTATIONS

15:50 – 16:30 **Rapid oral poster presentations 1**

“Influenza surveillance”, “Vaccine development”, “Diagnostic systems”

15:50 – 16:30 **Rapid oral poster presentations 2**

“Molecular biology of influenza”, “Influenza antivirals”

16:30 – 18:00 **POSTER PRESENTATIONS**

September 20, 2017

10:00 – 11:30 Business Session

11:30 – 11:50 Coffee break

11:50 – 13:00 Press-conference

10:00 – 13:00 WHO Regional Office for Europe Meeting (on invitations)

13:00 – 14:00 Lunch

Closing Plenary Session

14:00 – 14:30 100 Years Since 1918: Are We Ready for the Next Pandemic?

Daniel Jernigan

Centres for Disease Control and Prevention, Atlanta, USA

14:30 – 15:00 Global Initiative on Sharing of All Influenza Data (GISAID) – Connecting the Influenza Community

Andrey Komissarov

GISAID Database Technical Group, Research Institute of Influenza, Russia

15:00-15:30 mariCloud on-line service for real-time and global epidemiological surveillance of acute infections

Janne O. Koskinen

ArcDia International Oy Ltd, Finland

15:30-15:50 Adjuvants for influenza vaccines: challenges, perspectives and multi-component formulations

Yury M. Vasiliev

Institute of Highly Pure Biopreparations, Saint-Petersburg, Russia

15:50 Conference closure

Plenary Lectures

Biological diversity of avian influenza viruses in wild birds in the Asian part of Russia

Shestopalov A.^{1*}, Sharshov K.¹, Yurlov A.²

¹ *Scientific Research Institute of Experimental and Clinical Medicine,
Novosibirsk, Russia*

² *Institute of Systematics and Ecology of Animals, Novosibirsk, Russia*

The disease of animals and humans, called influenza, is caused by representatives of the family of orthomixoviruses: influenza viruses of genera A, B and C. Of these, only the influenza A (HAV) virus has a wide range of hosts, mainly waterfowl. The study of the biological diversity of HAV in wild birds in the territory of Western Siberia was carried out in the period 2007-2017. It was collected 3,375 specimens from birds of 8 orders: Anseriformes, Charadriiformes, Passeriformes, Ciconiiformes, Gruiformes Grebes, Pelecaniformes, birds of prey.

The main number of samples was collected from the birds of three orders - Anseriformes, Charadriiformes and Passeriformes. The percentage of specimens from the representatives of the Anseriformes was more than 70% of the total. 285 isolates of influenza A virus were isolated from the material, collected from Anseriform. In the period from 2007 to 2017, the percentage of virus carrying in the Anseriformes varied from 5.6 to 20. The order of the Charadriiformes is characterized by lower percentage of virus isolation. In our work, the virus carrying in the birds of this order averaged 1.4%.

Phylogenetic analysis of the M gene of all the isolated HAV strains showed that the viruses belong to the classical avian-like viruses. Regarding the period of isolation, all viruses on the dendrogram of the

M gene are located chaotically, which indicates the persistence of various variants of the M gene in the wild bird population of the south of Western Siberia.

The territory of the south of Western Siberia is located in the center of the Eurasian continent and is intersected by three main migratory bird migration routes, uniting migratory ways of birds from Europe, Africa, Asia and Oceania. The climate features of this region create favorable conditions for long-term preservation of the virus both in soil and water.

Thus, Western Siberia plays an important role in the persistence of avian influenza viruses, their evolution and geographical distribution in the Eastern Hemisphere.

* shestopalov2@ngs.ru

A universal influenza vaccine candidate based on attenuated influenza NS vector

Egorov A.*

*“Pharmenterprises” LLC Moscow, Russia
Research Institute of Influenza, Saint Petersburg, Russia*

We have previously shown that shortening the reading frame of the NS1 protein results in the attenuation of the virus in animals (Egorov A, et al., Transfectant Influenza A viruses with long deletions in the NS1 protein grow efficiently in Vero cells. [J Virol 1998 Aug; 72 (8): 6437-41]). In addition to attenuation, the removal of the immunosuppressive domains of NS1 leads to an increase in the immunogenicity of the virus due to an increased induction of the factors of innate immunity in the vaccine application zone (upper respiratory tract). This stimulates the immune response to the conserved epitopes of the internal structures of the virus, which, when occur naturally, are low immunogenic (Ferko B. et al., Immunogenicity and protection efficacy of replication-deficient influenza A viruses with altered NS1 genes. [J Virol. 78 (23): 13037-45].

Currently, we develop a novel universal influenza vaccine based on influenza A attenuated vector expressing conservative influenza A and B epitopes from the NS1 open reading frame. The vector is the influenza A/PR/8/34 virus reassortant, containing modern California-like H1N1pdm surface glycoproteins. A single intranasal immunization of mice or ferrets was well tolerated and sufficient to provide a heterologous

protection from different influenza A virus subtypes as well as influenza B viruses. The vaccine candidate was designed for production in 10-day old embryonated chicken eggs with the growth potential of 8-9 log EID₅₀/ml. A new simple method of vaccine purification was established to achieve > 90% viral recovery after the sterile filtration and lyophilization of the drug product. Therefore, a single component Universal Influenza Vectored Vaccine candidate shows good potential for clinical development.

* aevirol@gmail.com

“Live and not let the virus die” as therapy principle

Bovin N.*

Institute of Bioorganic Chemistry RAS, Moscow, Russia

Key problem of anti-viral therapy is the rapid mutation of viruses under drug pressure – the drug kills viruses and thereby facilitates its evolution. This is why “upside-down” principles in the search of new anti-influenza approaches appear in recent years, the principles based on the blocking of patient’s sialo-receptors rather than the pathogen. In particular, neuraminidase as the drug hydrolysing sialo-receptors in the respiratory tract, as well as an engineered 2-6 sialo-specific lectin that masks the receptor. However, any impact on human cell is a great risk to cause another illness.

We chose the third way - without killing the virus and without transforming the host tissues. It is a sweet cage for a virus particle that isolates it from the receptor and prevents infection. The specificity of binding is based on 6'SLN trisaccharide as key motif of the therapeutic. The efficacy of the coating is ensured by simple peptide moiety capable of self-assembling into a giant supramer enveloping the viral particle. This approach demonstrated a promising result in cell culture system, but its efficacy in ferrets was not so impressive. That's why we complicated the supramer by adding a second self-assembling element, capable of binding to neuraminidase. The results of the inhibition test of cell culture infection, and the results of the trial on the ferret model are reported.

* professorbovin@yandex.ru

mariCloud™ on-line service for real-time and global epidemiological surveillance of acute infections

Porjo N., Soukka J., Koskinen J.*

ArcDia International Ltd, Turku, Finland

Background: Epidemiological data available today (e.g. by WHO or national bodies) in acute infections is often collected only from hospitalized patients and limited set of sources, covers a limited selection of pathogens, and is rather old by the time it is published. There is a clear need for more accurate, real-time, and extensive epidemiological data from hospitals and primary care to e.g. i) enable more accurate diagnosis and treatment, ii) guide IVD use, iii) optimize antibiotic and anti-viral use, iv) improve epidemic and infection control, v) optimize health care resource usage and preparedness, vi) plan surgical operations especially in the immunocompromised, and vii) increase pandemic preparedness.

mariPOC® (ArcDia Int. Ltd, Finland) is an automated, LIS-connectable and CE-marked platform for rapid multianalyte POC-testing of infectious diseases. Currently four applications are available: mariPOC® *re-spi* targets 11 pathogens causing influenza like illnesses, *pharyn* targets two tonsillitis pathogens, *gastro* is a five-pathogen test for acute gastroenteritis, and CDI is a test for *C. difficile* GDH and Toxin antigens. Typically users run 1000–4000 multianalyte tests per year, producing not only diagnostic result for managing individual patients but also data about the local epidemiology. More than 35 test systems have been installed nationwide in Finland alone. Combined, these produce an unprecedented amount of epidemiological timeline data. We have developed hardware and software to send anonymised epidemiological data into a cloud

server for data integration and analysis, and means to display the data in web browsers to health care providers and infection control specialists.

Materials/methods: mariPOC® test systems are indirectly connected to the cloud server, typically using wireless connection, through a proxy that communicates with the test system using ASTM protocol on RS-232 serial transfer. mariCloud® is designed so that it can take in data from other devices as well, whether other automated analysers or lateral flow tests. Three major strategies were used to design patient data safety into the system: I) Isolate. There is only one way transfer of information, and the device is not open to the Internet. II) Anonymize. Only epidemiological data is transferred, no personal information is sent outside the core test system. III) Encrypt. Everything.

Results: mariCloud® service has been in pilot test use for the mariPOC respi data since influenza season 2016-2017. The service has been working robustly, and the first version of the user interface which is accessed through a web browser has received good feedback from the users.

Conclusions: Introduction of the real-time epidemiological service is expected to allow significant improvements in understanding of the epidemiological situation and its changes. By raising general awareness about circulating viral causes of respiratory infections it may even help health care to reduce the unnecessary antibiotic use. mariCloud® will also provide authorities and the health care system means to detect new epidemics and pandemics earlier. In the near future, the coverage of mariCloud® will be expanded to other countries where mariPOC® is available.

* janne.koskinen@arcdia.com

Controlling influenza and other respiratory virus infections using therapeutic interfering particles

Almond J. *

University of Oxford, United Kingdom

This presentation will describe the technology being developed by VirionHealth Ltd, a small Biotech company spun out from Warwick University based on work from Nigel Dimmock and Andrew Easton. The approach is to use defined homogeneous preparations of Defective Interfering particles of Influenza A to both treat established respiratory infections and to provide short duration prophylaxis during oPtbreaks. The DI particle VH244 will be described, including its mechanism of action, system of manufacture, and pre-clinical results in mice and ferrets. The product is broad spectrum, showing effectiveness against all sub-types tested, and is able to prevent and treat infection by respiratory viruses other than influenza A. This is a novel approach to influenza control that can be used seasonally but could also form an effective first line of defence in the event of pandemic.

* almond.jeffrey@yahoo.co.uk

Oral Abstracts

Influenza surveillance in Russia: status, challenges and future developments

Sominina A.^{1*}, Danilenko D.¹, Komissarov A.^{1,2}, Vasin A.^{1,3}

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*Saint-Petersburg State University, Saint-Petersburg, Russia*

³*Peter the Great St. Petersburg Polytechnic University*

Influenza surveillance (IS) is an important component of counteraction to epidemics, which provides the possibility of timely introduction of preventive measures and annual update of the strain composition of influenza vaccines. IS provides detailed antigenic and genetic characterization of circulated influenza viruses (CIV) and such important biological properties as susceptibility to antivirals, pathogenicity and transmissibility among humans. It is obvious that these properties of viruses should be studied before the epidemic start and it determine the need the early virus isolation and obtaining accurate genetic data in a very short period. It is difficult now to predict exactly which of seasonal epidemic strains of influenza will dominated in upcoming season. Continuous tracking of tendencies of virus circulation in southern hemisphere analysis of representative GISAID data and evaluation of post-epidemic population immunity level to each of CIV gives provides useful information for the forecasting of etiology of epidemic and its severity. Unfortunately, at present we do not have enough information on which of the mutations in the genes of CIV are responsible for the increased pathogenicity for humans and the ability of the virus for transmission in the human population because most of the data were obtained in animal models.

Full genome analysis (FGA) of CIV accompanied by detailed clinical data on severity of disease could be obtained in hospital surveillance

system initiated by Sanofi Pasteur in the frame of GIHSN. The proper integration of contemporary laboratory data with epidemiological surveillance is of key importance. In the last ten years influenza surveillance system in Russia significantly strengthened with the launch of national online system for entering of epidemiological and laboratory data, implementation of sentinel and hospital influenza surveillance. Increased capacity for PCR detection of both different subtypes of influenza A viruses including potentially pandemic strains and other respiratory pathogens, influenza virus isolation and capacity to perform antigenic cartography and next-generation sequencing improved IS system capacities. Analysis of long-term data has revealed certain regularities in the circulation of influenza viruses.

The main challenges in influenza surveillance are: incomplete geographic coverage, insufficient standardization of investigations in regional base laboratories, limited information on influenza associated mortality, insufficiency of investigations on early virus isolation in pre-epidemic period, on comparative data on FGA of CIV with detailed clinical information on severity of disease, limited access to the study of the circulation of influenza viruses among animals. Further improving surveillance should include better standardization of protocols and methods of investigation with development of national external quality control panels for PCR and HI test, timeliness of virus isolation and sharing, understanding severity of influenza (transmission, seriousness and impact), understanding of disease burden, risk groups and vaccine efficacy and effectiveness. Development of region-specific weekly epidemic thresholds both for incidence rate and hospitalization will promote the early detection of the epidemic start and severity. Further development of bioinformatics and modeling using NGS for the forecasting of evolution of influenza viruses, modeling of influenza and other ARI burden should increase influenza surveillance level. Support better vaccines and vaccine programs will contribute to decrease the incidence and hospitalization of patients with influenza.

Enhancement of the capacity for novel influenza virus identification, risk assessments data for national situational awareness/decisions, partnership with WHO initiated PIP using recommendation of TIPRA and PISA , should enhance the pandemic influenza preparedness level of the country.

*anna.sominina@influenza.spb.ru

Influenza in pregnant: the results of monitoring during epidemics 2012-2017, Moscow

Burtseva E.,* Trushakova S., Kisteneva L.

*D.I. Ivanovsky Institute of Virology, Federal Research Center of Epidemiology and Microbiology named after N. F. Gamaleya
Moscow, Russia*

The objective of this study was to assess the threat of influenza infection caused by A(H1N1)pdm09, A(H3N2) and B viruses in hospitalized pregnant women during 2012-2017 seasons. The women were screened based on the Global Influenza Hospital Surveillance Network (GIHSN) Protocol. Nasal and nasopharyngeal swabs were obtained from all hospitalized women. RT-rt-PCR was used to detect influenza virus infection in clinical materials.

Since 2009 in Russia influenza A(H1N1)pdm09 has been the main etiological agent of epidemics during 2009-2010, 2010-2011, 2012-2013 and 2015-2016; influenza A(H3N2) virus – during 2011-2012, 2014-2015 and 2016-2017; influenza B virus co-circulated during all epidemics. During the five past seasons, 3336 pregnant women were screened and 2566 of them were included in the study. In 1244 (48%) of pregnant women the influenza virus infection was found: 361 (29%) cases were typed as A(H1N1)pdm09, 471 (39%) as A(H3N2) and 403 (32%) – as B viruses.

Analysis of clinical symptoms and some complications of illness showed that myalgia, cough, breath, and cesarean section were more often noted in A(H1N1)pdm09 positive women; bronchitis, sore throat and urinary infection – in A(H3N2); bronchitis, laryngitis, sinusitis and otitis – in B infection. Pneumonia was diagnosed in 4,3% of pregnant women with influenza A(H1N1)pdm09 virus infection, meantime with A(H3N2) – in 0,65% and B virus infection – in 2,0%. Women with comorbidities had confirmed influenza more often in comparing with non-influenza

pregnants. Among positive and negative on influenza women the onset of any obstetrical problems there were found significant differences in hematometra in positive on influenza group (6 cases). There were 14 pregnant with vaccination in anamnesis only. Our results showed the important role of each type/subtype of influenza viruses in severity of illness in pregnant with followed hospitalization. One of the causes of it might be low vaccinated rate among pregnant. In Russia the pregnant have been included in the National Calendar of vaccination since May 2013.

Our investigations were supported by the Centro Superior de Investigación en Salud Pública (CSISP), Sanofi Pasteur and Fondation Merieux, France.

* elena-burtseva@yandex.ru

Collection of influenza and ARVI viruses of the Research Institute of Influenza (RII): significance for influenza surveillance in Russia, current state and perspectives of development

Eropkin M.*, Danilenko D., Konovalova N.

Research Institute of Influenza, Saint-Petersburg, Russia

The RII disposes two Museum Collections of Viruses on the basis of the laboratory of evolutionary variability where the lyophilized strains of viruses are kept in glass ampoules at +5°C. The collection of influenza viruses numbers about 7,000 strains of human viruses and also more 100 strains of animal viruses including the most modern human, avian, swine and other mammalian strains. The collection of ARVI-viruses contains adeno-, corona-, para-influenza-, RS-viruses – more 3,500 units of storage. Viruses are stored in accordance to the biosecurity regulations.

Each year, several hundreds of new strains are supplemented to the collection. Since 2004 some of the isolated strains were sequenced (partially or completely). The sequences were deposited in genomic databases GenBank, later in GISAID. Since 2015 the full sequencing was carried out by means of the NGS. The collections are used for purposes of scientific research, production of diagnostic preparations and reference strains for the production of vaccines. In recent years several strains from the RII collection were chosen by the WHO Collaborating Centre in London as the international reference strains. Since 2015 the RII entered into the Project “European Virus Archive goes Global” (EVAg), 2015-2019, supported by the EU grant. The purpose of the Project is to join the existing virus collections in form of the distributed virtual bank of viruses, to enhance the quality of isolation, characterization and storage of isolates, to establish the unified standards of quality for all members of the Project.

Plans for further development of the Collections comprise the creation of an interactive electronic database, transfer of long-term storage to -20°C, the creation of more comprehensive “passport of the strain” with the obligatory sequence data, development of the bank of rat antisera to the reference and representative epidemic strains. The improvement and constant replenishment of the Collections will provide an opportunity for more detailed and precise monitoring of influenza virus evolution on the territory of Russia, an operative response to the emergence of new, potentially pandemic strains and will finally contribute into the development and implementation of new vaccines and antiviral preparations.

* mikhail.eropkin@influenza.spb.ru

Sentinel surveillance of Influenza and other respiratory viruses in Cuba

Borroto-Gutiérrez S.*, Valdés V.

Pedro Kourí Institute of Tropical Medicine, Havana, Cuba

Background: Acute Respiratory Infections (ARI) in Cuba are the main cause of Medical Attendances (MA) and hospitalizations. As a tropical country, influenza and other respiratory viruses circulate all over the year. Objective: To describe the outcomes of respiratory viruses sentinel surveillance in Cuba during 2012-2016. Methods: Statistical departments from the municipal to national levels collect grouped information on ARI. Sentinel surveillance collect nasopharyngeal samples from hospitalized severe cases and Influenza Like Illness (ILI) cases and outbreaks, as well as from other risk groups under surveillance.

The National Reference Influenza and other Respiratory Viruses Laboratory (NRIRVL) performs the viral diagnosis using Real Time Polymerase Chain Reaction. Proportions of specimens according to the clinical diagnosis and year are calculated. Annual time series for the period are represented in graphics. Results: NRIRVL reported during 2012-2016 a total of 31554 samples tested; up to the total samples 8479 (26.9%) were positive to respiratory virus; 30% (2537) of them were positive to Flu virus. The majority of the positive samples (57.1%) were taken to patients diagnosed as Severe Acute Respiratory Infection (SARI) and 35.2% from patients with Influenza Like Illness (ILI). A small proportion came from other risk population under surveillance in the country: outbreaks (4.9%), pregnant women (1.9%), dead (0.7%) and travellers coming from other countries (0.2%). The year 2013 showed the higher detection of influenza virus circulating, with the longest epidemic period from week 15 to 32. Influenza A(H1N1) pdm09 was the dominant virus circulating during 2013 and 2015, meanwhile Influenza B was

dominant during 2012 and 2016. The year 2014 was atypical with more influenza circulation during the last quarter, mainly due to influenza A(H3). Conclusion: Influenza viruses increase circulation during rainy season from May to August, but are detected during the whole year with variations. Sentinel surveillance is useful to follow respiratory viruses' circulation in the country and allowed to decision makers the changing of influenza vaccination time.

