2017 ISV Annual Congress

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Dear Delegates,

On behalf of the International Society for Vaccines (ISV), it is a great pleasure and honor to welcome experts from around the globe and across diverse disciplines to the ISV 11th Annual Congress in Paris, France. We are particularly thrilled this year to have the 2017 ISV congress co-hosted by the Institute Pasteur. There is no more historic venue for the ISV community to come together and to advance vaccine science.

The high quality and number of attendees and abstract submissions are a testament to the outstanding level of science and impact that is the ISV Congress. It also highlights the importance of the dialogue and breakout sessions allowing for interactions that occur at the Congress amongst leading scientists representing diverse aspects of the vaccine field.

With the outbreaks of old pathogen foes, the large number of emerging and reemerging threats, the growing importance of vaccine technologies for immune therapy of cancer, the new and growing challenges and novel opportunities in the vaccine space, there has never been a more important time for the ISV Meeting. With an expanding outlook and vigor the ISV Congress continues to play an important role for the field through its tradition of presentations of cutting edge developments and providing a forum for the best minds and leaders and new blood in the fields of vaccinology and related scientific areas to interact and collaborate and grow their mission.

The ISV Congress occupies a unique position of being the world’s largest non-profit scientific conference in the field of vaccines covering all aspects of research and development for vaccine and immunotherapies. As such we represent all stakeholders in an unbiased and open manner focusing on our dedication to enhancing vaccine science for public good. The program is organized to facilitate presentation of the latest scientific findings from around the world as well as providing a forum for determining and advancing the best means to expedite vaccine development. We are excited to have high impact sessions covering topics that the ISV community recommended including AMR threats, Maternal and Neonatal Immunization, Vaccines for the elderly, Mucosal Vaccination, Structural Vaccinology and MAB technologies, Systems Vaccinology, Cancer Vaccines and Immune Therapy, Neglected and LMIC Diseases, Viral Vaccines, and a special section highlighting research from the Institute Pasteur, as well as much more. This year we have a special panel discussion focused on Public-Private Partnerships. Panelists from key sectors with diverse expertise and a global health perspective will guide this session.

The Congress also features a career development and mentoring session, to be held jointly with a “Meet the Fellows” session. Young scientists will be able to obtain advice from senior vaccinologists from the varied sectors (academia, industry, governmental research organizations, NGOs), and explore career options.

We would like to thank the many individuals from ISV, the Scientific Committee, the invited speakers, and the volunteer leaders for their efforts throughout the year in developing and contributing to this outstanding program. Special thanks are due to our President Margaret Liu, for her leadership and innumerable contributions to the program on multiple levels, Dr. Shan Lu and Ted Gibson at the ISV Congress Secretariat for their enormous efforts with all aspects of the Congress and handling the many logistical issues that arise with such a complex undertaking, and of course to the Institute Pasteur for hosting us in Paris.

Gratitude is also due our partners and sponsors, which include once again the Bill & Melinda Gates Foundation, plus the numerous organizations and companies who have enabled the ISV to both provide financial assistance enabling scientists from Lower and Middle Income Countries and trainees to attend and to keep registration costs low for all attendees.

Everyone is encouraged to attend the ISV annual general meeting on Friday afternoon, Oct. 6th. The Society welcomes members and non-members alike. We are continually expanding the areas of information and benefits offered to members and the greater vaccine community. Your personal involvement in the Society promotes increased collaboration and your scientific visibility.

Welcome to Paris! Enjoy the science, the people, and of course the City of Lights!

Sincerely,

Denise Doolan, ISV Congress Co-Chair, James Cook University, Australia
David Weiner, ISV Congress Co-Chair, The Wistar Institute, USA.
Christiane Gerke, ISV Congress Local Co-Chair, Institut Pasteur, France
Frédéric Tangy, ISV Congress Local Co-Chair, Institut Pasteur, France
Co-chairs of the 2017 ISV Congress will select presentations for possible publication at leading scientific journals in the field of vaccines including the publication of a special congress issue at one of these journals. Details will be announced during the congress.
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CONGRESS CHAIRS

David Weiner, Wistar Institute, USA
Denise Doolan, James Cook University, Australia
Christiane Gerke, Institut Pasteur, France
Frédéric Tangy, Institut Pasteur, France

SCIENTIFIC COMMITTEE

Peter Anderson, Statens Serum Institut, Denmark
Danilo Casimiro, Aeras, USA
Ian Frazer, Univ Queensland, Australia
Nathalie Garçon, Bioaster Technology Research Institute, France
Michael Good, Griffith University, Australia
Carlos Guzmán, Helmholtz Centre for Infection Research, Germany
Adrian Hill, University of Oxford, UK
Peter Hotez, National School of Tropical Medicine, USA
Kathrin Jansen, Pfizer, USA
Stefan Kaufmann, Max Planck Institute for Infection Biology, Germany
Florian Krammer, Icahn School of Medicine at Mount Sinai, USA
Marie-Paule Kieny, Inserm, France
Jerome Kim, IVI, South Korea
Hiroshi Kiyono, University Tokyo, Japan
Gary Kobinger, Centre for Research in Infectious Diseases, Canada
Wayne Koff, The Human Vaccines Project, USA
Odile Leroy, European Vaccine Initiative, Germany
Vish Nene, International Livestock Research Institute, Kenya
Stanley Plotkin, VaxConsult, USA
Allan Saul, GSK Vaccine Inst. Global Health, Italy
Jeffrey Ulmer, GSK Vaccines, USA
Tonya Villafana, MedImmune, USA
Yu-Mei Wen, Fudan University, China

CONGRESS SECRETARIAT

Shan Lu, UMass Medical School, USA
Email: shan.lu@umassmed.edu
Tel: +1-508-856-6791

Ted Gibson, UMass Medical School, USA
Email: edward.gibson@umassmed.edu
Tel: +1-508-856-1179

CONGRESS VENUE

Institut Pasteur, 25-28 Rue du Dr Roux, 75015 Paris, France; Tel: +33 1 45 68 80 00

CONGRESS WEBSITE

Conference website: www.isvcongress.org
ISV website: www.isv-online.org
REGISTRATION DESK
The registration/information desk will be located in the CIS Building and will remain open throughout the conference staffed during the following times:

- Thursday, 5 October  8:00am-7:00pm
- Friday, 6 October  8:00am-6:00pm
- Saturday, 7 October  8:00am-2:00pm

BADGES
For security reasons and catering purposes please make sure you wear your conference badge. Replacements for lost badges are available from the registration desk.

