ISAR News
Newsletter of the International Society for Antiviral Research

Editor Anthony Vere Hodge Guest editor Graciela Andrei
©International Society for Antiviral Research

Invitation to the 32nd ICAR, Baltimore, Maryland, USA

IN THIS ISSUE

ISAR PRESIDENT'S MESSAGE

Johan Neyts

This issue of ISAR News has been kindly put together by guest editor Graciela Andrei. I am sure that you will appreciate her efforts to put the spotlight on a number of most interesting articles.

In this issue, you will also find a short bio-sketch of the candidates that run either for positions in the ISAR board of directors or for secretary. I am most grateful to all candidates. It is of utmost importance for the success and future of ISAR/ICAR that dedicated and competent people are willing to take up the leadership of the society. Please kindly bring out your vote!

In this issue, you will also find text by Mike Bray in which he presents the speakers that have already accepted to talk at ICAR 2019. This line-up of excellent speakers that will cover a range of interesting topics should attract a large crowd to the meeting. Please help us to spread the information to all those that you believe may be interested in attending ICAR. You will find the biographies of the speakers also on our website www.isar-icar.com.

I would also like to kindly remind everyone to

subscribe (via the website) to the ISAR mailing. Please also follow our LinkedIn and Facebook page as well as the ISAR Twitter account (all accessible via the website).

Enjoy reading this issue of ISAR News,

Kind regards,
Johan Neyts

ISAR ELECTIONS

This year the Nominations Committee was charged with finding candidates to fill the office of secretary, currently held by Graciela Andrei, and three board seats, presently occupied by Andrea Brancale, Mike Bray and Kathie Seley-Radtke. Their terms expire at the end of the 2019 ICAR.

The ISAR membership will be voting for one Secretary and three Board of Directors (BOD) members. There are two candidates for Secretary (Jinhong Chang and Zlatko Janeba) and five candidates for Board of Directors members (Maaike Everts, Chris Meier, Luis Schang, Jessica Spengler and Subhash Vasudevan). ISAR members should vote for one Secretary and three BOD members. Please review the candidates' biographical sketches.

The election is being held electronically and will open soon. An e-mail will be sent to all ISAR members explaining how to vote. We strongly encourage all members of the society to vote, and wish the very best to this year's candidates.

Biographies of the candidates

Secretary

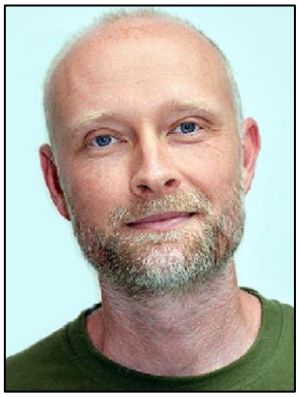
Dr Jinhong Chang, MD, Ph.D., is a Professor and Principal Investigator, Laboratory of Molecular Virology and Antiviral Research, at Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, Pennsylvania, USA.

Dr. Chang received her medical education and clinical training in Infectious Diseases as well as Ph.D. training in Virology at Peking University Health Science Center, Beijing, China. She received her postdoc training in Molecular Virology at Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.

Dr. Chang has more than 20 years of research experience in the areas of molecular virology, innate immunity and antiviral drug discovery, and has more than 90 publications in peer-reviewed journals and 6 patents. She established her independent translational research group at Drexel University, Philadelphia, Pennsylvania, in 2007 and joined Baruch S. Blumberg Institute in 2015. Her group has been focused on the development of antiviral and innate immune modulating agents for treatment of viral infections that cause hepatitis and hemorrhagic fever.

Dr. Chang has been a member of the Editorial Board of Antiviral Research since 2015, and will serve as an Editor of Antiviral Research starting November 2018.

Jinhong has been a member of ISAR since 2008. She has participated in reviewing ICAR abstracts for the past 5 years, and has been a member of the ICAR poster award committee since 2015. Dr. Chang served as a mentor for the Woman in Science program in 2016 and 2017.



Dr. Zlatko Janeba earned his Ph.D. in organic chemistry from the Institute of Organic Chemistry and Biochemistry

(IOCB) in Prague. He underwent postdoctoral training in the groups of Prof. Morris J. Robins (Brigham Young University) and Prof. Paul F. Torrance (Northern Arizona University). He spent three years at Moravek Biochemicals,

Inc. in California, and in 2008, he rejoined the research team of Prof. Antonín Holý at the IOCB. He established his Junior Research Group in 2010, and since January 2016, he has been the head of the Senior Research Group at the IOCB. Current research of his group involves design and synthesis of modified nucleosides, nucleotides, and other heterocyclic compounds, with a wide range of biological properties.

He is a member of International Society for Antiviral Research, International Society of Nucleosides, Nucleotides and Nucleic Acids, and International Society of Heterocyclic Chemistry (ISHC). Currently, Dr. Janeba is an associate editor of Antiviral Chemistry & Chemotherapy and serves as vice-chairman of the IOCB supervisory board and of the supervisory board of IOCB technology transfer office (TTO).

Board members

Dr Maaike Everts is an associate professor in the Division of Infectious Diseases of the Department of Pediatrics, University of Alabama School of Medicine at Birmingham. Maaike (pronounced "Micah") was born in Meppel, the Netherlands.

After receiving a master's degree in pharmaceutical sciences and a Ph.D. in pharmacokinetics and drug delivery from the University of Groningen, she moved to UAB (University of Alabama at Birmingham) for postdoctoral training with David Curiel in the Division of Human Gene Therapy, where she pursued her interest in targeted gene delivery for the treatment of cancer, using adenoviral vectors. She joined the UAB Department of Pathology in August 2005, continuing her research on targeted therapies using gene therapy and nanotechnology approaches.

Since 2009, Maaike has been the associate director of the Alabama Drug Discovery Alliance, a collaboration between UAB and Southern Research, with the goal of finding new small-molecule drugs for unmet medical needs in a variety of therapeutic areas. She also assists physician-investigators with the IND application process, and provides quality assurance for the UAB Vector Production Facility, which manufactures novel drugs for Phase I clinical trials. She is also the administrative director for the Antiviral Drug Discovery and Development Center, a multi-institutional consortium funded by a U19 grant from NIAID. Maaike joined ISAR in 2015. In 2016, she was invited to join the Women in Science committee and to be responsible for organizing the career development panel.

Prof. Chris Meier, born 1962 in Berlin, Germany, received a diploma and a doctorate (Ph.D.) in Chemistry from the University of Marburg, Germany. During his Ph.D. thesis, he worked in the group of Prof. Gernot Boche on the synthesis of so-called ultimate carcinogens formed from aromatic amines by metabolic steps, metabolites that are involved in the induction of carcinogenesis. He joined the Organic Chemistry Division at the Pasteur-Institute in Paris, France, headed by Prof. Jean Igolen and Prof. Tam Huynh-Dinh as a Post-Doc and started working on nucleoside chemistry and prodrugs. He returned to Germany joining the University of Frankfurt/Main in 1991 as an Assistant Professor under the mentorship of Prof. Joachim Engels. In 1996, he obtained the Habilitation in Organic Chemistry from the University of Frankfurt/Main, Germany. He was appointed as Associate Professor at the University of Würzburg, Germany and then in 1999 he joined University of Hamburg, Germany as a full professor. He is the Scientific Director of the Centre for Structural Systems Biology (CSSB) in Hamburg.

Moreover, he is the current President of the International Society on Nucleoside, Nucleotides and Nucleic Acids (IS3NA), after being Vice-President the previous two years. He received the Prusoff-Award in 2007 and the Antonín Holý-Award 2018 from the International Society on Antiviral Research (ISAR).

He was involved with ISAR since many years ago, serving as chair of the program committee "medicinal chemistry" and being a long-standing member of the poster award committee. Recently, he was awarded as being a Zhiquiang-guest professor from Shanghai University, China, and he has worked as an invited guest professor and visiting professor at the University of Montpellier II and Toulouse, France, and Shanghai, China. His research focuses are pronucleotide development, nucleoside chemistry, structure-based drug design of small molecule antivirals against *Bunya viridae* and hemorrhagic fever viruses, carbohydrate chemistry, phosphorylation methods in nucleoside chemistry and the synthesis of photocaged compounds, e.g. second messengers. He has published more than 235 scientific publications and is an inventor of record in 10 issued patents.

Dr Luis Schang is the Director of the Baker Institute of Cornell University College of Veterinary Medicine. He got his Médico Veterinario (Veterinary doctor) degree from the University of Buenos Aires, Argentina in 1987. He then received a Ph.D. on molecular virology from the University of Nebraska Lincoln before doing a postdoctoral training with the late Dr. Schaffer at the School of Medicine of the University of Pennsylvania. He moved to take an assistant professor position at the Faculty of Medicine and Dentistry of the University of Alberta in 2000, where he climbed the ranks to the full professorship and co-led international programs with the Helmholtz Association (Germany) and Zhejiang University before moving to Cornell University in 2016.

Luis attended his first ICAR in 2000 (Baltimore) and has been a member of the publications committee of our society since 2007, of the Poster awards committee since 2014, and of the Ambassador program (South America and Canada) since 2014. He became a co-chair of the publications committee, together with Anthony Vere Hodge, earlier this year and has regularly guest edited ISAR News issues.

Luis is a molecular virologist interested in using chemical biology to learn about the interactions between viruses and infected cells, interactions that determine the outcomes of all infections. He is particularly interested in identifying commonalities among unrelated viruses. Luis' group works with a number of established human pathogens, including herpes simplex virus 1 and 2 (HSV-1; -2), hepatitis C virus (HCV), and influenza A virus (IAV), and emerging viruses such as Zika virus. With the research team, Luis identifies or designs small molecules that inhibit the infectivity or replication of a variety of unrelated viruses and then uses these compounds as probes to identify commonalities among the affected viruses. This research answers fundamental questions on molecular virology while identifying biologically active small molecule scaffolds that may be developed as antivirals. Using this approach, his group has identified new families of compounds with antiviral activity against multiple unrelated viruses (broad-spectrum antiviral activity), as well as some compounds that act exclusively against single viruses. He is interested the most in compounds with novel targets and mechanisms of action, such as the first lipid targeting antiviral molecules, the RAFIs. His work has resulted in 67 publications and patents issued in the EEUU, the European Union, Canada, China and Japan.

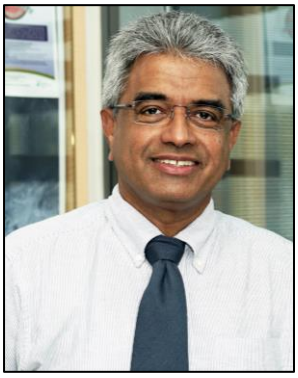
Luis is a member of the editorial board of several journals in the antiviral area, including the official publication of our society, Antiviral Research, as well as Antimicrobial Chemistry and Chemotherapy (ACC) and the Journal of Virology, the official journals of the American Society for Microbiology (ASM). He is also a section editor of PLOS One, and an editor of Virology Journal, and contributes reviewing manuscripts for a number of publications, including PLOS Pathogens, mBio, and eLife among many others. He reviews grants for the NIH, the Canadian Institute of Health Research, and several Argentinean, UK, Polish, Belgian, and Hong Kong agencies, among others.

Dr. Jessica Spengler, D.V.M., Ph.D., M.P.H., received her M.P.H. in infectious diseases in 2004 from the University of California, Berkeley, and then completed a California Epidemiologic Investigation Service (Cal-EIS) fellowship with the Vector-Borne Disease Section of the California Department of Health from 2004–2005. She received her Ph.D. (2011) and D.V.M. (2012) from the University of California, Davis.

Her graduate research on innate immune evasion by hantaviruses was performed on-site with the Special Pathogens Program at the Public Health Agency of Canada in Winnipeg, Manitoba, and at the NIH Laboratory of Virology, Rocky Mountain Laboratories (Hamilton, Montana). Since 2012,

Dr. Spengler has worked with the Viral Special Pathogens Branch at the Centers for Disease Control and Prevention in Atlanta, Georgia. Currently, she directs a translational research program utilizing biosafety level 4 laboratory facilities to identify, prevent, ameliorate, and control high-hazard zoonotic viral pathogens, including Ebola, Marburg, Nipah, Crimean-Congo hemorrhagic fever, and Rift Valley

fever viruses. This program investigates molecular mechanisms of pathogenesis, develops animal models of disease, and conducts in vivo screens of therapeutic and vaccine candidates for high-containment, high-consequence viral hemorrhagic fevers.



Prof. Subhash Vasudevan and Principal Investigator in the Program in Emerging Infectious Diseases, DUKE-National University of Singapore Medical School.

Subhash Vasudevan was born in Singapore (1959). He obtained his BSC Hons in Chemistry (La Trobe University, Australia; 1985) and Ph.D. in Biochemistry from The Australian National University (ANU) in 1989 followed by post-doctoral training at the Max-Planck Institute for Biophysics in Germany and Research School of Chemistry at ANU. He established his first independent research laboratory at James Cook University (Australia) when he was appointed as a lecturer in Biochemistry and Molecular Biology (1993) and rose through the ranks to become a Reader (~Assoc. Professor). It was during this period that he started working on dengue virus focusing mainly on the NS3 and NS5 proteins. In 2003, he moved to Singapore as the inaugural Unit Head of the Dengue Research Unit at the newly established Novartis Institute for Tropical Diseases and led an intensive effort to find directly acting antivirals against dengue virus serotypes. In 2008, he was recruited to the Signature Research Program in Emerging Infectious Diseases at the Duke-National University of Singapore Medical School where he currently pursues his research interests in dengue and Zika pathogenesis and antiviral drug discovery and development. He was appointed as an Editor of Antiviral Research (Elsevier) in 2009 and is a member of the Editorial Board of the Journal of Virology (2017-2021).

Recently, Subhash has started to become involved with ISAR to try to increase the membership from Asia and also hopes to attract and help organize together with Griffith University'sInstitute for Glycomics, the ICAR in the Gold Coast in Australia in 2021.

32nd ICAR, BALTIMORE, MARYLAND, USA

The International Society for Antiviral Research (ISAR) will host the 32nd International Conference on Antiviral Research (ICAR) at the Hyatt Regency, Baltimore Inner Harbor, USA. The conference will begin on Sunday, May 12, 2019, and will conclude on Wednesday, May 15, 2019. The abstract submission is now open.

Key Dates for 2019 ICAR

ISAR Awards Submission Deadline
December 17, 2018

TCFF Scholarship Application Deadline
December 17, 2018

Registration & Hotel Reservation Sites Open
January 7, 2019

Abstract Submission Deadline (Advertised)
January 11, 2019

Travel Awards Application Deadline
January 11, 2019

Abstract Acceptance Notifications Sent
February 15, 2019

Travel Awards Notifications Sent
February 18, 2019

Second Submission Site (Poster Only) Opens
February 18, 2019

Second Submission Site (Poster Only) Deadline
March 8, 2019

Second Submission Notifications Sent
March 14, 2019

Advance Rate Registration Deadline
April 5, 2019

Registration Cancellation Deadline
April 5, 2019

Hotel Reservations Deadline
April 20, 2019

32nd ICAR in Baltimore: program of invited speakers (Mike Bray, Kara Carter, Justin Julander, Johan Neyts)

In addition to the four ISAR award lectures, we'll have an excellent list of invited speakers for the next meeting, with interesting presentations on a wide range of topics. The following individuals have confirmed their participation.

Diane Griffin

On Sunday afternoon, one of the two keynote lectures will be given by Diane Griffin, Professor of Infectious Diseases and Neurology at the Johns Hopkins Bloomberg School of Public Health and vice-president of the US National Academy of Sciences. She will present her research findings on the pathogenesis of measles and persistent infection of the central nervous system. A second keynote speaker has been invited, but not yet confirmed.

Fred Hayden

Jeff Taubenberger

Florian Krammer

In a session on influenza, Fred Hayden, professor emeritus at the University of Virginia, will review approved antivirals and promising new drugs under development. Jeffery Taubenberger, deputy chief of the Laboratory of Infectious Diseases, NIAID/NIH, will discuss the legacy of the 1918 pandemic and current efforts to develop a universal flu vaccine.

Florian Krammer, an associate professor at the Icahn School of Medicine at Mount Sinai in New York City, will talk about his studies of antibody responses to antigenic sites in the influenza HA stalk and targets in other viral surface glycoproteins. An additional invitation is pending.

Bob Gallo

Marina Caskey

In the area of retroviral diseases, Bob Gallo, Director of the Institute of Human Virology at the University of Maryland Medical School, will review the global burden of HTLV-1 infection, the need for research and a public health response. Marina Caskey, an associate professor of clinical research at Rockefeller University, will discuss the development and evaluation of broadly neutralizing anti-HIV-1 antibodies. Additional invitations are pending.

Tim Block

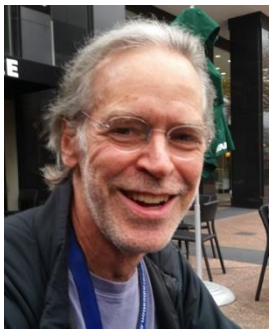
Vicki Olson

To permit coverage of a wide range of topics, we are organizing two sessions of invited lectures on "What's new in antiviral research." Tim Block, Director of the Hepatitis B Institute in Doylestown, PA will review current efforts to develop more effective, potentially curative therapies for chronic hepatitis B. Vicki Olson, Chief of the Poxvirus and Rabies Branch at the US CDC in Atlanta, will describe ongoing research on variola virus and the current status of countermeasures against a possible reintroduction of smallpox.

Bryan Cullen, Professor of Molecular Genetics and Microbiology at Duke University Medical Center, will discuss his research on potential applications of CRISPR/Cas9–mediated gene editing to the treatment of hepatitis B and other chronic DNA virus infections. Rhonda Cardin, Associate Dean for Research at the Louisiana State University School of Veterinary Medicine and an expert on cytomegalovirus disease, will review recent progress in CMV antivirals.

Bryan Cullen

Rhonda Cardin



Marc Collett

Subhash Vasudevan

Marc Collett, the founder and director of ViroDefense, which is devoted to developing antivirals against poliovirus, will describe the need to treat immunodeficient individuals chronically shedding vaccine-derived polioviruses in a future global eradication "endgame." Subhash Vasudevan from Duke-National University of Singapore will provide an update on the development of antivirals against flavivirus infections. Additional speakers are pending.

Amadou Sall

Emily Gurley

In a session on emerging viral diseases, Amadou Sall, scientific director of the Pasteur Institute in Dakar, Senegal, will review emerging viruses in Africa, other than Ebola. Emily Gurley, associate scientist at the Johns Hopkins School of Public Health, will describe her experience investigating outbreaks of Nipah virus infection in Southeast Asia and the development of behavioral and pharmaceutical interventions to prevent the spread of infection.

Maria Van Kerkhove

Scott Weaver

Maria Van Kerkhove, the MERS-CoV Technical Lead in the WHO High-threat Pathogens Unit, will give an update on the global status of the Middle East Respiratory Syndrome and the development of countermeasures for animals and humans. Scott Weaver, chair of the Department of Microbiology and Immunology at UTMB Galveston, will review recent experience with the introduction of West Nile, chikungunya and Zika viruses into the Western Hemisphere and discuss efforts to predict, prevent and control arbovirus epidemics.

Yan-Yi Wang

Kathie Seley-Radtke

In other sessions, Yan-Yi Wang, director of the Wuhan Institute of Virology, part of the Chinese Academy of Sciences, will present some of her research on innate antiviral responses and viral immune evasion, and will also describe the work of her institute, which includes China's first BSL-4 containment laboratory. Katherine Seley-Radtke, Presidential Research Professor at the University of Maryland, will chair a session on medicinal chemistry, and will give the opening presentation; invited speakers are pending.