* susana@ipk.sld.cu

Viral etiology of influenza-like illnesses in hospitalized children in Novosibirsk, Russia, in 2013 – 2017

Kurskaya O.^{1*}, Sobolev I.¹, Anoshina A.², Leonova N.², Ryabichenko T.³

¹ *Scientific Research Institute of Experimental and Clinical Medicine, Novosibirsk, Russia*

² *Pediatric hospital №6, Novosibirsk, Russia*

³ *Novosibirsk State Medical University, Novosibirsk, Russia*

Acute respiratory infections (ARIs) are one of the illnesses of highest morbidity and mortality in children worldwide. In Russia annually 25-35 million cases of influenza and other ARIs occurs. Children are on average infected two to three times more frequently than adults. ARIs pose considerable threat for public health because of high mortality rate ranging from 250 to 500 thousand people globally, especially in infants and elderly.

Influenza-like illness (ILI), a subset of ARIs, can be caused by a wide range of respiratory viruses, including influenza A and B viruses, parainfluenza virus, respiratory syncytial virus, adenovirus, human coronaviruses and rhinovirus. In the past decade, new viruses associated with ARIs have been detected such as human metapneumovirus, novel strains of coronaviruses (SARS-CoV, HCoV-NL63 and HKUI), human bocavirus. Clinical symptoms of ARIs are too similar to distinguish between etiologic agents. However identification of the viral pathogens is necessary for development of more effective prevention and control strategies.

The purpose of this study was to determine the prevalence, seasonality and clinical characteristics of ILI in hospitalized children in Novosibirsk during four epidemic seasons. We collected nasal and throat swabs from children 0–15 years of age presenting within 3 days of onset of illness with at least two of the following: fever, sore throat,

cough, rhinorrhea, nasal congestion, and hoarseness of voice. Detection of common respiratory viruses, including IFVA, IFVB, PIV 1-4, RSV, HMPV, HCoV-OC43, HCoV-229E, HCoVNL63, HCoV-HKU1, ADV, HRV and HBoV, was performed by using a real-time PCR Kit (Interlabservice, Russia) according to the protocols.

A total of 1463 samples collected from patients with ILI during 2013 - 2017 were enrolled in the investigation. The age distribution of patients was as follows: 20.9% of children were under 1 year, 1 - 3 years – 43,4%; 4 - 6 years – 16,5%; 7 – 15 years - 19.2%. Among 1463 samples 1050 were positive for at least one viral pathogen. Viral co-infections were detected in 10.3% of samples. The lowest positive rate was in the age group 7 – 15 years. No difference in the positive rate was observed between males and females. The most common viral pathogens were respiratory syncytial virus (31.9%), influenza virus (29.5%) and rhinovirus (20.2%). However, the proportion of respiratory viruses varied in different seasons and in different age groups.

* kurskaya_og@mail.ru

Influenza virus surveillance at the human-animal interface and characterization of avian influenza viruses A(H5N8) isolated in Russia in 2016-2017 years

Marchenko V.^{1*}, Susloparov I.¹, Kolosova N.¹, Goncharova N.¹, Komissarov A.^{2,3}, Fadeev A.², Gudymo A.¹, Danilchenko N.¹, Piankova O.¹, Maltsev S.¹, Svyatchenko S.¹, Durymanov A.¹, Ilyicheva T.¹, Mikheev V.¹, Maksyutov R.¹, Ryzhikov A.¹

¹ *State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Novosibirsk, Russia*

² *Research Institute of Influenza, Saint-Petersburg, Russia*

³ *Saint-Petersburg State University, Saint-Petersburg, Russia*

In the spring of 2016, wild birds mortality was observed during the monitoring of avian influenza virus activity in the Republic of Tyva. That outbreak was caused by influenza A(H5N8) virus. During the fall of 2016 and the first half of 2017 avian influenza viruses subtype A(H5N8) caused infection of poultry and wild birds in the European regions of Russian Federation and European countries. More than 40 regions of the Russian Federation have participated in the surveys and analysis of biosamples collected from human populations at risk (workers at poultry farms, pig farms, fishing farms) as well as analysis of biosamples collected from wild birds, poultry, seals and pigs (swabs, washings, and organ fragments) and from the environment. The biosamples were examined by real-time PCR on the presence of influenza virus RNA. The virus isolation was conducted in chicken embryos for the biosamples obtained from birds, seals and pigs. Virus isolation was conducted in MDCK cells for the biosamples obtained from pigs, seals and human. A total of 67 strains of avian influenza virus A(H5N8) were isolated from the birds and environment during the outbreaks in wild birds and

poultry. The strains were subtyped by PCR and HAI. Antigenically the new viruses are closely related to CVVs and reference viruses of the subclade 2.3.4.4. The full genome sequencing of newly isolated A(H5N8) viruses demonstrates that newly isolated strains belong to the clade 2.3.4.4, group B.

The studied strains showed a high degree of virulence in intranasally infected mice. Transmission of selected A(H5N8) viruses was studied in guinea pig model and the data obtained indicated very low respiratory transmission of the viruses. Drug resistance of all viruses to zanamivir and oseltamivir has been performed using the MUNANA protocol. The data indicated that all viruses are sensitive to neuraminidase inhibitors. Serological testing of human populations at risk indicated the presence of anti-(H5N8) antibodies in some samples while the clinical signs of the disease were not observed and virus was not isolated from biosamples from people having serum positive results were not detected.

* marchenko_vyu@vector.nsc.ru

Isolation of unusual strains of H1N1 swine influenza virus from a pig in Russia, 2016-2017

Sobolev I.^{1*}, Kurskaya O.¹, Murashkina T.¹, Leonov S.², Sharshov K.¹, Kabilov M.³, Alikina T.³, Alekseev A.¹, Shestopalov A.¹

¹ *Research Institute of Experimental and Clinical Medicine, Novosibirsk, Russia*

² *Institute of Experimental Veterinary of Siberia and the Far East, Novosibirsk, Russia*

³ *Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*

In the present study, we report the isolation and genome sequences of two swine influenza virus strains (A/swine/Siberia/1sw/2016 and A/swine/Siberia/4sw/2017), initially isolated from a swine pulmonary tissue sample. Sectional material from pigs was collected on private pig farm in Russia, 2016-2017 (in March 2016 and in April 2017). Influenza virus A was detected by real-time PCR. The strains were isolated on Madin–Darby canine kidney (MDCK) cells. Complete genome sequencing was performed in the SB RAS Genomics Core Facility (Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia) using MiSeq (Illumina).

Antigenic analysis of isolated swine influenza viruses was performed using polyclonal mice antisera against different A(H1N1) viruses (A/New Caledonia/22/99, A/Novosibirsk/653/2009, A/California/07/2009). Both isolates did not demonstrate cross-reactivity with mice antisera against 2009 pandemic influenza strain and seasonal influenza strains, circulated in human population in early 2000 and in the last prepandemic season.

Phylogenetic analysis showed that genes encoded surface glycoproteins (HA and NA) of the investigated strains are significantly

different from HA and NA genes sequences are represented in databases (GenBank, GISAID and IRD). The closest (90% identity) sequences of HA and NA belong to the seasonal influenza A (H1N1) virus isolated from humans in the USA in the 1970-80s. Also, according to the phylogenetic analysis, HA of H1N1 and H1N2 belonging to the phylogenetic groups 1.B.1 and 1.B.2 can be considered as phylogenetically closest to HA of investigated strains, along with the seasonal human influenza virus in the 70-80s. Thus, phylogenetic relationships shows that strains A/swine/Siberia/1sw/2016 and A/swine/Siberia/4sw/2017 refer to the subtype H1N1, but is not a pandemic variant of A (H1N1)pdm09. Wherein, the nucleotide sequences of the genes encoding the internal proteins of the virus (PB2, PB1, PA, NP, MP, NS) are similar to the genes of the influenza A (H1N1)pdm09 virus isolated from humans.

*sobolev_i@hotmail.com

Swine influenza surveillance in Russia: failures, advances, perspectives

Danilenko D.^{1*}, Komissarov A.^{1,4}, Petrova P.¹, Konovalova N.¹,
Komissarova K.¹, Fadeev A.¹, Vasilieva A.¹, Egorova A.¹, Zheltukhina A.¹,
Eropkin M.¹, Aliev A.², Pruglo V.³

¹ *Research Institute of Influenza, Saint-Petersburg, Russia*

² *State Veterinary Academy, Saint-Petersburg, Russia*

³ *Expert Council for Veterinary of the National Swine Producers Union,
Russia*

⁴ *Saint-Petersburg State University, Saint-Petersburg, Russia*

Swine influenza viruses (SIV) along with avian influenza viruses represent a constantly evolving threat for human and animal health. Their pandemic potential was proven by the last pandemic. Swine influenza surveillance in Russia is not conducted on a regular basis. To overcome this gap RII established the collaboration with veterinarians to gain insight on the evolution of SIV in Russia. This collaborative three-year effort allowed to identify SIV subtypes that circulate in pig population in European part of Russia.

Swabs were collected into commercially available VCM and tested in rt-PCR for influenza. Virus isolation was performed in MDCK cells and chicken eggs. Antigenic analysis of viruses was performed with the panel on antisera raised against representative swine, human and avian influenza viruses. Serum samples were also collected from approximately 600 samples and analyzed in hemagglutination inhibition (HI) test. The majority of influenza viruses belonged to A(H1N1)pdm09 and A(H3N2) subtypes and were related to human influenza viruses of the same subtypes that circulated in 2009-2012 that was confirmed by Sanger sequencing. Two strains belonged to A(H1N1) subtype and were antigenically related to A/Caledonia/20/99 human strain. No other influenza A subtypes were isolated from swabs.

The majority of swine serum samples analyzed in HI were negative for all subtypes of influenza A viruses analyzed (H1, H1pdm09, H2, H3, H5, H7, H9). Antibodies to classical A(H1N1) SIV were detected scarcely. On the contrary, most abundantly were found antibodies to A(H1N1)pdm09 and A(H3N2) human influenza viruses (more than 90% of all antisera that tested positive for influenza). This indicates the fact that swine populations are highly susceptible to infection with human influenza viruses. Interestingly, antibodies found in serum samples for A(H3N2) viruses reacted well with older human A(H3N2) viruses (A/Perth/16/2009-like), but did not interact with more recent A/Texas/50/2012, A/Switzerland/9715293/2013 or A/Hong Kong/4801/2014 viruses – the fact that indicates for slower evolution of SIV in comparison to human influenza viruses.

Further regular surveillance for SIV is urgently needed in order to provide insight into evolution of these viruses and to contribute for better pandemic preparedness.

* daria.baibus@gmail.com

The study of molecular-biological properties of H4N6 AIV subtypes isolated in Caspian seal population: the assessment of pathogenic potential in experimental infection in mice

Gulyaeva M.^{1,2*}, Sharshov K.¹, Sobolev I.¹, Alekseev A.Y¹,
Shestopalova L.², Shestopalov A.¹

¹*Scientific Research Institute of Experimental and Clinical Medicine,
Novosibirsk, Russia*

²*Novosibirsk State University, Novosibirsk, Russia*

Aquatic birds are supposed to be a natural reservoir of the most subtypes of AIV. It is assumed that a reason of influenza pandemics is a penetration of new virus subtypes or some of their genes from aquatic birds to other species. The high importance is to study influenza virus circulation in marine mammals, that are underestimated. In previous studies were shown, that marine mammals play a big role in influenza virus transmission between different species. The result of surveillance in the population of Caspian seals (*Phoca caspica*) in 2002 and 2012 is isolation and identification of 2 new AIV. During the study were shown that these strains belong to the low pathogenic AIV of H4N6 subtype. Biological properties of new viruses were studied in mice model (Balb/C). Both selected strains of H4N6 cause a disease in mice and replicated in their lungs without prior adaptation. Mice serum collected for HI test on 21 dpi had significant titers: 1/160 and more. HI test revealed that isolated viruses have similar antigenic properties. Extended morphological study shows that experimental infection in mice model cause pathological changes in lungs and some other organs in compare with a control group. The results analyzed during the study revealed a significant pathogenic potential of avian influenza virus of H4N6 subtype in mammals. The reported study was funded by RFBR according to the research project №17-04-01919.

* mgulyaeva@gmail.com

Identification of etiology of RVI using PCR-tests in order to manage etiotropic treatment

Lvov N.*

S. M. Kirov Military Medical Academy, Saint-Petersburg, Russia

Acute respiratory diseases in organized groups of people are characterized by outbreaks. Early etiology determination has critical importance in order to hold the anti-epidemic measures and take up effective etiotropic (specific antiviral) therapy. Currently, the highest specificity and sensitivity goes to PCR method.

We have analyzed the results of PCR-tests, which had been undertaken on purpose to verify the etiology of RVIs in young people from organized collectives. Examination included 301 patients with moderate and acute RVI. Samples for PCR-tests were taken from nasopharynx during admission in first 2 days from the onset.

PCR-tests revealed the etiology of RVI in 68,4% of cases. From the total amount of verified cases, influenza A(H1N1)pdm was indicated in 0,5%, influenza A(H3N2) in 28,6%, influenza B in 18,0%, adenoviral infection in 41,8%, metapneumovirus infection in 1,0%, parainfluenza of 4 type in 0,5%, rhinovirus infection in 9,2%, respiratory-syncytial infection in 0,5%.

Therefore, young people from organized groups mostly suffer from adenoviral infection (41,8%) and influenza (47,1%). This fact is in line with trends of recent years. It has already been used to achieve early clinical and epidemiological diagnostics and determine etiotropic management tactics.

* 05011912@mail.ru

**Positive association of supersaturation effects
in the human airways with influenza seasons in
Okinawa (2007-2012) – New method for analyzing
and forecasting in subtropical climate (first preliminary results)**

Ishmatov A.*

*Scientific Research Institute of Experimental and Clinical Medicine,
Novosibirsk, Russia*

Previously (Ishmatov, 2017a,b) we have hypothetically shown that under specific climatic conditions which are peculiar to the seasonal patterns of influenza (“humid-rainy” and “cold-dry”) the effect of supersaturation and condensational growth in the airways can act as additional common trigger for influenza, common colds and other respiratory infections. We hypothetically found the effects of supersaturation and condensational growth in the airways may lead to additional risk of acidification of epithelial lining fluid in the local areas of the respiratory tract, and, as a result, may lead to negative impacts on host cells and weakening of the defense mechanisms of the human airways (Ishmatov, 2017b), and moreover, we found this effect may lead to additional risk of deposition of infectious agents from inhaled air in the upper airways (Ishmatov, 2017a). Here we analyzed the statistical daily and hourly climate data in Okinawa (South Japan) (26.2 N latitude, 127.7 E longitude).

We have shown for the first time the intensity of the effects of supersaturation in the airways is strongly associated with main representative peaks of intensity/activity of influenza in Okinawa in the period of observation from Jan 2007 until Dec 2012 including 2009 pandemic. And we originally found that intensity of supersaturation effect in the human airways may play a critical role in the activity of influenza in subtropical climate. Our observation is the first one which clearly shows in the practice that the effect of supersaturation in the human airways may be used as a new method for understanding and

forecast the influenza activity in the subtropical and tropical zones. The results of the study may radically change the current understanding of influenza seasons in the world, because, there are many theories of the seasonality of influenza for different climatic zones, but so far none of the known theories provides a clear explanation for the tropical and subtropical climate.

I report no competing interests.

The study was partially supported by a grant of Russian Scientific Foundation (project No. 17-44-07001).

* ishmatoff@centercem.ru

Plants as factories for production of influenza vaccines

Blokhina E.¹, Mardanov E.¹, Kotlyarov R.¹, Stepanova L.²,
Tsybalova L.², Ravin N.^{1*}

¹ *Research Center for Biotechnology RAS, Moscow, Russia*

² *Research Institute of Influenza, Saint-Petersburg, Russia*

The use of plants as "biofactories" for the production of recombinant proteins is a promising alternative to traditional technologies based on the use of bacteria, yeast or animal cells. In recent years, methods for rapid and efficient transient expression of proteins in non-transgenic plants have been developed, based on the use of recombinant plant viral vectors. Conservative M2 protein of the influenza virus is one of the promising candidates for the development of "universal" influenza vaccine. However, to be immunogenic M2 must be linked to an adjuvant or carrier protein. As such partners we use flagellin of *Salmonella typhimurium*, a ligand of TLR5 receptors and an effective mucosal adjuvant, and virus-like particles formed by the core antigen of the hepatitis B virus (HBc).

We developed vector for expression of the target proteins in *Nicotiana benthamiana* plants based on the genome of the potato virus X. Recombinant protein consisting of the extracellular domain of the M2 protein (M2e) fused to flagellin was expressed in plants at the level of about 1 mg/g of leaf tissue. The recombinant protein showed a high immunogenicity when administered intranasally and protected mice from the lethal challenge with various influenza strains. Within the second approach, plant viral vector was developed for the expression of HBc antigen carrying an M2e peptide on the surface. This protein, M2eHBc, was expressed at the level of about 2% of the total protein; it formed virus-like particles in vivo. In immunological studies, purified M2e-HBc particles induced a high level of antibodies and protected mice from

influenza virus infection. Thus, plant-produced recombinant vaccine proteins based on M2e can become the basis of recombinant “universal” influenza vaccine.

The work was supported by the program “Nanostructures” of the Russian Academy of Sciences and RFBR grant 16-34-00976.”

*nravin@mail.ru

Effort made to create a universal influenza vaccine

Tsybalova L.^{1*}, Stepanova L.¹, Kuprianov V.², Shuklina M.¹, Korotkov A.¹,
Potapchuk M.¹, Blokhina E.², Kovaleva A.¹, Ravin N.²

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*Research Center for Biotechnology RAS, Moscow, Russia*

Control over influenza infection, presently loose, could be made efficient using multi-purpose vaccine, that would induce response against all (or most) influenza A subtypes. Many scientists are focused on development of such vaccines. The conservative viral proteins, such as ectodomain of M2 protein (M2e) or stalk of hemagglutinin (HA2), are a promising object for creation of cross-protective influenza vaccine.

In order to create candidate vaccine, we tested several recombinant proteins. One of them (used in vaccine Uniflu) contains four copies M2e genetically fused with core protein of hepatitis B virus (HBc4M2e). Eukariotic oligonucleotides (product Derinat) were added as adjuvant. The vaccine recently passed preclinical trial. The second recombinant protein Flag4M2eHA2-2 contains two viral peptides: M2e and HA2 (aa 76-130) genetically fused with bacterial flagellin. Uniflu was tested on mice and ferrets, while protein Flag4M2eHA2-2 on mice only. Mice were immunized 3 times with Uniflu intramuscularly, or 3 times with Flag4M2eHA2-2 protein intranasally. Ferrets were immunized twice with Uniflu intramuscularly. Anti-M2e IgG (IgG2a and IgG1) in sera and bronchoalveolar lavages (BAL) were determined in ELISA. In addition, intracellular cytokine staining assay was performed. Mice immunized with Uniflu were challenged intranasally with 5LD₅₀ mouse-adapted influenza viruses A/PR/8/34(H1N1), A/Aichi/2/68 (H3N2), A/Singapore/1/57 (H2N2) or A/California/07/09 (A/H1N1pdm 09). Ferrets were challenged with 6.5 - 7.0 log₁₀ TCID₅₀ virus A/Brisbane/53/07 (H1N1), then with virus A/Texas/50/2012 (H3N2) four months later.

Uniflu stimulated the production of high level anti-M2e IgG in sera and BAL. It induced IgG2a and IgG1 in comparable quantities. We determined the level of intracellular cytokines (IFN- γ and IL-4) immediately after the second boost of immunization, then in 150 days after it. These results demonstrated the formation of M2e-specific memory T-cells, and provided evidence for (i) activation of both CD4+ and CD8+ lymphocytes, as well as (ii) stimulation of immune responses and development of two pathways (Th1 and Th2). When challenged with each of viruses used in this study, immunized mice survived in 80% -100% of cases. As to virus reproduction in lungs, its drop was 1.2- 3.5 log₁₀. Experiments on ferrets gave similar results. We obtained considerable rise of anti-M2e IgG, and reduced clinical influenza symptoms in immunized animals.

Stronger protective effect was obtained after immunization with recombinant protein containing both peptides M2e and HA2. Having been challenged with 10LD₅₀ of viruses belonging to both phylogenetic groups, immunized animals demonstrated strong immune response, which kept them alive in 90% -100% of cases.