CONFERENCE BAGS & DELEGATE BOOKS
Please make sure that you insert a business card or name tag in your bag. Please also write your name in your delegate book and do not leave either your book or bag unattended at the conference at any time.

ORAL ABSTRACT PRESENTATIONS
Your presentation has been allocated a total of 15 minutes; this includes time for questions, so please keep the actual talk to 10-12 minutes to allow time for questions. Chairpersons will eliminate questions for speakers whose talk runs the full 15 minutes. Please take your presentation to congress staff at the time of registration to be loaded onto the projector.

POSTER SESSIONS
Due to the amount of poster applicants this year there will be two separate poster sessions.

Poster numbers P1-P60 will be part of Poster Session # 1 (please refer to your program to see your poster designation number). Posters should be set up between 9:00-9:30 on Thursday, October 5th. The dedicated poster session will take place on Thursday from 6:00PM-7:00PM and poster presenters should ensure that they stand by their posters during this time. Posters must be taken down by 1:30PM on Friday, October 6th.

Poster numbers P60 and above will be part of Poster Session # 2 (please refer to your program to see your poster designation number). Posters should be set up at 1:30PM on Friday, October 6th. The dedicated poster session will take place on Friday from 1:30PM-2:45PM and poster presenters should ensure that they stand by their posters during this time. Posters must be taken down by 4:00PM on Saturday, October 7th.

WELCOME RECEPTION
A welcome reception will take place on Thursday, October 5th in the CIS Building during poster session # 1. Complimentary hors d’oeuvres and drinks will be provided.
**LUNCH**

Lunch will be provided on Thursday, Friday and Saturday in the Social – Modules building at no cost for all attendees.

**COFFEE BREAKS**

Coffee breaks will be available in the CIS Building on Thursday, Friday and Saturday at no cost for all attendees.

**Wi-Fi**

Wi-Fi will be available at the conference free of charge. Your personalized password will be distributed at registration.

**GALA DINNER**

The Gala Dinner will take place on the evening of Friday, October 6th on board the Le Paris and includes dinner, drinks, and an excursion down the Seine River. Buses will be leaving the main entrance of the Institut Pasteur at 7:00PM. Staff will be available to escort you to the pick up area. The dinner will take place from 7:30PM-10:00PM. Buses will be leaving the pier at 10:30PM to bring you back to the Institut Pasteur.

Pre-purchased tickets are available in your badge holder.

**RECORDING OF SESSIONS**

Please be advised that no photography or video/sound recording of conference presentations is allowed to take place during the conference.

**EVALUATION FORM**

Your comments and views on the content and organization of the conference are highly valued and we would encourage you to complete an online evaluation form which will be emailed to you after the conference.

**MESSAGES**

Messages for delegates received at the registration desk will be posted on the message board near the registration area. You are welcome to use the message board to contact fellow delegates.
## Oral Program