The Chu Family Foundation Scholarships for Early Career Women in Science (Katherine Seley-Radtke)

Purpose

The Chu Family Scholarships were initiated by The Chu Family Foundation (TCFF) and ISAR to support the professional development of early career level women by providing funds to attend a specialized workshop, visit/work in another laboratory to obtain new skills, take a course, or acquire specialized training.

Award

Up to three awards will be given biannually to advance the careers of early career level women with potential for significant contributions in the field of basic, applied, and clinical aspects of antiviral research and antiviral drug development. Each award will consist of a $3,000 stipend, a 2-year ISAR membership and a commemorative plaque. The stipend must be used within a year of its award and the winners must present their research at the next International Conference for Antiviral Research (ICAR). As a result, the award should be used for both the proposed external training and attending the ICAR. However, if the total cost exceeds the $3,000 limit, applicants are still eligible to apply for the ICAR travel award funds. The funds are not meant, however, just to attend ICAR.

Eligibility

To be eligible to apply for the TCFF award, the early career level woman scientist must currently be either a (i) graduate student or (ii) hold a doctoral degree and have no more than four years of cumulative postdoctoral experience. The applicant must currently be doing graduate or postdoctoral research in the general field of antiviral research – this includes virology, chemistry, molecular biology or another virally-related focus. The criteria for selection includes, but is not limited to, the level of interest in antiviral research, the ability to do independent scientific work, the potential for a high level of scientific endeavor in antiviral research, as well as the extent of scientific accomplishments and scientific leadership/mentoring skills. Graduate students and postdoctoral candidates must be members of ISAR. A letter of support must be provided by a nominator, who may be the candidate's research project director, Department Chair, or Center Director. Click here (www.isar-icar.com) for information on how to become an ISAR member.

2019 TCFF Application Process

All grant applications must be submitted electronically by December 17, 2018.

Once submitted, nomination materials become the property of the Selection Committee and will not be returned. Winners will be selected by the TCFF Selection Committee by February 1, 2019 and will be informed of their status by email. The winners must present their research at the next ICAR meeting. (Note: Winners are still eligible to receive ICAR travel merit and assistance awards to attend ICAR).

Each candidate must submit:

- A Candidate Statement that describes her academic accomplishments and future career goals. The candidate should include an explanation of how the planned use of the award (workshop, training, specialized course, external lab visit, etc.) will help her career. The length of the Statement may not exceed two pages, single spaced. A Curriculum Vitae may be of any length.

- A letter of support from the candidate's research project director, Department Chair, or Center Director. Recommenders should describe how the planned use of the award (meeting, course, lab visit, etc.) will benefit the candidate's scientific and career development.

- Any candidate who wishes to use the award money to visit another scientist's laboratory must also submit a letter from the head of the laboratory indicating that the proposed visit is permissible.

- Any candidate who wishes to use the award money to attend a specialized course or workshop or course must provide a detailed description of the conference/course including the date, location, and other pertinent details, including a link to online information.

Additional Information

Candidates may apply more than one time but women who have previously won the award are not eligible for a second award. In addition, any candidate that has won a TCFF Scholarship from the International Society of Nucleosides, Nucleotides and Nucleic Acids (IS3NA) is ineligible to apply. In general, the career development activity should not be one for which the applicant's advisor is already funded. After completion of the training or course, etc., the recipient must provide a brief report of the work that was supported by the award to ISAR. This report will then be sent to The Chu Family Foundation.

For questions, please contact the TCFF Chair, Professor Katherine Seley-Radtke at kseley@umbc.edu, with the words The Chu Family Foundation Scholarship in the subject title.

Apply for a travel award to participate in the 32nd ICAR

This year, ISAR is pleased to announce the availability of a single type of travel grant with different levels of award based on region of origin of the applicant. These awards aim at stimulating the participation of students, postdocs and young researchers and provide them with the opportunity to be recognized for their scientific contribution to antiviral research. ISAR will grant the award based on quality of the submitted abstract and on need to support the travel to the meeting.

At least three independent reviewers will evaluate the scientific quality of the submitted abstract and abstracts will be ranked according to the scores provided by the reviewers.

Stipends will vary depending on the region of origin of the presenting author:

- Europe, Asia, Australia and South America: $800.00
- North America: $400.00
- Low / middle income country as defined by the World Bank: $1,500 and complimentary registration to the ICAR meeting

Registration to the meeting is mandatory for recipients of a travel award, but registration fee will be waived only for those coming from Low / middle income countries.

Please, check the ISAR website for selection criteria and eligibility requirements.

To apply for a travel award, please submit your abstract through the submission system. When you reach the final page of the submission form, you will be prompted to attach your CV (including publication records) and a nomination letter for the ICAR meeting by the Head of the Department and/or the Principal Investigator.

Please, keep in mind that abstracts submitted during the second submission period, are not eligible for travel awards.

Application Deadline for travel grants is January 11, 2019

Applicants will be notified by email by February 18, 2019 of award status and the travel award will be distributed at the meeting (receipt to be signed).

Recipients of a travel award are required to attend and actively participate during the entire conference.

VIROLOGY HISTORY, EVOLUTION AND ACTUAL IMPACT OF BIG DISCOVERIES

Introduction

One hundred years ago, just when the First World War was ending, the world faced another catastrophe, the Spanish flu pandemic. The 1918 to 1919 H1N1 influenza pandemic is among the most deadly outbreaks recorded in human history. About one third of the entire global population probably caught the Spanish flu and between 50 to 100 million in all corners of the world were killed. If one adjusts for population growth and extrapolates to the present population, this is equivalent to 200 million and 425 million today.

The Spanish flu, so named because in 1918 Spain was one of the few neutral countries providing reports of the outbreak decimated mainly adults aged 20 to 40. This is in contrast to most flu seasons, when deaths occur mainly in the elderly and children under five.

During 1918–19, the influenza pandemic had three distinct waves: the first wave (mid-1918) was exceedingly infectious but clinically mild, the second one (autumn 1918) was also highly infectious but with much higher mortality and the third wave (winter 1919) was associated with fewer cases and less mortality than the second wave. The three waves of infection are often assumed to share the same virus; however, there is recent evidence that the first and second waves of infection were probably caused by antigenically distinct influenza viruses and not by one virus that suddenly increased in pathogenicity between the two waves.

A century ago, the cause of the Spanish flu was unknown and the vaccine development was in a very early stage. There were no vaccines to protect against influenza virus infection, no antiviral drugs to treat the disease, and no antibiotics to treat secondary bacterial infections such as pneumonia. The main response to the Spanish flu consisted in non-pharmaceutical interventions such as school closings, restrictions on large gatherings, and isolation and quarantine. In the first decades of the 20th century, there was no healthcare notion. Many people had no access to doctors who either worked for themselves or were funded by charities or religious institutions.

The Spanish Flu can be regarded as an inflection point in the history of epidemic responses and global health. Following the mass death caused by the Spanish flu, health authorities realized that an individual could not be blamed for catching an infectious disease. Many governments embraced the concept of socialized medicine and recognized, as well, the need to coordinate public health at the international level, since contagious diseases had no borders.

Since the 1918-19 flu pandemic, tremendous advancements have been made to characterize the virus that caused the pandemic, and to treat or prevent influenza virus infections. Nevertheless, many questions remain unanswered and flu viruses continue to pose a serious public health threat. Therefore, we should remain alert and use the knowledge we have gained from the 1918-19 and other influenza pandemics to target our research efforts on preparedness planning for an eventual new deadly pandemic influenza, with focus on prevention, containment, and treatment.

Since the 1918-19 Spanish flu, other influenza pandemics, though less severe, have occurred in 1957, 1968, and 2009. Similar to the big earthquake that will hit California one day, influenza virus experts believe that a new influenza pandemic will inevitable appear at one point in the future but the big question is when this will happen. On the centenary of the 1918 pandemic, it is pertinent to wonder whether the world would be prepared for such an event. Unfortunately, we are not fully prepared to face a new influenza pandemic. First, we do not know which influenza virus will cause the next pandemic. Due to increase mobility, the virus can rapidly spread and today there is no way to develop promptly an effective vaccine against a pandemic virus. Further, differences in quality of health systems impede a prompt global response, and major gaps exist regarding surveillance data on emergence of influenza virus.

This year is not only the 100th anniversary of the Spanish flu pandemic of 1918 but it is also a century since some women got the right to vote, a century of the end of the First Word War, 70 years of the Universal Declaration of Human Rights, 50 years of May '68 protests, 40 years that Martin Luther King died and 70 years since the state of Israel was born. These events have unquestionably marked human history.

The Spanish flu, one of the most devastating events in humanity history, is also a central historical event in virology. To contribute to reflection on the evolution of virology having the 1918 pandemic as the central point, this session focuses on the impact of big discoveries in virology that took place at decennia's intervals before and after the Spanish flu. This year remembers not only the 100th anniversary of the 1918-19 flu pandemics but also 130 years of the discovery of the Leporipoxvirus myxoma virus, 80 years of the description of the Reed-Muench method, 70 years of the discovery of Coxsackie viruses, 40 years of freedom from smallpox, 30 years that human herpesvirus 6 was linked to *exanthema subitum*, and 20 years since the discovery of RNA interference. All these events have profoundly influenced virology research and we aim here to highlight their impact on past, present and future research.

1898 - Discovery of myxoma virus and evolution of myxoma virus research

David Evans
Department of Medical Microbiology & Immunology
The University of Alberta
Edmonton, Alberta
Canada
devans@ualberta.ca

The study of myxoma virus (MYXV) has offered some critically important insights into the biology and natural history of virus infections. In the document that follows, I'll try to summarize more than a century of myxoma research, highlighting some of these discoveries.

MYXV is a poxvirus, one of an extensive family of large DNA viruses that infect organisms ranging from insects to whales. It was first discovered in 1896 when a colony of European rabbits (*Oryctolagus cuniculus*) in Montevideo contracted a highly lethal disease that was called "myxomatosis" by its discoverer Professor G. Sanarelli (1). Infected animals exhibit multiple skin lesions characterized by tumour-like growths and die within 10 days of infection. Modern investigations have subsequently noted the high viremia, and death is often caused by the secondary infections arising from the profound immune suppression in infected animals. The high infectivity is also indicated by an early study showing that a single particle seems to correspond to an infectious unit (2).

The source of this and other sporadic South America infections long remained a mystery until the 1940's when myxomatosis was found to be endemic in native populations of wild rabbits, *Sylvilagus brasiliensis*. This species controls MYXV as a localized infection within a single infected site, although the tumours can still aid virus propagation through the medium of biting insect vectors (3; 4). Another strain of MYXV was subsequently discovered infecting populations of *Sylvilagus bachmani* in California (5). A third virus was also discovered that produces benign fibroma-like infections in *S. floridanus* rabbits across northeastern USA (6) and subsequently shown to provide immunological cross-protection against MYXV (7). Myxoma and Shope fibroma virus have all now been assigned to the taxonomic genus Leporipoxvirus.

The high mortality rate associated with MYXV infections did not escape the notice of researchers and others concerned with the environmental damage caused by *O. cuniculus* in the absence of its natural predators. The history and impact of these animals is especially well documented in Australia and the interested reader will find an authoritative description

in the book *Myxomatosis* (8). Briefly, in 1859 a shipment of 24 rabbits was released on an estate near the southeast tip of Australia and within the space of only a few decades, and in a stunning illustration of exponential population growth, succeeded in crossing the continent at a rate estimated at ~70 miles/year. The idea that these animals might be controlled with MYXV was first floated in 1919, but it would take a series of on-and-off again trials and government reviews before the disease finally gained a permanent foothold as a zoonotic infection of Australian rabbits in the early 1950's. These trials served an important role in demonstrating MYXV's limited host range, and also the critical importance of ecological factors such as rainfall and insect vectors in spreading the disease. At around the same time (1952) the disease was introduced into Europe and has since established a permanent presence there as well.

During the couple of decades following MYXV's introduction into Australia, researchers could watch a grand scale experiment that has since come to play an important role in our understanding of disease dynamics. Again, the interested reader can find a detailed description in Fenner and Ratcliffe (8). The virus released in 1950-51 spread rapidly, crossing the continent westward in only three years. One of the most important observations was that highly pathogenic MYXV strains had a strong tendency to die out, replaced by attenuated strains. For example the standard Australian laboratory strain produced a case-mortality rate of >99%, but within just 2-3 years in the field, this had been reduced to 50-70% in circulating MYXV strains (8). At the same time, it was also observed that the wild populations of rabbits were rapidly evolving resistance. The KM13 strain of MYXV was used to infect non-immune rabbits captured after each of a series of repeated epizootics. This strain produced ~90% mortality among rabbits captured at the beginning of the project, but by year seven this had been reduced to ~30% (9). Again European investigations observed similar trends (10).

MYXV research during 1965-80 was a continuation of this line of investigations. Meanwhile, smallpox was declared eradicated in 1978 and the focus in the poxvirus field was shifting heavily towards studying vaccinia virus as the standard model for poxvirus infections. The discovery that viruses can cause cancer created some interest in the tumour-like growths that characterize Leporipoxvirus infections, but subsequent research has shown that these are not transforming viruses and the phenotype is dependent upon a combination of immune suppression and virus-encoded cell growth factors (11). The early 1980's is when molecular biology began to be applied as a tool in MYXV research, much of it pioneered in the laboratory of Grant McFadden in Canada. David Strayer also contributed some early insights into the immunological properties of these viruses with his study of a hybrid between MYXV and Shope fibroma virus called malignant rabbit fibroma virus (12; 13).

MYXV offered an inviting model for exploring the complex array of genes that collectively promote disease, because, with the advent of poxvirus gene knockout technologies (14; 15), it became possible to selectively delete MYXV genes and then test the effect on disease in rabbits. Using this approach in combination with gene sequencing and rapidly evolving computational tools, a great number of MYXV "pathogenes" were subsequently identified. Some early examples were virus-encoded homologs of cellular serpins and the IFN-γ and TNF receptors. A common theme began to emerge of homologs of host genes being repurposed for more nefarious ends. The discovery that MYXV down-regulates MHC Class I molecules on the surface of infected cells has also provided a key insight into the immunosuppression that characterizes MYXV infections (16). Many excellent reviews summarize the extensive and still growing literature on this subject [e.g. (17-19)].

During the 1990's the first complete genome sequences were reported for vaccinia and variola viruses (20; 21), and the value of this approach was rapidly appreciated by researchers studying other poxviruses. The complete genome sequences for MYXV and Shope fibroma virus were reported in back-to-back papers in 1999 (22; 23). This work showed that the two viruses share a common ancestor but have long evolved in isolated environments where Shope fibroma virus has been attenuated through gene loss. An unanticipated discovery was that both viruses encode a photolyase, a DNA repair protein that provides protection from the damaging effects of ultraviolet light (24).

Through the first decade of the 21st century, there was a continuation of studies exploring MYXV pathogenesis while researchers like Peter Kerr and Tony Robinson continued to explore the biology of Australia's still evolving MYVX and rabbit strains. Some of this work was not without controversy when efforts to develop MYXV as a less virulent vaccine vector (25; 26) led to the discovery that incorporating one of the proposed genetic modifications in ectromelia virus could break pre-existing immunity (27). Public sensitivity to the release of genetically modified organisms has meant that few of these plans

have yet to come to fruition. During this period a collective understanding also began to emerge concerning the operations of cellular innate immune signalling networks. Among many other discoveries it was shown that MYXV can't block interferon-triggered STAT1 activation in resistant species, providing an important insight into the factors that determine MYXV host range (28). These signalling networks are essentially "burglar alarms" that some viruses will trigger, while others have evolved strategies to disarm.

Cancer research had been the big driver behind the study of cell-signalling networks. Cancer cells accumulate mutations that can disable these pathways and this plays a key role in helping tumours evade immune surveillance. For example, mutations that alter the expression of the apoptotic regulator Bcl-2 inhibit apoptosis and can thus block T-cell induced killing. Since many of these same systems provide defense against viruses, an important consequence is that viruses that would not infect non-transformed cells can sometimes infect cancer cells. This is the principle behind oncolytic virus technology. Peter Forsyth's laboratory was the first to illustrate the oncolytic potential of MYXV *in vivo*, by demonstrating its capacity to eradicate glioblastoma tumours in a xenografted mouse model (29). More recently MYXV-based virotherapies have shown particular promise for treating blood malignancies when used in conjunction with allogeneic hematopoietic cell transplants in mouse models (30). These procedures are hazarded by the risk of graft versus host disease, but MYXV treatment can control this when dormant viruses gain an opportunity to replicate in and disable activating T-cells. These same activated T-cells can deliver a virus payload to target cells, thus also killing any residual myeloma cells. Recent reviews summarize this exciting and still growing oncolytic virus literature [e.g. (31-33)].

This commentary began by summarizing the history of Australia's grand biological experiment and it seems only appropriate to conclude on the same note. In the last few years, Peter Kerr and his colleagues have used next generation sequencing technologies to determine the sequence of 30 Australian and 11 British MYXV strains (34-35). These studies have provided detailed insights into the evolutionary trajectories followed by different wild strains, including the propensity to accumulate gene duplications and deletions. Rather surprisingly, it has proven difficult to link specific mutations to changes in virulence, suggesting that the single-gene knockouts that are studied in the laboratory may not be reflective of the disease in the wild (36). Interestingly, when the disease phenotype is compared between modern and archived strains, modern strains seem to have reversed the trend towards attenuation seen in the 1950's. These viruses are more virulent and cause a different disease in laboratory rabbits called immune acute collapse and resembling in some ways septic shock syndrome (37). A similar disease called amyxomatous myxomatosis had been previously described in Europe, which like the Australian strains do not form the cutaneous lesions seen in classical infections in laboratory animals (38). Kerr *et al.* propose the attractive hypothesis that this phenotype reflects a way in which MYXV has adapted to maintain transmission in the face of enhanced host resistance to infection. However, although all of these viruses have been sequenced, there's no obvious genetic signature that would explain the altered disease phenotype. Clearly, we still have a lot more to learn about myxoma virus.

Acknowledgements. I'd like to thank Dr. R. Noyce for his comments on the manuscript and apologize to the many researchers whose work could not be cited due to space constraints. Research in my laboratory is supported by the Canadian Institutes for Health Research, Natural Sciences and Engineering Research Council of Canada, and Canada Foundation for Innovation.