*sovets@influenza.spb.ru

Novel mucosal influenza-vectored tuberculosis vaccines: preclinical and clinical evaluation

Stukova M.^{1*}, Khairullin B.², Shurygina A.-P.¹, Zabolotnykh N.³,
Vinogradova T.³, Buzitskaya Zh.¹, Pisareva M. ¹, Sansyzbay A.², Egorov A.¹

¹ *Research Institute of Influenza, Saint Petersburg, Russia*

² *Research Institute for Biological Safety Problems, Gvardeisky, Republic of Kazakhstan*

³ *Saint Petersburg Research Institute of Phthisiopulmonology, Saint Petersburg, Russia*

Today TB vaccine development represents a diversity of strategies including recombinant BCG, rationally attenuated *M. tuberculosis*, viral-vectored platforms, recombinant purified proteins, and novel adjuvants. The selection of appropriate methods for antigen delivery plays a key role in the development of effective tuberculosis vaccines. In this respect application of live attenuated influenza vaccines as a vector seems to be a promising strategy for mucosal immunization against various pathogens including *M. tuberculosis*. It is now well accepted that live viral vaccines administered by mucosal routes efficiently stimulate humoral and cellular mediated immune responses, in both mucosal and systemic compartments. Importantly, the viral vectors may have self-adjuvanting activities through the stimulation of innate immune systems.

We created several influenza vectored vaccine candidates expressing proteins (ESAT-6, Ag85A, HspX, TB10.4) representing different phases of mycobacteria life cycle. As a backbone we used a replicating-deficient influenza virus vector expressing partially deleted NS1 protein elongated at its carboxi-end with mycobacterial antigenic sequences. All obtained vectors were characterized by a stable high expression of target proteins and efficient growth in embryonated chicken eggs and Vero cells.

A set of comprehensive pre-clinical studies of several vaccine candidates was conducted in different animal models (mice, ferrets, guinea pigs, nonhuman primates). Vectors were capable of inducing a strong TB-specific Th1 immune response and provided a significant level of protection in mice and guinea pigs, when applied intranasally as prophylactic or immunotherapeutic schemes. Evaluation of sublingual vaccine delivery as an alternative to the intranasal route has been studied. Two Vero-cell manufactured vaccines reached their clinical evaluation in BCG-vaccinated healthy adult volunteers. Phase I trials showed that influenza vector vaccines administered intranasally or sublingually had good safety profile and stimulated balanced T-cell mediated immune response. A clinical Phase 2a trial in subjects with latent TB infection is initiated.

*marina.stukova@influenza.spb.ru

Parallel testing the subunit, adjuvanted, whole-virion inactivated and live influenza vaccines against homologous and heterosubtypical challenge

Gambaryan A.^{1*}, Boravleva E.¹, Kaplun A.,² Krasilnikov I. V.³

¹ *Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products, Moscow, Russia*

² *Lomonosov Moscow University of Fine Chemical Technology, Moscow, Russia*

³ *Saint-Petersburg Institute of Vaccines and Sera, FMBA, Saint-Petersburg, Russia*

Comparison of effectivity and safety of inactivated influenza vaccines, split vaccines with different adjuvants, and live attenuated influenza vaccines against homologous and heterosubtypical challenge was a goal of this study. Homologous and heterosubtypical protection against challenge with H1N1 and H5N1 influenza viruses in mouse model were studied. Addition of some adjuvants to split vaccine sometimes leads to paradoxal outcomes: they increased antibody levels but reduced the protective effect of vaccine. All tested adjuvants shift the ratio of IgG1 and IgG2a antibodies. Inactivated vaccines are effective against homologous challenge only, whereas immunity induced by live virus not only prevents infection with close related viruses but also provides partial protection against antigenically distant influenza viruses.

*al.gambaryan@gmail.com

GRIPPOL family vaccines: development history and perspectives

Puchkova N.^{*}, Nekrasov A.

“NPO Petrovax Pharm”, LLC, Russia, Moscow

Grippols – the first Russian subunit adjuvanted influenza vaccines developed via the ballast-free complex conception: purified virus antigens, and high-molecular safe adjuvant. Original water-soluble, biodegradable adjuvant Polyoxidonium (PO; INN: Azoximer bromide) complies with EMEA requirements; it collects antigens into virion-like structure, presents it to immune cells and stimulates specific immunity. In Grippols, PO provides low antigen dose (5 µg of HA/strain) high immunogenicity in all including subjects with low immune responsiveness (immunocompromized patients, young children, seniors), realizing antigen-sparing strategy and making vaccines safety profile greatest possible. Immunotropic adjuvant has no negative impact to vaccinee’ immunity and is safe in allergic and autoimmune subjects; vice versa it helps to respond adequately with no polyclonal stimulation, regardless of state condition, that was demonstrated by studies. Post-approval surveys included healthy subjects, pregnant women, patients with diabetes, asthma, broncho-pulmonary diseases, and seniors with cardiovascular illnesses. Observational studies included schoolchildren and adults and showed good tolerability, high efficiency and prophylactic profit. Protective antibody titer achieved on the 8-10th post flu shot day allowing vaccination till epidemiological morbidity rise.

Vaccines name describes the history towards quality advance according to requirements changing. Being approved in 1996 in Russia, Grippols for more than 10 years still the vaccines of National Immunization Schedule, showing good compatibility with other vaccines. In 2009 15 mln doses of pandemic H1N1 mono-Grippols was

produced. Now Grippols are supplied all over the Russia, in CIS countries and Iran. More than 200 mln Grippol® and about 118 mln Grippol® plus doses were manufactured for Russia and abroad.

Grippols history continues by quadrivalent vaccine comprising 2 influenza subtype A and 2 influenza types B antigens to improve protective potential. Grippol Quadrivalent had passed the clinical trials Phase II-III. Seroconversions, seroprotections, multiplication factor correspond to EMEA requirements. Vaccine will be entered into Russian market in 2018. Grippol Quadrivalent will be the first adjuvanted quadrivalent vaccine.

*info@petrovax.ru

Manufacturing technology development and pre-clinical and clinical trials of pandemic and seasonal influenza vaccines

Nurpeisova A.^{1*}, Khairullin B.¹, Kassenov M.¹, Makbuz A.³, Volgin N.¹, Sarsenbaeva G.¹, Bogdanov N.¹, Isagulov T.¹, Sagymbay A.¹, Abitay R.¹, Shurygina A.-P.², Buzitskaya Zh.², Stukova M.², Sansyzbay A.¹

¹ *Research Institute for Biological Safety Problems, Gvardeiskiy, Kazakhstan*

² *Research Institute of Influenza, Saint Petersburg, Russia*

³ *Kazakhstan National Agrarian University, Almaty, Kazakhstan*

The WHO recommends each country to develop and increase the production capacity of its own vaccines. Over the past years Research Institute for Biological Safety Problems has developed manufacturing technology for pandemic and seasonal influenza vaccines used in the Republic of Kazakhstan. Pandemic influenza vaccines Refluvac® against swine flu, Kazfluvac® against avian influenza and trivalent seasonal influenza vaccine were objects of this study.

The technology of vaccine candidate preparation was based on production of whole virions adsorbed on aluminum hydroxide and used for manufacturing of monovalent pandemic vaccines. During manufacturing technology scaling new approaches of virus purification were used allowing to obtain viral concentrate with a reduced ovalbumin concentrations. Trivalent seasonal influenza vaccine was obtained by ultracentrifugation and is adjuvant-free.

Pre-clinical studies of candidate vaccines confirmed the safety, high level of immunogenic and protective activity. Toxicological studies have demonstrated the absence of general toxicity, allergic properties and immunotoxicity of the developed vaccines. The results of phases I and II clinical trials showed good tolerability, low reactogenicity and safety of vaccines when administered intramuscularly to healthy volunteers aged 18 to 60 years. Immunogenicity indicators of the vaccines developed by

the Research Institute for Biological Safety Problems are in line with the European requirements of the CPMP EMEA for influenza vaccines.

Original Kazakhstan pandemic vaccines Kazfluvac® (hemagglutinin strain A/AstanaRG/6:2/2009 (H5N1) - 7.5 µg) and Refluvac® (hemagglutinin strain NIBRG-121xp A(H1N1) – 3.75 µg) were registered for the Republic of Kazakhstan and can be easily deployed. A phase III clinical trial of the trivalent seasonal influenza vaccine is ongoing.

*ainurnurpeisova@mail.ru

The protective effect of associated virus-bacterial vaccines based on live attenuated influenza vaccine (LAIV) and streptococcal recombinant peptides in mouse model of post-influenza *S. pneumoniae* infection

Desheva Y. ^{1,2*}, Leontieva G. ¹, Kramskaya T. ¹, Sychev I. ¹, Rekstin A. ¹, Grabovskaya K. ¹, Rudenko L. ¹, Suvorov A. ^{1,2}

¹ *Institute of Experimental Medicine, Saint Petersburg, Russia*

² *Saint-Petersburg State University, Saint Petersburg, Russia*

Influenza is often complicated by secondary bacterial infection with *S. pneumoniae* and other bacteria. The insufficient immunogenicity of carbohydrate vaccines stimulating the search for compounds to improve the immunostimulatory effects of bacterial antigens.

CBA mice were immunized intranasally with H7N3 LAIV combined with Group B streptococcus (GBS) surface proteins P6 and ScaAB. Also, we used the H1N1pdm09 LAIV in combination with chimeric protein (PSPF), composed of the fragments of three surface proteins of *S. pneumoniae* (Suvorov A., et al., 2015). We evaluated protective effect of mono- or combined vaccine preparations against sub-lethal challenge with potential pandemic H7N9 and lethal challenge with drift variant of pandemic H1N1 influenza virus under pneumococcal super-infection conditions.

Combined H7N3 LAIV+GBS vaccination provided advantageous protection against double infections with H7N9 and *S. pneumoniae* serotype 3 and significantly improved viral and bacterial clearance from the lungs of mice. The study of early protection against lethal challenge with distant A/South Africa/3626/13(H1N1)pdm virus showed that H7N3 LAIV and the ScaAB protected about 20% of the immunized animals on day 5 after the first vaccination. This may be explained by elevated IFN alpha expression which was observed when stimulated THP-1 cell

line. The H1N1 LAIV in combination with PSPF effectively prevented lethality after challenge with 10 LD₅₀ of A/South Africa/3626/13 (H1N1) pdm followed by pneumococcal infection. LAIV alone also significantly increased animal survival.

For successful protection of mice against post-influenza pneumococcal infection, the recombinant streptococcal polypeptides can be administered directly by mixing with LAIV. The use of LAIV decreased a manifestation of homologous and heterologous influenza virus infection both after the booster immunization, and soon after vaccination, and contributed to reduce pneumococcal contamination in the lungs.

*desheva@mail.ru

Bioprocessing of influenza vaccines

Lundgren M.

GE Healthcare Life Sciences, Uppsala, Sweden

Vaccines are becoming increasingly important for prevention of a large number of diseases. The manufacturing processes and technologies are important to enable rapid and scalable production of safe and efficacious influenza vaccines and become critical for a fast response during epidemic or pandemic outbreaks.

This presentation will cover the development and performance of a new platform for propagation and purification of live influenza virus with emphasis on vaccine production both in seasonal and pandemic situations. The work can be seen as an example of a modern generation vaccine production process, where influenza virus is propagated in mammalian cell lines growing on microcarriers. The virus particles are subsequently purified using a number of robust and scalable filtration and chromatography techniques. This procedure has been successfully verified with several different influenza vaccine strains.

Furthermore, an alternative scalable purification platform for egg-based influenza vaccines will be presented.

* mats.lundgren@ge.com

Development of trivalent recombinant influenza vaccine based on virus-like particles

Maksyutov R.¹, Piankova O.¹, Maltsev S.¹, Tregubchack T.¹, Gudymo A.¹,
Danilchenko N.¹, Ots A.², Karpova E.², Nekrasov M.², Ryzhikov A.¹

¹ *Federal Budgetary Research Institution State Research Center of Virology
and Biotechnology VECTOR, Rospotrebnadzor, WHO H5 Reference
Laboratory, Koltsovo, Russia*

² *LLC "Nanolek", Russia*

We have developed a candidate seasonal trivalent influenza vaccine based on the virus-like particles (VLP) produced in mammalian producer cell. The producer cells themselves were prepared when a synthetic DNA encoding three influenza virus proteins, hemagglutinin (HA), neuraminidase (NA) and M1 protein, were integrated into the cellular genome. Three plasmids carrying the cassettes of synthetic genes of HA, NA and M1 of influenza viruses A(H1N1), A(H3N2) and B were constructed. The identity of three gene cassettes was proved by restriction analysis and by sequencing. Three vectors constructed were used for transduction of mammalian cells and three clones of cells producing A(H1N1), A(H3N2) and B influenza VLPs were selected according to their level of antigen production and the rate of cell propagation. The supernatants of three producer cell lines were harvested, VLPs were concentrated and purified. Immunization of mice and ferrets with trivalent VLPs elicited a humoral immune response and caused significant protection against three homologous influenza viruses A(H1N1), A(H3N2) and B. Influenza vaccines based on recombinant viral proteins that are assembled into non-infectious VLPs in mammalian cell culture have advantages in terms of immunogenicity, biosafety and manufacturing processes. This flexible mammalian influenza VLP system is the alternative to the conventional reverse genetic vaccine platform because it has no limitations related to manipulations with live virus.

* maksyutov_ra@vector.nsc.ru

Development of new multiplex diagnostic methods for sepsis

Klotchenko S.^{1*}, Plotnikova M.¹, Lebedev K.¹, Taraskin A.¹, Vasin A.^{1,2}

¹Research Institute of Influenza, Saint-Petersburg, Russia

²Peter the Great St. Petersburg Polytechnic University,
Saint-Petersburg, Russia

Sepsis is a life-threatening dysfunction of the internal organs caused by a poorly regulated response to infection (bacterial, fungal, viral and parasitic). It is associated with Systemic Inflammatory Response Syndrome (SIRS). Sepsis can also be a fatal consequence of numerous viral infection types. Without timely treatment, sepsis can lead to septic shock, multiple organ failure, and death. According to the WHO, sepsis is one of the main causes of maternal and neonatal morbidity and mortality. A key factor in positive outcomes is timely and accurate diagnosis. Presently, the number of molecular markers used for sepsis diagnosis is extremely limited.

The aim of this work was to develop a method for the early determination of a patient's SIRS/sepsis status, thus allowing improved outcomes. A select group of molecular sepsis markers was identified for use in a multiplex test system (biochip) for the sepsis diagnosis. Based on published data, 12 proteins of particular importance were chosen as potential molecular markers of SIRS and sepsis: C-reactive protein (CRP); PCT; lipopolysaccharide-binding protein (LBP); lipocalin 2 (NGAL); transforming growth factor beta 1 (TGF- β 1); tumor necrosis factor (TNF); interleukin 1 receptor antagonist (IL-1RA); four interleukins (IL-6, IL-8, IL-10, IL-18); and troponin T (TnT). Monoclonal antibody and recombinant protein pairs, corresponding to the markers, were purchased commercially and tested for specificity by ELISA.

A novel method was used to make an aldehyde-based substrate for immobilized protein biochips. The chips were printed and preliminary

testing on a sample of patients in St. Petersburg with inflammatory soft tissue infections (likely to progress to sepsis) showed that in the group of patients with a sepsis diagnosis, a significant increase in the levels of serum CRP, PCT, LBP, NGAL, IL-6, IL-8 and IL-18 was observed. These proteins can be preliminarily considered as potential biomarkers of SIRS and sepsis.

*sergey.klotchenko@influenza.spb.ru

Microarray Based Approach for Multiparameter Analysis of Influenza Virus

Heydarov R.N.* , Fesenko E.E., Shaskolskiy B. L., Mikhailovich V. M.

Engelhardt Institute of Molecular Biology, RAS, Moscow

The study was aimed at developing a reliable and relatively fast microarray based approach for multiparameter analysis of influenza virus genome. The technique developed includes multiplex asymmetric PCR followed by hybridization with specific oligonucleotide probes immobilized in microarray 3D gel pads.

The method allows simultaneous identification of the following:

- 1)** viral type, A and B;
- 2)** subtype variants of hemagglutinin (16) and neuraminidase (9);
- 3)** mutations in the viral M2 gene leading to resistance to amantadine and remantadine (S31N, V27A, L26F, and A30T);
- 4)** mutation in the NA gene (H274Y, in N1 subtype) that causes resistance to neuraminidase inhibitors (oseltamivir and zanamivir);
- 5)** nonsense mutations in the PB1-F2 open reading frame (stop-codons in positions 12 and 58) and N66S mutation;
- 6)** structural variations in NS1 protein C-terminal region (length variation types: LVT(-10), LVT(-13), LVT(+7); and terminal 4-amino acid sequence variations: RSEV, RSKV, KSEV, ESKV, ESEV, EPEV);
- 7)** highly pathogenic avian variants H5 and H7.

As many as 54 specific primers are used to simultaneously amplify viral cDNA prepared in advance by reverse transcription. Fluorescent thymidines are incorporated into the amplification products during

multiplex PCR. The resulting labeled amplicons are hybridized with 174 oligonucleotide probes immobilized into microarray gel pads. Capturing and processing of fluorescence images were performed with a microarray reader equipped with specialized software. Total analysis can be performed in 6-8 hours.

The approach developed was validated using a panel of 21 viral reference stains, and it was successfully used to analyze 42 clinical samples of human influenza viruses. The microchip based array could be considered as a reliable alternative technique to conventional sequencing.

* rustam.heydarov@gmail.com

Automated mariPOC® platform enables highly sensitive and rapid antigen detection and subtyping of influenza viruses

Antikainen P.,¹ Suomala P.,² Klotchenko S.,³ Vasin A.,³ Koskinen J.^{1*}

¹*ArcDia International Ltd, Turku, Finland*

²*Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB), Mikkeli, Finland*

³*Research Institute of Influenza, St. Petersburg, Russia*

Background: mariPOC® (ArcDia International Ltd, Finland) is an automated diagnostic system for rapid testing of acute infectious diseases such as respiratory and gastrointestinal infections. mariPOC® respi is a multianalyte test that identifies 11 pathogens causing respiratory tract infections. The influenza A virus (IAV) method has shown 77–92% sensitivity and 98–100% specificity compared to PCR or DFA (Ivaska et al 2013; Tuuminen et al. 2013; Sanbonmatsu-Gámez et al. 2015). We recently redesigned and re-validated the method to be even more sensitive and to bridge the gap against PCR and DFA (direct fluorescent antibody). The method targets a highly conserved epitope within the nucleoprotein antigen. Furthermore, we have preliminary showed that the platform allows subtyping of influenza infections.

Material/methods: Analytical sensitivity of the new mariPOC® IAV method was studied against the old IAV method with dilution series of purified seasonal IAV subtypes (H1N1, H1N1 swine and H3N2). IAV subtype recognition of the new IAV test was also studied against potentially pandemic IAV subtypes (H2N2, H5N1, H7N3, H7N9 and H9N2).

The performance of the new IAV test and a LF test (CerTest Biotec, ZV862001P) was compared with 198 frozen nasopharyngeal aspirates that were leftovers from routine diagnostics (ISLAB, Finland). The samples were collected during the influenza season of 2015–2016. The samples

were pretreated and analysed according to manufacturers' instructions. The LF results were analysed blind by two persons and documented by photography. Samples with discrepant results were resolved using PCR and/or DFA.

Prototype methods for subtyping of influenza viruses on mariPOC® platform were developed using antibody reagents (developed in the Research Institute of Influenza, St. Petersburg) specific to hemagglutinin.

Results: The new mariPOC® IAV test showed order of magnitude better analytical sensitivity (1.5 ng/ml for H1N1) than the old IAV test with seasonal IAV subtypes. The new diagnostic IAV test also recognised well all the other tested IAV subtypes.

In a retrospective study with clinical samples, the new IAV test detected 13 IAV true positive samples and no false positives (table). The LF test had sensitivity of 77% (10/13) compared to the new mariPOC® IAV test. Specificities for the new mariPOC® IAV test and the LF test were 100% (185/185) and 96% (178/185), respectively.