### Thursday October 5\(^{th}\), 2017

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<td>Opening Talk: The future of vaccines and immunotherapy:</td>
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<td>PLENARY SESSION 1: Innovative Clinical Designs and Human Challenge</td>
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<td>Session Chairs: Denise Doolan, James Cook University and Frédéric Tangy, Institut Pasteur</td>
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<td>10:30-11:00</td>
<td>[PL1.1] How to Tackle the Most Successful Pathogen on Earth:</td>
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<td>Tuberculosis vaccine and biomarker development</td>
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<td>Stefan Kaufmann, Max Planck Institute for Infection Biology</td>
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<td>11:00-11:30</td>
<td>[PL1.2] Phase IIb Test of Concept studies for HIV</td>
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<td>Peter Gilbert, Fred Hutchinson Cancer Research Center</td>
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<td>[PL1.3] Lessons learned from Ebola Vaccine R&amp;D during a public health emergency</td>
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<td>Session Chairs: Kathrin Jansen, Pfizer; and Shabir Madhi, University of Witwatersrand</td>
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<td>13:30-13:55</td>
<td>[O1.1] Progress on Clinical trials of RSV, influenza and pertussis vaccines in pregnancy</td>
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<td>13:55-14:20</td>
<td>[O1.2] Perinatal and neonatal infection prevention: Group B streptococcus vaccines</td>
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<td>Session Chairs: Armelle Phalipon and Frédéric Tangy, Institut Pasteur</td>
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<td>Concurrent Session 3 <em>(François Jacob Building)</em></td>
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<td>Structural Vaccinology and Protective Monoclonal Antibodies</td>
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<td>Session Chairs: Florian Krammer, Icahn School of Medicine at Mount Sinai; and Joon Haeng Rhee, Chonnam National University Medical School</td>
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<td>13:30-13:55</td>
<td>[O3.1] Lesson from the analysis of the immune response to <em>Plasmodium falciparum</em></td>
<td>(CIS Building)</td>
<td>Antonio Lanzavecchia, ETH Zurich</td>
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<td>13:55-14:20</td>
<td>[O3.2] Epitope-focused vaccine design to protect against Zika and dengue virus simultaneously</td>
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<td>Felix Rey, Institut Pasteur</td>
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<td>14:20-14:35</td>
<td>[O1.3] Updates on the Development of a Multivalent Group B Streptococcal vaccine</td>
<td>Annaliesa Anderson, Pfizer</td>
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<td>[O2.3] Institut Pasteur Vaccinology Course</td>
<td>Armelle Phalipon, Frederick Tangy, Insitut Pasteur</td>
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<td>[O3.3] Analysis of antibody repertoires reshaped by vaccination with respiratory syncytial virus post fusion protein</td>
<td>Gerald Schneikart, GSK</td>
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<td>14:35-14:50</td>
<td>[O1.4] Induction of epithelial cell-neutralizing antibodies against cytomegalovirus (CMV) in adults vaccinated with an enveloped virus-like particle (eVLP) containing an optimized form of glycoprotein b for prophylactic vaccination against CMV</td>
<td>Joanne M. Langley, Dalhousie University</td>
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<td>[O2.4] IMVACC: a web-based international master in vaccinology</td>
<td>Jean-Pierre Kraehenbuhl, HSeT</td>
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<td>[O3.4] A novel approach to unravel the effective human antibody response induced by natural infection or vaccination against respiratory syncytial virus (RSV)</td>
<td>Emanuele Andreano, University of Siena (IT)</td>
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<td>14:50-15:05</td>
<td>[O1.5] The Importance of RSV F protein conformation in stimulation of protective immune responses in animals previously infected with RSV</td>
<td>Trudy Morrison, University of Massachusetts Medical School</td>
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<td>[O2.5] A bacterial protease inhibitor as vaccine adjuvant</td>
<td>Lorena Coria, Universidad Nacional de San Martin</td>
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<td>14:50-15:00</td>
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<td>[O3.5] Engineering transient production of HIV-1 broadly neutralizing antibodies by DNA encoded monoclonal antibody technology (dMabs)</td>
<td>Megan Wise, Inovio Pharmaceuticals</td>
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<td>15:05-15:20</td>
<td>[O1.6] Epitope-specific antibody responses to HSV-2 glycoprotein D immunization differs depending on the adjuvant</td>
<td>Sita Awasthi, University of Pennsylvania</td>
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<td>[O2.6] Developing a universal flu vaccine: multifunctional T cells and multidisciplinary consortia</td>
<td>Ajibola Omokanye University of Gothenburg</td>
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<td>[O3.6] Conversion of a DNA-encoded monoclonal antibody (dMab) into scFv-Fc format improves expression and protects against lethal Zika virus challenge following in vivo gene delivery in a mouse model</td>
<td>Stephanie Ramos, Inovio Pharmaceuticals</td>
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<td>[O2.7] Vaccine approaches against leishmaniasis</td>
<td>Rafael de Freitas e Silva, Aggeu Magalhães Institut</td>
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<td>[O2.8] Lessons for developing countries – introduction of 6 new vaccines into a national program in less than a decade</td>
<td>Chandrakant Lahariya, World Health Organization</td>
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<td>15:30-16:00</td>
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<td>PLENARY SESSION 2: Vaccines as Solutions to AMR Threats</td>
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<td>16:00-16:10</td>
<td>[PL2.1] Introductory Overview</td>
<td>Danilo Casimiro, Aeras</td>
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<td>16:10-16:40</td>
<td>[PL2.2] The role of vaccines in fighting antimicrobial resistance</td>
<td>Kathrin Jansen, Pfizer</td>
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<td>[PL2.3] Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine</td>
<td>Louis Picker, Oregon Health &amp; Science University</td>
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<td>17:10-17:25</td>
<td>[PL2.4] A recombinant fimbrial prototype vaccine administered by intradermal route protects Aotus nancymae non-human primates from ETEC diarrhea</td>
<td>Geneviève Renaud-Mongénie, Sanofi-Pasteur</td>
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<td>17:25-17:40</td>
<td>[PL2.5] Safety, tolerability and immunogenicity of ExPEC4V (JNJ-63871860) vaccine for prevention of invasive extraintestinal pathogenic Escherichia coli disease: a phase 1, double-blind, placebo-controlled study in healthy Japanese participants</td>
<td>Patricia Ibarra de Palacios, Janssen Vaccines</td>
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<td>[PL2.6] New promising targets for synthetic Omptin-based peptide vaccine against Gram-negative pathogens</td>
<td>Valentina Feodorova, Saratov Research Veterinary Institute</td>
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<td>Poster Session # 1: Authors present at their posters</td>
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<td>Welcome Reception (CIS Building)</td>
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<td>[PL3.1] Vesicular Stomatitis Virus Vectors – a promising vaccine platform</td>
<td>Heinz Feldmann, NIAID, National Institutes of Health</td>
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<td>08:25-08:50</td>
<td>[PL3.2]</td>
<td>Adolfo García-Sastre, The Icahn School of Medicine at Mount Sinai</td>
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<td>08:50-09:05</td>
<td>[PL3.3] A Phase 1 clinical trial of a Hantaan virus, Puumala virus, and Hantaan/Puumala virus DNA vaccine delivered by disposable syringe jet injection: preliminary findings</td>
<td>Jay Hooper, US Army Medical Research Institute of Infectious Disease</td>
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<td>09:20-09:35</td>
<td>[PL3.5] A single-round infectious particle Zika virus vaccine candidate</td>
<td>Karin B. Sundstrom, Duke-NUS Medical School</td>
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<td>09:35-09:50</td>
<td>[PL3.6] Durability and correlates of vaccine protection against Zika virus in rhesus monkeys</td>
<td>Peter Abbink, Beth Israel Deaconess Medical Center</td>
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<td>09:50-10:05</td>
<td>[PL3.7] A synthetic, consensus DNA vaccine against Zika virus, GLS-5700, is highly immunogenic in humans and induces antibody responses that are protective in a passive transfer mouse challenge model</td>
<td>Emma Reuschel, The Wistar Institute</td>
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<td>[PL4.1] A.I.R vaccines – A synthetic self-amplifying RNA-based vaccine platform</td>
<td>Stephanie Erbar, BioNTech RNA Pharmaceuticals</td>
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<td>11:10-11:35</td>
<td>[PL4.2] Delivery systems for RNA Therapy, RNA vaccines and in vivo gene editing</td>
<td>Daniel Anderson, Massachusetts Institute of Technology</td>
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<td>11:35-11:50</td>
<td>[PL4.3] DNA-launched RNA replicon vaccines induce potent anti-Ebola virus immune responses that can be further improved by protein or MVA boosts</td>
<td>Karl Ljungberg, Karolinska Institute</td>
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<td>11:50-12:05</td>
<td>[PL4.4] Thermostable plasmid DNA launches a live-attenuated yellow fever vaccine platform that induces protection in vivo</td>
<td>Kai Dallmeier, University of Leuven</td>
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| 12:05-12:20  | [PL4.5] A combination heroin-HIV vaccine abrogates nociceptive and locomotive effects of heroin and induces cross-reactive antibodies to other abused prescription opioids and to the V2-loop of the HIV-1 envelope protein  
Gary Matyas, Walter Reed Army Institute of Research |
| 12:30-13:30  | Lunch (“Social – Modules” Building)  
Sponsored By: VGXI, Inc. |
| 13:30-14:45  | Poster Session # 2: Authors present at their posters  
(CIS Building) |
| 14:30-15:00  | Coffee Break (CIS Building)  
Sponsored By: Eurogentec |
| 14:45-15:45  | ISV Annual General Meeting  
(CIS Building) |
| 16:00-18:15  | Concurrent Session 4 (CIS Building)  
Vaccines for the Elderly  
Session Chairs: Tonya Villafana, Medimmune; and Britta Wahren, Karolinska Institute |
| 16:00-18:15  | Concurrent Session 5 (Duclaux Building)  
Mucosal Vaccination  
Session Chairs: Linda Klavinskins, King’s College London; and Hiroshi Kiyono, University of Tokyo |
| 16:00-18:15  | Concurrent Session # 6 (François Jacob Building)  
Systems Vaccinology and Computational Vaccinology  
Session Chairs: Annie de Groot, EpiVax Inc. |
| 16:00-16:25  | [O4.1] HZ/su: an innovative approach to the prevention of Herpes Zoster  
Jacqueline Miller, GSK |
| 16:00-16:25  | [O5.1] Cutting edge of the mucosal immune system for the development of oral and nasal vaccines  
Hiroshi Kiyono, University of Tokyo |
| 16:00-16:25  | [O6.1] Human Vaccines Project: decoding the human immune system to accelerate next generation vaccine development  
Stacey Wooden, Human Vaccines Project |
| 16:25-16:50  | [O4.2] RSV vaccines in the elderly: a challenge for development, the MEDI7510 story  
Tonya Villafana, Medimmune |
| 16:25-16:50  | [O5.2] Challenges in developing mucosal vaccines against global infections  
Cecil Czerkinsky, CNRS-INSERM-University of Nice |
| 16:25-16:50  | [O6.2] Systems approaches highlight the many facets of interferons in infections and in vaccine mediated protection  
Rafick-Pierre Sékaly, Case Western Reserve |
| 16:50-17:15  | [O4.3] Vision for adult vaccination  
Kathrin Jansen, Pfizer |
| 16:50-17:15  | [O5.3] Flagellin-based recombinant divalent vaccines induce protective immune responses in a Porphyromonas gingivalis and Fusobacterium nucleatum mixed infection model in mice  
Joon Haeng Rhee, Chonnam National University Medical School |
| 16:50-17:15  | [O6.3] Milieu Intérieur: defining the boundaries of a healthy immune response for a better understanding of disease  
Darragh Duffy, Institut Pasteur |
| 17:15-17:30  | [O4.4] Benefits of high dose Fluzone® Influenza vaccine for anti-neuraminidase immune responses in the elderly  
Anne-Gaelle Bebin-Blackwell, University of Georgia |
| 17:15-17:30  | [O5.4] Food grade live oral mucosal vaccine (LacVax™:OmpA) against Shigella: affordable strategies for effective immunisation  
Priti Desai, B.V. Patel PERD Centre |
| 17:15-17:30  | [O6.4] Computational vaccine design for humans and animals using the iVAX toolkit  
Frances Terry, EpiVax Inc. |
| 17:30-17:45 | [O4.5] Zoster vaccine effectiveness against incident herpes zoster and risks of vaccine failure in elderly in the UK Maria Alexandridou, P95 | [O5.5] Efficacy of novel epithelial stem cell-based AIDS vaccine to induce mucosal immune responses and protect against repeated low dose SIV challenge Marie-Claire Gauduin, Texas Biomedical Research Institute | [O6.5] Computational vaccinology for the design of personalized cancer therapies: from infectious disease to cancer vaccines Guilhem Richard, EpiVax Inc. |