References

1. Sanarelli, G., 1898 Das myxomatogene Virus. Beitrag zum Stadium der Krankheitserreger ausserhaulb des Sichtbaren. Zbl. Bakt. (Abt. 1) 23: 865.
2. Parker, R. F., 1940 Studies of the Infectious Unit of Myxoma. J Exp Med 71: 439-444.
3. Aragao, H. d., 1942 Sensibilidade do coelho do mato ao virus do mixoma; transmissao pelo "Aedes scapularis" e pelo ""Stegomyis". Brazil-med 56: 207.
4. Aragao, H. d., 1943 O virus do mixoma no coelho do mato (Sylvilagus minensis), sua transmissao pelos Aedes scapularis e aegypti. Mem. Inst. Osw. Cruz. 38: 93.
5. Marshall, I. D., and D. C. Regnery, 1960 Myxomatosis in a California brush rabbit (Sylyilagus bachmani). Nature 188: 73-74.
6. Shope, R. E., 1932 A transmissible tumor-like condition of rabbits. J. Exp. Med. 56**:** 793-802.
7. Jacotot, H., A. Vallee and B. Virat, 1958 [Immunization against infectious myxoma virus by inoculation of Shope fibroma virus]. Ann Inst Pasteur (Paris) 94**:** 282-293.
8. Fenner, F., and F. Ratcliffe, 1965 Myxomatosis. University Press, Cambridge Eng.
9. Marshall, I. D., and F. Fenner, 1958 Studies in the epidemiology of infectious myxomatosis of

rabbits. V. Changes in the innate resistance of Australian wild rabbits exposed to myxomatosis. J Hyg (Lond) 56**:** 288-302.
10. Fenner, F., and I. D. Marshall, 1955 Occurrence of attenuated strains of myxoma virus in Europe. Nature 176**:** 782-783.
11. Opgenorth, A., D. Strayer, C. Upton and G. McFadden, 1992 Deletion of the growth factor gene related to EGF and TGF alpha reduces virulence of malignant rabbit fibroma virus. Virology 186**:** 175-191.
12. Block, W., C. Upton and G. McFadden, 1985 Tumorigenic poxviruses: genomic organization of malignant rabbit virus, a recombinant between Shope fibroma virus and myxoma virus. Virology 140**:** 113-124.
13. Heard, H. K., K. O'Connor and D. S. Strayer, 1990 Molecular analysis of immunosuppression induced by virus replication in lymphocytes. J Immunol 144**:** 3992-3999.
14. Mackett, M., G. L. Smith and B. Moss, 1982 Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc Natl Acad Sci U S A 79**:** 7415-7419.
15. Panicali, D., and E. Paoletti, 1982 Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. Proc Natl Acad Sci U S A 79**:** 4927-4931.
16. Boshkov, L. K., J. L. Macen and G. McFadden, 1992 Virus-induced loss of class I MHC antigens from the surface of cells infected with myxoma virus and malignant rabbit fibroma virus. J Immunol 148**:** 881-887.
17. Nash, P., J. Barrett, J. X. Cao, S. Hota-Mitchell, A. S. Lalani *et al.*, 1999 Immunomodulation by viruses: the myxoma virus story. Immunol Rev 168**:** 103-120.
18. Werden, S. J., M. M. Rahman and G. McFadden, 2008 Poxvirus host range genes. Adv Virus Res 71**:** 135-171.
19. Spiesschaert, B., G. McFadden, K. Hermans, H. Nauwynck and G. R. Van de Walle, 2011 The current status and future directions of myxoma virus, a master in immune evasion. Vet Res 42: 76.
20. Goebel, S. J., G. P. Johnson, M. E. Perkus, S. W. Davis, J. P. Winslow *et al.*, 1990 The complete DNA sequence of vaccinia virus. Virology 179**:** 247-266, 517-263.
21. Shchelkunov, S. N., S. S. Marennikova, V. M. Blinov, S. M. Resenchuk, A. V. Tetmenin *et al.*, 1993 [Entire coding sequence of the variola virus]. Dokl Akad Nauk 328**:** 629-632.
22. Cameron, C., S. Hota-Mitchell, L. Chen, J. Barrett, J. X. Cao *et al.*, 1999 The complete DNA sequence of myxoma virus. Virology 264**:** 298-318.
23. Willer, D. O., G. McFadden and D. H. Evans, 1999 The complete genome sequence of shope (rabbit) fibroma virus. Virology 264**:** 319-343.
24. Bennett, C. J., M. Webb, D. O. Willer and D. H. Evans, 2003 Genetic and phylogenetic characterization of the type II cyclobutane pyrimidine dimer photolyases encoded by Leporipoxviruses. Virology 315**:** 10-19.
25. Kerr, P. J., H. D. Perkins, B. Inglis, R. Stagg, E. McLaughlin *et al.*, 2004 Expression of rabbit IL-4 by recombinant myxoma viruses enhances virulence and overcomes genetic resistance to myxomatosis. Virology 324**:** 117-128.
26. Mackenzie, S. M., E. A. McLaughlin, H. D. Perkins, N. French, T. Sutherland *et al.*, 2006 Immunocontraceptive effects on female rabbits infected with recombinant myxoma virus expressing rabbit ZP2 or ZP3. Biol Reprod 74**:** 511-521.
27. Jackson, R. J., A. J. Ramsay, C. D. Christensen, S. Beaton, D. F. Hall *et al.*, 2001 Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. J Virol 75**:** 1205-1210.
28. Wang, F., Y. Ma, J. W. Barrett, X. Gao, J. Loh *et al.*, 2004 Disruption of Erk-dependent type I interferon induction breaks the myxoma virus species barrier. Nat Immunol 5**:** 1266-1274.
29. Lun, X., W. Yang, T. Alain, Z. Q. Shi, H. Muzik *et al.*, 2005 Myxoma virus is a novel oncolytic virus with significant antitumor activity against experimental human gliomas. Cancer Res 65**:** 9982-9990.
30. Villa, N. Y., C. H. Wasserfall, A. M. Meacham, E. Wise, W. Chan *et al.*, 2015 Myxoma virus suppresses proliferation of activated T lymphocytes yet permits oncolytic virus transfer to cancer cells. Blood 125**:** 3778-3788.
31. Chan, W. M., and G. McFadden, 2014 Oncolytic Poxviruses. Annu Rev Virol 1**:** 119-141.
32. Villa, N. Y., M. M. Rahman, G. McFadden and C. R. Cogle, 2016 Therapeutics for Graft-versus-Host Disease: From Conventional Therapies to Novel Virotherapeutic Strategies. Viruses 8**:** 85.
33. Oliva, S., M. Gambella, M. Boccadoro and S. Bringhen, 2017 Systemic virotherapy for multiple myeloma. Expert Opin Biol Ther 17**:** 1375-1387.
34. Kerr, P. J., M. B. Rogers, A. Fitch, J. V. Depasse, I. M. Cattadori *et al.*, 2013 Genome scale evolution of myxoma virus reveals host-pathogen adaptation and rapid geographic spread. J Virol 87**:** 12900-12915.
35. Kerr, P. J., I. M. Cattadori, M. B. Rogers, A. Fitch, A. Geber *et al.*, 2017b Genomic and phenotypic characterization of myxoma virus from Great Britain reveals multiple evolutionary pathways distinct from those in Australia. PLoS Pathog 13**:** e1006252.

36. Liu, J., I. M. Cattadori, D. G. Sim, J. S. Eden, E. C. Holmes *et al.*, 2017 Reverse Engineering Field Isolates of Myxoma Virus Demonstrates that Some Gene Disruptions or Losses of Function Do Not Explain Virulence Changes Observed in the Field. J Virol 91.
37. Kerr, P. J., I. M. Cattadori, J. Liu, D. G. Sim, J. W. Dodds *et al.*, 2017a Next step in the ongoing arms race between myxoma virus and wild rabbits in Australia is a novel disease phenotype. Proc Natl Acad Sci U S A 114**:** 9397-9402.
38. Marlier, D., D. Cassart, C. Boucraut-Baralon, F. Coignoul and H. Vindevogel, 1999 Experimental infection of specific pathogen-free New Zealand White rabbits with five strains of amyxomatous myxoma virus. J Comp Pathol 121**:** 369-384.

Failure and success to combat the Great Influenza of 1918

John S. Oxford
j.oxford@qmul.ac.uk
Emeritus Professor of Virology
Blizard Institute, 4 Newark Street, Whitechapel
London, E1 2AT

Alfred Crosby, who wrote a pioneering book about the Spanish Influenza, concluded that the "physicians of 1918 were participants in the greatest failure of medical science in the 20th Century, and, if absolute numbers of dead are the measure, of all time" (1). But viewing the outbreak, which killed as many as 100 million and caused serious illness in another 300 million (2, 3), a century later, the scientific response looks less bleak. Remarkably individual scientists in the UK and USA produced their own batches of bacterial vaccines to prevent serious pneumonia and death (4, 5). Also local outbreaks, particularly in the USA, introduced layered public health approaches of social distancing, cotton masks, hygiene and school closure in some cities to the benefit of their citizens (6). Local doctors used passive antibody taken from patients who had recovered, and others desperately set up tents where patients were treated with steam, oxygen and eucalyptus (4). Rather the failures were more the result of poor medical infrastructure and leadership to combat epidemics in many countries of the world, including England.

Some early warning signals of pre-pandemics in 1918 were ignored

There was no epidemic of any infection in the British Army during the first two years of the Great War. However, there were continuing small numbers of cases of typhoid, paratyphoid, respiratory infection and cerebrospinal fever and even smallpox. But suddenly in December 1916 and during the ensuing months, an influenza like illness called Purulent Bronchitis (7, 8). The pathologists at Etaples, (8-10) having isolated pneumococcus and streptococcus from many of the victims of the disease there, began to formulate a complex vaccine. They grew streptococcus, pneumococcus and the controversial *B.influenzæ* (Pfeiffer's bacillus) bacterium on agar, suspended them to about 200 million organisms per millimetre and heat-treated them at 55^0C for 30 minutes. They added preservative and bottled up the 'new vaccine' in small vials ready for injection into soldiers. Is it quite remarkable that small groups of researchers could take this initiative? Of course, the technology of bacterial vaccine production on a small scale, say 200,000 doses, is technically quite simple. By taking some elementary precautions (such as the preparation of a 'clean room' free of other bacteria), a vaccine could be made – even in a 'field laboratory' in a wooden shed, such as that used by Dr Hammond at Etaples (4).

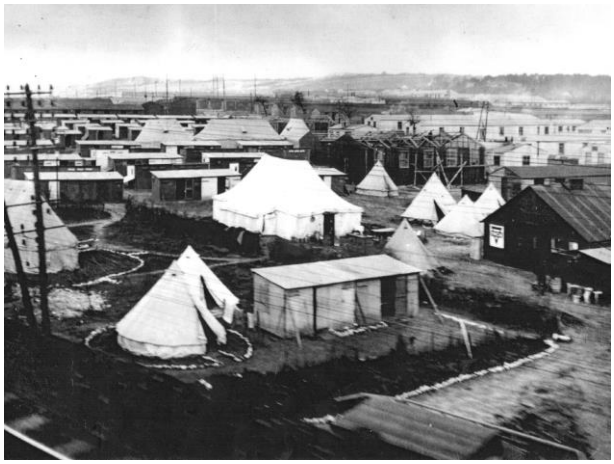
View of a laboratory at the time of the Spanish Influenza. Source: Imperial War Museum London with permission granted

In retrospect, we now know that perhaps half the victims of pandemic influenza, then and now, die because of deep-seated super infection with respiratory bacteria like streptococcus and staphylococcus (9). Today, polyvalent pneumococcus vaccines are stockpiled for use in pandemics.

In the event, the end of the winter that year, in March 1917, coincided with the end of the 'epidemic' of purulent bronchitis. The vaccine project, not unexpectedly, was put on hold. These authors must have had extensive discussions among themselves, and with their superiors in the RAMC (Royal Army Medical Corps), about the possibility of their "small epidemic" involving only a few hundred men spreading more widely amongst the tens and thousands of soldiers in the Camp at Etaples and later outside, and that it was influenza. We presume that there were no cases in the civilian countryside around Etaples at that time but unfortunately, the medical records have

been destroyed by the passage of time. The world would react differently now, armed as it is with new knowledge and hindsight! A virologist or pathologist today would fit together the scenario of an emerging influenza pandemic using China as a current model. Today, the World Health Organisation is on full alert, and every nation in the world has been asked to plan for a pandemic of bird influenza A (H5N1) or (H7N9). As with the earlier "pre-pandemic" outbreaks at Etaples in 1917, China today has had fewer than a thousand deaths in a population of two billion and H5N1 or H7N9 are not spreading in the community.

But we appreciate now from remarkable and brave 'gain of function' animal experiments with H5N1 that the virus would only need 4-5 mutations in the HA gene to enable spread (10). We postulate that a similar scenario could have happened on the Western Front and also that mutations of the 1916 H1N1 virus, perhaps in the USA army camps, completed the evolution of the Darwinian virus which then spread back to Europe and to the rest of the world.



Camp at Etaples. Source: Imperial War Museum London with permission granted

A large clinical study of "universal" vaccines in Canadian soldiers

By 1918, WH Eyre, who had provided us with the early descriptions of the 1916-1917 outbreaks, published a clinical description of the effects of the autumn influenza outbreak in soldiers of the New Zealand Expeditionary Force, and of the measures taken to attenuate their disease (5). The soldiers in this study were spread among three general hospitals, seven training camps, a convalescent camp, a discharge depot and city billets. It is a snapshot of young men who survived the War, only to be confronted with an even more dangerous enemy. For use in this study, Eyre and his colleagues formulated a mixed catarrhal vaccine (MCV) containing pneumococcus, staphylococcus and *H. influenzæ*. Out of a total strength of 21,759 men, approximately 16,104 received full prophylactic vaccination and approximately 5,700 were left uninoculated or had only received one dose of the vaccine. The risk of death in severe and complicated cases in the two groups was 8 per cent in those who received full vaccination and 27 per cent in those who did not. Of course, such a vaccine would not prevent infection by the virus but could reduce secondary bacterial infection.

We can view influenza A as a billion-casualty virus

Influenza A viruses have caused worldwide death and serious pneumonia from 1916 to the present day in yearly epidemics, as well as in the pandemics of 1918, 1957, 1968, and 2009. But especially only now with new antivirals to the virion endonuclease (11) to supplement neuraminidase inhibitors such as Tamiflu, together with the development of new universal vaccines with the broader immune response (12, 13), and well tested pandemic plans, the world can become a safer place (14).

References

1. Crosby, AW. American's forgotten Pandemic: the influenza of 1918. *Cambridge University Press* 1989: 377pp.
2. Stuart-Harris, CH, Schild, GC, Oxford, JS. Influenza, the Viruses and the Disease. Edward Arnold, London (1985): 264p.
3. Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. *Bulletin of the History of Medicine 2002; **76**: 105-15.*
4. Hammond JAR, Rolland W, Shore THG. Purulent bronchitis a study of cases occurring amongst the British troops at a base in France. *Lancet* 1917; **ii**: 41–45.
5. Eyre JWH, Cronin E, Lowe E. Report upon the autumn influenza epidemic (1918) as it affected the NZEF in the United Kingdom. *Lancet* 1919; **193**: 553-60.
6. Markel H, Lipman HB, Navarro A. Non-pharmaceutical interventions implemented by US cities during the 1918-1919 Influenza Pandemic. *Journal of the American Medical Association* 2007*; **298**: 644-654.*
7. Oxford JS, Sefton A, Jackson R, Innes, W, Daniels RS, Johnson NPAS. World War I may have allowed the emergence of "Spanish" influenza. *Lancet Infectious Diseases* 2002; **2**: 111–14.
8. Gill D, Putkowski J. The British Base Camp at Etaples 1914-1918. Etaples: *Musee Quentovic* 1997; 80p.
9. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *Journal of Infectious Disease* 2008; **198**: 962-70.

10. Herfst S, Schrauwen EJ, Linster M, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 2012; **336**: 1534-41.
11. Omoto S *et al*. Characterisation of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil, *Nature* 2018; **8**: 9633.
12. Yewdell JW. To dream the impossible dream: universal influenza vaccination. *Current opinion in Virology* 2013; **3**: 316-21.
13. Fiers W, De Filette M, Birkett A, Neirynck S, Min Jou W. A "universal" human influenza A vaccine. *Virus Research* 2004; **103**: 17-76.
14. Oxford JS and Gill D. Unanswered questions about the 1918 influenza pandemic: origin pathology, and the virus itself. *Lancet Infectious Diseases* 2018; on line June 20th: 1-7.

Influence of Reed-Muench Median Dose Calculation Method in Virology in the Millennium

M. A. Ramakrishnan and D. Muthuchelvan
Division of Virology, ICAR-Indian Veterinary Research Institute, Mukteswar, Uttarakhand – 263 138, India.
maramakrishnan@gmail.com

Almost all the virological research starts with titration of virus in animals, embryonated chicken eggs, or tissue cultures. Although several methods have been available for titration of viruses, the formula described by Reed and Muench in 1938 (1) is still immortal and continuously being applied by researchers because of its ease of use. This current short review focuses on the development and use of the Reed-Muench method purely from the virologist's view.

1. Introduction

Virus infectivity can be measured either by quantal or quantitative assays. The quantal assay is based on all or none/yes or no principle, for examples live or dead in infected animals or embryonated chicken eggs, presence or absence of cytopathic effect (CPE) in cell culture etc. The infectious unit is measured in terms of median dose such as 50% lethal dose (LD_{50}) in animals, 50% embryo infectious dose (EID_{50}) in embryonated chicken eggs, 50% tissue culture infectious dose ($TCID_{50}$) in cell culture, etc. In the case of quantitative assays, the infectivity is measured by an accurate or absolute unit such as plaque forming unit (PFU), focus forming unit (FFU), pock unit, etc.

The plaque assay has been considered as a gold standard for virus titration. However, there are viruses which will produce only CPE but not plaques in cell culture. These viruses need to be measured in terms of $TCID_{50}$. Originally, Trevan introduced the term median lethal dose (LD_{50}) in 1927 (2). In the LD_{50} assay, in a particular dilution (virus concentration), 50% animal will die whereas 50% of the animal will remain live. Many times, we may encounter the question from students asking why does the virus needs to be measured in terms of median dose and not by other values. The detailed statistical explanation for the selection of median rather than mean or mode can be found in the classical literatures (2, 3). In brief, virus titer can be measured in other units such as 10% and 90% or 25% and 75% infectivity but the error rate will be more at these values compared to 50% (2).

There are several formulas available to estimate median lethal dose (1, 4-18). However, two long-lasting methods, Reed-Muench and Spearman-Karber, are still being used by most researchers (1, 7, 16).

2. Reed-Muench Method

Lowel Jacob Read (8 January 1886 – 28 April 1966) graduated in electrical engineering (1907) and earned a Ph.D. in mathematics (1915). For a couple of years, he taught Math and Physics at the University of Maine (1915-7) and for a short period (1917-8), he served as a chief of the Bureau of Tabulation and Statistics of the War Trade Board. After that, for many years (1918 – 1956) he was affiliated with the Johns Hopkins University at the various positions – Associate Professor, Professor, Dean, Vice President, and President. When he was appointed as a chair of the Department of Vital Statistics in 1930, he renamed the department by coining the new terminology "biostatistics". Dr. Reed's popular contributions includes 1) Pearl-Reed logistic curve theory of population growth, 2) Reed-Merrell short method for constructing an abridged life table, 3) Reed-Frost theory of epidemics, and 4) Reed-Muench method of measurement of LD_{50} (19, 20).

Hugo Muench Jr (17 October 1894 – 16 November 1972) received his Medical degree (1918) from Washington University and Doctorate of Public Health (1932) from Johns Hopkins School of Hygiene and Public Health. He chaired the Department of Biostatistics, Harvard School of Public in 1946–1961. He also served there as Professor and Emeritus Professor. Before that, he was a staff member of the International Health Division, Rockefeller Foundation, New York City (20, 21)

For the median dose calculation, the Reed-Muench method is still preferred by many researchers because of its relative ease of use. Further, reducing the dose range is possible as this method required just above and below 50% values for measuring median dose. Initially, this method has been used for the endpoint estimate at the Yellow Fever Laboratory at the Rockefeller Foundation. Later, the Reed and Muench duo simplified this method. In the Reed-Muench original article, they explained the method for

calculation of protective serum. When it is applied to the virus titration, the formula needs to be modified accordingly. Because of this, many authors quoting the formulas in different ways especially in using +/- symbol for dilution and dilution factor. The following formula is taken from (22) as it is simple and clear.