		LF test		
		Positive	Negative	Total
mariPOC®	Positive	10	3	13
	Negative	7	178	185
	Total	17	181	198

So far we have been able to construct prototype methods for subtyping of H2N2, H5N1, H9N2 and H7N9 influenza viruses. Analytical sensitivities have been < 10 ng/ml of purified inactivated virus for the best methods.

Conclusions: The new mariPOC® IAV test was shown to be analytically ten times more sensitive than the old diagnostic IAV test. The test showed broad IAV subtype recognition suggesting that it is likely able to detect any new arising pandemic variants. The new test was

clinically significantly more sensitive than the LF test. Our results indicate that the new mariPOC® IAV test enables the detection of seasonal as well as pandemic IAV subtypes with improved sensitivity. The magnitude of the improvement suggests that in clinical use the new test considerably bridges the gap to PCR. We also showed that mariPOC® platform allows further subtyping of the samples which may be of epidemiological interest and important in case a new pandemic breaks out.

* janne.koskinen@arcdia.com

Etiological laboratory diagnostics of ILI in surveillance and clinical practice

Yatsyshina S.*

*Central Research Institute for Epidemiology of Rospotrebnadzor (CRIE),
Moscow, Russia*

The complexity of influenza diagnosis is based on the fact that the clinical picture may differ from the classical one, especially in case of mixt-infection. Moreover, similar ILI symptoms in the first days of onset, may cause adenovirus, respiratory syncytial virus, metapneumovirus, parainfluenza virus, bocavirus, coronavirus, rhinovirus, *M.pneumoniae* and *C.pneumoniae*. In this regard, rapid laboratory tests for confirmation of influenza, are particularly important and in demand in medical practice and surveillance. Real-Time PCR provides reliable information on the prevalence of influenza, the effectiveness of vaccination, helps to determine the etiology of ARI, and promotes the use of therapy.

We present the results of application of AmpliSens Real-Time PCR kits (CRIE, Russia), which demonstrate their good analytical characteristics and high diagnostic value. PCR screening of biologic samples increases several times the number of Influenza virus isolates, allows identify the potentially highly pathogenic Avian Influenza viruses H5, H7, H9 and new subtypes. In our study the etiology of ILI in children with classical flu symptoms was determined by PCR in 181 of 211 (85.8%) cases; Influenza virus RNA was identified in 132 (62.6%), of which 88 were A(H3N2), 4 were A(H1N1)pdm09 and 40 - B.

The diagnostic characteristics (sensitivity, specificity, likelihood ratio for test positive (LR+) and negative (LR-)) of immunochromatographic (ICA) assay ("Influenza A+B" Vegal Pharmaceutica, Spain) relative to Real-Time PCR were estimated. The diagnostic sensitivity of ICA was 58% for Influenza A (H3N2) and 20% for Influenza B, specificity - 97.5% and 97.7%, respectively. It was shown, that ICA can often definitively rule in

an Influenza A (H3N2) ($LR+=22.99$), but is moderately useful in ruling out an Influenza A (H3N2) ($LR-=0.43$). ICA often does not definitively rule in Influenza B ($LR+=8.6$) and is useless in terms of ruling out an Influenza B ($LR-=0.8$). In some swabs with the Influenza viral RNA of 1×10^5 copies/ml and more, ICA gave negative results. Similar studies are needed for Influenza virus A (H1N1)pdm09 and other rapid tests.

* svetlana.yatsyshina@pcr.ms

Effects of PA-X deficient viruses in chicken embryos and implications on yield of candidate vaccine viruses (CVVs)

Hussain S.^{1*}, Turnbull M.², Wise H.², Beard P.^{2,3}, Lo A.⁴, Vervelde L.², Kovacikova K.², Engelhardt O.⁵, Digard P.²

¹ *The Francis Crick Institute, London, United Kingdom*

² *The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, United Kingdom,*

³ *The Pirbright Institute, Surrey, United Kingdom*

⁴ *University of Cambridge, Cambridge, United Kingdom*

⁵ *National Institute for Biological Standards and Control, Hertfordshire, United Kingdom*

The PA-X protein of influenza A virus has proposed roles in host cell shut off and viral pathogenesis. Although most strains of virus are predicted to encode PA-X, strain-dependent variations in activity have been noted, with certain laboratory-adapted isolates seemingly lacking effective shut off function.

We found that PA-X protein from A/PR/8/34 (PR8) strain had substantially lower repressive activity against cellular gene expression in mammalian and avian cells compared with PA-X from the avian strains A/Turkey/England/50-92/91 (T/E) (H5N1) and A/chicken/Rostock/34 (FPV). Loss of normal PA-X expression, either by mutation of the frameshift site or by truncating the X-ORF, had little effect on the infectious virus titre of PR8 or PR8 7:1 reassortants with the T/E segment 3 grown in embryonated hens' eggs. However, in both virus backgrounds, mutation of PA-X led to decreased embryo mortality and lower overall gross pathology; effects that were more pronounced in the WT PR8 strain than the T/E PA reassortant, despite the low shut off activity of the PR8 protein. Following purification of viruses from allantoic fluid, the PA-X mutant viruses displayed an increased ratio of HA to NP and

M1 compared to their WT counterparts, suggesting an effect on virion composition. When the PA-X gene was mutated in the background of poorly growing PR8 6:2 vaccine reassortant analogues containing the HA and NA segments from pdm2009 viruses or an avian H7N3 strain, HA yield increased by around 2-fold. This suggests that the PR8 PA-X protein may harbour a function unrelated to host cell shut off and that disruption of the PA-X gene has the potential to improve the HA yield of vaccine viruses.

*saira.hussain@crick.ac.uk

Human T-cell immunity to emerging highly-pathogenic viruses

Liu W.*

*National Institute for Viral Disease Control and Prevention, Chinese Center
for Disease Control and Prevention (China CDC), Beijing, China*

The newly emerging influenza viruses such as H5N1, H5N6, H7N9, and H10N8 pose great threat to public health. During the acute phase of human infection, virus-specific T-cells against these viruses not only contribute to the virus clearance, but also may lead to immunopathogenesis. By the collaboration with different hospitals in China, we recruit the seasonal influenza virus and avian influenza virus infected patients and investigate their T-cell immune features during the acute infection and the convalescence phase. We also investigate the cross-immune responses in the healthy population against different avian influenza viruses. The immune mechanism and factors discovered in our study will benefit the prediction of the disease outcome and the development of the vaccines.

* liujun@ivdc.chinacdc.cn

Defining RNA structured regions and finding RNA structured regions in H1N1 influenza virus

Shneider A.^{1*}, Chursov A.¹, Fridlyand N.²

¹ CureLab Oncology, Inc., Dedham, MA, USA

² Laboratory of Translational Biology, School of Biosciences and Biotechnology, University of Camerino, Italy

RNA structures play a crucial role in influenza biology. RNA region may not have one particular structure in a certain location. Instead, it may have the RNA region to be involved in forming several alternative structures each playing its crucial role. However, although it is commonly understood what the RNA structure is, the literature provides no satisfactory definition of what RNA structured region is. Instead, most publications consider a “structured region” as a region with the highest percentage of nucleotides paired via W-C bonds. As a result, it creates a bias when loops in a “clover leaf” are not considered to be elements of the structured region. We propose a definition of a structured RNA region based on the premise that both stems and loops within important RNA structures should be conserved among functional RNA molecules sharing high sequence homology. In addition, we present a computational approach to identify RNA regions possessing evolutionarily conserved secondary structures.

Applying this method to H1N1 influenza mRNAs revealed previously unknown structured regions that are potentially essential for viral replication and/or propagation. Evolutionary conservation of RNA structural elements may explain, in part, why mutations in some nucleotide positions within influenza mRNAs occur significantly more often than in others. We found that mutations occurring in conserved nucleotide positions may be more disruptive for structured

RNA regions than single nucleotide polymorphisms in positions that are more prone to changes. These results suggest a novel approach to the rational design of live attenuated vaccines: *in silico* prediction and *in vitro* introduction of mutations, which disrupt evolutionarily conserved RNA secondary structures in viruses and/or bacteria.

* ashneider@curelab.com

Structural bases of PB1 derived peptide mechanism of action

Zabrodskaya Y.^{1*}, Shaldzhyan A.¹, Egorova M.¹, Lebedev D.²,
Petrova-Brodskaya A.¹, Shurygina A.-P.¹, Bobkov D.^{1,3}, Gorshkov A.¹,
Bondarenko A.¹, Matusevich O.¹, Egorov V.¹

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*Petersburg Nuclear Physics Institute named by B.P.Konstantinov*

of National Research Centre «Kurchatov Institute», Saint-Petersburg, Russia

³*Institute of Cytology RAS, Saint-Petersburg, Russia*

Influenza A neuraminidase and the ion channel M2 are the most often targets for antiviral drugs. However, influenza resistance to existing drugs determines the requirement for new drugs development and searching for new targets. A promising target is the influenza virus polymerase complex. Previously we have proposed a number of peptides, corresponding to the influenza virus polymerase complex PB1 subunit. Their antiviral activity was tested in cell culture [1]. The most promising drug was a short peptide, PB1 subunit N-terminal region fragment. We hypothesized that this peptide affects the PB1 N-terminus [2], which is critical for the influenza virus polymerase complex formation [3].

By means of surface plasmon resonance we determined the peptide-PB1 binding constant in the model peptide system. The temporal parameters of such an interaction were estimated by time-resolved small-angle X-ray scattering (experiment #LS2508 @ID02, ESRF, Grenoble). The optimal drug formulation was found. Next, confocal microscopy and flow cytometry were used to study the peptide transport in cell culture.

Thus, as it was shown in the model system, the peptide interaction parameters and its transport through the membrane features allow us to continue the study with in vivo experiments. The work was carried

out with the financial support of the state contract №14.N08.11.0080 (Federal Target Program « The Russian Federation pharmaceutical and medical industry development for the period until 2020 and beyond»).

[1] O. V Matusevich et al., "Synthesis and antiviral activity of PB1 component of the influenza A RNA polymerase peptide fragments," Antiviral Res., vol. 113, pp. 4–10, Jan. 2015.

[2] V. V Egorov et al., "Structural Features of the Peptide Homologous to 6-25 Fragment of Influenza A PB1 Protein," Int. J. Pept., vol. 2013, p. 370832, 2013.

[3] S. Chang et al., "Cryo-EM structure of influenza virus RNA polymerase complex at 4.3 Å resolution," Mol. Cell, vol. 57, no. 5, pp. 925–35, Mar. 2015."

* zabryaka@yandex.ru

RNA secondary structure analyses of the NS segment of influenza A virus

Baranovskaya I.^{1*}, Petrova-Brodskaya A.¹, Egorova A.¹,
Vorobyov E.¹, Vasin A.^{1,2}

¹Research Institute of Influenza, Saint-Petersburg, Russia

*²Peter the Great St. Petersburg Polytechnic University,
Saint-Petersburg, Russia*

Background

Secondary structure elements of viral genomic RNAs play important roles in regulation of transcription, in several steps of the viral replicative cycle and pathogenesis. Previously, it was demonstrated that two regions (nucleotide positions 82-148 and 497-564) in the positive-sense RNA of the NS segment fold into a multi-branch loop or hairpin structures. These regions are located near 5' and 3' splice sites of the NS segment and potentially may influence open reading frame shift switch between the NS1 and NEP genes. The purpose of our study is to analyze structural properties of the aforementioned RNA regions.

Methods

Based on RNA secondary structure prediction of the NS segments of more than 40 different strains of influenza A virus, we have chosen single nucleotide positions which may play roles in hairpin structure formation and stability. Using site-directed mutagenesis, we obtained several combinations of mutations in the corresponding regions of the NS segment.

Results

Here, we experimentally compared four variants of the in vitro-transcribed RNAs, in terms of their secondary structure manifestations,

by electrophoretic separation. As a result, the analyzed RNA variants were found to correspond to their predicted structural shapes.

Conclusion

Further, based on these results, we plan to perform (in vivo) experiments with infection of cultivated cells with viruses assembled by “reverse genetics” in order to better understand the role of these RNA structures in the viral life cycle.

*irina.baranovskaja.1992@gmail.com

**Immunochemical and virus neutralization properties
of a recombinant human immunoglobulin A
specific to influenza virus A hemagglutinin**

Aliev T.^{1*}, Argentova V.¹, Dement'yeva I.², Klotchenko S.³, Sergeeva M.³,
Shtro A.³, Taraskin A.³, Toporova V.⁴, Pozdnyakova L.²,
Dolgikh D.⁴, Sveshnikov P.², Vasin A.^{3,5}, Kirpichnikov M.¹

¹*Moscow State University, Faculty of Biology, Moscow, Russia*

²*Russian Research Center for Molecular Diagnostics and Treatment,
Moscow, Russia*

³*Research Institute of Influenza, Saint-Petersburg, Russia*

⁴*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow,
Russia*

⁵*Peter the Great St. Petersburg Polytechnic University, Saint-Petersburg,
Russia*

Immunoglobulin A (IgA) plays important role in mucosal immunity providing defense against various viral and bacterial pathogens. At mucosal sites it acts either as a dimer or a secretory IgA. By binding to FcαRI receptors on the surface of neutrophils, dimeric IgA activates uptake of opsonized pathogens and neutrophil-mediated death of cells infected by viruses.

We used variable domains of a broadly neutralizing antibody FI6 to influenza virus A (IV) hemagglutinin (HA) to develop expression constructs for the production of monomers and dimers of IgA1 and IgA2m1 isotypes in CHO cells. Recombinant IgA were isolated from cultural medium using KappaSelect (GE Healthcare Life Sciences) affinity columns. HA binding patterns of IgA analysed using immunoblotting appeared to be similar to FI6 IgG. Indirect ELISA showed that all four recombinant IgAs bound to 11 viruses of H1N1 and H3N2 subtypes. The IgA binding profiles correlated with IgG binding. The antibody dimer of IgA1 isotype was tested for anti-viral neutralizing activity in vitro and in vivo. The strongest anti-viral activity with IC50 ranging

from 0.1 to 0.5 µg/ml was demonstrated on MDCK cells infected with IV of H1N1 subtype. Animal tests were carried out on mice challenged with one of the following IV: A/California/7/09 (H1N1)pdm09, A/Puerto Rico/8/34 (H1N1), and A/Aichi/2/68 (H3N2). In a prophylactic setting, antibodies (IgA1 dimer or corresponding IgG) were administered 1 h prior to infection, while in a therapeutic setting antibodies were given 1-2-3 days p.i. In both settings, antibodies were given using intranasal or intraperitoneal routes. Oseltamivir was used as a positive control. Intranasal administration of IgA1 dimer showed highest protection among antibodies tested with 7-8 day survival rate matching that of oseltamivir.

The results obtained evidence that the IgA counterpart of the broadly neutralizing antibody FI6 is characterized by high affinity, cross-specificity, and significant anti-viral activity.

This work was supported by the subsidy RFMEFI60714X0060 of the Ministry of Education and Science of Russian Federation.

* ta12345@list.ru

Multi-contact ligands as one of the ways to overcome viral resistance

Tsvetkov V.^{1*}, Serbin A.², Bolshchikov B.²

¹Research Institute of Influenza, Saint-Petesburg, Russia

²A.V. Topchiev Institute of Petrochemical Synthesis RAS, Moscow, Russia

A solution of resistance problem today is one of the most vital tasks in the development of drugs with antiviral activity. One of the possible approaches to this may be the transition from small molecules as ligands, capable of interacting with only one site of potential targets, to molecules with a large conformational capacity, having the opportunity to contact with possible targets in several areas. In favor of this assumption is the fact that probability (P) of a single one-point mutation (M) should be many orders more than probability of series of mutations (M1, M2, ... Mn) occurred in many mutative centers simultaneously: $P(M_i) \gg P(M_1M_2...M_n) = P(M_1)P(M_2)...P(M_n)$. To create such multi-contact ligands, it is possible to use natural and synthetic oligomers or polymers. Taking into account the fact that with increase of the ligands size the complexity of its penetration into the cell increases, it is reasonable to look for potential targets for them outside the cell. The most suitable target for such ligands can be structures that are exposed on envelope of virus, and participate in the entry process of binding to the cell and penetration into it. In this investigation, a hemagglutinin protein (HA), involved in adsorption and fusion, was employed as a potential target.

The study of its surface accessible for binding reveals the presence of cavities of considerable volume, as well as zones of a powerful hydrophobic and a positively charged electrostatic potentials. In the current research, a step wise strategy from creation of 3D models, using quantum chemistry, to studying the ligand-target binding by procedure of docking and molecular dynamics, was developed for the oligomers, carrying both hydrophobic anchors and a set of negatively charged

groups, and capable of forming multiple contacts on the surface of HA. The simulation showed that multi-contact binding can lead to the wrapping of the oligomers around the surface of the protein, which make impossible the conformational rearrangements of HA during the fusion process.

* v.b.tsvetkov@gmail.com

New strategies for effective delivery of antiviral siRNA against influenza A virus

Timin A.,^{1*} Petrova-Brodskaya A.,^{2,3} Muslimov A.,^{3,4}

Lepik K.,⁴ Vasin A.,^{2,3} Sukharukov G.^{1,2,5}

¹*RASA center in Tomsk, Tomsk Polytechnic University, Tomsk, Russia*

²*Peter the Great St. Petersburg Polytechnic University, Saint-Petersburg, Russia*

³*Research Institute of Influenza, Saint-Petersburg, Russia*

⁴*Pavlov First Saint Petersburg State Medical University, Saint-Petersburg, Russia*

⁵*School of Engineering and Materials Science, Queen Mary University of London, United Kingdom*

The effectiveness of many biologically active compounds is limited by their low selectivity, bioavailability, limited solubility, etc. There are methods used to increase the drug compounds' biological activity, for example, chemical modification. Alternatively the methods of targeted delivery based on the use of various carriers (nanoparticles, liposomes, micelles, etc.) are widely used nowadays. They provide effective delivery of bioactive molecules to target cells or organs and can be functionally modified to increase their bioavailability. A high potential of micro-containers obtained using "Layer-by-Layer" technology for targeted delivery of biologically active compounds was demonstrated. RNA interference (RNAi) by synthetic siRNA targeting virus genes is a perspective way for antiviral therapy, but its implementation into clinical therapy is complicated because of difficulties with delivery of naked siRNA.

The purpose of this work was to create micro carriers for the delivery of siRNA. New hybrid micro containers with low toxicity, high biocompatibility, ability to protect the encapsulated material from the aggressive external environment were obtained. The toxicity and

delivery capacity of micro containers was estimated in comparison with commercial reagent Lipofectamine 2000 and polycationic systems. In vitro studies of therapeutics micro containers with siRNA against IAV A/PR/8/34(H1N1) conducted on the A549 cell line demonstrated strong virus inhibition comparable with oseltamivir antiviral effect. For treatment with siRNA's targeting NP gene dose-dependent effect was shown. Besides, we tested siRNA targeted most conservative genes PA, NP, M and NS and evaluated the contribution of each of siRNAs separately and together in viral reproduction reducing. These results possess a great potential of siRNA microencapsulation technology for development of new anti-viral drugs delivery systems.

The work was supported by Russian Science Foundation Pr.№ 17-73-10023

* timin@tpu.ru

The efficacy of the influenza treatment with antiviral drugs with different mechanism of action

Malyshev N.* , Popov A.

*Federal Research Center of Epidemiology and Microbiology
named after N. F. Gamaleya, Moscow, Russia*

An open prospective comparative study of the efficacy of influenza monotherapy with antiviral drug versus combination therapy of two anti-viral drugs with different mechanisms of action (within the limits of registered medical use for the drug) was performed in 200 patients with influenza A (H1N1) pdm09. In all patients, the clinical diagnosis was confirmed by the indication of the virus in nasopharyngeal washings by polymerase chain reaction with pre-reverse transcription (RT-PCR) in real time. All patients were randomly divided into 4 groups of 50 people, they were equivalent in terms of admission to hospital, age, sex, and the length of treatment from the onset of the disease. Group 1 received monotherapy with Umifenovir 800 mg/day in 4 divided doses for 5 days; Group 2 — monotherapy with Oseltamivir 150 mg/day in two doses for 5 days; Group 3 — Umifenovir according to the described scheme in combination with Kagocel 72 mg/day in 3 doses for the first 2 days and 36 mg/day in 3 doses in the following 2 days; Group 4 — Oseltamivir according to the described scheme in combination with Kagocel 72 mg/day in 3 doses for the first 2 days and 36 mg/day in 3 doses in the following 2 days.