**Saturday, October 7th, 2017**

| 08:00-10:00 | PLENARY SESSION 5: Neglected and Low and Middle Income Countries (LMIC) Diseases **Session Chairs:** Allan Saul, GSK; and Sarah Gilbert, University of Oxford |
| 08:00-08:25 | [PL5.1] Prioritizing vaccine development for neglected diseases of LMIC Allan Saul, GSK |
| 08:25-08:50 | [PL5.2] The “Antipoverty Vaccines” for Neglected Tropical Diseases Peter Hotez, Baylor College of Medicine |
| 08:50-09:15 | [PL5.3] Affordable vaccines for global health: the Hilleman approach Davinder Gill, Hilleman Laboratories |
| 09:15-09:30 | [PL5.4] First evaluation in humans of a chemically attenuated *P. falciparum* whole parasite blood-stage vaccine Danielle Stanisic, Griffith University |
| 09:30-09:45 | [PL5.5] Novel subunit-vaccine approaches against tuberculosis Manish Gupta, Jawaharlal Nehru University |
| 09:45-10:00 | [PL5.6] Discovery of Crimean-Congo Hemorrhagic Fever Virus vaccine candidate antigens by protein microarray scanning and verification of the determined antigens by recombinant ELISA Sultan Gulce-Iz, Ege University |
| 10:00-10:30 | Coffee Break (CIS Building) **Sponsored By:** CTL Europe Gmbh |

**Concurrent Session 7:** **Cancer Vaccines and Immunotherapy** (CIS Building)  
**Session Chairs:** David Weiner, The Wistar Institute; and Joon Haeng Rhee, Chonnam National University Medical School

**Concurrent Session 8:** **Viral Vaccines** (Duclaux Building)  
**Session Chairs:** Christiane Gerke, Institut Pasteur; and Ted Ross, University of Georgia Center for Vaccines and Immunology