In the above formulas, it is noteworthy to clarify the following points. 1) Proportional (or proportionate) distance also known as interpolated value or difference of logarithms, 2) minus (–) symbol will be applicable for log of dilution and dilution factor, 3) Log ID is an endpoint dilution i.e. in that minimum dilution 50% of the experimental unit will be infected, for example death of animal, embryos etc. In case of $TCID_{50}$, it is the minimal dose required for infecting 50% of the wells (3 out of 6 wells etc.) and not 50% of the cell population. Thus, a well showing 100% CPE and a well showing indication of CPE will be equally scored as plus; if no CPE, then as minus, 4) virus titer is reciprocal of the end dilution.

The below Table along with the notes explain the Reed-Muench method and its principles.

Log_{10} virus dilution	Mice		Cumulative totals			% mortality
	Died	Survived	Died	Survived	Total	
-1	10	0	42	0	42	42/42 × 100 = 100
-2	10	0	32	0	32	32/32 × 100 = 100
-3	10	0	22	0	22	22/22 × 100 = 100
-4	6	4	12	4	16	12/16 × 100 = 75.0
-5	4	6	6	10	16	06/16 × 100 = 37.5
-6	2	8	2	18	20	02/20 × 100 = 10.0
-7	0	10	0	28	28	0/28 × 100 = 0.00

a) Proportional distance = (75-50)/(75-37.5) = 0.67; log10 50% end point dilution = -4 + (0.67 × -1) = -4.67; 50% end point dilution = 10-4.67; the titer of the virus = 104.67 LD_{50}/ml.
b) Reed and Muench method uses cumulative (accumulated) values rather than observed individual values because it aids in equalizing chance variation (23).
c) The cumulated value is calculated on the assumption that if animals die at low virus concentration (high dilution) that would also die when inoculated with a high concentration of virus (low dilution). Therefore, the cumulative value for death is summed up from bottom to top (high dilution to low dilution). Similarly, if animal survives at a high concentration of virus (low dilution) will also survive at low concentration of virus (high dilution) and therefore cumulative values are summed up from top to bottom.
d) If 50% mortality is observed in a particular dilution, that is an endpoint dilution and proportional calculation is not required.

3. The other side of the Reed-Muench Methods

Although this method is popular among researchers, very similar methods were available before that (24, 25). Several researchers compared different median calculation methods and observed that the more recent Spearman-Karber method is superior (26 -28).

Concluding Remarks

As any other biological assays, the method of Reed and Muench also has merits and demerits in application. However, this method served several virological breakthroughs and is still recommended by international agencies for vaccine related works.

References

1. Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. American Journal of Epidemiology 27, 493–497.
2. Trevan, J.W., 1927. The Error of Determination of Toxicity. Proceedings of the Royal Society B: Biological Sciences 101, 483–514.
3. Armitage, P., Allen, I., 1950. Methods of estimating the LD_{50} in quantal response data. J Hyg (Lond) 48, 298–322.
4. Bliss, C.I., 1938. The determination of the dosage-mortality curve from small numbers. Q J Pharm Pharmacol 11, 192–216.
5. Bruce, R.D., 1985. An up-and-down procedure for acute toxicity testing. Fundam Appl Toxicol 5, 151–157.
6. Chinedu, E., Arome, D., Ameh, F.S., 2013. A new method for determining acute toxicity in animal models. Toxicol Int 20, 224–226.
7. Kärber, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie 162, 480–483.
8. Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.
9. Lorenz, R.J., Bögel, K., 1973. Laboratory techniques in rabies: methods of calculation. Monogr Ser World Health Organ 321–335.
10. Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol. 54, 275–287.
11. Miller, L.C., Tainter, M.L., 1944. Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper. Experimental Biology and Medicine 57, 261–264.
12. Ott, W.H., 1947. Quantitative assay of the intravenous toxicity of streptomycin in mice. Journal of the American Pharmaceutical Association (Scientific ed.) 36, 193–197.
13. Ramakrishnan, M.A., 2016. Determination of 50% endpoint titer using a simple formula. World J Virol 5, 85–86.
14. Rispin, A., Farrar, D., Margosches, E., Gupta, K., Stitzel, K., Carr, G., Greene, M., Meyer, W., McCall, D., 2002. Alternative methods for the median lethal dose ($LD_{(50)}$) test: the up-and-down procedure for acute oral toxicity. ILAR J 43, 233–243.

15. Saganuwan, S.A., 2016. The new algorithm for calculation of median lethal dose (LD_{50}) and effective dose fifty (ED_{50}) of Micrarus fulvius venom and anti-venom in mice. International Journal of Veterinary Science and Medicine 4, 1–4.
16. Spearman, C., 1908. The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae. British Journal of Psychology, 1904-1920 2, 227–242.
17. Thompson, W.R., 1947. Use of moving averages and interpolation to estimate median-effective dose: I. Fundamental Formulas, Estimation of Error, and Relation to Other Methods. Bacteriol Rev 11, 115–145.
18. Weil, C.S., 1952. Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions in Their Use. Biometrics 8, 249. https://doi.org/10.2307/3001557
19. Kiser, C.V., 1966. Lowell J. Reed (1886 - 1966). Population Index 32, 362–365.
20. Thomas, K.K., 2016. Cultivating Hygiene as a Science: The Welch-Rose Report's Influence at Johns Hopkins and Beyond. American Journal of Epidemiology 183, 345–354.
21. AJPH, 1946. News from the Field. American Journal of Public Health and the Nations Health 36, 825–836.
22. Burleson, F.G., Chambers, T.M., Wiedbrauk, D.L., 1992. Introduction to quantal virus assays, in: Virology. Elsevier, pp. 53–57.
23. Leland, D.S., French, M.L.V., 1988. Virus Isolation and Identification, in: Laboratory Diagnosis of Infectious Diseases Principles and Practice. Springer New York, New York, NY, pp. 39–59.
24. Behrens, B., 1929. Zur Auswertung der Digitalisblätter im Froschversuch. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie 140, 237–256.
25. Dragstedt, C.A., Lang, V.F., 1928. Respiratory stimulans in acute cocaine poisoning in rabbits. J Pharmacol Exp Ther 32, 215.
26. Finney, D.J., 1952. Statistical method in biological assay. Charles Griffin & Company Limited, London.
27. Kappenman, R.F., 1987. Nonparametric estimation of dose-response curves with application to ED_{50} estimation. Journal of Statistical Computation and Simulation 28, 13–28.
28. Myers, L.E., McQuay, L.J., Hollinger, F.B., 1994. Dilution assay statistics. J. Clin. Microbiol. 32, 732–739.

The discovery of coxsackieviruses and evolution of coxsackievirus research

Brett L. Hurst[a,b]
[a]Institute for Antiviral Research, Utah State University, Logan, UT;
[b]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah.
brett.hurst@usu.edu

The story of coxsackievirus (CV) is intertwined with the history of poliovirus and difficult to separate from the related virus. In 1947, an outbreak of poliomyelitis was identified in upstate New York. Gilbert Dalldorf and Grace M. Sickles were actively searching for poliovirus that could be adapted to mice (1). In 1948, they recovered a virus from acute-phase fecal specimens of two boys with flaccid paralysis that produced paralysis in suckling mice and hamsters. Further study of the viruses showed that paralysis was caused by infection and destruction of muscle tissue rather than damage to the central nervous system, as was common with poliovirus. Because the patients were located in Coxsackie, New York, the virus was eventually named coxsackievirus (CV) to distinguish it from the ongoing poliovirus epidemic. However, one year after the initial identification of coxsackieviruses, Edward Curnen, Ernest Shaw and Joseph Melnick discovered another novel virus that produced unique pathology in mice, which they named coxsackievirus B (2).

Coxsackieviruses are classified into groups A or B depending upon the type of paralysis induced in suckling mice. Coxsackieviruses in group A (CVA) produce a myositis-based flaccid hind limb paralysis while those in group B (CVB) produce a spastic paralysis and more generalized infection in newborn mice (3). Members of the CVB family have been observed to cause damage to the heart, brain, pancreas and fat tissue of newborn mice (4). While all of the CVs fall within the *Picornaviridae* family and enterovirus genus, they are then separated according to species. The CVs are split into the A, B, and C species of enterovirus as shown in Table 1 along with the associated illnesses attributed to CV infection.

CV infections are responsible for a wide range of diseases ranging from hand-foot-and-mouth disease (HFMD) to pericarditis in infants (5, 6). CVs are the primary agent of (HFMD) as well as a potential cause of acute meningoencephalitis and flaccid paralysis (6, 7). CV infections are reported and tracked through the National Enterovirus Surveillance System (NESS) in the United States as well as the Polio Eradication Initiative for the World Health Organization (WHO) (8, 9).

In addition to the diseases for which CV infection is a known cause, CV infection has been implicated in the onset of type 1 diabetes (T1D). While the link between

CVs and T1D is not definitive, CVB4 has been shown to infect the thymus in mice as well as fetal thymus organ cultures (10-12). CVs may cause rapid and complete destruction of pancreatic beta cells leading to fulminant T1D (13). More research is needed for conclusive evidence that CV infection is a potential factor in the development of T1D.

Table 1. List of coxsackieviruses by species, types, and associated illnesses or symptoms[1].

Enterovirus Species	Type	Associated illnesses and symptoms
A	CVA2, CVA3, CVA4, CVA5, CVA6, CVA7, CVA8, CVA10, CVA12, CVA14, CVA16	Poliomyelitis, herpangina, meningitis, paralysis, exanthema, hand-foot-and-mouth disease (HFMD), pneumonitis, hepatitis, infantile diarrhea
B	CVA9, CVB1, CVB2, CVB3, CVB4, CVB5, CVB6	Pleurodynia, meningitis, paralysis (rarely), systemic infection (infants), myocarditis, pericarditis, upper respiratory illness, pneumonia, rash, hepatitis
C	CVA1, CVA11, CVA13, CVA17, CVA19, CVA20, CVA21, CVA22, CVA24	Poliomyelitis, myalgia, Guillain-Barré syndrome, hepatitis, mild respiratory disease, vomiting, diarrhea

[1] Adapted from Field's Virology Sixth Edition (3).

The genome of coxsackievirus is similar to those of other enteroviruses. The single-stranded positive-sense RNA genome encodes for a single polyprotein, which is then enzymatically cleaved by viral proteases 2A and 3CD (14). A total of 11 proteins are separated into four structural proteins (VP1-VP4) and seven non-structural proteins (2A-2C, 3A-3D).

The CV life cycle follows the similar steps to other enteroviruses. The virus first attaches to a cell surface receptor, endocytosis, uncoating, translation, proteolytic processing, genome replication, assembly, virion maturation, and release. Virus is released through either lytic or non-lytic mechanisms (14).

Several receptors have been implicated in the entry of CVs into host cells. While many experiments were required to demonstrate the roles of each receptor in coxsackievirus infection, only a brief overview will be presented here. Intracellular adhesion molecule 1 (ICAM-1) has been implicated for infection with CVA13, CVA18, and CVA21 (15). ICAM-1 normally functions in the process of extravasation of neutrophils into surrounding tissues (16). The $\alpha_v\beta_3$ vitronectin receptor has been shown to allow infection of CVA9 in green monkey kidney cells (17). It is an integrin expressed by platelets, bone reabsorbing cells, and osteoclasts that mediates cell adhesion to the extracellular matrix (18). Decay-accelerating factor (DAF, also known as CD55) has been shown to be important for CVA21, CVB1, CVB3, and CVB5 (19, 20). DAF is a widespread regulatory membrane protein responsible for dissociation of the complement pathway (21). Studies have shown that insertion of the coxsackievirus and adenovirus receptor (CAR) will render nonpermissive cells susceptible to infection by CVB (22). CAR normally functions as a cell adhesion molecule and may have a role in scaffolding (23). Heparan sulfate has also been shown to aid in CVB3 infection in cells lacking CAR and DAF (24). The exact role of each of these receptors is a focus of ongoing research and the receptors specific to each CV may influence tissue tropism and pathogenesis (25).

Compounds that target the CV life cycle have focused on either the viral proteins or essential host factors. Compounds targeting the viral proteins tend to have less side effects while those targeting host factors decrease the likelihood of the virus developing antiviral resistance (14). Amiloride was repurposed as an antikaliuretic diuretic and has shown the ability to act as a non-nucleoside inhibitor of the CVB3 3D polymerase (26). Pirlindole was initially approved as an antidepressant but also displayed activity on the 2C protein of CVB3 (27). OSW-1 was isolated from the plant *Ornithogalum saundersiae* and binds to the host oxysterol-binding protein (OSBP) which is essential for replication of many enteroviruses (28). It has been shown to inhibit both CVB3 and CVA21 (29). Despite these advances, no specific antiviral therapy has been developed for CV infection and treatment remains supportive in nature.

The role that the vaccine played in the near-eradication of poliovirus infections signifies that an effective vaccine may be invaluable in combatting enterovirus and more specifically CV infections. Recent advances with rhinovirus vaccines have shown broad-range efficacy in primates and may be useful in developing a vaccine for CVs (30). The Food and Drug Administration (FDA) of China approved the first inactivated whole virus vaccine for EV71 infection in December of 2015 (31). Both of these examples provide evidence that a vaccine for CV infection, while difficult, is feasible and requires further research. A multivalent vaccine for HFMD would be a major milestone for CV research (32, 33).

Since their discovery over 70 years ago, CVs have been identified as a significant human pathogen responsible for a number of diseases and illnesses. Recent advances have increased the understanding of the life cycle and potential antiviral targets for CV infection. Development of a vaccine in the coming years may be the most realistic option for controlling

CV infections. Additional research may provide evidence of the link between CV infection and T1D as well.

References

1. Dalldorf G, Sickles GM. 1948. An Unidentified, Filtrable Agent Isolated From the Feces of Children With Paralysis. Science 108:61-2.
2. Melnick JL, Shaw EW, Curnen EC. 1949. A virus isolated from patients diagnosed as non-paralytic poliomyelitis or aseptic meningitis. Proc Soc Exp Biol Med 71:344-9.
3. Knipe DM, Howley PM. 2013. Fields virology, 6th ed. Wolters Kluwer/Lippincott Williams & Wilkins Health, Philadelphia, PA.
4. Melnick JL. 1983. Portraits of viruses: the picornaviruses. Intervirology 20:61-100.
5. Lugo D, Krogstad P. 2016. Enteroviruses in the early 21st century: new manifestations and challenges. Curr Opin Pediatr 28:107-13.
6. Anastasina M, Domanska A, Palm K, Butcher S. 2017. Human picornaviruses associated with neurological diseases and their neutralization by antibodies. J Gen Virol 98:1145-1158.
7. Huang HI, Shih SR. 2015. Neurotropic Enterovirus Infections in the Central Nervous System. Viruses 7:6051-66.
8. Abedi GR, Watson JT, Pham H, Nix WA, Oberste MS, Gerber SI. 2015. Enterovirus and Human Parechovirus Surveillance - United States, 2009-2013. MMWR Morb Mortal Wkly Rep 64:940-3.
9. Wassilak S, Orenstein W. 2010. Challenges faced by the global polio eradication initiative. Expert Rev Vaccines 9:447-9.
10. Brilot F, Geenen V, Hober D, Stoddart CA. 2004. Coxsackievirus B4 infection of human fetal thymus cells. J Virol 78:9854-61.
11. Michaux H, Martens H, Jaidane H, Halouani A, Hober D, Geenen V. 2015. How Does Thymus Infection by Coxsackievirus Contribute to the Pathogenesis of Type 1 Diabetes? Front Immunol 6:338.
12. Jaidane H, Halouani A, Jmii H, Elmastour F, Abdelkefi S, Bodart G, Michaux H, Chakroun T, Sane F, Mokni M, Geenen V, Hober D, Aouni M. 2017. In-utero coxsackievirus B4 infection of the mouse thymus. Clin Exp Immunol 187:399-407.
13. Ohara N, Kaneko M, Nishibori T, Sato K, Furukawa T, Koike T, Sone H, Kaneko K, Kamoi K. 2016. Fulminant Type 1 Diabetes Mellitus Associated with Coxsackie Virus Type A2 Infection: A Case Report and Literature Review. Intern Med 55:643-6.
14. Baggen J, Thibaut HJ, Strating J, van Kuppeveld FJM. 2018. The life cycle of non-polio enteroviruses and how to target it. Nat Rev Microbiol 16:368-381.
15. Colonno RJ, Callahan PL, Long WJ. 1986. Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses. J Virol 57:7-12.
16. Lyck R, Enzmann G. 2015. The physiological roles of ICAM-1 and ICAM-2 in neutrophil migration into tissues. Curr Opin Hematol 22:53-9.
17. Roivainen M, Hyypia T, Piirainen L, Kalkkinen N, Stanway G, Hovi T. 1991. RGD-dependent entry of coxsackievirus A9 into host cells and its bypass after cleavage of VP1 protein by intestinal proteases. J Virol 65:4735-40.
18. Horton MA. 1997. The alpha v beta 3 integrin "vitronectin receptor". Int J Biochem Cell Biol 29:721-5.
19. Shafren DR, Dorahy DJ, Ingham RA, Burns GF, Barry RD. 1997. Coxsackievirus A21 binds to decay-accelerating factor but requires intercellular adhesion molecule 1 for cell entry. J Virol 71:4736-43.
20. Shafren DR, Bates RC, Agrez MV, Herd RL, Burns GF, Barry RD. 1995. Coxsackieviruses B1, B3, and B5 use decay accelerating factor as a receptor for cell attachment. J Virol 69:3873-7.
21. Lublin DM, Atkinson JP. 1989. Decay-accelerating factor: biochemistry, molecular biology, and function. Annu Rev Immunol 7:35-58.
22. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. 1997. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275:1320-3.
23. Patzke C, Max KE, Behlke J, Schreiber J, Schmidt H, Dorner AA, Kroger S, Henning M, Otto A, Heinemann U, Rathjen FG. 2010. The coxsackievirus-adenovirus receptor reveals complex homophilic and heterophilic interactions on neural cells. J Neurosci 30:2897-910.
24. Zautner AE, Korner U, Henke A, Badorff C, Schmidtke M. 2003. Heparan sulfates and coxsackievirus-adenovirus receptor: each one mediates coxsackievirus B3 PD infection. J Virol 77:10071-7.
25. Evans DJ, Almond JW. 1998. Cell receptors for picornaviruses as determinants of cell tropism and pathogenesis. Trends Microbiol 6:198-202.
26. Harrison DN, Gazina EV, Purcell DF, Anderson DA, Petrou S. 2008. Amiloride derivatives inhibit coxsackievirus B3 RNA replication. J Virol 82:1465-73.
27. Ulferts R, de Boer SM, van der Linden L, Bauer L, Lyoo HR, Mate MJ, Lichiere J, Canard B, Lelieveld D, Omta W, Egan D, Coutard B, van Kuppeveld FJ. 2016. Screening of a Library of FDA-Approved Drugs Identifies Several Enterovirus Replication Inhibitors That Target Viral Protein 2C. Antimicrob Agents Chemother 60:2627-38.