Criteria for the clinical efficacy of antiviral therapy were the time of temperature normalization, decrease of intoxication, catarrhal symptoms, frequency of the main clinical manifestations, and the frequency of complications development after the end of treatment in comparison with the state before it began. In assessing the duration of the symptomatology, the end point was the absence of symptoms of

the disease within 24 hours. The study showed that the combination of etiotropic drugs Oseltamivir (Tamiflu®) and Umifenovir (Arbidol®) with the antiviral agent Kagocel® makes it possible to significantly improve the therapeutic efficacy compared with the corresponding monotherapy, which is expressed by a decrease in the frequency of manifestation of the main clinical indicators, a reduction in the duration of clinical symptoms, and a decrease in the frequency of complications. Good tolerability of treatment by patients was noted.

*manikolai@yandex.ru

Some Prospects toward Poly-Inhibitors of Influenza and other Human Viruses

Serbin A.^{1,2*}, Egorov Yu.¹, Karaseva E.¹, Alikhanova O.¹

¹ *Biomodulators & Drugs RC, Health RDF, Moscow, Russia*

² *A.V. Topchiev Institute of Petrochemical Synthesis, Moscow, Russia*

Bio-expansion of human population raises the human-occupying viruses counter intervention - evolution that is fatally promoted now by anthropogenic self-impacts: virus-transfer migrations, techno-contaminations, gene engineered co-genesis of viruses (terrorism incl.), etc. On the contrary, current development of antivirals/vaccines is far from optimal completeness and efficiency, allowing progression of viral resistance. In way to possible solutions for the problem we explored design - synthesis of original antivirals capable of poly-inhibiting effects due to rational combinations of functionally-synergetic structures cooperated on water-soluble oligomers.

Three generations based on poly-succinic carboxylate-type oligomers with own immune-adjuvant activity were newly advanced by covalently linked vectors/anchors to direct antiviral interventions. Accounting similarity in potential targets within life cycles of different viruses, the present report is focused mainly on orthomixoviridae, herpes and retro-viridae families. The first generation was designed for competition with similarly (negative) charged cell receptors involved in the virions adsorption. Stepwise enhancing a negative charge via conversion toward bio-mimetic derivatives leads to significant inhibition of influenza (SI -> 10000) and cytomegalovirus (SI -> 7500) viruses, as well as HIV-1 (SI -> 500). The second way is tropism to cholesterol-enriched raft-domains, the selective portals to the viral entry. Even single cholestene-related anchor per oligomer molecule can promote a considerable inhibition of influenza viruses (SI -> 5400) and HIV-1 (SI -> 1000).

The next are envelope proteins of influenza (HA) and HIV (gp41) – sensitive to pendant anchors multi-connectable with fusion-initiating pre-hairpin domains. A powerful inhibition was achieved against both influenza (SI-> ≥ 10000) and HIV (SI-> 10000). Computational modeling the correspondent mechanism was completed by Vladimir B. Tsvetkov [special report].

* serbin@ips.ac.ru

Poster Abstracts

Phylogenetic analysis of surface proteins of the influenza A(H1N1)pdm09 viruses isolated in 2015-2016 in Moscow

Krasnoslobodtsev K.,* Alkhovsky S., Burtseva E., Kirillova E.,
Fedyakina I., Kolobukhina L., Merkulova L., Lvov D.

*Federal Research Center of Epidemiology and Microbiology
named after N. F. Gamaleya, Moscow, Russia*

Influenza A(H1N1)pdm09 virus dominated in 2015-2016 epidemic season in Russia. Previous study shows that its active circulation in epidemic seasons increases morbidity and hospitalization rate. Some cases influenza infections were fatal. 12 strains isolated from autopsy material were studied using NGS on illumine platform.

All 12 strains isolated on MDCK cell and characterized as A/California/7/2009-like in HI test. DNA library was done using TruSeq DNA Sample Prep Kit v2 according to manufacture protocol. Genome sequences were obtained on MiSeq (Illumina) using MiSeq Reagents Kits v2. Bioinformatic analysis was done using CLC Genomic Workbench 7.0 and MEGA 6 software.

Phylogenetic analysis of surface protein genes HA and NA was done. According to the obtained data 11 viruses belong to 6B.1 sub-clade and bear amino acid substitutions S84N, S162N, I216T, except one virus (A/IIV-Orenburg/30-B/2016) that are belong to 6.B clade.

There are 4 strains bear amino acid substitution in 222 position of HA1 (D222N/G/Y)

All viruses have no any substitutions that are can lead to reduced sensitivity to neuraminidase inhibitors (oseltamivir, zanamivir, peramivir). It was confirmed by (NI) test.

The data indicated that all obtained viruses belong to A/California/7/2009-like, the component of the 2015-2016 influenza vaccines. Some strains bear amino acid substitution in receptor-binding site (222 position of HA1). There are no resistance strains to known neuraminidase inhibitors among them.

The work was supported by the Centers for Disease Control and Prevention, Atlanta, USA. CoAg: U51IPOOO527-05

* kkg_87@mail.ru

Overview of 2016 – 2017 influenza season in Georgia

Machablishvili A.* , Karseladze I., Gavashelidze M., Zakhashvili Kh.

National Center for Disease Control and Public Health, Tbilisi, Georgia

Background. Routine monitoring of Influenza Like Illnesses (ILI) and Severe Acute Respiratory Infections (SARI) is an important tool for tracking changes in globally circulating influenza viruses. The aim of this study was to describe influenza activity during 2016-2017 season in Georgia.

Methods. Epidemiological data and specimens were obtained from ILI/SARI sentinel sites. Real-time RT-PCR was performed for influenza virus detection and Sanger sequencing for genetic characterization of HA and NA genes. In addition, sequences of Georgian strains were downloaded from GISAID.

Results. Totally 954 ILI/SARI samples were tested at NIC from week 40, 2016 to week 20, 2017; 372 (39%) specimens found positive for influenza viruses: 204 A/H3N2 and 168 B Victoria lineage. Influenza detection was similar in samples obtained from ILI (37%, 130/351) and SARI (40%, 242/603) sites. Bimodal distribution of influenza activity was observed during the season: first wave was caused by influenza A/H3N2 with detection median week 51 (IQR weeks 50-52) and the second by influenza B with median week 9 (IQR weeks 7-11). Influenza detection was highest in age group 5-14 year old (58%, 104/178) followed by 15-29 years old group (54%, 60/111). Six SARI cases had lethal outcome, five deaths were associated with influenza A/H3N2 and one with flu B. All fatal cases were persons >50 years and suffered with at least one

chronic disease. All sequenced Georgian A/H3N2 viruses belonged to 3C.2a1 subclade characterized with HA amino acid substitutions N171K in HA1 and I77V and G155E in HA2 compared to vaccine virus A/Hong Kong/4801/2014. Georgian A/H3N2 clustered in two subgroups: first carrying R142G substitution in HA1 and second with amino acid substitutions K92R, N121K, H311Q in HA1. NA genes of Georgian strains showed same pattern of clustering as observed for HA gene. HA and NA genes of influenza B Victoria lineage viruses fell into clade1A, the vaccine virus B/Brisbane/60/2008 clade. B viruses carried additional amino acid changes in NA gene.

Conclusions. 2016-2017 season data demonstrate that influenza is major cause of ILI/SARI cases in Georgia, especially in children 5-14 years and young adults aged 15-29 years old. Genetic analyses of our strains revealed genetic variability of influenza viruses circulating in Georgia and need for routine surveillance.

* a_machablashvili@hotmail.com

Surveillance and seasonality of Influenza and other respiratory viruses in Cuba, 2011-2016

Valdes O.,* Acosta B., Mune M., Arencibia A., Martínez J., Gonzalez G.,
Roque R., Hernández B., Oropesa S.

Tropical Medicine Institute Pedro Kouri, Habana, Cuba

Respiratory infections require a rapid etiological diagnosis for efficient management of cases. Seasonal epidemics of influenza and respiratory syncytial virus (RSV) are responsible for significant morbidity and mortality worldwide. We investigated the frequency of respiratory viruses responsible for respiratory tract infections between January 2011 and December 2016. This study was conducted for simultaneous detection and identification of 17 respiratory viruses using multiplex real-time PCR. Of these 20441 cases, 48% (9807/10633) were positive for at least one pathogen. Respiratory syncytial virus was the most frequently detected pathogen (14.4%) followed by human rhinovirus (12%), Influenza A (9.1%), Influenza B (3.4%). The other respiratory viruses: human metapneumovirus, human parainfluenza virus 1-4, human coronaviruses 229E, OC43 and HKU1, human adenovirus, enteroviruses and human bocavirus were found in less than 2.2% of the total samples. The positive rates among the following subtypes were: 5.7% for pH1N1, 2.8% H3. Influenza A was the predominant virus during the 2011-2012 and 2013-2015 seasons. Influenza B was the primary virus during the 2012-2013 and 2015-2016 seasons. The epidemic period of HRSV infections occurred from July to November.

* odalys@ipk.sld.cu

Immunological aspects of severe syndromes the flu in pediatrics

Afanasieva O.,* Golovacheva E.

Research Institute of Influenza, Saint-Petersburg, Russia

The real threat of a rise in mortality in pediatric patients in the epidemic period creates the development of severe syndromes — croup and bronchial obstruction caused by influenza viruses, which is especially often observed during circulation of the pandemic influenza virus in Saint-Petersburg.

Objective: to compare the dynamics of cytokine responses in life-threatening syndromes, croup and/or bronchial obstruction, the flu with the expectation of informative factors that not only predict the development of severe syndromes, but also to assess the adequacy of therapy.

In epidemic seasons 2013-2016 where observed 85 children with croup syndrome and 156 children with the syndrome of bronchial obstruction with verified influenza with study of the content of serum IL-1 β , IL-4, IL-8 and IFN- γ in the dynamics of the disease by ELISA with calculation of odds ratio IL-4/IFN- γ and IL-8/IFN- γ .

Results. In children with croup syndrome with flu in the beginning of the disease was noted a moderate increase of IL-4 coupled with a high level of IL-1 β (100 PG/ml), whereas the obstructive syndrome was determined by a moderate increase of IL-1 β (50 PG/ml) and significant increase of IL-4 and IL-8. The decline in these indicators is 2-3 times on the second day correlated with the decrease of the temperature and

the relief of stenosis of the larynx, whereas in patients with airflow obstruction changes in the level of these cytokines in the blood was not observed on the background of low-grade fever. In addition, patients with croup at a low concentration of IFN- γ (<30 pg/ml) early in the disease with increasing ratios of IL-4/IFN- γ and IL-8/IFN- γ more than three times on the second or third day of the disease predicted the recurrence of stenosis of the larynx, that is, the return syndrome without re-infection due to remaining in the body of pathogens that in 9% of cases was observed for 5-6 days in the period of convalescence from the flu. When ratios of IL-4/IFN- γ and IL-8/IFN- γ more than 3 early in the disease predicted the development of virus-induced bronchial obstruction, due to Th2-type immune response. It is shown that the rational use of antivirals in the acute phase contributed to the switch from Th2 to Th1 type immune response by reducing antigen load, and sequential interferon reduces the risk of recurrence and complications.

Conclusion: Thus, the dynamics of the ratios of IL-4/IFN- γ and IL-8/IFN- γ may not only predetermine the development of severe syndromes of influenza, and to improve the strategy of the specific and interferon therapy in children, including the period of the disease.

* olga-afanaseva57@mail.ru

Etiology of severe acute respiratory tract infection in hospitalized patients in Belarus, 2016-2017

Sivets N., Shmialiova N.*, Gribkova N., Lapo T.

*Republican Research & Practical Center
for Epidemiology & Microbiology, Minsk, Belarus*

Objective: To investigate etiology of severe acute respiratory infection (SARI) in hospitalized patients in Belarus.

Methods. The nasopharyngeal swab samples were collected from 539 patients with SARI between October 2016 - May 2017 as defined by the World Health Organization. Common respiratory pathogens including influenza virus A and B, respiratory syncytial virus (RSV), parainfluenza viruses 1, 2, 3 and 4, human bocavirus, human metapneumovirus, rhinovirus (RV), adenovirus, mycoplasma pneumoniae (MP) and chlamydia pneumoniae were detected by PCR. The results were analyzed to explore the prevalent pathogens among different age group and the seasonal distribution.

Results. Respiratory pathogens were identified in 234 cases (39,7%). The detection rates of respiratory pathogens by age group were: 63,2% (0-4 years), 50,0% (5-14 years), 38,0% (15-17 years), 43,5% (18-29 years), 26,4 (30-64 years) and 25,6% (over 65 years).

The positive rate of different respiratory pathogens in patients with SARI was found related to age. The most frequently detected pathogens among aged group 18-29, 30-64 and over 65 years were influenza viruses (41,3, 30,4 and 50% respectively). MP was the most common pathogen in children aged 5-14 and 15-17 years (42,1 and 36,4% respectively). RV and influenza viruses were the most prevalent among the children 0-4 years (27,2 and 29,1% respectively).

When the seasonal distribution was considered, MP and RV were most frequently detected during the pre-epidemic period. In the epidemic 68,3% of the SARI cases were associated with influenza viruses, predominantly A (H3N2). RV and RSV were the most common pathogens in the post-epidemic period, whose detected rate were 28,1 and 26,5%, respectively.

Conclusion: Influenza virus A was the most common pathogen in respiratory tract infection in hospitalized patients with SARI among most age groups. Different pathogens were prevalent in different ages groups with respective seasonal distribution.

* shmelevanataliya@mail.ru

Mucosal influenza vectored tuberculosis vaccine enhances priming of innate and adaptive T-cell immunity to *Mycobacterium tuberculosis*

Shurygina A.-P.,^{1*} Vasilyev K.,¹ Zabolotnykh N.,² Vinogradova T.,²
Romanovskaya-Romanko E.,¹ Buzitskaya Zh.,¹ Sansyzbay A.,³
Khairullin B.,³ Stukova M.¹

¹ *Research Institute of Influenza, Saint Petersburg, Russia*

² *Saint Petersburg Research Institute of Phthiopulmonology,
Saint Petersburg, Russia*

³ *Research Institute for Biological Safety Problems, Gvardeisky,
Republic of Kazakhstan*

Pulmonary tuberculosis (TB) remains a serious health problem worldwide. The necessity to develop new TB vaccines is determined by highly variable efficacy of the widely used BCG (Bacille Calmette-Guérin) vaccine in preventing adolescent and adult pulmonary tuberculosis. Understanding the vaccine-induced immune mechanisms involved in long-term control of TB is of high priority in the development of novel vaccine and effective vaccination strategies.

The aim of the study was to investigate post-vaccination cellular immune response to the mucosal administration of a candidate booster vaccine based on the attenuated influenza vector expressing *M. tuberculosis* proteins ESAT-6 and Ag85A (Flu/ESAT-6_Ag85A) by the means of multicolor flow cytometry. Adaptive cellular immune response was analyzed following ex vivo stimulation with recombinant Ag85A

protein three weeks after the single vaccination of C57/black/6 mice compared to BCG administered subcutaneously.

A study of the dynamics of innate immunity cells showed a significant increase in the proportion of macrophages, monocytes and neutrophils in the lungs of mice 24-48 hours after immunization. In addition, the relative number of antigen-presenting cells expressing CD83 and CD86 activation markers was increased.

Three weeks after Flu/ESAT-6_Ag85A or BCG immunization an increase in the percentage of CD4 central memory T-lymphocytes (TCM) in the murine lungs and spleen was shown in both groups. A statistically significant difference with the BCG group was registered in the percentage of TCM in spleen ($p < 0.01$). Moreover, the influenza vector provides accumulation of CD8 effector cells in lungs more effectively than BCG. The advantage of the candidate vaccine was also shown in the formation of CD4 and CD8 effector memory cells (TEM) producing two cytokines (IFN- γ and IL-2, IFN- γ and TNF- α) and TEM population which produces all three cytokines simultaneously after the in vitro stimulation with Ag85A protein. These cells can be considered as potential immunological markers of protection in tuberculosis infection.

Our findings show that intranasal immunization with new influenza vector vaccine expressing mycobacterium antigens induces both innate and adaptive functionally active antigen-specific T-cell immune response.

* ann-polin@yandex.ru

Real-time RT-PCR determination of viral load in ferret nasal swabs with specimen collection control during experimental infection with influenza A virus

Petrov S.,^{1*} Sergeeva M. ¹, Grischenko V. ^{1,2}, Tsybalova L.¹

¹Research Institute of Influenza, Saint-Petersburg, Russia

*²Peter the Great Saint-Petersburg Polytechnic University,
Saint-Petersburg, Russia*

The preclinical testing of influenza vaccine includes an estimation of protection efficacy during influenza infection of laboratory animals. The assessment can be made not only by survival rate of immunized animals during lethal infection, but also by the detection of the viral load in the tissues and swabs of animals and by the clinical symptoms during non-lethal infection. Ferrets represent the animal model most close to the human. The classical method for influenza viral load estimation in nasal swabs of ferrets is virus isolation in chick embryos or MDCK cell culture, however, this method has low sensitivity. Moreover, peculiarities of manipulations during nasal swab collection from ferrets can lead to false-negative results if no specimen collection control is presented.

In the present work, a real-time RT-PCR panel for the evaluation of influenza virus in nasal swabs of ferrets with material collection control is developed and applied. To identify the influenza virus, the pre-existing panel of primers and probes from CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel was used. The control panel was developed to use the same PCR profile as for virus detection. The mRNA of RNAase P, which is species-specific and does not vary quantitatively from age, sex, functional state of the organism, was chosen as control target RNA.

During experimental infection of ferrets, the nasal swabs were collected on days 1, 3, 5 and 7 post infection. Clinical symptoms were observed until the day 6. The infectious virus was isolated from swabs only on day 1. By real-time RT-PCR method the virus was detected up to the day 7, though the threshold Ct values increased with time, indicating a reduction in the viral load. The threshold Ct values of RNAase P mRNA didn't significantly change from capture to capture and from animal to animal, indicating a uniform quality of the swab material.

Research was supported by the grant for young PhD from the Government of Saint-Petersburg.

* sergey.petrov@influenza.spb.ru

Development of multiplex real-time PT-PCR assays for seasonal A/H3N2, B, pandemic A/H1pdm09 and avian A/H5, H7, H9 influenza viruses detection

Sivets N.*, Shmialiova N., Gribkova N.

*Republican Research & Practical Center for Epidemiology & Microbiology,
Minsk, Belarus*

Molecular diagnostic techniques are rapid and sensitive methods for the detection and identification of influenza viruses. Our National Reference Centre for Influenza monitors the circulation of influenza virus in Belarus. Samples from patients with symptoms of respiratory infection are sent in for influenza virus diagnosis and differentiation of virus (sub) types A/H1N1pdm09 A/H3N2, B and also avian A/H5, H7, H9 influenza viruses. Therefore, our research is aimed to speed up and simplify the diagnostic procedure by the introduction of multiplex format for real-time PCR.

Methods:

Primers and probes for Influenza A and B viruses were selected from highly conserved regions of specific gene targets within the matrix protein (M) and nucleoprotein (NP) respectively. Primers and probes for detection and differentiation of seasonal influenza A/H3, A/H1pdm09 and A/H5, A/H7, A/H9 viruses are targeted for conserved regions of their respective hemagglutinin (HA) genes. Influenza-specific primers were designed to be 19–25 nucleotides long with annealing temperatures of approximately 62–66°C (GC content of 45–60%). Sizes of targeted regions are between 100–200 nucleotides. The PCRs were established with

plasmid standards. All concentration of the components were optimized for conditions of multiplex PCR. The PCR runs were performed with real-time PCR systems (Rotor Gene 6000, Corbett research, Australia).