**Concurrent Session 9:** **One Health** (François Jacob Building)  
**Session Chairs:** Ed Rybicki, University of Cape Town; and Nathalie Garcon, Bioaster
| 10:30-10:55 | [O7.1] Personalized cancer vaccines  
Gerald Linette, University of Pennsylvania |
|-------------|---------------------------------------------------------------------------------
| [O8.1] The biology of antibody enhanced disease: evidence of increased risk after CYD-TDV vaccination  
Scott Halstead, Uniformed Services University of the Health Sciences |
| [O9.1] Evaluation of ChAdOx1 MERS vaccine in camels in Saudi Arabia  
Naif Alharbi, King Abdullah International Medical Research Center  
10:30-10:45 |
| 10:55-11:20 | [O7.2] Cancer vaccines and immunotherapy- where are we today and where do we go from here?  
Adil Daud, UCSF Medical Center |
| [O8.2] Long-term efficacy of a Hepatitis E vaccine  
Jun Zhang, Xiamen University |
Jianping Li, University of Connecticut  
10:45-11:00 |
| 11:20-11:35 | [O7.3] Clinical and immunologic biomarkers for regression of high grade cervical dysplasia and clearance of HPV16/18 infection after immunotherapy with VGX-3100 in a Phase IIb Clinical Trial  
Kimberly Kraynyak, Inovio Pharmaceuticals |
| [O8.3] DNA and protein co-delivery vaccines induce potent immune responses able to delay SIV/SHIV acquisition  
George Pavlakis, National Cancer Institute at Frederick |
| [O9.3] The application of NHEJ-CRISPR/Cas9 and Cre-Lox system in the generation of multivalent vaccines against avian influenza virus  
Pengxiang Chang, The Pirbright Institute  
11:00-11:15 |
Elizabeth Duperret, The Wistar Institute |
| [O8.4] Preclinical assessment of multivalent vaccine vectors against filoviruses  
Sarah Sebastian, University of Oxford |
| [O9.4] Immune Engineered H7N9 influenza hemagglutinin overcomes poor vaccine immunogenicity  
Annie De Groot, EpiVax Inc.  
11:15-11:30 |
| 11:50-12:05 | [O7.5] DNA-based cancer vaccines designed by SynCon® technology break tolerance in genetically diverse pre-clinical models  
Jian Yan, Inovio Pharmaceuticals |
| [O8.5] Assessment of protective immunity elicited by chimeric hemagglutinin-based universal influenza virus vaccines against pandemic H1N1 infection in preclinical ferret studies  
Wen-Chun Liu, Icahn School of Medicine at Mount Sinai |
| [O9.5] OMV based vaccine formulations against shigatoxin producing Escherichia coli strains are both protective in mice and immunogenic in calves  
Matias Fingermann, Instituto Nacional de Producción de Biológicos  
11:30-11:45 |
| 12:05-12:20 | [O7.6] Synthetic immunogens drive potent antigen specific anti-tumor immune responses  
Bernadette Ferraro, Inovio Pharmaceuticals |
| [O8.6] Improved Correlation between Dengue vaccine Clinical Efficacy and Protection in NHP using an intravenous, high-dose challenge model  
Veronique Barban, Sanofi Pasteur |
| [O9.6] Impact of obesity on humoral and cellular immune responses to vaccination against tick borne encephalitis  
Ursula Wiedermann, Medical University Vienna  
11:45-12:00 |
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Maria Isaguliants, Riga Stradins University |
|              | [O8.7] Induction of binding and functional antibody responses to HIV-1 envelope V2 peptide with army liposome formulation as the adjuvant
Mangala Rao, Walter Reed Army Institute of Research |
|              | [O9.7] Vaccine batch to vaccine batch comparison by consistency testing (VAC2VAC)
Hilde Depraetere, European Vaccine Initiative 12:00-12:15 |
| 12:45–14:00  | Lunch (“Social – Modules” Building) Sponsored By: Pfizer |
| 13:15–14:00  | Career Development Panel and “Meet the ISV Fellows” (during lunch) (CIS Building) |
| 14:00–14:30  | ISV Award Ceremony Chairs: Denise Doolan and David Weiner (CIS Building) |
| 14:30–15:30  | PLENARY SESSION 6: Public Private Partnerships Session Chairs: Margaret Liu, ProTherImmune; and David Weiner, The Wistar Institute (CIS Building) |
| 14:30–14:40  | [PL6.1] Nathalie Garcon, Bioaster |
| 14:50–15:00  | [PL6.3] Odile Leroy, European Vaccine Initiative |
Laurence Torcel-Pagnon, Sanofi Pasteur |
| 15:10–15:50  | OPEN DISCUSSION WITH ALL PARTICIPANTS (CIS Building) |
| 15:50–16:00  | Closing Remarks: Denise Doolan and David Weiner Introduction to 2018 ISV Annual Congress – Ted Ross, University of Georgia (CIS Building) |
Invited Speakers

Daniel Anderson  Cecil Czerkinsky  Adil Daud  Darragh Duffy  Stephanie Erbar  Heinz Feldmann

Nathalie Garçon  Peter Gilbert  Davinder Gill  Scott Halstead  Paul Heath  Peter Hotez

Kathrin Jansen  Stefan Kaufmann  Marie-Paule Kieny  Jerome Kim  Hiroshi Kiyono  Gary Kobinger

Wayne Koff  Antonio Lanzavecchia  Odile Leroy  Gerald Linette  Shabir Madhi  Jacqueline Miller

Louis Picker  Felix Rey  Allan Saul  Rafick-Pierre Sékaly  Tonya Villafana  Jun Zhang
Daniel Anderson

Professor Daniel G. Anderson is a leading researcher in the field of nanotherapeutics and biomaterials. He is appointed in the Department of Chemical Engineering, the Institute for Medical Engineering and Science, the Koch Institute for Integrative Cancer Research, and the Harvard-MIT Division of Health Science and Technology at MIT. The research done in Prof. Anderson’s laboratory is focused on developing new materials for medicine. He has pioneered the development of smart biomaterials, and his work has led to advances and products in a range of areas, including medical devices, cell therapy, drug delivery, gene therapy and material science. Prof. Anderson received a B.A. in mathematics and biology from the University of California at Santa Cruz and a Ph.D. in molecular genetics from the University of California at Davis. His work has resulted in the publication of over 300 papers, patents and patent applications. These patents were the basis for the foundation of a number of companies in the pharmaceutical, biotechnology, and consumer products space, and have led to a number of products that have been commercialized or are in clinical development.

Abstract [PL4.2]:
High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing RNA delivery and gene editing systems. Libraries of degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to deliver RNA, both in vitro and in vivo. A number of delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer

Cecil Czerkinsky

Cecil Czerkinsky currently serves as Research Director for the French National Institutes of Health and Medical Research (Inserm) at the Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), a joint CNRS-INSERM-University of Nice research center located in the Sophia-Antipolis Science Park on the French Riviera.

Prior to returning to France in 2014, he served as Deputy Director General of the International Vaccine Institute in South Korea, and as Professor at the University of Göteborg, Sweden.

He has published over 180 articles in the areas of experimental and clinical immunology, with a main focus on mucosal immunology and vaccine science.

During his career, Cecil Czerkinsky held various academic appointments, including faculty positions in Sweden and the US. He has served as adviser for various national, foreign and supranational agencies and foundations. He graduated from Nice and Lyon Medical and Dental Schools (France) and holds a PhD from the University of Gothenburg (Sweden).

Abstract [O5.2]:
As of today, all vaccines but one (oral polio vaccine) recommended by the Expanded Program on Immunization are administered by way of injections. As such, these vaccines induce immunity in blood and in peripheral tissues but are relatively inefficient for eliciting immune responses in mucosal tissues. On the other hand, mucosal (oral, sublingual, nasal, rectal) administration of a number of experimental as well as few licensed enteric vaccines have been shown to be more efficient for inducing mucosal intestinal immune responses, especially in young infants.

Since the first licensure of the Sabin oral polio vaccine over half a century ago, only 8 mucosal vaccines (for 4 disease indications) have been licensed and all but one are given orally. While mucosal vaccines offer programmatically attractive tools for facilitating vaccine deployment, their development remains hampered by several factors:
- Lack of knowledge regarding the functional properties of the mucosal immune system during early life;
- Lack of mucosal adjuvants, limiting mucosal vaccine development to live-attenuated or killed whole virus/bacteria vaccines;
-Lack of correlates/surrogates of mucosal immune protection;
-Limited knowledge of the factors contributing to mucosal vaccine underperformance in undernourished children from lesser developed countries.