28. Strating JR, van der Linden L, Albulescu L, Bigay J, Arita M, Delang L, Leyssen P, van der Schaar HM, Lanke KH, Thibaut HJ, Ulferts R, Drin G, Schlinck N, Wubbolts RW, Sever N, Head SA, Liu JO, Beachy PA, De Matteis MA, Shair MD, Olkkonen VM, Neyts J, van Kuppeveld FJ. 2015. Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein. Cell Rep 10:600-15.
29. Albulescu L, Strating JR, Thibaut HJ, van der Linden L, Shair MD, Neyts J, van Kuppeveld FJ. 2015. Broad-range inhibition of enterovirus replication by OSW-1, a natural compound targeting OSBP. Antiviral Res 117:110-4.
30. Lee S, Nguyen MT, Currier MG, Jenkins JB, Strobert EA, Kajon AE, Madan-Lala R, Bochkov YA, Gern JE, Roy K, Lu X, Erdman DD, Spearman P, Moore ML. 2016. A polyvalent inactivated rhinovirus vaccine is broadly immunogenic in rhesus macaques. Nat Commun 7:12838.
31. Yi EJ, Shin YJ, Kim JH, Kim TG, Chang SY. 2017. Enterovirus 71 infection and vaccines. Clin Exp Vaccine Res 6:4-14.
32. Klein M, Chong P. 2015. Is a multivalent hand, foot, and mouth disease vaccine feasible? Hum Vaccin Immunother 11:2688-704.
33. Aswathyraj S, Arunkumar G, Alidjinou EK, Hober D. 2016. Hand, foot and mouth disease (HFMD): emerging epidemiology and the need for a vaccine strategy. Med Microbiol Immunol 205:397-407.

Forty years of freedom from smallpox – and the continuing threat

Mike Bray[1] & Graciela Andrei[2]
[1]Chevy Chase, MD, USA
mikebrayavr@gmail.com
[2]Rega Institute for Medical Research
KU Leuven
Herestraat 49, box 1030
3000 Leuven, Belgium
graciela.andrei@kuleuven.be

Smallpox has a unique place in the history of medicine. Caused by the orthopoxvirus *Variola*, it was one of the most lethal diseases, devastating humankind for millennia. It is also the only human viral disease to have been eradicated, thanks to a massive vaccination campaign launched by the World Health Organization in 1967. The earth has now been free of smallpox for 40 years, since the last case of smallpox occurred in 1978 (Figure 1 and Figure 2).

Triumph over humanity's greatest scourge

The origin of smallpox is uncertain. At the time of its eradication, variola virus had no animal reservoir, and depended for its persistence entirely on person-to-person transmission. Running the clock backwards, sufficient numbers of humans in large enough groups to maintain the virus did not exist before about 5,000 BC, indicating that it must have "jumped" to humans from an animal host after that time (1). The earliest physical evidence of smallpox are the pustules on the faces of Egyptian mummies from around the 3rd century BC (2).

The frightening nature of the disease led to early efforts to develop a means of protection. Variolation, the inoculation of material from smallpox lesions, was used in Africa and Asia well before 1000 AD. The practice was introduced into Great Britain by Lady Mary Wortley Montagu, the wife of the ambassador to Constantinople, in the early 1720s, and by mid-century it had become a standard practice in many parts of Europe and the British colonies (3). However, the procedure was traumatic, producing a febrile illness a large cluster of pustules at the inoculation site, and a small percentage of those variolated developed full-blown smallpox.

In the 1790s, the British country doctor Edward Jenner, without any understanding of immunity or awareness of the existence of viruses, succeeded in developing a new, safer method of protection against smallpox (4). Jenner had been variolated as a boy, and routinely performed it as part of his medical practice. In the course of his work in rural Gloucestershire, he noticed that milkmaids and others who had developed cowpox, a pustular disease of cattle, were somehow protected against smallpox. To experimentally confirm his observation, he scarified the arm of an 8 year-old boy, James Phipps, with material from cowpox lesions on the hand of a milkmaid, Sarah Nelmes. Six weeks later, he variolated the boy, and found that he had no reaction. Jenner's "mental leap," realizing that protection against variolation equated to protection against smallpox, earned him immortality in the history of medicine.

The new procedure, named vaccination from the Latin *vacca* (cow), was rapidly introduced throughout Europe and the New World, where it led to a decline in smallpox, but the disease persisted in much of Africa and Asia. We now understand that infection with one orthopoxvirus confers cross-immunity against other members of the genus. Sometime in the 19th century, cowpox virus was replaced in vaccine manufacture by the virus we now term vaccinia, which may originally have been horsepox virus.

The world's last cases of smallpox

The WHO-sponsored eradication campaign began to zero in on the last remaining areas of endemic smallpox in the 1970s. At that time, there were still

two types of the disease, a severe form (variola major), and a milder form (variola minor). The last person in the world to contract variola major was a three-year-old girl, Rahima Banu, who lived on Bhola Island in Bangladesh, who became ill in 1975 (5). She was isolated at home until she was no longer infectious, and happily survived. Smallpox eradication workers initiated a house-to-house vaccination campaign and visited every house, public meeting area, school, and doctor within five miles, to ensure the illness did not spread.

In 1977, Ali Maow Maalin in Somalia was the last person to develop variola minor (6). On October 12, he was in close contact with two smallpox patients, and 10 days later, he developed a fever, followed by a rash. He was first diagnosed with malaria, then chickenpox, and smallpox was finally recognized on October 30. He was isolated and made a full recovery. Ali died of malaria in 2013, while working in the polio eradication campaign.

A year after the conclusion of the global eradication campaign, WHO officials were alarmed to hear of an outbreak of smallpox in England (3). Janet Parker, a medical photographer at Birmingham University Medical School, worked one floor above the laboratory where Henry Bedson was conducting smallpox research. She came down with a fever on August 11th, 1978 and developed a rash four days later. It was not August 24th that smallpox was finally diagnosed, and she was moved to an isolation hospital; she died two weeks later. A total of 341 persons who had been in contact with her were vaccinated or placed under surveillance. Her mother developed smallpox on September 7, despite having been vaccinated on August 24. A subsequent investigation indicated that Janet had most likely been infected via an airborne route through the building's air-duct system. Bedson blamed himself for the escape of the virus and committed suicide, and his laboratory was closed. The event led the WHO to call for the world-wide destruction of all remaining stocks of variola virus and the restriction of research to only a few laboratories.

The continuing threat of smallpox

Following the declaration of global eradication, stockpiles of smallpox vaccine were greatly reduced; no effort was made to develop safer vaccines for persons with impaired immunity; and no attempt was made to identify antiviral drugs for the treatment of smallpox or of vaccination complications. Variola virus is now stored and used for WHO-approved research only at the Centers for Disease Control and Prevention in Atlanta, Georgia, and the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia.

The elimination of naturally occurring smallpox did not remove the threat of variola virus as a potential bioterrorist weapon. Indeed, the cessation of vaccination has rendered most of the current world population vulnerable to the disease, while rapid progress in biotechnology has made it possible to artificially create variola virus, or a similar pathogen, in the laboratory. The risk of an accidental release of variola virus from one of the two WHO-approved research laboratories is considered negligible. Instead, concern focuses on the possible reemergence of smallpox if scientists acting independently, employed by a terrorist organization or part of a covert, state-sponsored biowarfare effort, would use modern molecular methods to reconstruct the virus, based on published sequence data (7). Undeclared secret or misplaced stocks of variola virus might also exist.

The feasibility of creating variola virus *de novo* was demonstrated by the recent construction of infectious horsepox virus entirely from chemically synthesized DNA (8; 9). Because such work requires sophisticated knowledge of virological methods, the assembly reactions are inefficient, and the necessary infrastructure is not widely accessible, terrorists might instead attempt to acquire the naturally occurring monkeypox virus, a zoonotic agent maintained in various animal species in west and central Africa (10). Monkeypox virus causes a smallpox-like disease in humans, and although it is much milder than smallpox, the virus could potentially be modified in the laboratory to increase its virulence, through the insertion of genes encoding human cytokines that interfere with protective immune responses.

In addition to their possible use in bioterrorism, both variola virus and a modified monkeypox virus could be further "weaponized" through the introduction of genes encoding peptide or protein toxins. Terrorists armed with such agents might release them clandestinely as aerosols in densely populated urban centers and transportation hubs, from which infected travelers might carry the disease to hundreds or thousands of locations worldwide, resulting in a global health catastrophe.

Scientific progress has thus paradoxically increased the threat of a return of smallpox. Fortunately, advances in the laboratory are also leading to the development of safer vaccines and of new antiviral drugs that could be used to prevent or treat the disease. The same medications could also be used to prevent or attenuate complications from the traditional vaccine. Scientists and public health officials should recognize the threat of a potential return of smallpox and decide on appropriate countermeasures.

Progress in developing safer smallpox vaccines

The vaccines used in the global eradication campaigns were prepared using traditional methods, in which live virus was harvested from lesions on the skin of calves. These "first-generation" vaccines proved highly efficacious in protecting against different variola virus strains, as well as against related orthopoxviruses, but

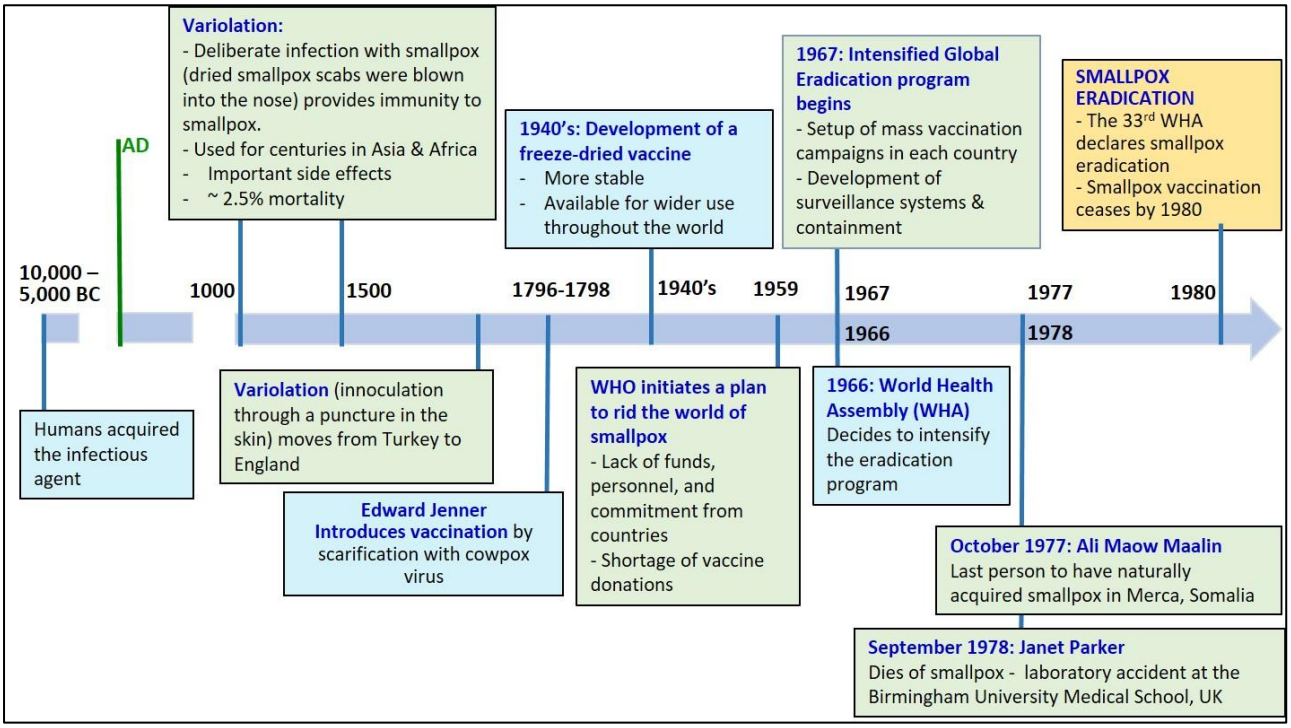


Figure 1. History of smallpox from its origin until its eradication in 1980 - the triumph over one of the humankind's greatest scourges

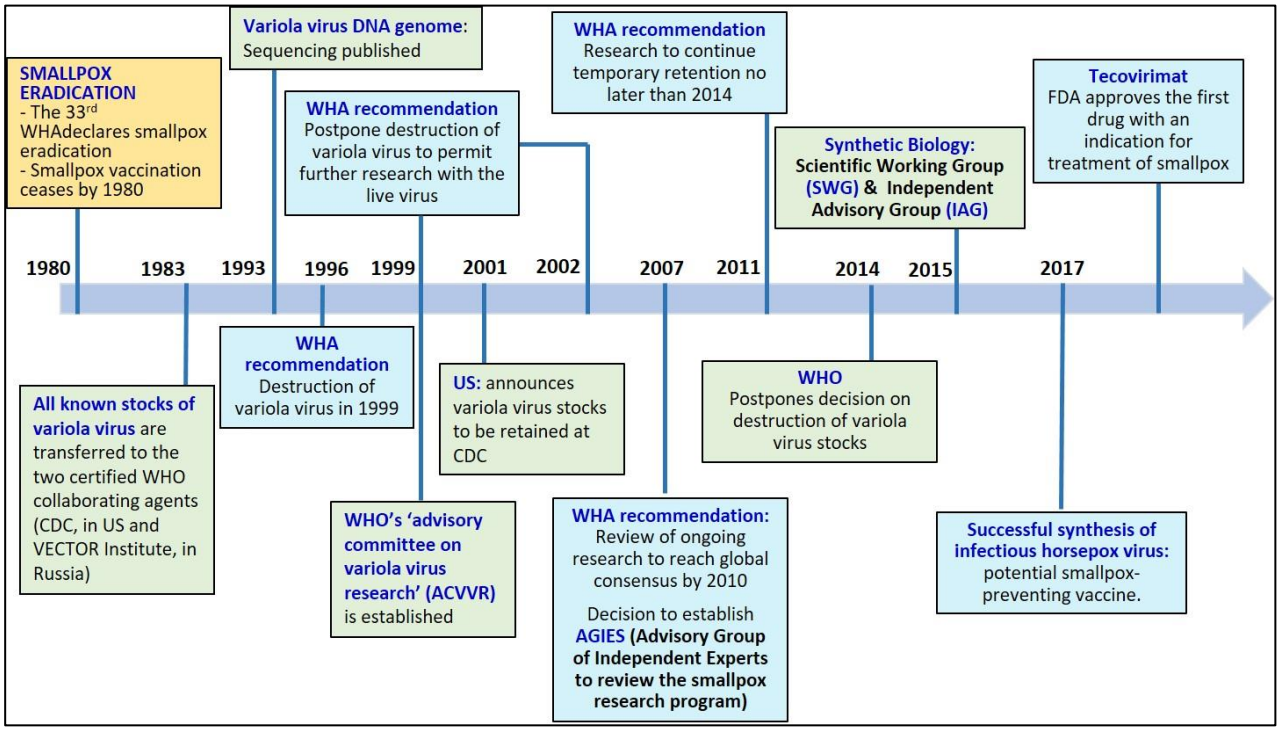


Figure 2. Timeline in the smallpox post-eradication era and the threat of reemergence of smallpox or a similar human disease in the future

they are no longer in production. Instead, they have been replaced by 2nd- and 3rd-generation vaccines, in an attempt to improve manufacturing processes, product uniformity and safety profiles (11; 12).

Tissue-culture-based 2nd-generation vaccines, prepared in tissue culture, still present the same risks of adverse events as the original vaccines, i.e. the unintentional transfer of live vaccinia virus to other sites on the body or to other persons, generalized vaccinia, eczema vaccinatum, progressive vaccinia and myopericarditis. The 2nd-generation vaccine ACAM200, now stored in large quantities in the US Strategic National Stockpile, is not recommended for young children (<12 months of age), people with exfoliate skin diseases, individuals with impaired immune systems, pregnant or breastfeeding women, patients with central nervous system disorders, heart disease, or those allergic to vaccine components. ACAM2000 is also contraindicated for persons with intimate or household contacts who have any of these conditions, due to the risk of inadvertent infection. This leaves a considerable proportion of the human population excluded from vaccination.

Third-generation smallpox vaccines, based on replication-deficient vaccinia virus strains, are not associated with adverse events, but their immune reactivity seems to be lower. Vaccines such as ®Imvamune could be given to individuals with immune deficiencies and atopic dermatitis. Work continues on 4th-generation DNA and protein vaccines.

First- and second-generation smallpox vaccines are highly effective when administered prophylactically, however they have questionable efficacy if administered to non-immunized persons who have been exposed to variola virus, but have not yet developed signs of disease.

The need for antivirals

In the late 1990s, the WHO committee overseeing variola virus research decided that protection against a possible return of smallpox would require the development of effective antiviral drugs, in addition to vaccines. Similarly, the U.S. Institute of Medicine recommended the development of at least two orally available therapeutics with different mechanisms of action. At that time, Vaccinia Immune Globulin for intravenous administration (VIGIV), containing high titers of anti-vaccinia neutralizing antibody, was the only anti-poxvirus therapeutic approved by the FDA, and only for treatment of adverse events from the smallpox vaccine.

Currently, ®TPOXX (tecovirimat, ST-246) and ®Vistide (cidofovir) are stockpiled in the USA. Tecovirimat, developed by Siga, inhibits the addition of a second outer membrane to developing orthopoxvirus virions, preventing their exit from the cell. It is orally bioavailable, with negligible toxicity. Tecovirimat proved efficacious against a wide range of orthopoxviruses (vaccinia, cowpox, ectromelia, rabbitpox, monkeypox, and variola viruses) in laboratory animals. It did not compromise the development of antibodies when combined with vaccination, and proved effective as post-exposure treatment. Tecovirimat received its first global approval on 13 July 2018 for the treatment of smallpox in adults and children weighing ≥ 13 kg under the US FDA's Animal Rule, in which marketing approval is based on efficacy in relevant animal models (13; 14).

The other stockpiled medication, cidofovir, would be much less useful in the setting of a smallpox outbreak, as it requires intravenous infusion, coupled with supplemental hydration and probenecid, to prevent nephrotoxicity. To overcome the drawbacks of an intravenous drug, a lipid conjugate of the cidofovir molecule (brincidofovir, CMX-1000) developed by Chimerix, has been synthesized. This prodrug has both improved oral bioavailability and increased antiviral potency, which are attributable to its more efficient cellular uptake, facilitated by the lipid moiety.

Brincidofovir has desirable characteristics in an emergency, considering its oral bioavailability, potent anti-variola activity and the need for only a short course of therapy. Because its mechanism of action differs from that of tecovirimat, the two drugs prove synergistic when used in combination. In addition, brincidofovir has a large clinical-safety database, no evidence of nephrotoxicity, hematologic or bone marrow toxicity, consistent PK data in healthy and infected subjects (15). Brincidofovir received orphan drug designation, and the focus of Chimerix is to get the antiviral accepted into the US Strategic National Stockpile as an emergency treatment for smallpox.

Antiviral drugs are a crucial part of preparedness for a smallpox outbreak. They can be used for

- prophylaxis, to prevent disease in non-vaccinated individuals and in populations unable to be vaccinated;
- post-exposure prophylaxis, to treat persons who have been exposed to variola virus, but are not yet ill;
- therapy for individuals who have developed smallpox disease or another zoonotic poxvirus disease, such as monkeypox or cowpox; and
- combination with vaccines to prevent smallpox disease and prevent vaccination complications.

For as long as 2nd-generation smallpox vaccines continue to be used, two antivirals with different mode of action will be needed to treat severe cases of vaccine

adverse events and to reduce the emergence of drug-resistant viruses.