Results:

The result of the study was the development of two diagnostic kits «Flu-gene» and «SARI-gene». The multiplex PCR method established in our study showed high sensitivity, reproducibility and specificity. This indicates a promising application of our method for monitoring influenza A viruses and influenza B virus, subtypes of the influenza A virus (H3N2, H1N1pdm09, H5, H7, H9) in respiratory samples from patients with signs symptoms of respiratory infection.

* sivets_n@mail.ru

Water-soluble [60]fullerene derivatives can suppress efficiently three influenza virus subtypes

Voronov I.^{1*}, Martynenko V.¹, Chernyak A.², Peregudov A.³,
Kushch A.⁴, Schols D.⁵, Troshin P.⁶

¹ *Institute for Problems of Chemical Physics
of Russian Academy of Sciences, Chernogolovka, Moscow region, Russia*

² *Science Center in Chernogolovka RAS, Chernogolovka,
Moscow Region, Russia*

³ *INEOS RAS, Moscow, Russia*

⁴ *D.I. Ivanovsky Institute of Virology, Federal Research Center of
Epidemiology and Microbiology named after N. F. Gamaleya, Moscow,
Russia*

⁵ *Rega Institute for Medical Research, Leuven, Belgium*

⁶ *Skolkovo Institute of Science and Technology, Moscow, Russia*

Fullerenes and their derivatives show exciting biological properties ranging from radical scavenging and neuroprotective activities and going all the way to photodynamic and chemotherapy of different types of tumors and viral infections. In particular, there are several reports highlighting the activity of certain fullerene derivatives against influenza virus [PLoS One, 2013, 8, e66337; Chem. Commun., 2011, 47, 8298; J. Huazhong Univ. Sci. Technol. Med. Sci., 2008, 28, 243].

Unfortunately, the development of medicinal chemistry of fullerenes has been strongly hampered by poor availability of their water-soluble forms. In particular, design of individual water-soluble fullerene derivatives can be particularly challenging. Recently we have elaborated efficient methodology for facile synthesis of various water-soluble fullerene derivatives from readily available precursor C₆₀Cl₆

[Chem. Commun., 2012, 48, 5461; Chem. Commun., 2012, 48, 7158; Org. Biomol. Chem., 2016, 15, 773; Org. Biomol. Chem., 2007, 5, 2783].

Here we present two new water-soluble fullerene derivatives (WSF) synthesized via Friedel-Crafts arylation of C₆₀Cl₆ with the dimethyl ester of phenylethylmalonic acid with subsequent cleavage of the protecting ester groups under acidic conditions. Compositions and molecular structures of all compounds were proved by ESI-MS and NMR spectroscopy.

The antiviral action of WSF was evaluated by in-vitro assay using cell culture infected with a certain virus. It has been shown that WSF effectively suppress A/H1N1, A/H3N2 and influenza B viruses outperforming significantly amantadine (Symmetrel®), rimantadine (Flumadine®) and ribavirin while being comparable to Zanamivir (Relenza®). A notable activity against human immunodeficiency virus and herpes simplex virus was also revealed. The obtained results point towards a big potential of further exploration of fullerene derivatives as promising antiviral drug candidates.

* voronovii@gmail.com

Water-soluble amino acid fullerene derivatives as potential anti-influenza drugs

Zhilentkov A.^{1*}, Peregudov A.², Balzarini J.³, Schols D.³, Troshin P.⁴

¹ *Institute for Problems of Chemical Physics of Russian Academy of Sciences, , Chernogolovka, Moscow region, Russia*

² *A. N. Nesmeyanov Institute of Organoelement Compounds of RAS, Russia*

³ *Rega Institute for medical research, Leuven, Belgium*

⁴ *Skolkovo Institute of Science and Technology, Moscow, Russia*

Fullerene derivatives have demonstrated a diverse spectrum of antiviral activities suppressing with a decent efficiency such viruses as herpes simplex virus [Antiviral Res., 2010, 86, 1, A70], cytomegalovirus [Russ. Chem. Bull. Int. Ed., 2011, 60, 6, 1172], human immunodeficiency virus [J. Am. Chem. Soc., 1993, 115, 6506], hepatitis C virus [Bioorg. Med. Chem. Lett., 2016, 26, 4565], Ebola-related viruses [Nature Chemistry, 2016, 8, 50] and, importantly, also influenza viruses [PLoS ONE, 2013, 8, 11, e66337].

Here we present a new family of water-soluble fullerene derivatives with five amino acid residues attached to the carbon cage around one of the pentagonal rings. It should be emphasized that the application of amino acids as solubilizing groups for the fullerene molecules ensures biocompatibility of the designed compounds. The synthesis of the target amino acid fullerene derivatives has been performed using chlorofullerene C₆₀Cl₆ as a precursor. The synthesized compounds were characterized by NMR spectroscopy and electrospray mass spectrometry. Additionally, the aggregation behavior of these compounds in aqueous solutions has been explored using dynamic light scattering.

In a full agreement with our expectations, the designed amino acid fullerene derivatives have shown low cytotoxicity and acute toxicity in mice (LD50 between 500 and 900 mg/kg). Some of the synthesized compounds demonstrated a potent antiviral activity against a series of subtypes of influenza A and also influenza B viruses with EC50 values ranging from low micromolar to high nanomolar concentrations. The best lead compounds outperformed significantly commercial drugs such as oseltamivir carboxylate (Tamiflu®) and zanamivir (Relenza®) tested under the same conditions. To conclude, low toxicity and strong antiviral action of amino acid fullerene derivatives makes them promising drug candidates for suppressing severe influenza infections.

* zhilenkow91@inbox.ru

Cytisine derivatives inhibit replication human influenza viruses *in vitro*

Fedorova V.^{1*}, Tsypiyheva I.², Koval'skaya A.², Petrova P.²,
Borisevich S.², Zarubaev V.¹

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*Ufa Institute of Chemistry, Ufa, Russia*

Influenza is currently one of the most common diseases in the world. It causes epidemics and pandemics and leads to a significant morbidity and mortality in people of all age groups. There are several drugs for influenza treatment. In some cases they are not effective because of appearance of resistant strains. Therefore, there is a need to search for new compounds that are effective against viruses resistant to the action of already available drugs. At first, we evaluated *in vitro* the antiviral activity of 40 synthetic derivatives of cytosine against different subtypes of influenza A (A/PR/8/34(H1N1), A/California/7/09(H1N1) pdm09, A/Aichi/2/68/(H3N2), A/Mallard/Pennsylvania/10249/84(H5N2)) and influenza B (B/Malaysia/2506/04, B/Florida/04/06). According to the received data, 3 of tested derivatives have antiviral effect against all subtypes of the influenza A and one - against all subtypes of influenza A and B viruses. Next, we used a time-of-addition assay to identify when in the virus life cycle an inhibitor acts. Reduction in viral titer indicates that tested compound acts later in the replication cycle and may be affecting processes such as viral budding and release. We also performed an electron microscopic study of influenza-infected cells treated with cytosine derivative. It was showed that infected cells in the presence of tested compound release less virions comparing to drug-free specimens. Obtained data serve as the basis for further development of the compounds of this group as a means for the treatment of influenza infection.

* vikusik1510@rambler.ru

The expression system for the production of recombinant hemagglutinin-specific monomers and dimers of human immunoglobulin A in CHO cells

Argentova V.^{1*}, Aliev T.¹, Toporova V.², Rybchenko V.¹,
Dolgikh D.², Kirpichnikov M.¹

¹Moscow State University, Faculty of Biology, Moscow, Russia

*²Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry,
Moscow, Russia,*

The use of recombinant hemagglutinin-specific immunoglobulin A (IgA) for the prophylaxis and treatment of influenza virus infection might have promising future prospects. One of the tasks that should be resolved is the development of reliable expression system for the production of IgA in eukaryotic cells. IgA is presented by two isotypes, IgA1 and IgA2m1 differing in glycosylation patterns and interchain disulfide bond location. For mucosal application the most prominent therapeutic candidate could be IgA dimer consisting of two monomers linked by J chain.

Here we focused on expression of monomers and dimers of IgA1 and IgA2m1 isotypes of a broadly neutralizing FI6 antibody to influenza A hemagglutinin. We used bi-promotor vectors carrying antibody heavy and light chain genes to obtain stable CHO cell lines producing IgA monomers. These lines were used for transfection with pcDNA 3.1 harbouring J chain coding sequence. The resulting cell lines producing IgA1 and IgA2m1 dimers were selected using G418 pressure and several rounds of fluorescent clone selection combined with limiting dilution method. Productivity of clones expressing IgA1 and IgA2m1 dimers

exceeded that of monomers by 28-38 % and 45-47 %, respectively. Cell lines producing IgA1 dimer demonstrated the highest productivity among all lines tested.

To further increase the production yield of cell lines expressing IgA1 dimers we studied the influence of orientation of heavy and light chain genes in a bi-promoter vector. Also, we introduced introns in a constant domain of IgA1 heavy chain. The combination of introns with uni-directional transcription of heavy and light chain genes resulted in 3-fold increase in antibody production yield.

This work was supported by the subsidy RFMEFI60714X0060 of the Ministry of Education and Science of Russian Federation.

* vicarg@rambler.ru

Not far from the tree: the peptide from hemagglutinin which interacts with the whole protein

Egorov V.*, Zabrodskaia Y., Taraskin A., Egorova M.

Research Institute of Influenza, Saint-Petersburg, Russia

Introduction

The problem of specific effects on the structure and activity of proteins is relevant in the development of new drugs problem. The advantage of using peptide-based drugs is the possibility of using the native mechanisms of protein-protein interactions specificity and metabolism [1]. We proposed an original algorithm for searching of peptide modulators of the structure and function of proteins based on the target proteins structure elements. Using this algorithm we found the peptide capable for specific interaction with influenza A hemagglutinin.

Results

By means of molecular dynamics simulations using GROMACS [2] we found a number of rigid regions in H1 hemagglutinin. Using the analysis of labile hydrogen bonds we proposed a primary structure of the potential inter-monomer interaction competitor. By SPR analysis, electronic microscopy, gel-electrophoresis and hemagglutination inhibition reaction we showed that the peptide found is capable to interact with hemagglutinin and change its functional properties.

It is planned to study the applicability of this peptide as a basis for the development of an antiviral drug.

Conflict of Interests statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

References:

[1] P. Vlieghe, V. Lisowski, J. Martinez, and M. Khrestchatisky, "Synthetic therapeutic peptides: science and market.," *Drug Discov. Today*, vol. 15, no. 1–2, pp. 40–56, Jan. 2010.

[2] B. Hess*, C. Kutzner, D. van der Spoel, and E. Lindahl, "GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation," 2008.

* sondyn@yandex.ru

Studying the role of a new, theoretically predicted, type A influenza viral protein

Plotnikova M.^{1*}, Klotchenko S.¹, Taraskin A.¹, Vasin A.^{1,2}

¹ *Research Institute of Influenza, Saint-Petersburg, Russia*

² *Peter the Great Saint-Petersburg Petersburg Polytechnic University,
Saint-Petersburg, Russia*

The influenza A virus (IAV) causes millions of acute respiratory infections globally every year. The genome of IAV contains 8 segments of single-stranded, negative-sense RNA. The genome encodes more than 16 viral proteins. In 1980, when an 8th RNA segment of the A/PR/8/34 (H1N1) influenza strain was sequenced from a non-coding negative-sense RNA strand, an open reading frame (ORF), potentially capable of encoding protein, was revealed. There is, however, no experimental evidence of such a protein (NEG8) to date.

Analysis has shown that this ORF has been conserved since 1948. In some strains, it is potentially capable of encoding a protein of unknown function. Cytotoxic T cells responses to NEG8 epitopes have been shown, which implies the existence of the protein. Two transmembrane domains are predicted in the NEG8 structure. One of these could play the role of a signal peptide, an N-glycosylation site, phosphorylation site(s), or a leucine motif. This discovery indicates that our understanding of the IAV genome is incomplete. The ORF is the first evidence that IAV may have a bidirectional genome.

The aim of this work was to generate the NEG8 protein and to study its possible roles. For cloning, a consensus sequence from the IAV/ New Caledonia/20/1999 (H1N1) strain was selected, and pAcGFP1-N3

vector (which carries GFP) was used. The construct was accumulated in E. coli, and A549 cells were transfected in order to determine, via GFP, the possible localization of NEG8. Cells were analyzed by confocal microscopy. In single cells, it was shown that the NEG8 protein is localized primarily in the cytoplasm and is condensed near the decaying nuclear envelope of dividing cells. Expression of the NEG8 was confirmed by immunofluorescence with antibodies to GFP. The data showed that the GFP signals, in cells transfected with construct, were located outside the nucleus, and indicated the creation of dense intracellular structures similar to vesicles.

This work was supported by RFBR grant 16-34-01110.

* marina.plotnikova@influenza.spb.ru

Antiviral activity of small interfering RNAs

Bondarenko A.^{1*}, Petrova-Brodskaya A.¹, Timin A.², Muslimov A.³,
Semenova A.⁴, Afanasiev M.⁵, Gorshkov A.¹, Vasin A.¹

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*RASA center in Tomsk, Tomsk Polytechnic University, Tomsk, Russia*

³*First I. P. Pavlov State Medical University, Saint-Petersburg, Russia*

⁴*Saint Petersburg State Chemical Pharmaceutical Academy,
Saint-Petersburg, Russia*

⁵*Saint Petersburg State University, Saint-Petersburg, Russia*

In the last several decades, gene therapy methods have been actively studied. Such study aims to develop fast, highly precise, and potent methods for the regulation of epigenetic processes in the cell's metabolism. Such precision, ideally, should allow gene therapy to be applied individually to the patient. One potential therapeutic strategy is gene silencing by RNA interference (RNAi). These are small interfering RNA duplexes (siRNA) which play the main role in RNAi process. Here, we show that two siRNAs homologous to the of PA and NP protein genes of pandemic influenza A virus H1N1 downregulate viral replication. We also analyzed various systems for siRNA delivery into cells.

In this study, siRNAs were selected from a large group for further study. These molecules, NP-717, NP-1004, NP-1155, NP-1341, NP-1496 and PA-1630, are homologous to the aforementioned NP and PA regions. Treatment effectiveness was studied in Madin-Darby canine kidney cells (MDCK) and in human alveolar adenocarcinoma cells (A549). In order to studying treatment effectiveness, cells were transfected with siRNAs. For the delivery of siRNAs, four carrier systems were used: commercial Lipofectamine reagent RNAiMax (Lip); chitosan (Ch); SiO₂

microcapsules (Mc); and polyethyleneimine (PEI) as a control. Two hours post transfection, the cells were infected with H1N1 A/PR/8/34 virus (M.O.I. 0.01) and incubated for 48 hours. Hemagglutination assay (HA) and enzyme-linked immunosorbent assay (ELISA) for quantitative determination of viral NP and PA were used to evaluate the siRNA's antiviral activity. The delivery efficiency of Lip, Ch, and Mc was studied using fluorescently labeled siRNA duplexes with PEI as a control.

It was shown that viral production was lower in both siRNA treated cells and in supernatants from treated cells than in cells which were not treated with siRNAs. The most effective delivery system was Mc. These results can potentially be applied to methods which aim to provide influenza prevention and treatment. Acknowledgement: This work is supported by the Russian Science Foundation under grant 15-15-00170.

* andrew.b.bondarenko@gmail.com

Influence of alloxan-induced diabetes mellitus on influenza infection induced by influenza virus A/H1N1pdm09

Aleksandrov A.*, Shtro A.

Research Institute of Influenza, Saint-Petersburg, Russia

Diabetes mellitus is a confounding factor for many diseases, including influenza. The aim of present study was to investigate the influence of alloxan-induced diabetes on the severity of pandemic influenza infection.

White outbred mice were used, divided into groups: A – intact mice, B - alloxan-treated without influenza, C – influenza-infected and D - alloxan-treated and influenza-infected.

The highest lethality was found in group D (influenza+diabetes) - 77% comparatively to the group C (only influenza) - 40%. Mean lifetime was 10+/-3 days and 12+/-2 days, consequently.

In our work we noticed the homeostasis change to increase of thrombocytes level, what can be a reason for the inflammation reaction and oxidative stress. Pathological excessive of concentration of granulocytes can amplify the inflammation process. But pathological decreasing of granulocytes is prolong disease. Our experiment shows decreasing absolute level granulocytes in group with alloxan-induced diabetes infected mice at acute phase of influenza infection, when the level of lethality is highest.

* forphchemistry@gmail.com

Deletion of the hypervariable domain of carrier protein flagellin changes the type of specific T-cell response to conserved sequences of M2 and HA2 proteins of influenza A

Shuklina M.*, Stepanova L.

Research Institute of Influenza, Saint Petersburg, Russia

Bacterial flagellin, which is a Toll-like 5 receptor ligand (TLR5), is a promising carrier for low immunogenic epitopes, such as the influenza virus M2e peptide, which provides an effective immune response. Recombinant fusion protein (Flg-HA2-2-4M2e) containing consensus sequence of HA2 and 4 copies of ectodomain of M2 protein (M2e) was constructed.

Flagellin contains three domains: two conserved and one variable domain located in the inner part of the molecule (154-431 amino acids). Based on protein structure it has been proposed that the variable domain deletion will not lead to changes in the properties of flagellin as an adjuvant. Instead of the variable region, another sequence, for example an epitope, can be included. Therefore, the recombinant protein FlgSh-HA2-2-4M2e was constructed in which the hypervariable flagellin domain was replaced by the HA2 protein fragment of influenza virus and 4 copies of the M2e were attached to the C-terminal.

Evaluation of specific T-cell response was performed using multiparametric analysis of intracellular staining TNF α and IFN γ in stimulated murine splenocytes. Splenocytes were stimulated with the synthetic M2e peptide and inactivated influenza viruses. The percentage of CD4 $^{+}$ and CD8 $^{+}$ T-cells producing IFN γ , TNF α and the double IFN $^{+}$ TNF $^{+}$ population was estimated.

Immunization with the Flg-HA2-2-4M2e protein caused a high level of specific TNF α production by CD4 $^{+}$ T-cells and a complete absence of IFN γ . At the same time, for the FlgSh-Ha2-2-4M2e, the opposite result was shown, a high level of IFN γ production in response to stimulation of the M2e peptide and a minor production of TNF α . Also only for FlgSh-Ha2-2-4M2e was the presence of double populations of IFN γ and TNF α producing simultaneously. Stimulation with the virus showed similar results.

* ma.shuklina@yandex.ru

Properties of influenza viruses B isolated in Russia during epidemic seasons 2014-2017

Zheltukhina A.^{1*}, Konovalova N.¹, Komissarov A.^{1,2},
Fadeev A.¹, Eropkin M.¹, Lobova T.¹

¹Research Institute of Influenza, Saint-Petersburg, Russia

²Saint-Petersburg State University, Saint-Petersburg, Russia

Influenza B viruses along with influenza A viruses cause seasonal influenza epidemics. They are represented by two lineages: Victoria and Yamagata that co-circulate in different proportions in the world. In the last three influenza seasons in Russia the role of influenza B viruses in epidemic period was different. They represented 55% and 51% of all circulating human influenza viruses in seasons 2014-2015 and 2016-2017 respectively, but counted only for 14.7% in 2015-2016, which was predominantly A(H1N1)pdm09 season.

MDCK cell line was much more permissive for the isolation of influenza B viruses compared with chicken embryos. The overall rate of viruses isolated in CE in the last three seasons did not exceed 20%. Most of the strains isolated in 2014-2015 were of Yamagata origin. Antigenic and genetic analyses of these strains showed that they were closely related with the reference strain B/Phuket/3073/2013 and belonged to genetic clade 3 and thus did not correspond to the vaccine strain recommended by WHO B/Massachusetts/02/2012. In the last two epidemic seasons the majority of isolated influenza B viruses belonged to Victoria lineage and were antigenically and genetically similar to the reference strain B/Brisbane/60/2008 and belonged to genetic clade 1A. However, the recommended vaccine strain for the season 2015-2016 was of Yamagata lineage and did not match with the circulating B/Victoria

viruses. In 2016-2017 the WHO recommended vaccine B component for the trivalent vaccines was B/Brisbane/60/2008 and it matched with influenza B viruses that circulated in Russia.