There are now good reasons to believe that with the development of safe mucosal adjuvants and that of programmatically feasible intervention strategies - alternative routes of vaccine administration; mucosal-systemic prime-boost; macro- and micro-nutrient supplementation; use of anti-inflammatory agents - could enhance the efficacy of current mucosal vaccines, especially in lesser developed countries which are often co-endemic for concurrent infections and undernutrition.

Addressing these issues in relation to programmatic feasibility will require strategic partnering between vaccinologists, formulation scientists, vaccine manufacturers, and policy researchers.

Adil Daud

Adil Daud is an expert in the therapy and development of drugs for melanoma. His fellowship in medical oncology was at the Memorial Sloan Kettering Cancer Center and was a faculty member at the Moffitt Cancer Center in Tampa, Fl. He is currently Professor of Medicine and Dermatology at the University of California, San Francisco. He is the director of the Melanoma Program at the Helen Diller Family Cancer Center. Over the last decade, he has been involved in several key clinical trials, focusing on novel targeted therapy and immunotherapy for melanoma and for other tumors. His recent research has focused on the microenvironment as it related to the immune system.

Abstract [O7.2]:
Cancer Immunotherapy has lead to tremendous advances in the last 5 years doubling or in some cases tripling survival in solid tumor malignancies and blood cancers. Critical to these advances has been the use of immune checkpoint blockers such as PD-1/PDL-1 and CTLA-4 antibodies. Some of the specific features of cancer that enable effective immunotherapy are coming into view-these include a Th-1 biased CD4 help, M1 polarized macrophages, permissive NK cells and crucially the presence of activated CD8+ cells within the tumor microenvironment. Most tumors however (60% of melanomas and 60-80% of lung and head and neck cancers and >90% of breast cancers) lack one or more elements of a productive immune response. Several strategies have emerged to address these immune deficits which may arise from the developmental trajectories of cancer. These include the concept of in-situ vaccination as well as novel strategies to recruit T cells and NK cells as well as alternative checkpoints.

Darragh Duffy

Throughout my research career I have been interested in how a better understanding of fundamental immunology can be applied to novel solutions for improving human health. For this reason I pursued a PhD in immunological memory, and with progression of my career moved closer to translational research to deliver research findings to the clinic. During my post-doctoral positions I worked on translational vaccine development projects for HIV and TB to understand how different vaccination strategies differentially impact innate and adaptive immunity. More recently I have focused on biomarkers that are predictive of spontaneous clearance and response to therapy for Hepatitis C patients. This approach is helping us to understand what is required for a protective immune response to HCV while at the same time giving us unique tools to help manage therapeutic decisions. My current focus as scientific manager of the LabEx Milieu Interieur project at Institut Pasteur, Paris is to understand the genetic and environmental determinants of a healthy immune response, and also apply these findings to disease settings to develop new diagnostic and therapeutic approaches.

Abstract [O6.3]:
Susceptibility to infections, disease severity, and response to vaccines are highly variable from one individual to another. Medical practices and public health policies typically take a ‘one size fits all’ model for disease management and vaccine development. This approach ignores individual heterogeneity in immune responses that likely impacts the response to therapy or the efficiency and development of side
effects secondary to vaccine or treatment administration. Due to the complexity of immune responses at
the individual and population level, it has been challenging thus far to define the borders of a healthy
immune system as well as the parameters (genetic, epigenetic, and environmental) that drive its naturally-
occurring variability. In particular, such assessments require large sample sizes, consensus for defining
“healthy”, and standardized protocols for sample recruitment. In this context, the Milieu Intérieur
Consortium initiated in September 2012 a cross-sectional healthy population-based study of 1,000
healthy volunteers, split equally by sex (1:1 sex ratio) and stratified across five-decades of life. The
overall aim of the Milieu Intérieur study is to assess the factors underlying immunological variance within
the general healthy population. The primary objective is to define genetic and environmental factors that
contribute to the observed heterogeneity in immune responses. This is being realized through the
characterization and integration of (i) lifestyle and medical history (ii) genome-wide SNP genotyping and
whole-exome sequencing (iii) metagenomic diversity based on sequence analysis of bacterial, fungal and
viral populations in fecal and nasal samples; (iv) induced transcriptional and protein signatures by whole
microbes, microbial-associated molecular pattern (MAMP) agonists, medically relevant cytokines, or
stimulators of the T cell response; and (v) variability in levels of circulating immune cell populations based
on flow cytometry. In parallel a number of disease specific studies lead by consortium members, and a
pilot study on healthy donors in collaboration with Institut Pasteur Senegal have been initiated. These
results will lay the foundations for a better understanding of immune response variability helping to
support new precision vaccination strategies.

Stephanie Erbar

I have studied biology at the University of Marburg with special focus on virology, mostly on principal
research using BSL-4 classified viral agents. My main focus during the work at the institute of virology in
Marburg was to understand the pathology and tropism of a relatively new identified paramyxovirus named
Nipah. The virus was discovered in the year 2000 and the mechanism of infection, spreading and
especially the observed overcoming of the blood-brain-barrier in humans was not known. During this
research we were able to show that the selective infection of endothelial cells of the BBB is a crucial
mechanism to get into the brain in humans. In 2013 I started to work in Mainz at the BioNTech AG and
since then focus on establishing the RNA platform that was built for cancer therapy for the usage as a
prophylactic vaccine platform against infectious diseases (A.I.R© vaccines). My team mainly uses
Influenza A as a model system for the development of our platform, as we have the ability to perform
challenge infections in mice using this viral system, to show the protective potency of our vaccines. As
our platform is build modular it can be used for every emerging or re-emerging virus that comes up in the
population. BioNTech RNA Pharmaceuticals GmbH aims to solve the problem of long production times in
case of new emergencies and wants to contribute to new global vaccination programs that need in-time
delivery of cost-effectively produced vaccines by the usage of RNA to keep the health of the population.