References

1. Barquet N, Domingo P. 1997. Smallpox: the triumph over the most terrible of the ministers of death. Ann Intern Med 127:635-42.
2. Fenner F. 1993. Smallpox: emergence, global spread, and eradication. Hist Philos Life Sci 15:397-420.
3. Behbehani AM. 1983. The smallpox story: life and death of an old disease. Microbiol Rev 47:455-509.
4. Esparza J, Nitsche A, Damaso CR. 2018. Beyond the myths: Novel findings for old paradigms in the history of the smallpox vaccine. PLoS Pathog 14:e1007082.
5. Foster SO, Hughes K, Tarantola D, Glasser JW. 2011. Smallpox eradication in Bangladesh, 1972-1976. Vaccine 29 Suppl 4:D22-9.
6. 2002. From the Centers for Disease Control and Prevention. 25th anniversary of the last case of naturally acquired smallpox. JAMA 288:2533.
7. Koblentz GD. 2017. The De Novo Synthesis of Horsepox Virus: Implications for Biosecurity and Recommendations for Preventing the Reemergence of Smallpox. Health Secur 15:620-8.
8. Noyce RS, Evans DH. 2018. Synthetic horsepox viruses and the continuing debate about dual use research. PLoS Pathog 14:e1007025.
9. Noyce RS, Lederman S, Evans DH. 2018. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS One 13:e0188453.
10. Kabuga AI, El Zowalaty ME. 2018. A review of the monkeypox virus and a recent outbreak of skin rash disease in Nigeria. J Med Virol (Epub ahead of print).
11. Sanchez-Sampedro L, Perdiguero B, Mejias-Perez E, Garcia-Arriaza J, Di Pilato M, Esteban M. 2015. The evolution of poxvirus vaccines. Viruses 7:1726-803.
12. Jacobs BL, Langland JO, Kibler KV, Denzler KL, White SD, et al. 2009. Vaccinia virus vaccines: past, present and future. Antiviral Res 84:1-13.
13. Hoy SM. 2018. Tecovirimat: First Global Approval. Drugs 78:1377-82.
14. Grosenbach DW, Honeychurch K, Rose EA, Chinsangaram J, Frimm A, et al. 2018. Oral Tecovirimat for the Treatment of Smallpox. N Engl J Med 379:44-53.
15. Foster SA, Parker S, Lanier R. 2017. The Role of Brincidofovir in Preparation for a Potential Smallpox Outbreak. Viruses 9.

Human herpesvirus 6, a unique herpesvirus that can integrate into chromosomes, identified as a causal agent of *exanthema subitum* (roseola)

Graciela Andrei
Rega Institute for Medical Research
KU Leuven
Herestraat 49, box 1030
3000 Leuven, Belgium
graciela.andrei@kuleuven.be

Until 1986, only five human herpesviruses were known, i.e. herpes simplex virus 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV). Human herpesvirus 6 (HHV-6), in 1986 by Dharam Ablashi, Robert Gallo and Zaki Salahuddin in the Laboratory of Tumor Cell Biology at the US National Cancer Institute (1). Following this discovery, herpesvirus virology remained in the spotlight for a decade. HHV-6 was first isolated from two AIDS patients with cancer and four patients with other lymphoproliferative disorders (1). Antigenic analysis demonstrated that all six isolates were closely related, and sera from all six HHV-6 positive patients reacted immunologically with each virus isolate. HHV-6 was found to be morphologically similar to different members of the herpesvirus family though it was unique from the known human and nonhuman primate herpesviruses by host range, *in vitro* biological features, and antigenic properties. HHV-6 was originally named human B-lymphotropic virus (HBLV) because it was initially found in B-cells of infected persons and further and was shown to effectively infect freshly isolated human B cells inducing large, refractive mono- or binucleated cells with nuclear and cytoplasmic inclusion bodies. However, subsequent research revealed that CD4+ T cells were the major cell type infected by HHV-6 (2).

Primary infection with HHV-6 can either be asymptomatic or be associated with roseola. In 1988, Koichi Yamanishi's group discovered that HHV-6 was the causative agent of *exanthema subitum* or roseola in infants (3). A virus isolated from the peripheral blood lymphocytes of patients with *exanthema subitum* and cultured in cord blood lymphocytes proved to be antigenically related to human HHV-6. Morphological characteristics determined by thin-section electron microscopy, resembled those of herpesviruses. When convalescent-phase serum samples were tested against HHV-6 antigen, seroconversion was shown, indicating that the newly isolated virus was identical or closely related to HHV-6 and the causal agent for *exanthema subitum*.

By the early 1990's, it became clear that HHV-6 isolates recovered from various geographic regions and clinical settings could be stratified into two well-defined groups that differed in their molecular,

epidemiological and biological properties. At that time, there was a consensus to designate such groups as two variants of the same species: HHV-6A and HHV-6B, based on two main factors: a) the interspecies divergence of nucleic acids was low and (ii) there was a partial knowledge of differences on their epidemiology and pathogenic potential (4). However, based on subsequent research on the biology and pathogenic mechanisms of HHV-6A and HHV-6B viruses in the next years, several authors began to suggest that the two variants should be recognized as distinct viruses (4). In 2012, the herpesvirus subcommittee of an international taxonomy group proposed that the two HHV-6 variants should be formally recognized as distinct viruses. Several epidemiological, biological and immunological differences between HHV-6A and HHV-6B have been outlined and scientists and clinicians were urged to differentiate between these two viruses when possible for clarity in biological and clinical distinctions between them (4).

In 1990, Frenkel and colleagues isolated a new human herpesvirus from CD4+ T cells purified from peripheral blood mononuclear cells of a healthy individual (RK), following incubation of the cells under conditions promoting T-cell activation while the virus could not be recovered from non-activated cells (5). The RK virus proved to be genetically related but distinct to HHV-6 and was designated as the prototype of a new herpesvirus, the seventh human herpesvirus (HHV-7) (5). Another HHV-7 isolate (strain JI) was recovered from a patient with the so-called chronic fatigue syndrome (6). Subsequently, HHV-7 was isolated from the saliva of a significant number of healthy adults (7). HHV-7 has a selective tropism for CD4+ T lymphocytes, the glycoprotein CD4 being an essential element of the cellular membrane receptor for HHV-7.

The last known human herpesvirus, i.e. HHV-8, was discovered by means of molecular techniques in Kaposi's sarcoma (KS) lesions in 1994 (8) and in B-cell lymphomas of the abdominal cavity in 1995 (9). The sequences of Kaposi's sarcoma associated herpesvirus (KSHV) or HHV-8 were found to be homologous to, but distinct from, capsid and tegument protein genes of the γ-herpesviruses EBV, the prototype of the genus lymphocryptovirus (γ1-herpesvirus) and herpesvirus saimiri, the prototype of the rhadinovirus genus (γ2-herpesvirus). HHV-8 was found to be strongly associated with Kaposi's sarcoma, multicentric Castleman's disease and primary effusion lymphoma and classified as a member of the γ-herpesvirinae subfamily.

HHV-6A, HHV-6B and HHV-7 are members of the *Roseolovirus* genus in the subfamily β-herpesvirinae and are genetically related to HCMV, the type species of human β-herpesviruses. Amino acid identity is about 50% between HHV-6 and HHV-7 and approximately 90% between HHV-6A and HHV-6B (10). HHV-6A, HHV-6B and HHV-7 are widely distributed in the human population with HHV-6B primary infection occurring usually between 6 months and 3 years and later on for HHV-6A and HHV-7. The prevalence of HHV-6B and HHV-7 is over 90% in the general population but it has to be precisely determined for HHV-6A.

Although most of the infections caused by HHV-6 are asymptomatic, though HHV-6B primary infection is sometimes associated with *exantema subitum* (rosela infantum or sixth disease). In some cases, HHV-6B primary infection may be linked to more severe conditions such as meningoencephalitis, respiratory or digestive syndromes, and macrophage activating syndrome. Symptomatic HHV-6A primary infections are less well-documented compared to those caused by HHV-6B. Clear primary infections with HHV-7 are poorly documented, except for a few potential cases of *exanthema subitum*, HHV-6A and HHV-6B also cause opportunistic infections in patients with impaired immunity. For instance, the most common presentations include encephalitis, neurocognitive impairment, and delayed engraftment in hematopoietic stem transplant recipients. As summarized in Table 1, diseases associated with HHV-6A and HHV-6B infections depend on the status of the viral infection.

HHV-6A and HHV-6B replicate efficiently in human cord blood mononuclear cells and peripheral blood mononuclear cells as well as in various T-cell lines but the viruses can also establish latency in these cell lines. Recent work from Benedikt Kaufer's lab demonstrated that the cellular nuclear domain 10 (ND10) complex, as part of an intrinsic antiviral response plays an important role in suppressing HHV-6A lytic replication and the silencing of the virus genome in latently infected cells (11).

HHV-6 and HHV-7 share with HCMV several genes encoding products involved in viral replication, which serve as targets for antivirals. Therefore, many drugs active against HCMV show some activity in vitro against HHV-6 and HHV-7, including ganciclovir, foscarnet and cidofovir. In contrast, acyclovir, used to treat α-herpesviruses, is not effective *in vitro* against HHV-6 or HHV-7. Mark Prichard's team at University of Alabama developed a new method to assess antiviral activity against all DNA viruses using an automated format and qPCR to measure viral DNA accumulation (12).

Among FDA approved drugs, foscarnet displayed the highest selectivity index for HHV-6B. The pipeline drugs filociclovir (cyclopropavir) and CMX-001 (brincidofovir) showed promise with relatively low EC_{50}'s (50% effective concentration). However, it is unknown how well those drugs are able pass the blood-brain barrier. Valganciclvoir, the oral prodrug form of

ganciclovir, has some efficacy during HHV-6 reactivations in bone marrow transplant patients, but is not as efficacious as for HCMV. As for HCMV, HHV-6B GCV escape mutants have been reported in transplant recipients. The ganciclovir-resistant viruses have alterations in the U69 protein kinase, homologous to the HCMV UL97 protein kinase, required for phosphorylation of GCV as well as in the DNA polymerase, U38 (13). No antiviral agents have been officially approved for the treatment of HHV-6 and there is no consensus for initiating antiviral treatment for HHV-6A and HHV-6B, as studies on antiviral treatment are limited to a few clinical case patients. There is a need for developing and conducting well-designed studies to validate treatments for infections caused by HHV-6A, HHV-6B and HHV-7.

A unique feature of HHV-6A and HHV-6B is their ability to integrate into human chromosomes as a form of latency occurring in approximately 1% of the general worldwide population. If the virus integrates in a germline cell, the offspring are born with a full copy of the HHV-6 genome in every nucleated cell. HHV-6 is unique among human herpesviruses since it specifically and efficiently integrates into telomeres of chromosomes during latency rather than forming episomes. The telomeric repeats of HHV-6A (which are identical to the human telomere sequences and are located at the end of the viral genome) are dispensable for virus replication, but are crucial for integration and maintenance of the virus genome in latently infected cells (14).

Chromosomal integration of HHV-6 (ciHHV-6) was originally considered to be a "dead end" form of latency but integrated HHV-6 is now known to be capable of producing virions. (15). Mothers carrying ciHHV-6 were demonstrated to infect their non-ciHHV-6 children through the placenta (16). Also, a group led by Peter Medveczky at the University of South Florida demonstrated that the ciHHV-6 virus can be activated by stimulating the integrated cells with chemicals (17).

The most convenient method to determine if a patient has ciHHV-6 is to perform a whole blood quantitative PCR DNA test for HHV-6. An HHV-6 viral load of >500,000 copies per ml in the absence of an acute illness can then be attributed to ciHHV-6. The viral load of a non-ciHHV-6 individual can occasionally be >500,000 copies per ml of whole blood in cases of extreme illness – during encephalitis or an acute episode of graft-versus-host disease or extreme drug hypersensitivity. A suspicion for ciHHV-6 can be confirmed by testing the patient's parents (at least one of the two would have a high positive result). Other tests such as FISH analysis can confirm ciHHV-6. PCR can also be done on fingernails or hair follicles of ciHHV-6 individuals and only ciHHV-6 positive persons would have a positive PCR test on a fingernail or hair follicle. Das and Munoz proposed to determine the ciHHV-6 status of the donors/recipients before organ transplant using a single pre-transplant qPCR test on whole blood, and carefully monitor patients with ciHHV-6 or ciHHV-6+ organs for signs of active HHV-6 infection (18). Evaluation of plasma PCR for HHV-6 is not very useful to assess viral reactivation in individuals with ciHHV-6 due to the high background level of DNA from lysed cells and only assays that measure viral mRNA can reveal whether the virus is replicating.

Table 1. Diseases associated with HHV-6 in function of the status of viral infection

Congenital infection
- Impairment of neurophysiological development in infants*
Primary infection
- Exanthema subitum (roseola infantum or sixth disease) for HHV-6B and to be confirmed for HHV-6A
- Fever
- Seizures
- Mild respiratory and digestive symptoms
- Thrombocytopenia
- Viral-like syndrome with mononuclear cells in blood
- Encephalitis
- Hepatitis, colitis
- Macrophage activating syndrome*
- Temporal lobe epilepsy*
Reactivation
- Fever
- Skin rash
- Thrombocytopenia, leukopenia, anemia
- Myelosuppression
- Encephalitis, neurocognitive deficit
- Hepatitis, colitis, gastroenteritis
- Retinitis
- Pneumonitis
- Drug-induced hypersensitive syndrome (DRESS)
- Temporal lobe epilepsy*
- Graft-versus-host disease (GVHD)*
- Thrombotic microangiopathy*
Chronic infection
- Multiple sclerosis*
- Hashimoto's thyroiditis*
- Myocarditis, chronic cardiomyopathy*
- Rapid progression towards AIDS in HHV-6A infected HIV-positive patients*
- Alzheimer's disease*
Chromosomal integration
- Increased risk for angina pectoris*
*Suggested association to be confirmed Adapted from (10)

There has suddenly been a lot of interest in HHV-6 by the scientific community, as HHV-6 along with HHV-7, appear to be central to the progression of Alzheimer's disease (AD). Two recent papers in *Neuron* provide new evidences of a link between herpesviruses and AD. Using transgenic AD mice that express human amyloid-β (Aß) and 3D human neural cell culture, Eimer and colleagues (19) demonstrated that infection with HHV-6A, HHV-6B or HSV-1 dramatically accelerated the deposition of Aß peptide. Aβ oligomers were shown to bind herpesvirus surface glycoproteins, accelerating β-amyloid deposition and leading to protective viral entrapment activity in 5XFAD mouse and 3D human neural cell culture infection models. This protective viral entrapment of the reactivated pathogens prevents these viruses from infecting other brain cells. However, this 'protective' effect leads to the formation of Aβ fibrils and then Aβ plaques (a pathological hallmark of Alzheimer's disease).

The team led by Joel Dudley, based at Mount Sinai, New York (20), inspected AD brain samples for 515 known human viruses. Noticeably, they discovered that in AD brains, HHV-6A and HHV-7 transcripts were increased, although there was also evidence of over-representation of HSV-1 and HSV-2 transcripts. The researchers also found higher levels of HHV-6A (and HSV-2) DNA, pointing to active viral replication in AD brain. Moreover, the presence of HHV-6A and HHV-7 proved to be significantly associated with severity of dementia and brain pathology. It should be noted that, HHV-6 and HHV-7, similarly to HSV-1 and HSV-2, are well-known causes of viral encephalitis, in particular in immunocompromised individuals, and have also been linked to demyelinating brain disease. These studies have brought herpesviruses to the front as crucial contributors in the development of AD, which will increase hopefully the interest in the search of novel antivirals against herpesviruses.

References

1. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, et al. 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234:596-601.
2. Takahashi K, Sonoda S, Higashi K, Kondo T, Takahashi H, et al. 1989. Predominant CD4 T-lymphocyte tropism of human herpesvirus 6-related virus. J Virol 63:3161-3.
3. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, et al. 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1:1065-7.
4. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, et al. 2014. Classification of HHV-6A and HHV-6B as distinct viruses. Arch Virol 159:863-70.
5. Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, et al. 1990. Isolation of a new herpesvirus from human CD4+ T cells. Proc Natl Acad Sci U S A 87:748-52.
6. Berneman ZN, Gallo RC, Ablashi DV, Frenkel N, Katsafanas G, et al. 1992. Human herpesvirus 7 (HHV-7) strain JI: independent confirmation of HHV-7. J Infect Dis 166:690-1.
7. Wyatt LS, Frenkel N. 1992. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. J Virol 66:3206-9.
8. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, et al. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865-9
9. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 332:1186-91.
10. Agut H, Bonnafous P, Gautheret-Dejean A. 2016. Human Herpesviruses 6A, 6B, and 7. Microbiol Spectr 4.
11. Sanyal A, Wallaschek N, Glass M, Flamand L, Wight DJ, Kaufer BB. 2018. The ND10 Complex Represses Lytic Human Herpesvirus 6A Replication and Promotes Silencing of the Viral Genome. Viruses 10.
12. Keith KA, Hartline CB, Bowlin TL, Prichard MN. 2018. A standardized approach to the evaluation of antivirals against DNA viruses: Polyomaviruses and lymphotropic herpesviruses. Antiviral Res 159:122-9.
13. Agut H, Bonnafous P, Gautheret-Dejean A. 2017. Update on infections with human herpesviruses 6A, 6B, and 7. Med Mal Infect 47:83-91.
14. Wallaschek N, Sanyal A, Pirzer F, Gravel A, Mori Y, et al. 2016. The Telomeric Repeats of Human Herpesvirus 6A (HHV-6A) Are Required for Efficient Virus Integration. PLoS Pathog 12:e1005666.
15. Endo A, Watanabe K, Ohye T, Suzuki K, Matsubara T, et al. 2014. Molecular and virological evidence of viral activation from chromosomally integrated human herpesvirus 6A in a patient with X-linked severe combined immunodeficiency. Clin Infect Dis 59:545-8.
16. Hall CB, Caserta MT, Schnabel KC, Shelley LM, Carnahan JA, et al. 2010. Transplacental congenital human herpesvirus 6 infection caused by maternal chromosomally integrated virus. J Infect Dis 201:505-7.
17. Arbuckle JH, Medveczky MM, Luka J, Hadley SH, Luegmayr A, et al. 2010. The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro. Proc Natl Acad Sci U S A 107:5563-8.

18. Das BB, Munoz FM. 2017. Screening for chromosomally integrated human herpesvirus 6 status in solid-organ donors and recipients. J Heart Lung Transplant 36:481.
19. Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, et al. 2018. Alzheimer's Disease-Associated beta-Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. Neuron 99:56-63 e3.
20. Readhead B, Haure-Mirande JV, Funk CC, Richards MA, Shannon P, et al. 2018. Multiscale Analysis of Independent Alzheimer's Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus. Neuron 99:64-82 e7.

The discovery of RNAi and its role as an antiviral mechanism

Ben Berkhout
Laboratory of Experimental Virology, Amsterdam UMC,
University of Amsterdam,
The Netherlands
b.berkhout@amc.uva.nl

The RNA interference (RNAi) mechanism for gene suppression was discovered in 1998 in the nematode *Caenorhabditis elegans* upon introduction of short double-stranded RNA segments (1). These RNA molecules are processed in the cell and trigger degradation or translational inactivation of mRNAs with a complementary nucleotide sequence. This discovery of RNA-mediated regulation of gene expression at the post-transcriptional level won its discoverers Andrew Fire and Craig Mello the 2006 Nobel Prize in Physiology. Key components of the RNAi mechanism are well-conserved among different kingdoms, from plants to animals. In mammals, the RNAi mechanism constitutes an additional level of regulated gene expression performed by a large set of regulatory small double-stranded microRNAs (miRNA). These miRNAs mature with the help of the cellular endonucleases, Drosha and Dicer, and subsequently interact with the Argonaute protein as the key mRNA-slicer component of the RNA-induced silencing complex (RISC).