One of the strains of the last epidemic season: B/Astrakhan/2/2017 had distinct antigenic properties. The antiserum raised against egg-propagated B/Malaysia/2506/2004 reference virus recognized this strain at titres within 2-fold of the titre with the homologous virus. At the same time the antiserum raised against egg-propagated B/Brisbane/60/2008 reference virus recognized this virus also at titres within 2-fold of the titre with the homologous virus which makes this isolate unusual because the absolute majority of recent B Victoria viruses have lost the ability to be recognized by the antiserum raised against B/Malaysia/2506/2004 virus. The genetic analysis of this strain is currently ongoing.

* alena.zheltuhina@influenza.spb.ru

MicroRNA-mediated gene expression regulation during innate immune response in human cells

Stepanov G.^{1,2*}, Komissarov A.^{3,4}, Sergeeva M.³, Grudinin M.³,
Semenov D.¹, Richter V.¹

¹*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

²*Novosibirsk State University, Novosibirsk, Russia.*

³*Research Institute of Influenza, Saint-Petersburg, Russia*

⁴*Saint-Petersburg State University, Saint-Petersburg, Russia*

The aim of this study was to determine the possible role of microRNAs in the innate immune response of human cells. Whole-transcriptome analysis of gene expression in MCF-7 cells (human breast adenocarcinoma) transfected with immune-stimulating artificial analogues of box C/D RNA revealed transcriptional activation of the innate immunity genes and genes of the apoptotic cascade. Among upregulated genes were found genes encoding known miRNAs, such as miRNA-21, miRNA-1228 and miRNA-599. The similar increasing in miRNA-21 was found in other cultured cancer human cells. The list of targets of these miRNAs includes genes encoding transcription factors. Further analysis revealed that genes downregulated after box C/D RNA transfection were under control of these transcription factors. These results suggest that miRNAs activated by innate immune response impair expression of a vast group of genes by inhibiting the synthesis of transcription factors.

We analyzed upregulation of miRNA-21 in the course of viral infection. Lung adenocarcinoma cell line (A549) infected by influenza A/H3N2 virus 2-24 hpi was used as a model. It was found that after

8 hours post infection there was a significant increase in both miRNA-21 precursor and its mature form.

Thus, the data on the miRNA activation by innate immune response of human cells suggest that these regulatory RNA is involved in the control of cell survival during viral infection and play appreciable role in antiviral immunity.

* stepanovga@niboch.nsc.ru

Genetically stable cold-adapted influenza A vector with the insertion of *Mycobacterium tuberculosis* antigens ESAT-6 and Ag85A

Pulkina A.^{1,2*}, Sergeeva M.¹, Romanovskaya-Romanko E.¹,
Kuchur O.³, Stukova M.¹

¹ *Research Institute of Influenza, Saint-Petersburg, Russia*

² *Peter the Great Saint-Petersburg Polytechnic University,
Saint-Petersburg, Russia*

³ *Saint Petersburg University, Saint Petersburg, Russia*

Attenuated influenza viruses represent the advantageous platform for development of vectored vaccines against various respiratory pathogens including tuberculosis. In this study, recombinant influenza A virus A/PR8/HK-NS80-E85A, bearing ESAT-6 and Ag85A antigens of *M. tuberculosis* in the NS1 open reading frame, was generated by reverse genetics. A/PR8/HK-NS80-E85A virus contained surface antigens HA and NA from A/PR/8/34 (H1N1) virus and internal proteins from a cold-adapted donor strain A/Hong Kong/1/68/162/35 (H3N2). The NS1 protein of the recombinant virus was shortened to 80 amino acids, followed by ESAT-6 and Ag85A (199-261) fragment in a single reading frame. This construction resulted from a deletion in the modified NS gene, which originally contained full-length Ag85A and the 2A cleavage site after ESAT-6. The cause of the deletion was probably a disturbance of the conservative RNA secondary structure in 495-564 fragment of the NS gene segment, which may play a role in host adaptation of the influenza virus. The resulting virus variant was genetically stable and preserved the insert during serial passages in chicken embryos. Despite the modification of the NS gene and the insertion of heterologous antigens, A/PR8/HK-NS80-E85A virus inherited the cold-adapted and

temperature-sensitive phenotype of the master donor strain, on the basis of which it was constructed. When MDCK cell culture was infected by the recombinant virus A/PR8/HK-NS80-E85A, the expression of the NS1 protein, carrying heterologous insert of expected molecular weight was shown by Western blot analyses. Immunofluorescent staining and Western blot also demonstrated the expression of ESAT-6 antigen in the infected cells. Thus, the obtained recombinant virus possesses attenuation markers and carries M. tuberculosis antigens so representing a perspective vaccine candidate.

Research was supported by “UMNIK” program of FASIE (Russia) and the student’s grant from the Government of Saint-Petersburg.

* pureska@mail.ru

Trends in influenza A epidemic process in Russia from 2009 to 2017

Karpova L. *, Volik K., Stolyarov K., Popovtseva N.,
Stolyarova T., Sominina A.

Research Institute of Influenza, Saint-Petersburg, Russia

The data of the NIC of the Russian Federation on the weekly morbidity, hospitalization, and le-thal outcomes from influenza in 59 Russian cities were analyzed. The current morbidity was compared with weekly epidemic thresholds for cities, Federal Districts and the Russian Federation as a whole, and with the base lines (using the method of moving epidemics) (Tomas Vega, 2013), and the results of virological diagnosis.

Out of 8 epidemics of influenza in Russia from 2009 to 2017, in 4 cases the influenza A (H1N1)pdm09 virus prevailed in the etiology of diseases and 4 influenza A (H3N2) virus. The dominant strain of the epidemic was the dominant strain, which accounted for more than 50%. 2 epidemics of influenza A (H1N1) pdm09 were mono, and 2 more combined etiology, and all epidemics of A (H3N2) -combined etiology. Epidemics of influenza A (H1N1) pdm09 spread throughout Russia from west to east, except for the 2009-10 pandemic that ran from east to west. In the epidemic of influenza A (H3N2), the main pathogen also spread from west to east, and the accompanying strains - in the same or reverse direction. The morbidity and mortality rate among those infected with influenza and ARVI in the epidemic of influenza A (H1N1) pdm09 decreased, and in epidemic A (H3N2) - increased. The main risk groups were elderly people, among them morbidity and mortality increased

in the influenza epidemic of both subtypes. Among the deceased, the proportion of people older than 65 years increased in the epidemic of influenza A (H1N1) pdm09 (7.8 times) and in the epidemic A (H3N2), but less (2.5 times). Pandemic influenza remains the leading cause of death.

Risk factors for deaths in the epidemic of influenza A (H3N2) remained diseases of the cardiovascular system, and the role of diseases of internal organs, organs of breathing and immunodeficiency increased. In the epidemic of influenza A (H1N1) pdm09, the proportion of people with diseases of the cardiovascular system (7 times) and internal organs (4 times) increased among the deceased. Thus, the trend of decreasing the intensity of epidemics of influenza A (H1N1) pdm09 from high to medium (coefficient of the trend line, $k = -0.09$) and, conversely, increasing the intensity of epidemics A (H3N2) from low to the mean ($k = 0.22$).

* epidlab@influenza.spb.ru

Laboratory surveillance of influenza and other ARVI in St. Petersburg in 2016-2017 epidemic season

Musaeva T.*, Eder V., Pisareva M.

Research Institute of Influenza, Saint Petersburg, Russia

Multiple agents that cause acute respiratory viral infections are the reason of difficult clinical diagnosis. Laboratory surveillance of influenza and other respiratory viruses is crucial to determine which viruses actually circulate in the population, the timing of their activity, trends and impact on the epidemiological picture of the entire season.

The use of PCR diagnostics allows determining the etiology of pathogens in a real time mode and summarizing the obtained data into weekly reports. These give an opportunity to track the spread of influenza and respiratory viral infections and recognize the onset of an influenza epidemic at an early stage.

From the beginning of October 2016 to the middle of July 2017, 1598 specimens from children and 981 from adult patients with symptoms of acute respiratory viral infection were tested real-time RT-PCR.

According to the results of the study 480 (48.9%) out of 981 specimens from adult patients were positive for viral respiratory pathogens, of which 179 (37.3%) were influenza A virus, 101 (21.0%) - influenza type B virus, 94 (19.6%) – adenovirus, 6 (1.3%) - parainfluenza viruses, 16 (3.3%) - metapneumovirus, 1 (0.2%) - bocavirus, 17 (3, 5%) - RSV, 62 (12.9%) - rhinovirus, and 4 (0.8%) - coronavirus cases.

Laboratory surveillance data show that 1022 (64%) specimens from children were positive for influenza and non-influenza viruses: influenza A - 309 (30.2%), influenza B - 128 (12.5%), RSV - 263 (25.7%), rhinovirus - 137 (13.4%), metapneumovirus - 49 (4.8%), parainfluenza viruses (type 1 - 4) - 37 (3.6%), adenovirus - 48 (4.7%), bocavirus - 37 (3.6%), and coronavirus - 14 (1.4%) positive cases.

The evident dominance in circulation of influenza A viruses of subtype H3N2 (89.3%) and influenza B viruses of B/Victoria lineage (96.7%) was registered during 2016-2017 epidemic season in St. Petersburg. Among non-influenza respiratory viruses, prevalent circulation of RSV was marked in children; and rhinovirus and adenovirus - in adults.

Well established surveillance system with a strong laboratory component allows improving the knowledge of seasonal and cyclic variations in incidence of viral infections, prevalence of etiologic agents that is useful not only in clinical management of patients, but is essential in planning the outbreak control and preventive strategies.

* tamilamusaeva94@mail.ru

A study of circulating adenovirus diversity in the military

Amosova I.^{1*}, Timoshicheva T.¹, Egorova A.¹,
Musaeva T.¹, Lvov N.²

¹The Research Institute of Influenza, Saint-Petersburg, Russia

²The S.M. Kirova Military Medical Academy, Saint-Petersburg, Russia

The contribution of adenovirus (AV) infections to the overall structure of acute viral respiratory infections among young people of draft age can reach as high as 64.6%. Wide dissemination, the incidence of AV-associated pneumonias and lethal outcomes in some complicated infections illustrate the urgency of studying the antigenic diversity of AVs circulating among the military.

Objective: to study the genetic diversity of AVs that cause illness among military.

Materials and methods: 991 nasopharyngeal swabs from patients hospitalized in military health facilities with symptoms of acute respiratory infections from 2014 to 2017 were examined. Nucleic acids of non-influenza respiratory viruses were detected by real-time PCR using the “AmpliSens ARVI-screen-FL” kit. Sanger sequencing was performed using forward and reverse primers matching the fiber gene.

Results: AVs were detected in 326 samples. In 80 of those, AVs were present in combination with other respiratory viruses, as follows: 26 with respiratory syncytial viruses (RSV); 49 with rhinoviruses; 2 with bocaviruses; 1 with RSV and rhinovirus; 1 parainfluenza virus; and 1 with metapneumovirus. PCR detection of AVs was comprised of 6-30 cycles; PCR detection of heterologous viruses of AVs was comprised of 16-34

cycles. Thirty one samples wherein AVs were detected in cycles 6 to 10 (high titer) were sequenced. Thirty AVs belonged to group E (serotype 4), and 1 AV belonged to group B (serotype 7).

Conclusion: Serotype 4 and serotype 7 adenoviruses are a frequent cause of acute respiratory viral outbreaks in military. The development of AV-specific preventive measures could significantly reduce these indicators.

*amosova.23@mail.ru

Neutralizing epitopes in receptor binding site of hemagglutinin influenza B viruses Yamagata lineage

Sorokin E.^{1*}, Tsareva T.¹, Komissarov A.^{1,2}, Komissarova K.¹,
Egorova A.¹, Sominina A.¹

¹*Research Institute of Influenza, Saint-Petersburg, Russia*

²*Saint Petersburg University, Saint Petersburg, Russia*

Influenza is one of the most important infectious diseases in both industrial and developing countries. Since the mid-1970s, influenza B virus has formed two antigenically distinct lineages, “B/Victoria/2/87-like” and “B/Yamagata/16/88-like,” referred to as the Victoria and Yamagata lineages, respectively. Group-specific antigens have been conserved. We studied the neutralizing epitope sites specific for B/Yamagata group strains.

To identification of neutralizing epitopes of influenza B virus Yamagata lineage, panel of 8 monoclonal antibodies (Mab) to hemagglutinin molecule of B/Massachusetts/2/12 were used to select escape mutants (EM) of the virus. These MAbs were highly specific and reacted with viruses Yamagata lineage only without any reactivity with B/Victoria-like strains. The mutants are designated by the MAbs number (1G4, 1G9, 2B10, 3B12, 3C2, 4E11, 5B11, 5F11) and (in parentheses) the clone number.

Analysis of the predicted amino acid sequences of MAb-induced EMs of influenza B/Massachusetts/2/12 virus identified a single amino acid substitutions at positions G141=>E (loop-140) for EMs 1G4, 2B10 and 3C2; N202=>K (helix-190) for EMs 4E11. EMs 1G9/2 and 3B12/1 had a single amino acid substitution G237=>R and P240=>Q (loop-240).

Escape mutants 1G9/1 and 3B12/2 had a double mutations – G237=>R/ G141=>E and P240=>Q/N202=>K, respectively.

Additionally, number of MAb-induced EMs had only double mutations. EMs 5B11/1 and 5B11/3 had changes at loop-140 (R136=>I) and at loop-240 (P240=>S) while EMs 5B11/2 had two amino acid substitutions at loop-140 (R136=>I and G141=>E). EMs 5F11 had two amino acid changes at loop-140 (R136=>I) and helix-190 (D196=>G).

As is known, the receptor-binding site (RBS) is located at the top of the membrane-distal domain formed by 190-helix (HA1 194 to 203) at the top, 240-loop (HA1 237 to 242) as the left edge, and 140-loop (HA1 136 to 143) as the right edge. Our results indicate that all identified virus neutralizing epitopes are involved in the receptor-binding pocket of the influenza B virus hemagglutinin and affect the antigenic specificity of HA. Our data suggested that monoclonal antibodies are capable of recognizing epitopes in different antigenic regions of the RSB and neutralizing antibodies probably function by interfering with receptor binding.

* evgeniy.sorokin@influenza.spb.ru

Synthesis and antiviral activity of novel polycarboxylic fullerene derivatives.

Kraevaya O.^{1*}, Peregudov A.², Troyanov S.³, Kushch A.⁴,
Schols D.⁵, Troshin P.^{1,6}

¹ *Institute for Problems of Chemical Physics of Russian Academy of Sciences, , Chernogolovka, Moscow region, Russia*

² *A. N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences, Moscow, Russia*

³ *Moscow State University, Department of Chemistry, Moscow, Russia*

⁴ *Federal Research Center of Epidemiology and Microbiology named after N. F. Gamaleya, Moscow, Russia*

⁵ *Rega Institute for Medical Research, Leuven, Belgium*

⁶ *Skolkovo Institute of Science and Technology, Moscow, Russia*

Along with HIV, tuberculosis and healthcare associated infections, influenza is one of the most prevalent and dangerous viral diseases. World Health Organization reports that worldwide influenza epidemics results annually in about 3 to 5 million cases of severe illness and up to 500 000 deaths. Therefore, prevention and medication of influenza represents the key strategic issue that has to be addressed for sustainable development of the society.

Two main groups of anti-influenza drugs are represented by neuraminidase inhibitors and inhibitors of the viral M2 protein. However, viral strains showing resistance to both types of drugs have already emerged. Therefore, there is an urgent need for developing principally new anti-influenza drugs.

Water-soluble fullerene derivatives have a big potential for

biomedical applications owing to their efficient antitumor action, antiviral, antibacterial and neuroprotective activities. Our recent reports showed the potential of water-soluble fullerene derivatives to be applied for efficient suppression of influenza viruses, human immunodeficiency virus, herpes simplex virus and cytomegalovirus [Chem. Commun., 2012, 48, 5461; Chem. Commun., 2011, 47, 8298; Chem. Commun., 2012, 48, 7158; Org. Biomol. Chem., 2016, 15, 773].

Here we report the synthesis, characterization and investigation of antiviral properties of a big family of novel highly water-soluble fullerene derivatives C₆₀Ar₅X, which were synthesized from chlorofullerene C₆₀Cl₆ under Friedel-Crafts arylation conditions [following Org. Biomol. Chem., 2007, 5, 2783]. The obtained water-soluble fullerene derivatives comprised 5 to 10 carboxylic groups in their molecular frameworks.

The designed fullerene-based compounds showed low cytotoxicity and acute toxicity in mice. Some compounds exhibited a potent anti-influenza activity (EC₅₀ < 200 nM), outperforming commercial antiviral drugs such as Oseltamivir, Zanamivir, Ribavirin and Rimantadine tested under the same conditions.

* okraevaya@inbox.ru

Whole-genome analysis of Influenza A(H3N2) viruses circulating in Russian Federation in 2016-2017 epidemic season

Fadeev A.^{1*}, Komissarova K.¹, Egorova A.¹, Kotenko A.¹, Pisareva M.¹,
Eder V.¹, Musaeva T.¹, Komissarov A.^{1,2}, Somnina A.¹

¹Research Institute of Influenza, Saint-Petersburg, Russia

²Saint Petersburg University, Saint Petersburg, Russia

BACKGROUND: Whole-genome sequencing is a powerful tool evaluating the impact of circulating influenza viruses and determining possible factors that could influence the course of the current and future epidemics. Whole-genome characterization of influenza A(H3N2) viruses from 30 regions of Russia in 2016-2017 epidemic season was performed.

RESULTS: In 2016-2017 epidemic season according to real time RT-PCR results influenza A(H3N2) was responsible for 79.8%, and B (Victoria lineage) for 20 % of all influenza cases.

We performed the whole-genome sequencing on Illumina Miseq for 77 A(H3N2) samples from 30 regions of Russian Federation.

According to phylogenetic analysis of the hemagglutinin gene most of the sequenced viruses belonged to 3C.2a clade, bearing S144R/K and N121K substitutions. Some 3C.2a viruses also had N122D substitution leading to the loss of potential N-glycosylation site. 18 viruses clustered in 3C.2a1 genetic subgroup, bearing characteristic N171K substitution in HA1 and two substitutions in HA2 – I61V and G139E. Many of the amino acid substitutions in antigenic sites in influenza A(H3N2) virus occurred in antigenic site A.

All sequenced viruses possessed S245N substitution in neuraminidase associated with potential N-glycosylation site appearance. Both substitutions in NA are not associated with any reduction in susceptibility to NA inhibitors.

Whole-genome phylogenetic analysis showed that most of the sequenced viruses consistently clustered in two groups corresponding to 3C.2a and 3C.2a1 on HA tree, except for 5 viruses. Two types of 1+7 singleton intra-group reassortants were identified: three with HA gene belonging to 3C.2a clade and all other segments to 3C.2a1, and two with PA gene belonging to 3C.2a1-related cluster and all other segments to clade 3C.2a.

Full-genome analysis of 4 viruses from Northwestern part of Russia revealed a substitution in segment 2 resulting in 11 aa truncated PB1-F2 protein. These viruses belonged to subgroup 3C2a.1 according to phylogenetic analysis of HA gene, possessed I58V substitution in HA1 and clustered together on phylogenetic trees for all other segments of influenza genome.

CONCLUSION: The whole-genome analysis provides more detailed information on molecular evolution and variability of all segments of influenza virus genome.

* afadeew@gmail.com

Pre-clinical studies of live cold-adapted influenza pre-pandemic vaccines in mouse model

Rekstin A.^{1*}, Zaytceva M.², Doroshenko E.¹, Rudenko L.¹

¹ *Institute of Experimental Medicine, Saint Petersburg, Russia*

² *Institute of Toxicology, Saint Petersburg, Russia*

1. Background.

Recent emergence of a new H7N9 virus and threat of transmission of avian viruses to human elevated the need for research and development of live attenuated cold-adapted influenza vaccines against newly appeared influenza viruses. The following presentation describes the preclinical studies of new H1N1, H7N3, H5N2, H2N2 live attenuated influenza vaccine (LAIV) candidates in mice. The objectives of this study were: 1. Demonstration that cold-adapted (ca) reassortant influenza A vaccine candidates are indistinguishable from the parental A/Leningrad/134/17/57 (H2N2) Master Donor Strain (MDS) virus with regards to replication efficiency in upper and lower respiratory tract of mice. 2. Demonstration of safety of LAIV candidates in toxicology studies in rodents. 3. Demonstration of immunogenicity of LAIV candidates at different doses in mice. 4. Demonstration of protective efficacy of cold-adapted (ca) reassortant influenza A vaccine candidates in mice against a homologous wild-type virus challenge.