Abstract [PL4.1]:

Vaccines are one of the most effective methods of controlling infectious diseases. However, todays
vaccine production is facing difficult challenges, as the concepts are not fast and flexible enough to allow
quick responses for the efficient control and prevention of global outbreaks of newly emerging and re-
emerging viruses. The large Ebola virus outbreak in West Africa in 2014 is only one example, showing
that areas with resource-limited setting need to come into the focus of vaccination programs and that the
risk of worldwide propagation of deadly viral threats is getting higher with proceeding globalization. To
meet this challenge, BioNTech RNA Pharmaceuticals GmbH is developing an innovative synthetic
Amplified Immune Response (A.I.R) vaccine platform against Infectious Diseases that is characterized by
low costs, short manufacturing times, high flexibility, thermostability and the feasible production of at least
100,000 RNA-based human vaccination doses per week. The production of self-amplifying RNA (saRNA)
is totally cell-free and the big advantage over conventional vaccines is the induction of both B cell- and T
cell-responses. The administration of in vitro transcribed saRNA results in higher antigen expression than
delivery of comparable amounts of mRNA, correlating rather to the final subgenomic transcript copy
number than to initially transferred RNA amounts. However, antigen expression is still transient as innate
immunity effectively prevents persistent replication. BioNTech’s A.I.R vaccines are combined with
formulations specifically designed and preclinically tested for different application routes, to protect saRNA from degradation in vivo. The feasibility of A.I.R vaccine platform is analyzed at the preclinical stage mainly using influenza as model system in mice. BioNTech was able to show protection against live viral challenge after prime-boost as well as after single vaccination by using submicrogram quantities.

Heinz Feldmann

Heinz Feldmann is the Chief of the Laboratory of Virology and the Chief Scientist of the Rocky Mountain Laboratories’ BSL4 facility in the Division of Intramural Research at the National Institute of Allergy and Infectious Diseases, National Institutes of Health. His expertise is in high containment viruses, field studies and outbreak management His research interests are viral hemorrhagic fevers and other emerging zoonotic viruses with the focus on vaccine development and pathogenesis.

Abstract [PL3.1]:

Vesicular stomatitis virus (VSV), a rhabdovirus, has been established as a promising vaccine vector over the past years. Rose and colleagues developed the reverse genetics system allowing easy manipulation of the VSV genome with optimized expression of foreign antigens. The ability of VSV to grow to high titers, to induce strong innate and adaptive immune responses and the limited pre-existing immunity to VSV in the human population make this system a promising vaccine platform. We and others have utilized this system to generate vaccine vectors against emerging zoonotic diseases such as Ebola virus disease and Lassa fever. The vectors have shown promising use as prophylactic and emergency vaccines as exemplified by the ring vaccination approach applied in Guinea during the West African Ebola outbreak. This presentation will introduce the platform and summarize the key aspects of pre-clinical and clinical development.

Work on vaccines for emerging zoonotic diseases at NIAID is funded by the Intramural Research Program.

Nathalie Garçon

Nathalie Garçon is currently the chief executive (CEO) and scientific officer (CSO) of BIOASTER. She joined BIOASTER, the French technology research institute for infectiology and microbiology as chief scientific officer in July 2014. In this role, Dr Garçon directs the scientific aspect of the institute with the objective of accelerating the access to new technologies in infectious diseases that patient can benefit from. She was promoted to CEO in April 2015. Dr Garçon is a biological Pharmacist by training, and following the successful completion of two PhDs, one in pharmaceutical science and one in immunotoxicology / immunopharmacology, Dr Garçon moved to the UK for 1 year as a postdoctoral research fellow (the Royal Free Hospital London) undertaking research on liposomes in vaccines.

She then moved to the USA where she spent 4 years at Baylor College of Medicine in Houston, Texas, first as a postdoctoral research fellow, then as research assistant professor, working on vaccine delivery systems and immunopotentiators.

Dr Garçon joined SmithKline Beecham Biologicals now GlaxoSmithKline Vaccines in 1990, where she set up and led the vaccine adjuvant and formulation group. She moved from this position, to head of formulation technologies, head of research, vice president, head of global research and North America RD; and vice president, head of the global adjuvants and delivery systems centre for vaccines. In her last role within GSK vaccines before joining BIOASTER, Dr Garçon hold the position of vice president, head of adjuvants and technologies innovation center, where she provided leadership within GSK vaccines in the fields of new vaccines technologies, from discovery to registration and commercialization. Dr Garçon expertise in vaccinology extends from research to manufacturing, in particular immunology, adjuvant and formulation technologies, analytical methods, animal experimentation and toxicology/safety evaluation and testing. Dr Garçon is the 2014 laureate of the Stanley Plotkin award for lifetime achievement in the
field of vaccines and vaccine technologies. She has authored over 70 papers and book chapters, and holds more than 100 patents.

Abstract [PL6.1] not available at time of print 2017-09-25

Peter Gilbert

Dr. Gilbert is Member and Head of the Biostatistics, Bioinformatics, and Epidemiology Program of the Vaccine and Infectious Disease Division at the Fred Hutchinson Cancer Research Center, and Research Professor in the Department of Biostatistics at the University of Washington. Dr. Peter Gilbert has more than 20 years of experience in the design and analysis of randomized clinical trials for preventive vaccines against HIV, malaria, dengue, and other pathogens. Through his work as Principal Investigator of the Statistical Data Management Center for the HIV Vaccine Trials Network (HVTN), this research has centered on the design and analysis of HIV vaccine efficacy trials and related efficacy trials (e.g., for broadly neutralizing monoclonal antibodies), for which Dr. Gilbert has provided statistical science leadership. Dr. Gilbert has developed novel statistical methods for vaccine (and monoclonal antibody) efficacy trials, especially in the areas of surrogate endpoint/correlates of protection evaluation and sieve analysis of pathogen sequences, conducted within the fields of semiparametric inference, survival analysis, and causal inference. Dr. Gilbert's public health service includes chairing Data Safety Monitoring Boards and membership on the FDA's Advisory Committee for Vaccines and Related Biological Products (2008–2012).

Abstract [PL1.2]:

A primary goal of HIV prevention research is development of an HIV vaccine with durable efficacy to prevent HIV infection, and to develop biomarker correlates of that efficacy that would enable iterative improvement of the vaccine regimen and bridging. Accordingly, the two current HIV vaccine efficacy trials – HVTN 702 of an ALVAC-gp120 bivalent clade C prime-boost vaccine regimen versus placebo in South African men and women and HVTN 705/ VAC89220HPX2008 of a heterologous mosaic Ad26/gp140 clade C prime-boost vaccine regimen versus placebo in sub-Saharan African women – are designed to learn about efficacy durability and correlates. This talk discusses the design of these efficacy trials, with focus on how the statistical analysis of these trials can yield knowledge about immunological correlates of risk and vaccine efficacy and about viral sequence correlates of vaccine efficacy.