Among other things, RNAi was shown to act as innate mechanism to suppress the activity of transposons or "jumping" genes, which have been linked to induction of diseases like Duchenne muscular dystrophy in humans. But RNAi was also proposed as antiviral mechanism, initially in plants but later on in mammals, although the latter claim remained disputed for some time.

The relevance of the RNAi concepts for the field of virology was actually obvious from the start. Sometime before that Nobel Prize discovery, early findings from the plant virus field already hinted at a novel RNA-based mechanism called RNA silencing that controls gene expression at the post-transcriptional level. A key finding was the accumulation of characteristic short interfering RNAs (siRNA) in virus-infected plants (2). RNA silencing, which uses the cellular RNAi components for function, plays an important role in mediating cellular defence against infection by plant viruses. Plants defective in RNA silencing/RNAi are more susceptible to infection by viral pathogens (3). Plant viruses in turn have evolved potent anti-RNAi mechanisms by expression of proteins or transcripts with RNAi-suppressor activity (4, 5). The importance and conservation of the RNAi antiviral response is underscored by the observation of trans-kingdom suppression with a human viral protein in plants and vice versa (6, 7). RNAi is also a major antiviral mechanism in insects like Drosophila melanogaster (8), but it remained uncertain for quite some time if it executed a similar function in higher eukaryotes (9). As said, the complete machinery is present in mammalian cells to execute the critical miRNA program.

Furthermore, we also know that RNAi can be induced by synthetic siRNAs against viral pathogens, but this does not prove that the RNAi mechanism executes an essential antiviral program in real life. One reason for not immediately accepting this scenario was that mammals already have two robust antiviral defense systems: the innate interferon and adaptive immune system. Another reason was the absence of the typical siRNA signature in early studies of virus-infected cells (10).

But the tide has recently turned in favor of RNAi-mediated antiviral immunity in mammals. First, a study on virus-infected embryonic stem cells, which lack a functional interferon system, revealed the characteristic virus-specific siRNA signature that decreases in abundance upon differentiation of the cells (11). Second, such a viral siRNA signature was also apparent in cells infected with a mutant virus that does not encode the RNAi suppressor protein (12). Third, a mutant virus lacking the RNAi suppressor protein does not replicate, but can be rescued in RNAi-deficient cells (11). Thus, the cellular RNAi program could be linked to a potent antiviral response.

Not unimportantly, these studies also provided the reasons why the mammalian antiviral RNAi had remained obscure for so long. Virulent virus strains encode potent RNAi suppressors that mask the siRNA signature and a 10-fold higher siRNA level is scored in undifferentiated pluripotent cells that express less interferon. The masking effect was confirmed in a

recent study on the human enterovirus 71 by comparing the wild-type virus to a mutant lacking the 3A suppressor protein (13). This mutant showed a severe replication deficiency that could be overcome by Dicer-deficiency. To exclude an interferon effect, Qiu et al. used mouse cells lacking a functional interferon receptor and drug that specifically blocks this pathway (13). Thus, mammals seem to have developed at least three antiviral mechanisms: RNAi in undifferentiated cells on top of the innate and adaptive immune systems. Recent evidence indicates that invertebrates have also evolved multiple antiviral pathways (14), yet plants seem to rely exclusively on the RNAi mechanism.

RNAi was developed into a powerful research tool that allows scientists to suppress the expression of specific genes with synthetic double-stranded RNA. More recently, CRISPR-Cas methods have surpassed the RNAi technique, especially if a true knock-out phenotype is required. RNAi was also developed as a new therapy approach for a variety of diseases, including viral infections. RNA viruses and the transcripts of DNA viruses can be targeted, but one could also consider inhibition of critical cellular co-factors. One could address acute and persistent infections, e.g. caused by the respiratory syncytial virus or human immunodeficiency virus (15, 16). Because the attack is sequence-specific, one can expect viral escape to occur by selection of a single point mutation, and this was indeed observed in prolonged HIV culture experiments. But similar to the clinical success of current anti-HIV regimens that consists of multiple drugs, combinatorial RNAi approaches may be developed to prevent viral escape (17).

Not uncommon, clinical translation of the RNAi knowledge turned out to be a rocky path because of unexpected side effects, the requirements to shield the therapeutic RNA from degradation and to achieve targeting of the right organ and cellular uptake. But recently the first drug (Onpattro produced by Alnylam) was approved to treat a hereditary form of nerve degeneration. We still have to await the first antiviral RNAi drug.

References

1. Fire, A., et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806-811 (1998).
2. Hamilton, A.J. & Baulcombe, D.C. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286, 950-952 (1999).
3. Voinnet, O., Pinto, Y.M. & Baulcombe, D.C. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc Natl Acad Sci U S A 96, 14147-14152 (1999).
4. Hemmes, H., Lakatos, L., Goldbach, R., Burgyan, J. & Prins, M. The NS3 protein of Rice hoja blanca tenuivirus suppresses RNA silencing in plant and insect hosts by efficiently binding both siRNAs and miRNAs. RNA 13, 1079-1089 (2007).
5. Andersson, M.G., et al. Suppression of RNA interference by adenovirus virus-associated RNA. J Virol 79, 9556-9565 (2005).
6. Schnettler, E., et al. The NS3 protein of rice hoja blanca virus complements the RNAi suppressor function of HIV-1 Tat. EMBO Rep 10, 258-263 (2009).
7. Qian, S., et al. HIV-1 Tat RNA silencing suppressor activity is conserved across kingdoms and counteracts translational repression of HIV-1. Proc Natl Acad Sci USA 106, 605-610 (2009).
8. Czech, B., et al. An endogenous small interfering RNA pathway in Drosophila. Nature 453, 729-731 (2008).
9. Cullen, B.R., Cherry, S. & tenOever, B.R. Is RNA interference a physiologically relevant innate antiviral immune response in mammals? Cell Host Microbe 14, 374-378 (2013).
10. Parameswaran, P., et al. Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. PLoS Pathog 6, e1000764 (2010).
11. Maillard, P.V., et al. Antiviral RNA interference in mammalian cells. Science 342, 235-238 (2013).
12. Li, Y., Lu, J., Han, Y., Fan, X. & Ding, S.W. RNA interference functions as an antiviral immunity mechanism in mammals. Science 342, 231-234 (2013).
13. Qiu, Y., et al. Human virus-derived small RNAs can confer antiviral immunity in mammals. Immunity 46, 992-1004 e1005 (2017).
14. Poirier, E.Z., et al. Dicer-2-Dependent Generation of Viral DNA from Defective Genomes of RNA Viruses Modulates Antiviral Immunity in Insects. Cell Host Microbe 23, 353-365 e358 (2018).
15. DeVincenzo, J., et al. Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). Antiviral Res 77, 225-231 (2008).
16. Knoepfel, S.A., Centlivre, M., Liu, Y.P., F., B. & Berkhout, B. Selection of RNAi-based inhibitors for anti-HIV gene therapy. World Journal of Virology 1, 79-90 (2012).
17. Ter Brake, O., Konstantinova, P., Ceylan, M. & Berkhout, B. Silencing of HIV-1 with RNA interference: a multiple shRNA approach. Mol Ther 14, 883-892 (2006).

EMERGING DISEASES: A VIEW FROM THE SOUTH

Interventions to control of *Aedes aegypti* in Latin America and the Caribbean: systematic review, qualitative study with in-depth interviews with experts and policy dialog with decision-makers.

Ariel Bardach MD, María Belizán MSc, Agustín Ciapponi, MD
Institute for Clinical Effectiveness and Health Policy - National Scientific and Technical Research Council (IECS-CONICET)
Buenos Aires, Argentina
abardach@iecs.org.ar

The *Aedes aegypti* (AA) mosquito is the main vector transmitting diseases like dengue, chikungunya, Zika and yellow fever. In Latin America and the Caribbean, dengue disease burden increased in the recent years, and the co-existence of two diseases in 2013 (dengue and chikungunya) and 2014 (dengue and Zika) poses a new challenges in Latin America, as expressed by the World Health Organization (WHO) (1). WHO has identified the most important macro determinants for the spreading social, demographic and political circumstances, like the absence of effective programs, the rise in numbers of populations, bad sanitary conditions, deterioration of health systems and the worsening of the environmental situation (2).

The objective of this experience was to systematically review interventions used in Latin-America and worldwide, to identify the experiences, barriers and facilitators in the implementation of interventions for the control of AA in Latin America and The Caribbean (LA&C), to seek experts' opinions regarding the action needs to prevent and control infections as Zika, dengue and chikungunya, and to do a deliberative policy dialogue in which decision makers could discuss these matters. Three manuscripts were generated, the policy dialogue report has been accepted for publication in Cadernos de Saúde Pública, the systematic review is currently under peer review in PLoS Neglected Tropical Diseases, and the qualitative study is also under peer review in the former journal.

The systematic review (SR) summarized the information identified in the LA&C region regarding the interventions for vector control over 15 years. We conducted a comprehensive literature review in main databases and an assessment of the methodological quality of the studies included. Most information came from Brazil, Argentina, Cuba, Mexico and Peru. The randomized control trials identified were of moderate or low methodological quality. In this region, we found several interventions that showed to be effective in varying degrees. Insecticide-treated materials may reduce the entomological indices, both in experimental and quasi-experimental studies, although in many trials not reaching statistical significance. The evidence also indicated that health education and community engagement, which assess knowledge and prevention-related attitudes, have proven to be an effective intervention. Other interventions have also proven to be useful.

For this systematic review, we searched literature databases like MEDLINE, EMBASE, CENTRAL, SOCINDEX and LILACS from January 2000 to September 2016. We included experimental, quasi-experimental and observational studies; economic assessments and qualitative studies. We assessed the incidence and morbidity/mortality of AA-related diseases and the following entomological indices: Breteau (containers), House, and Pupae per Person. We used GRADE methodology for assessing quality of evidence.

Of a total number of 1,826 complete-text assessed studies, 75 were included, and 9 cluster randomized clinical trials were included for meta-analysis. We did not identify any interventions supported by a high certainty of evidence. Health education and community engagement may probably reduce the entomological indices, as well as insecticide-treated materials. Likewise, the use indoor residual spraying and the management of containers may also improve them, although the confidence interval includes both a reduction and an increase in the indices. We found low certainty of evidence supporting the use of ovitraps or larvitraps, and integrated epidemiological surveillance to improve entomological indices and decrease the incidence of dengue. The degree of implementation of these interventions was variable in the different countries and settings.

All in all, we found that, although many interventions were carried out in this region, only a handful provided scientifically sound evidence about their effectiveness. Most did not extend to whole cities and were not sustained beyond two years. It remains important to engage and educate the community, to work with the authorities to achieve the implementation of integrated actions between the health sector and other sectors at the national and regional level.

The second manuscript, that was part of this endeavor, is entitled "Experiences, barriers and facilitators in the implementation of AA control interventions in Latin America and the Caribbean: qualitative study". The study used inductive inquiry consistent with the grounded theory approach (3). It included in-depth semi-structured interviews with 19 stakeholders from 9 countries (Argentina, Brazil, Colombia, Cuba, Honduras, Panamá, Paraguay, Peru and Puerto Rico). Data collection was performed from July 2016 to July 2017. We undertook thematic analysis, and then we developed matrices to facilitate comparisons across the transcript materials and to retain the context of the data. Finally, we abstracted and interpreted data.

From the interviewees' point of view, initiatives to control the infections transmitted by the mosquito are usually started as a reaction to outbreaks. Interventions are mainly oriented to eliminate adult mosquito forms. Barriers for the successful implementation of interventions were classified in six levels (Biophysical, Health System, other factors such as Health, Governance, Community and Public Opinion). Regarding the biophysical conditions as climate conditions, and natural and built environment, a barrier underlined was the unpredictable occurrence of outbreaks due to the climate change. Main barriers identified within the Health System were the lack of material resources and capable personnel; that public health priorities are hardly ever included in vector control, programs discontinuity, lack of preventable initiatives and lack of budget allocation. Other factors different from health with a great impact in prevention deal with garbage collection, education or built environment, and traditionally do not prioritize public health problems and also may lack of resources. Governance level barriers relate to discontinuity of programs, lack of budget allocation, the absence of effective functional link between political decisions and experts' recommendations, and lack of programmatic coordination with local government. At community level, the lack of information and education conspires against the risk perception and awareness; moreover, there is a perceived reluctance to implement preventive actions. Regarding the public opinion, we identified that media messages may operate for or against public policies.

There is a need to strengthen the implementations and continuity of vector control program in Latin America. This study contributes to identify main barriers that may inform priority actions in order to improve the prevention of vector-borne diseases.

Lastly, we undertook a policy dialogue that was described in the manuscript "Workshop on the prioritization of interventions for the control of the AA mosquito in LA&C: policy dialogue". We narrate the experience of carrying out a workshop between decision makers and experts from LA&C on the prioritization of interventions and research for the control of the AA mosquito. Through a deliberative process done in collective instances, assisted by the results of the aforementioned projects, a consensus list of priorities for implementation of vector control strategies and regional development of key research lines was developed. It was agreed that the best strategy is integrated vector management or Integrated Management Strategy, disaggregated into: a) Chemical control; b) Biological control; c) Environmental management; d) Community participation; and e) Integrated surveillance. The fundamental and non-transferrable stewardship role of the state and inter-sectoral coordination between state agencies and civil society actors was highlighted.

It was proposed to prioritize the following research lines: to analyze the vector capacity of AA and associated factors; to assess community components of interventions; the incorporation of technology to vector control and monitoring; the most efficient modes of integrated surveillance; entomological indicators with better predictive capacity and resistance to insecticides.

The methodology of the "policy dialogue" allowed us to disseminate, validate and enrich the results of the previous instances of the research within the framework of which it was convened. This work dynamics guided future lines that would allow to offer a more consistent evidence on the vector control of AA in LA&C. Thus raised, this dialogue between decision makers and experts was translated into two main priorities for the region: strategies for vector control and a research agenda, which allows for pooling efforts, enhancing capacities, orienting resources towards these intervention, and research priorities. The participants, as well as the representatives of the Pan American Health Organization (PAHO), welcomed this initiative and undertook to follow up on the proposals that emerged, as well as, where possible, establish a network of collaboration and active communication to facilitate the objective.

This collective, deliberative and collaborative methodology can be not only enriching in the implementation of strategies, but it is also an efficient way to triangulate research techniques and strategies to obtain more solid results and broader consensus, in this case, on priorities in the field of vector control of AA in this region.

References

1. Pan American Health Organization (PAHO) and World Health Organization (WHO). Tool for the Diagnosis and Care of Patients with Suspected Arboviral Disease. 2017.
2. Chang MS, Christophel EM, Gopinath D, Abdur RM. Challenges and future perspective for dengue vector control in the Western Pacific Region. Western Pacific surveillance and response journal: WPSAR 2011; 2(2): 9.
3. Glaser BG SA. The Discovery of Grounded Theory: Strategies for Qualitative Research: Transaction Publishers. 283. 2009.

Zika Virus Epidemic in Brazil: then, now, and the future

Cybele García
Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN-Consejo Nacional de Investigaciones Científicas y Técnicas, 1428 Buenos Aires, Argentina
cybele.garcia@gmail.com

Zika virus (ZIKV) was first isolated in 1947 in Uganda and has been circulating in human populations from West Africa and Asia since the mid-1950s. Little attention was devoted to ZIKV, probably due to the mildness of its symptoms and the fact that it was geographically restricted, being a neglected disease for most of its history. However, this started to change in 2013 when the landing of ZIKV in French Polynesia was associated with increased rates of Guillain–Barre syndrome, an autoimmune condition that affects the peripheral nervous system and can be triggered by infections (1; 2).

In 2015, only two years after the Polynesian outbreak, an unprecedented epidemic was registered in Brazil. There, ZIKV became an extraordinary health burden due to the novel correlation between the infection of pregnant women and severe brain malformations in newborns. Brazil registered an average of 156 cases of microcephaly per year between 2010 and 2014 — but by the end of 2015, the number had risen 20-fold. Pernambuco was the first state to report an unusual number of microcephaly cases in newborns, with 10 times more reports than the average for the entire country in the previous five years. The number of reported cases continued to increase. Out of 10,867 registered between November 2015 and the end of 2016, 7,023 were located in the Northeast — and of the 10 states with the highest number of microcephaly cases related to ZIKV infection, nine were from the Northeast (3).

Evidence of a causal link between ZIKV and microcephaly was formally accepted in April 2016. The mechanism by which ZIKV crosses the placenta is still unclear, but its neurotropism and ability to destroy neural cells have been clearly demonstrated. ZIKV infection induces abnormal mitosis and apoptosis of human neural progenitor cells (NPCs), causing disruptive lesions in the fetal central nervous system (CNS). NPCs are the primary target of the ZIKV, and this may partly explain the high number of abnormalities seen in the CNS and detected by neuroimaging examinations (4). However, microcephaly is just one of the many ZIKV-related birth complications, now referred to as congenital Zika syndrome (CZS). About 20% of the children born with CZS have normal head sizes, and children born without any abnormality were later found to have developed brain damage and developmental problems. Evidence is now accumulating that subtle, but destructive brain, ear and ocular injuries can also occur in infants with a normal head size at birth. The expanding range of anomalies may be difficult or impossible to diagnose prenatally, and there remains uncertainty about the long-term neurocognitive effects of ZIKV exposure. In addition, ZIKV infections during any trimester of pregnancy may result in CZS, even if asymptomatic. At present, ZIKV is considered a new member of the teratogenic congenital infections (5; 6).

The ZIKV outbreak in Brazil triggered large mobilization of the technical and scientific apparatus, society, and the media. Mandatory notification of ZIKV commenced in February 2016; however, some of the reported ZIKV cases were not confirmed, and misdiagnoses between dengue, ZIKV, and chikungunya might have occurred early in the epidemic. In addition, accurate laboratory diagnosis was challenging due to the short viremia period, and to cross-reactivity among dengue and ZIKV in serodiagnostics.

The unprecedented nature of the disease raised doubts and expectations in the population. Fear and anxiety became part of the daily lives of pregnant women and their families especially in Brazil, but all South American countries were affected. Despite a notable increase in scientific focus, the path of CZS leaves some puzzling questions unanswered.

From a phylogenetic point of view, there are two main lineages of ZIKV: African and the Asian lineages. It is estimated that the Asian lineage entered the Americas in 2013, and since then the ZIKV genome has experienced several mutations. The functional implications of these genomic changes are an active topic of study, while the rapid geographic dispersion was attributed to the ubiquitous presence of its vector: the mosquitoes of the genus *Aedes*. Moreover, the finding that ZIKV is also sexually transmitted not only expanded the transmission channel but also broadened the geographic reach to no tropical countries.

By the end of 2017, ZIKV infections had been reported in approximately 84 countries and across all the main continents; a total of 23 countries reported cases of microcephaly and other congenital anomalies potentially caused by ZIKV infection. Notably, 95% of all CZS cases in 2015 and 2016 were reported in Brazil, of which more than 75% were from the Northeast region of Brazil (3).