2. Materials and methods.

Virus Replication Kinetic and Neuroinvasion potential. Female CBA mice, 6-8 weeks of age were inoculated with 100 MID50 of A/17/California/2009/38 (H1N1), A/17/mallard/Netherlands/00/95

(H7N3), A/17/Vietnam/1203/04 (H5N1), A/17/turkey/Turkey/05/133 (H5N2), A/17/California/66/395 (H2N2), A/17/Tokyo/67/326 (H2N2) and A/Leningrad/134/17/57 (H2N2) MDS as well as wild-type parent viruses and the viral loads were measured in respiratory and brain tissues collected at 3 and 6 days post-infection (dpi). Mice were inoculated intranasally with 50µl volume. Tissue homogenates were prepared using a disruptor and clarified supernatants titrated on eggs at permissive temperature to determine infectious virus concentration.

Immunogenicity and Protection Studies. To assess the immunogenicity of LAIV candidates, groups of animals were inoculated with 1000 and 100 MID50 of each virus intranasally, after collecting a pre-immunization blood sample. A second blood sample was collected at 28 dpi and on the same day animals received a second intranasal inoculation of the same virus that was used for priming at 0 dpi. A third blood sample was collected on dpi 56. To assess protection, all animals were infected on dpi 42 with 100 MID50 of wild-type parent virus by intranasal route. Four animals from each group were euthanized on 45 dpi and respiratory and systemic organs harvested for virus titration. HAI antibody titers were determined for individual serum samples collected on days 0, 28, and 42. Sera were tested for reactivity against homologous influenza viruses.

Toxicology Studies. Evaluation of the acute toxicity of LAIV strains was performed after intraperitoneal administration of viruses in mice and intradermal administration in guinea pigs after single dose administration of preparation. The control animals received sterile saline. During the course of the study all animals were observed daily for clinical signs of toxicity, behavior, food and water uptake, weight change. After 7 days the animals (mice) were sacrificed and organs (brain, heart, liver, kidney and spleen) were collected for histopathology study.

3.Results.

It was shown that H1, H5, H7 and H2 LAIV were indistinguishable from parental H2N2 MDS in terms of replication in lungs and noses of mice. All tested strains showed no neuroinvasive capacity and failed to infect brains. Single dose administration of LAIV strains in volume maximal for injection did not cause death of animals, change of behavior or significant weight loss. The candidate vaccine preparations were well tolerated. The dynamics of weight change in vaccinated animals was similar to that of the control group, which received placebo.

LAIV candidates were immunogenic, elicited antibody response confirmed by HI and ELISA assays. Vaccinated mice were completely protected against homologous wild-type challenge strains.

4. Conclusions

Live attenuated cold-adapted (ca) influenza vaccines are an effective means for the control of influenza, most likely due to their ability to induce both humoral and cellular immune responses. In our study we confirm that new LAIV candidates are safe, immunogenic and confer protection in mice. Based on the results of pre-clinical studies the permits for Phase I clinical studies were obtained from the Russian Federation Ministry of Health.

This study was supported by PATH.

* arekstin@yandex.ru

Patterns of the circulation dynamics of the influenza viruses A(H3N2), A(H1N1) and type B. Cuba, 1983-1984 to 2004-2005

Oropesa S.^{1*}, González E.¹, Alonso I.³, Savón C.¹, Mirabal M.⁴, Mayo O.⁵, Armas L.², Pupo M.⁶, Valdes O.¹, Mune M.¹, González G.¹, Arencibia A.¹, Borroto S.², Martínez J.⁷, Hernández B.¹, Gonzalez G.¹, Roque R.¹

¹ *Centro Nacional de Influenza, Pedro Kourí Institute of Tropical Medicine, Havana, Cuba*

² *Departamento de Epidemiología, Pedro Kourí Institute of Tropical Medicine, Havana, Cuba*

³ *Ministerio de Salud Pública, Habana, Cuba*

⁴ *Instituto Carlos J. Finlay, Habana, Cuba*

⁵ *Instituto José Antonio Echevarría, Habana, Cuba*

⁶ *Universidad de Ciencias Informáticas, Habana, Cuba*

⁷ *Escuela Latinoamericana de Medicina, Habana, Cuba*

Introduction. Since the 1983-1984 season, in Cuba, systematically co-circulate the influenza virus A(H3N2), A(H1N1) and type B. Its continuous genomic and antigenic changes produce variations in its prevalence. The aim of this study was to define patterns in the circulation dynamic of these virus, revealed through its interseasonal variations, from 1983-1984 to 2004-2005.

Materials and Methods. Three series were constructed with the percentage prevalence by season of the influenza virus A(H3N2), A(H1N1) and type B, related with the total of 7252 positive paired serums (4128 of H3N2, 1602 H1N1 and 1522 Type B), by hemagglutination inhibition assay. The difference between the percentages of each season against the precedent were found and studied using descriptive methods. They were also categorized by its quartiles in low, moderate, high and very high. SPSS 20 and Excel 2007 software were employed.

Results. There were similarities found in the interseasonal variations among the means of the A(H3N2) and A(H1N1) virus, with values of 13,2 and 12,1% respectively and more lower in type B (8,2%). The standard deviations were similar:10,4% for the A(H3N2), 9,4% the A(H1N1)and 7,0% for type B, which showed a very high variability respect to its means (85,7%); lower(near 77%) in A(H3N2) and A(H1N1). The ranges of these series were ample: A(H3N2) from 0,1–38,0%, A(H1N1) between 0,5-33,4% and of type B from 0,4 to 27,7%. The circulation by categories of each type/subtype conformed five periods: one of five seasons (1983-1984 to 1988-1989), another of three (1990-1991 to 1993-1994)and three of four seasons, that alternated with the previous.Table 1.

Conclusions. The categorization applied to the percentage prevalence of the influenza virus and the calculation of its differences provides a new and useful approach to the pattern sin its circulation dynamic from1983-1984 to 2004-2005, which constitute a contribution to the knowledge of the disease in Cuba and in other tropical regions.

Conflicts of interest: Not declared

* s.oro@ipk.sld.cu

Morbidity attributed to the influenza virus A(H3N2), A(HN1) and type B. Cuba, 1983-1984 to 2004-2005

Oropesa S.^{1*}, González E.¹, Alonso I.³, Savón C.¹, Mirabal M.⁴, Mayo O.⁵, Armas L.², Pupo M.⁶, Valdes O.¹, Mune M.¹, González G.¹, Arencibia A.¹, Borroto S.², Martínez J.⁷, Hernández B.¹, Roque R.¹

¹ *Centro Nacional de Influenza, Pedro Kourí Institute of Tropical Medicine, Havana, Cuba*

² *Departamento de Epidemiología, Pedro Kourí Institute of Tropical Medicine, Havana, Cuba*

³ *Ministerio de Salud Pública, Habana, Cuba*

⁴ *Instituto Carlos J. Finlay, Habana, Cuba*

⁵ *Instituto José Antonio Echevarría, Habana, Cuba*

⁶ *Universidad de Ciencias Informáticas, Habana, Cuba*

⁷ *Escuela Latinoamericana de Medicina, Habana, Cuba*

Introduction. Cocirculation of Influenza virus type subtypes is a factor of great impact in the morbidity by acute respiratory infections. Our purpose was to estimate the morbidity attributed to the virus A(H3N2), A(H1N1) and type B in Cuba from 1983-84 to 2004-2005 with the application of a statistical model.

Materials and methods. Four series were constructed, one with the morbidity rates by ARI, from which an ascending trend was eliminated, associated with the health services, subtracting its regression straight line ($R^2=0,98$), and the other three percentage series with the 4 128 paired serum to A(H3N2), 1602 to A(H1N1) and 1522 to type B, from 7252 positives samples using hemagglutination inhibition. A Poisson regression model was applied taking the morbidity by ARI as the dependent variable and the viral percentages as predicting covariables,

represented with the central values of the first and second quartiles, the third and the fourth. The resulting equation was transferred to the original series of the morbidity by ARI.

Results. The model reach a significant fit ($p < 0.001$) and its coefficients attributed to influenza 5.1 millions, 36.7% of the 95.5 millions reported in the 22 seasons. From them, 23.4 millions (67.0%) corresponded to A(H3N2), 5,9 millions (17%) to A(H1N1) and 5,6 millions (16%) to type B, with respect to the total of ill persons attributed to influenza. The season means were 1066.8, 270.1 and 255.3 thousands associated to A(H3N2), A(H1N1) and B and the standard deviation in the same order were 283.3, 158.1 and 120.3 thousands.

Conclusions. It was demonstrated the impact of influenza in the morbidity ARI, with the application for the first time in Cuba of an statistical model, and the predominance of subtype A(H3N2), with an important participation of A(H1N1) and of type B. The knowledge provided is of value for the understanding and control of influenza in Cuba. Conflicts of interest: Not declared

* s.oro@ipk.sld.cu

Cloning, expression and purification of antigens of *Mycobacterium tuberculosis*

Rusakovich G.^{1, 2*}, Grudin M.²

¹ Saint-Petersburg State University, Saint-Petersburg, Russia

² Research Institute of Influenza, Saint-Petersburg, Russia

Mycobacterium tuberculosis is one of the most threatening of human infection diseases. World Health Organization (WHO) statistics indicate that one-third of the world's population is currently infected. Tuberculosis is rated the second most common infectious disease and has the highest mortality rate of any infectious disease in the world. In addition, people may already be infected with multidrug-resistant strains of *Mycobacterium tuberculosis*.

Development of new generation vaccines may control thereby improve disease the patient care. In particular, vaccination against tuberculosis or early detection and treatment can limit the number of people with large risk. Nevertheless the most promising tuberculosis antigens are necessary to be investigated.

Laboratory of gene engineering and expression recombinant proteins in Influenza Research Institute works with antigens of *Mycobacterium tuberculosis* Ag85a, TB10.4, ESAT-6, Hsp X (Acr). Two of them (Ag85a and HspX) were already obtained in *E.coli*. The antigens chosen for the study have a high immunogenicity and their epitopes are recognized by the immune system. Thus, the aim of this study is to clone, express and purificate the *Mycobacterium tuberculosis* antigens for further research on vaccine development.

* georgerusakovich@gmail.com

Adjuvants for development of influenza vaccines production

Krasilnikov I. V.*

*St.Petersburg Institute of Vaccines and Sera FMBA,
Saint-Petersburg, Russia*

Influenza viruses are adept in human populations. Indeed, they have the capacity to evade the immune system through mechanisms of mutations (antigenic drift) and major variations in surface protein expression (antigenic shift). When a major change occurs, the risk of a human pandemic arises. Effective vaccination strategies are required to combat future influenza pandemics.

A major challenge for current vaccine development is the fact that many new subunit vaccines based on highly purified naïve and recombinant proteins are poorly immunogenic and mobilize insufficient immune responses for protective immunity. Adjuvants are therefore needed in vaccine formulations to enhance, direct and maintain the immune response to vaccine antigens. Few adjuvants are currently approved for human use that mainly induce humoral immunity, and there is therefore an unmet medical need for development of effective and safe adjuvants that in addition can stimulate cellular or mucosal immunity, or combinations thereof, depending on the requirements for protection against the specific disease.

Adjuvants have been considered for a long time to be an accessory and empirical component of vaccine formulations. However, accumulating evidence of their crucial role in initiating and directing the immune response has increased our awareness of the importance

of adjuvant research in the past decade. Nevertheless, the importance of adjuvants still is not fully realized by many researchers working in the vaccine field, who are involved mostly in the search for better target antigens. The choice of a proper adjuvant can be determinant for obtaining the best results for a given vaccine candidate, but it is restricted due to properties of adjuvant compounds and questions of intellectual property. Consequently, in most cases the selected adjuvant continues to be the aluminum salt, which has a record of safety, but predominantly constitutes a delivery system. Ideally, new strategies should combine immune potentiators and delivery system by mixing both compounds or by obtaining structures that contain both properties. Vaccine delivery systems are important components of adjuvants that allow proper delivery of antigens to antigen-presenting cells. Moreover, they often possess intrinsic immunopotentiating activity and/or can be customized towards a given immunological profile by the appropriate combination with immunopotentiating compounds. Corpuscular adjuvants based on natural terpenoid compounds correspond to listed requirements.

The results of experiments with samples of inactivated influenza vaccines containing corpuscular adjuvants, immunogenicity and protective activity in the model mice were presented.

* kiv06@mail.ru

Author Index

A

Abitay R. 61
Acosta B. 101
Afanasyeva O. 102
Afanasyev M. 123
Aleksandrov A. 125
Alekseev A. 44
Alekseev A.Y. 48
Aliev A. 46
Aliev T. 85, 117
Alikhanova O. 93
Alikina T. 44
Alkhovsky S. 97
Almond J. 28
Alonso I. 149, 151
Amosova I. 138
Anoshina A. 40
Antikainen P. 71
Arencibia A. 101, 149, 151
Argentova V. 85, 117
Armas L. 149, 151

B

Balzarini J. 114
Baranovskaya I. 83
Beard P. 76
Blokhina E. 52
Bobkov D. 81
Bogdanov N. 61
Bolshchikov B. 87
Bondarenko A. 81, 123

Boravleva E. 58
Borisevich S. 116
Borroto-Gutiérrez S. 38
Borroto S. 149, 151
Bovin N. 25
Burtseva E. 34, 97
Buzitskaya Zh. 56, 61, 106

C

Chernyak A. 112
Chursov A. 79

D

Danilchenko N. 42, 66
Danilenko D. 31, 36
Dement'yeva I. 85
Desheva Y. 63
Digard P. 76
Dolgikh D. 85, 117
Doroshenko E. 146
Durymanov A. 42

E

Eder V. 136, 144
Egorov A. 23, 56
Egorova A. 46, 83, 138, 140, 144
Egorova M. 81, 119
Egorov V. 81, 119
Egorov Yu. 93
Engelhardt O. 76
Eropkin M. 36, 46, 128

F

Fadeev A. 42, 46, 128, 144

Fedorova V. 116
Fedyakina I. 97
Fesenko E.E. 69
Fridlyand N. 79

G

Gambaryan A. 58
Gavashelidze M. 99
Golovacheva E. 102
Goncharova N. 42
González E. 149, 151
Gonzalez G. 149
González G. 149, 151
Gorshkov A. 81, 123
Grabovskaya K. 63
Gribkova N. 104, 110
Grischenko V. 108
Grudin M. 130, 153
Gudymo A. 42, 66
Gudymo A. S. 42
Gulyaeva M. 48

H

Hernández B. 101, 149, 151
Heydarov R.N. 69
Hussain S. 76

I

Ilyicheva T. 42
Isagulov T. 61
Ishmatov A. 50

K

Kabilov M. 44

Kaplun A. 58
Karaseva E. 93
Karpova E. 66
Karpova L. 134
Karseladze I. 99
Kassenov M. 61
Khairullin B. 56, 61, 106
Kirillova E. 97
Kirpichnikov M. 85, 117
Klotchenko S. 67, 71, 85, 121
Kolobukhina L. 97
Kolossova N. 42
Komissarov A. 31, 42, 128, 130, 140, 144
Komissarova K. 46, 140, 144
Konovalova N. 36, 46, 128
Korotkov A. 54
Koskinen J. 26, 71
Kotenko A. 144
Kotlyarov R. 52
Kovacikova K. 76
Kovaleva A. 54
Koval'skaya A. 116
Kraevaya O. 142
Kramskaya T. 63
Krasilnikov I.V. 58, 154
Krasnoslobodtsev K. 97
Kuchur O. 132
Kuprianov V. 54
Kurskaya O. 40, 44
Kushch A. 112, 142

L

Lapo T. 104

Lebedev D. 81
Lebedev K. 67
Leonova N. 40
Leonov S. 44
Leontieva G. 63
Lepik K. 89
Liu W. 78
Lo A. 76
Lobova T. 128
Lundgren M. 65
Lvov D. 97
Lvov N. 49, 138

M

Machablshvili A. 99
Makbuz A. 61
Maksyutov R. 42, 66
Maltsev S. 42, 66
Maltsev S. V. 42
Malyshev N. 91
Marchenko V. 42
Mardanova E. 52
Martínez J. 101, 149, 151
Martynenko V. 112
Matusevich O. 81
Mayo O. 149, 151
Merkulova L. 97
Mikhailovich V. M. 69
Mikheev V. 42
Mirabal M. 149, 151
Mune M. 101, 149, 151
Murashkina T. 44
Musaeva T. 136, 138, 144
Muslimov A. 89, 123

N

Nekrasov A. 59
Nekrasov M. 66
Nurpeisova A. 61

O

Oropesa S. 101, 149, 151
Ots A. 66

P

Peregudov A. 112, 114, 142
Petrova-Brodskaia A. 81, 83, 89, 123
Petrova P. 46, 116
Petrov S. 108
Piankova O. 42, 66
Pisareva M. 56, 136, 144
Plotnikova M. 67, 121
Popov A. 91
Popovtseva N. 134
Porjo N. 26
Potapchuk M. 54
Pozdnyakova L. 85
Pruglo V. 46
Puchkova N. 59
Pulkina A. 132
Pupo M. 149, 151

R

Ravin N. 52, 54
Rekstin A. 63, 146
Richter V. 130
Romanovskaya-Romanko E. 106,

132

Roque R. 101, 149, 151

Rudenko L. 63, 146

Rusakovich G. 153

Ryabichenko T. 40

Rybchenko V. 117

Ryzhikov A. 42, 66

S

Sagymbay A. 61

Sansyzbay A. 56, 61, 106

Sarsenbaeva G. 61

Savón C. 149, 151

Schols D. 112, 114, 142

Semenova A. 123

Semenov D. 130

Serbin A. 87, 93

Sergeeva M. 85, 108, 130, 132

Shaldzhyan A. 81

Sharshov K. 21, 44, 48

Shaskolskiy B. L. 69

Shestopalov A. 21, 44, 48

Shestopalova L. 48

Shmialiova N. 104, 110

Shneider A. 79

Shtro A. 85, 125

Shuklina M. 54, 126

Shurygina A.-P. 56, 61, 81, 106

Sivets N. 104, 110

Sobolev I. 40, 44, 48

Sominina A. 31, 134, 140, 144

Sorokin E. 140

Soukka J. 26

Stepanova L. 52, 54, 126

Stepanov G. 130

Stolyarova T. 134

Stolyarov K. 134

Stukova M. 56, 61, 106, 132

Sukharukov G. 89

Suomala P. 71

Susloparov I. M. 42

Suvorov A. 63

Sveshnikov P. 85

Svyatchenko S. 42

Sychev I. 63

T

Taraskin A. 67, 85, 119, 121

Timin A. 89, 123

Timoshicheva T. 138

Toporova V. 85, 117

Tregubchack T. 66

Troshin P. 112, 114, 142

Troyanov S. 142

Trushakova S. 34

Tsareva T. 140

Tsvetkov V. 87

Tsybalova L. 52, 54, 108

Tsypiyheva I. 116

Turnbull M. 76

V

Valdes O. 101, 149, 151

Valdés V. 38

Vasilieva A. 46

Vasilyev K. 106

Vasin A. 31, 67, 71, 83, 85, 89,
121, 123

Vervelde L. 76
Vinogradova T. 56, 106
Volgin N. 61
Volik K. 134
Vorobyov E. 83
Voronov I. 112

W

Wise H. 76

Y

Yatsyshina S. 74
Yurlov A. 21

Z

Zabolotnykh N. 56, 106
Zabrodsкая Y. 81, 119
Zakhashvili Kh. 99
Zarubaev V. 116
Zaytceva M. 146
Zheltukhina A. 46, 128
Zhilenkov A. 114