The link between ZIKV and microcephaly spurred some government leaders in Latin America to suggest that women should postpone having babies for a few years, ignoring the fact that, in the region, more than one-half of the pregnancies are not intended; rates of sexual violence are high; and while abortion is legally

restricted, women seek clandestine abortions, or self-induce the termination of pregnancy, mostly under unsafe conditions. In Brazil, abortion is allowed to save a woman's life or in cases of rape. In the absence of a treatment that prevents ZIKV from crossing the placenta, and of a legal abortion policy, women have no legal framework to exercise the choice of continuing or terminating a pregnancy following a ZIKV infection. When presenting with an ultrasound showing problems in fetal development; she would have to carry on the pregnancy and face the risk of delivering a child with congenital problems, or put her own health at risk by inducing an abortion.

Although ZIKV outbreaks were registered in similar proportions across many geographic areas, informed cases of CZS have been clustered, indicating an asymmetric distribution among human populations. This peculiarity opened several questions: why does the symptom distribution of such a widely spread virus vary so significantly by region and apparently also over time? Are there specific factors prevalent in the Northeast region or among certain populations that increased the severity of ZIKV during pregnancy? Are there significant cofactors in that population that help to determine the observed pattern? Currently there is no evidence that such factors exist.

The global biomedical research reaction to understand CZS was remarkable. From these coordinated efforts, the necessity to strengthen and support epidemiological surveillance, which can not only help direct prevention but also provide information of scientific value for the future, became evident. ZIKV infection numbers dropped dramatically, and CZS patients born in 2017 are almost inexistent in South America. A plausible explanation for this pattern may be the immunization of the vast majority of the at-risk population. However, specific serological analyses remain necessary to confirm this.

In addition to genetic background, environmental context can be a factor that affects the immune status and response to ZIKV infection (7). In human populations, environmental factors that impact immune status are strongly related to socioeconomic position. Housing conditions, malnutrition, and coinfection susceptibility amongst other variables differ by region as well as by population and may contribute to differing responses to ZIKV infection.

When analyzing this relationship for Recife (Pernambuco), a city that was severely affected, it was found that cases of reported microcephaly in 2015 and 2016 were largely concentrated in areas with more impoverished living conditions (8). Communities with lower socioeconomic status have more degraded housing, which facilitates ZIKV transmission by enhancing mosquito reproduction and human access. In fact, dengue virus prevalence in the same area of Brazil has been found to be strongly related to living conditions. Although this ecological vector-based hypothesis provides a fertile area for further study and could describe a potential increase in infection cases, it may fall short of explaining why geographic regions, which are characterized by similar levels of ZIKV infection and pregnancy, are impacted differently by CZS. Two other hypotheses, which associated the CZS epidemic with the use of the larvicide pyriproxyfen or vaccine administration during pregnancy, were dismissed by a recent case-control study in Recife.

Coinfections with other flaviviruses transmitted by the same vector (*Aedes* mosquito) could play a role in the immune response to ZIKV infection. Dengue virus, for example, is endemic in South America, and its seroprevalence is found in more than 90% of the population within CSZ cluster regions. It is known that after initial infection with dengue virus, posterior exposures to other dengue serotypes could result in life-threatening complications due to the phenomenon known as antibody-dependent enhancement. Antibodies do protect against infection with the same serotype but not when infected with any one of the other three serotypes. The antibody-dependent enhancement phenomenon describes a process in which antibodies generated during the first infection bind to the new virus but fail to efficiently neutralize it. Instead, the phenomenon facilitates virus entry to target cells. Since ZIKV is structurally close to dengue virus, several studies have examined their cross-reactivity. The studies demonstrated, that antibodies generated after initial dengue infection, bind with high levels of affinity to ZIKV, significantly enhancing pathogenesis and ultimately failing to counteract the virus. The mechanism for this viral enhancement is thought to be mediated by immunoglobulin G engagement of Fc gamma receptors (FcγR), in agreement with other antibody-dependent enhancement examples.

Nutrition is an additional cofactor tied to both socioeconomic and demographic conditions, which are positively correlated with income as well as development levels, and has been demonstrated to interfere with immunity. Malnutrition due to the insufficient intake of nutrients leads to impairment of immune function for several innate and adaptive pathways. Further studies may explore the link between these specific cofactors and cases of birth defects produced after ZIKV infection.

To date, no drugs have been approved for the treatment of ZIKV or other *Flavivirus* infections. Treatment of ZIKV infection primarily consists of supportive measures and rest because ZIKV is usually a self-limiting infection. However, persistent infection in the male reproductive tract and sexual transmission, ability to cross the placenta during pregnancy and infect the developing fetus causing congenital malformations, and association with Guillain-Barré

syndrome in adults are unusual clinical features of ZIKV. Although the incidence of ZIKV cases has declined, the threat is not gone. *Ae. Aegypti* reigns in Brazilian cities and currently transmits dengue, Zika, chikungunya, and Mayaro virus, and is competent to transmit the strains of the yellow fever virus circulating in the country; indeed, in 2018 urban yellow fever was recorded in Brazil, 76 years after urban transmission had been successfully eliminated. A new epidemic of ZIKV is possible, and could be exacerbated by climatic conditions.

In conclusion, ZIKV provides an example of how important is the constant dialogue between experimental, epidemiological and viral vigilance work. After ZIKV emerged as a medical concern at the end of 2015, answers to many questions came from the collaborative work of virologists, immunologists, ecotoxicologists, environmental ecologists, epidemiologists, geneticists, neuroscientists, and developmental biologists, among others. To better understand why CZS is not homogeneously distributed among human populations, more interdisciplinary studies are needed. Future advances in understanding the role of cofactors in ZIKV infection should result from these interdisciplinary efforts.

References

1 Baud, D., Gubler, D. J., Schaub, B., Lanteri, M. C. & Musso, D. An update on Zika virus infection. The Lancet 390, 2099-2109, (2017).

2 Petersen, L. R., Jamieson, D. J., Powers, A. M. & Honein, M. A. Zika Virus. New England Journal of Medicine 374, 1552-1563, (2016).

3 Barbeito-Andrés J, Schuler-Faccini L, Garcez PP. Why is congenital Zika syndrome asymmetrically distributed among human populations? PLoS Biol. 16(8):e2006592. (2018).

4 Tang, H. et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. Cell Stem Cell 18, 587-590, (2016).

5 França, G. V. A. et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. The Lancet 388, 891-897, (2016).

6 Yuan, L. et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. Science 358, 933-936, (2017).

7 Franchini, A. M. & Lawrence, B. P. Environmental exposures are hidden modifiers of anti-viral immunity. Current Opinion in Toxicology 10, 54-59, (2018).

8. Albuquerque MF, Souza WV, Mendes AD, Lyra TM, Ximenes RA, Araújo TV, Braga C, Miranda-Filho DB, Martelli CM, Rodrigues LC. Pyriproxyfen and the microcephaly epidemic in Brazil - an ecological approach to explore the hypothesis of their association. Mem Inst Oswaldo Cruz. 111(12):774-776. (2016).

NextGen RESEARCHERS

This section of ISAR News aims to highlight the career of Ph.D. students, postdocs and young investigator awardees at past ICAR meetings.

Johanna Huchting, postdoctoral scientists, Institute for Organic Chemistry, University of Hamburg (johanna.huchting@chemie.uni-hamburg.de)

Johanna, you studied chemistry and did your Ph.D. with Chris Meier in Hamburg, on a topic more related to carbohydrate chemistry. For your Postdoc, you then started working in the antiviral field, first in the Hamburg lab and then at Rega Institute in Leuven. What is the focus of your research now?

Looking at recent developments in antiviral nucleotides, highly innovative and successful drug candidates keep popping up. I believe that we're far from having exploited the chemical space for this class of compounds, and this is also where the focus of my research is set. My background in carbohydrate chemistry provides a perfect basis, since the most important and straightforward techniques to synthesize nucleosides use methods of carbohydrate chemistry.

Moreover, I studied phosphorylated carbohydrates and their synthesis. So, when I moved to nucleotide chemistry, I already had experience in phosphorylation reactions and product isolation, which is often very challenging. During my Postdoc in the Meier lab, I further had the opportunity to learn from excellent experts in nucleotide chemistry and to combine curiosity-driven research with the latest innovations in prodrug technologies. This enabled me to confidently and successfully take on challenging projects. The same, of course, holds true for when I joined the Virology and Chemotherapy lab at the Rega Institute. Being trained by virology experts of this prestigious institution, I now understand more of the biological side of antiviral research and hope to combine "the best of both worlds" in my research. So, to answer your question, my current project aims to contribute to

the understanding of intracellular activation processes of antiviral pseudobases. Recently, we identified a novel intracellular nucleotide-metabolite of de-fluoro-favipiravir, which I also presented at the ICAR2018 in Porto. I want to understand its relevance and hope to gain further insight into the relationship between chemical structure and mode of action of antiviral pseudobases and nucleoside analogues.

You moved from Hamburg in Germany to Leuven in Belgium during your Postdoc. Could you please tell us about this experience and how your stay abroad influenced your career?

In my research, I combine my experience in chemical synthesis of phosphorylated biomolecules that I gained in Chris Meier's lab in Hamburg, with the skills I developed during my stay in Lieve Naesens' group in Leuven. For my first research stay at Rega Institute, I was awarded the Chu Family Foundation Scholarship at the 2016 IS3NA Roundtable in Paris, and this turned out to be an extremely valuable and rewarding experience for me. Working at the interface of two disciplines, I wanted to be able to speak both, the language of Chemistry and the language of Biology. Not only so that I'd be able to follow the details of antiviral studies, to better understand the interpretation of results and the limitations of experiments, but also because I believe that open and efficient communication is the basis of a productive and enjoyable collaboration. Since my passion is nucleotide chemistry and antiviral drug discovery, my choice of where I wanted to learn the biological and virological approach was very easy.

The Rega Institute's prestigious past, leading to the discovery of prominent anti-HIV drugs, and its vibrant present with many important contributions i.e. to the discovery of drug-leads for the treatment of newly emerging viral pathogens, make this place an excellent research institution. I got the chance to work with inspiring scientists and to learn from the experts of their field. This way, I could also expand my network in antiviral research beyond Rega Institute, since the lab fosters many international collaborations. Moreover, Leuven is a lovely town and I got the chance to stay at the Groot Begijnhof. This picturesque place dates from the 13th century and is listed as UNESCO world heritage, and I had never before experienced the special atmosphere this place creates. It perfectly mixed with the pioneering research and state-of-the-art facilities at KU Leuven and Rega Institute. After my first, three-month stay, I was awarded another scholarship to do a follow-up project for 6 month in Lieve's group. Our collaborative project was very successful and yielded a publication in J. Med. Chem., and I had the chance to present some of our results at ICAR 2017 in Atlanta and ICAR2018 in Porto, supported by Travel Awards from ISAR.

What does ISAR mean for you? Did ICAR meetings motivate your research?

During my Ph.D., I attended two big carbohydrate chemistry conferences. I loved the inspiring atmosphere of these meetings and the opportunity to get direct feedback regarding my research. After I had just started my PostDoc project, I attended the ICAR meeting for the first time in Rome (2015). I was impressed with the science that was presented and it was there where I met Lieve Naesens and many more researchers from the Rega Institute for the first time. This started our close collaboration on anti-influenza virus nucleotides and consequently gave me the opportunity to get funding and go abroad. Through ISAR, I have had the opportunity to get to know people who have supported my career and I'm extremely grateful for their encouragement and mentorship. ISAR has enabled me to join ICAR meetings by their generous support with Travel Awards, for which I'm very grateful! The network, that ISAR creates, connects top-notch international labs and the openness, to new members and young researchers, fosters a very positive, supportive and inspiring atmosphere. In the future, I hope not only to contribute to science, but also to introduce new members and young researchers to the Society.

How do you find the experience of supervising students and what advice can you give to students who want to start a Ph.D.?

Teaching and mentoring students is an extremely rewarding part of being a scientist. Students, taking their first steps into research, bring lots of enthusiasm and curiosity. This is what drives research and it creates an atmosphere that I want to foster in a lab. Yet, research poses big challenges: The initial approach to answering a scientific question rarely works out, one has to start over again and again. Frustration is a big part of daily business and one needs enormous diligence, persistence and self-motivation to do research. I believe that open communication with mentors and experienced lab partners as well as being part of a team can help to overcome these challenges. Talk to advisors and fellow students, identify accomplishments, especially those that don't present themselves as such at first sight, and celebrate them together!

Angela Corona, postdoctoral scientist
Department of Life and Environmental Sciences, University of Cagliari
(angela.corona@unica.it)

Could you please tell us about your career and your mobility as a researcher?
When I was a master student in Pharmaceutical Chemistry, I wasn't planning to do research, I was just fascinated by the fact that there is the possibility to do something that can make a positive effect on our society. Therefore, I chose a thesis on drug synthesis, COX-2 inhibitors. That's where everything started. When talking with colleagues and friends, they said often to me "I think you could be happy doing that".

My thesis supervisor collaborated with a group that screened antivirals, so I applied for a Ph. D. position in Prof. Tramontano's group. I had a project on the development of HIV-1 RNase H inhibitors. It was an exciting topic and allowed me to have international collaborations quite soon. The lab did, and still does, many collaborations; therefore, I had the opportunity to go to other labs to do part of my project and learn new methods.

I went for the first time abroad, to the US, at the HIV Drug Resistance Program of the NCI in Frederick. Le Grice's lab has been a really friendly place, I have been encouraged to work hard on my project, everyone in the lab was happy to help me and share with me their techniques and methods. I met friends with whom I am still in contact. After that experience, I came back much more self-confident and independent in my lab work. After that, during the last year of the Ph. D., I spent three months in Paris at the Laboratory of Biology and Applied Pharmacology, in Cachan. I had my first biosafety level 3 training there, and the opportunity to perform the last experiments to conclude my Ph. D. thesis.

How did your stay abroad influence your career?
My stay abroad was a great opportunity and completely changed the way I perceived doing research. I understood more clearly what a shared effort to meet a goal means, sharing complementing tasks and knowledge with generosity. It has not been easy. I am a family person, tightly joined with my relatives and my home, and I missed them deeply.

However, travelling so far, meeting face-to-face other scientists showed me that a good network of collaborations with people known personally could allow good science to be performed and reach good results. In addition, it is always good to know that there is the possibility to ask or even to move to perform experiments complementary to the work done in the home lab. For example, recently I have been at IrsiCaixa in Badalona, where I found a friendly team, and I did and learnt a lot. To be abroad for a definite project pushed me to work really hard to get all my work done in the limited amount of time I had. It showed me my potentials and my limits, teaching me also that sometimes it is better to slow down a little bit.

What are your thoughts about the future of antiviral research?
I think that antiviral research is a field that is giving a great help on facing deadly challenges. Since the scientific community in the field is not so big, this could help others to join the efforts, build working teams and meet common goals. I see that this academia can play an important role because one can devote efforts on exploring new targets and strategies that are not strictly governed by mere economic logic. That would help a lot in case of neglected diseases.

What is the focus of your research for the moment?
I have several projects I am working with, after the doctorate; it has been hard to find a focus. I would like to focus more on a clinic-related study of drug resistance.

What do you consider your most significant research accomplishments today?
The result, that I considered most significant, is the result obtained on the development of RNase H inhibitors of HIV-1, because, gradually, we are optimizing a few compounds, which proved to be active against commonly selected drug-resistant strains. It is not like finding the new first-line drug, but, if it would work, it could be a good side option in case of therapeutic failure.

What has been your large disappointment? How did you cope with it?
It is a difficult question for me. Even if I think carefully, I cannot find something that deeply disappointed me. I never pursued an objective as absolutely essential for my happiness. I have a number

of regrets, mainly related to the personal life and interpersonal relationships, but it is a good way to remember that everyone can make mistakes, and we must have the strength to face the consequences.

What are your expectations for the coming years?
I am not the person that looks far into the future; I never did, speaking about work. It allows me to be more free and calm to make decisions. My expectations for coming years are to teach some classes in my university, and work hard on a subject I really like.

What is your involvement in the organization of Innovative Approaches for novel Antiviral Agents Summer School? What are the goals of this summer school?
I went to the first Summer School as a Ph. D. student, it was my first year, and I found it extremely useful and exciting. This time, the fourth meeting, I am in the organizing committee, mainly looking after logistics and correspondence with the students. It is nice to be in contact with them. From the emails they send, they look already enthusiastic to participate, and I am looking forward to the School to start because it will be not only good science but also a lot of fun. It was meant, since the first summer school, to be a perfect mix. Lectures of eminent scientists in the morning, and poster and oral presentations of the students in the afternoon - a mixed background of all the disciplines involved in antiviral development.

A highly interactive environment, in which every moment, from breakfast to dinner, are shared with other students and teachers. Every student has the opportunity to learn, to present his/her project, get suggestions, and, most importantly, to confront himself/herself with other students facing the same difficulties that the Ph. D. implies. The environment may encourage them to ask for advice from the experienced scientists that are around them, ready to answer. This will make them more aware about the future, the possibilities and the opportunities. How to distinguish them and how to choose.

How do you find the experience of supervising students?
Supervising students is exciting, challenging, and frustrating. They are a spring of enthusiasm and they can get fascinated by research and devote themselves to learn and grow, but they can also be bored and careless, no matter how hard I try to involve them in the experiments. On the other hand, it can give great satisfaction.

What advice can you give to students who want to start a Ph.D.?
Mmmm, the Ph.D. is like a marathon, mental and physical, try not to get too tired too early. Get passionate about your project, and try not to be phagocytized by it. Do sports and save time for your friends. Take any occasion to meet new people and learn.

How did you succeed to manage your professional and personal life?
I did not! I am always behind in something, always late, always dwelling on things to do. But I am lucky enough to have on my side a person who loves me and supports me, and a lot of friends, they know me enough well to face my hyper organizing temperament with humor. The point is not to let the professional life overcome the personal one, try to balance the two. I may say that a researcher is indeed difficult because it is hard to "switch off" the brain from a working project which excites (or worries) me when I arrive home. I am slowly learning.

What does ISAR mean for you? Did ICAR meetings motivate your research? Did ISAR help you and/or support you in your scientific career?
I must say that I think that ISAR is a great society, very friendly, especially with young scientists. Since the beginning, it tries to involve and help them. It has some characteristic aspects that I particularly like, first, the high level of interdisciplinary links among the members. This is something that, as a pharmaceutical chemist, made me feel at home since the first meeting I attended. It was in 2014 in Raleigh, only four years ago. The meetings have a high level of science and, every time, I come home with many new ideas, sometime even completely new projects to start. In the lab, some colleagues make fun of me about that. With time, the meeting has become even nicer, because now I know more people, and we remember each other year after year. This is also possible because it is not a big meeting. It is a nice network incubator, I think. The Society offers many opportunities to young scientists and to women in particular. I have been supported many times in attending meetings by ISAR and, thanks to the Women in Science program, also in doing research abroad, which helped a lot to improve my skills that I could use once back home. It has been a worthwhile and fantastic experience.

ISAR News is an official publication of the International Society for Antiviral Research. It is published four times a year as a free article in *Antiviral Research* (http://www.journals.elsevier.com/antiviral-research) and is posted on the ISAR website (http://www.isar-icar.com).

Except where stated otherwise, all photographs are published with permission from ISAR.

ISAR News is prepared by the ISAR Publications Committee: Anthony Vere Hodge and Luis Schang (co-chairs), Andrea Brancale, Mike Bray, Rhonda Cardin, Kara Carter, José Esté, Brian Gowen, Justin Julander, Jason Peng, Joana Rocha-Pereira, Aruna Sampath, Ashoke Sharon, Sietske Speerstra, Bart Tarbet and Simon Tucker.