Davinder Gill

Dr. Davinder Gill has served as the Chief Executive Officer at Hilleman Laboratories since 2012. Dr. Gill comes with two decades of R&D and business experience in Global Pharma and Biotech companies. At Hilleman Labs, Dr. Gill is responsible for Company Strategy, Product Development, Fund Raising and Public Affairs.

Dr. Gill started his career in 1998 at Millennium Pharmaceuticals in Cambridge, Massachusetts in the area of Biotherapeutics. In 2003, he moved to Wyeth, Collegeville, Pennsylvania where he served in roles of increasing responsibility as Vice President of Biological Technologies. At Wyeth, Dr. Gill was instrumental in creating and leading first-of-their-kind R&D Laboratories in Ireland and Scotland in collaboration with local governments. After the acquisition of Wyeth in 2009, Dr. Gill served as Vice President and Head, Global Biotherapeutics at Pfizer, New York where he successfully led an international team and was a member of Pfizer CEO Council. In 2012, Dr. Gill was appointed CEO at Hilleman Laboratories.

Dr. Gill holds a Ph.D in Chemical Engineering from University of Houston, Texas and is an alumnus of the Indian Institute of Technology. He completed a Cancer Research Institute (New York) Fellowship at the Massachusetts General Hospital/Harvard Medical School in Boston, Massachusetts.
In addition to his role as CEO at Hilleman Labs, Dr. Gill is also a member of The Wellcome Trust (London, UK) Affordable Healthcare Initiative as well as the National Committee on Public Health, Confederation of Indian Industry (New Delhi, India).

Abstract [PL5.3]:

Many first-generation vaccines were developed without the specific needs of low-income countries in mind. This is a much-needed exploration of how to tackle delivery of life-saving vaccines without the requirement for large bulk shipments, expensive warehousing and costly, difficult-to-maintain cold chains. Hilleman Laboratories aims to bridge the gap in vaccine equity by establishing clinical proof-of-concepts for promising vaccines. We seek to contribute to forward-thinking science and to move new solutions from bench to bedside quickly and effectively. This talk will highlight the approach undertaken by Hilleman Laboratories to develop affordable and effective vaccines for diseases that commonly affect people in low-income countries, especially children.

Scott Halstead

Scott B Halstead, M.D. is an independent consultant and Adjunct Professor, Department of Preventive Medicine and Biometrics, Uniformed University of the Health Sciences, Bethesda, Maryland. From 2010-16 he served as Senior Advisor, Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Korea. From 3002-2010 he was Director, Supportive R&D, Pediatric Dengue Vaccine Initiative. After obtaining an MD he did hospital training in Internal Medicine and from 1957-68 served in the U.S. Army Medical Corps at the Department of Virus and Rickettsial Diseases, 406th Medical General Laboratory, Sagami Ono, Japan, the Department of Virus Diseases, Walter Reed Army Institute of Research, the Virology Department, SEATO Medical Research Laboratory, Bangkok, Thailand and the Yale Arbovirus Research Unit, New Haven, Connecticut. From 1968-1983, he was Professor and Chair, Department of Tropical Medicine and Medical Microbiology, John Burns School of Medicine, University of Hawaii at Manoa. From 1983-1995, at the Rockefeller Foundation, he served successively as Associate, Deputy and Acting Director of the Health Sciences Division. From 1995-1999, he was Scientific Director, Infectious Diseases Program, U.S. Navy, Bethesda, Maryland and Senior Scientist, Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland. Dr. Halstead is a world leader in research on dengue and other arthropod-borne viral infections. He has published over 400 scientific papers and book chapters on many areas of flavivirology, vaccine research and in international health development. In 1990, while with the Rockefeller Foundation, he co-founded the Children’s Vaccine Initiative. Dr. Halstead graduated from Yale University with a B.A. in 1951 and from Columbia University with a M.D. in 1955.

Abstract [O8.1]:

Dengvaxia, a live-attenuated tetravalent chimeric yellow fever dengue vaccine, has completed phase III per protocol analyses on 32,568 children in 10 dengue-endemic countries, vaccinated and controls, ages 2 – 16 years. After 2 years observation, there were 32 dengue infection events among 682 children vaccinated as seronegatives compared with 27 events among 365 controls, 36.6% efficacy, p= 0.08. Vaccinated seronegatives were poorly protected. These children acquired dengue infections despite the documentation that all vaccinated children raised broad dengue neutralizing antibodies. Over a surveillance period of 5 – 6 years, among 22,630 children given Dengvaxia, 854 experienced a virologically documented acute dengue clinical illness of whom 300 were hospitalized. During year 3, dengue hospitalizations occurred at a high rate among 2 -5 year olds, 51% of whom were seronegative. Throughout the world, first dengue infections in seronegative young children are usually inapparent and only infrequently mild. During years 3 – 6, a total of 66 vaccinated children in this age group were hospitalized. These children likely were seronegative when vaccinated. Hospitalizations of young children during a first dengue infection cannot be labeled as “normal.” These are instances of vaccine enhanced dengue disease.
Dengvaxia has been shown to raise broad dengue neutralizing antibodies, similar to those passively acquired from dengue-immune mothers that protect infants from dengue disease. But these same maternal antibodies at lower concentrations efficiently enhance dengue infections to a severe and life-threatening disease. Recent studies implicate dengue NS1 as a direct viral toxin, demonstrating how dengue infections enhanced by actively or passively acquired enhancing antibodies can produce the same vascular permeability syndrome. Thus, the identical clinical dengue disease would be expected to occur in placebos who are experiencing a second heterotypic dengue infection and among vaccinees, experiencing vaccine antibody enhanced dengue infections.

**Paul Heath**

Paul Heath is a Professor and Honorary Consultant in Paediatric Infectious Diseases at St George’s University Hospitals NHS Foundation Trust and St George’s, University of London, where he co-leads the Paediatric Infectious Diseases Research Group and is the Director of the Vaccine Institute. His training in paediatrics and infectious diseases was at the Royal Children’s Hospital, Melbourne, Australia, the John Radcliffe Hospital, Oxford and St George’s Hospital, London. His particular research interests are in the epidemiology of vaccine preventable diseases, in clinical vaccine trials, particularly in at-risk groups, and in perinatal infections. He coordinates a European neonatal infection surveillance network (neonIN: https://www.neonin.org.uk) and other recent work includes national surveillance on neonatal meningitis and invasive GBS and Listeria infections, maternal immunisation trials and studies of different vaccine schedules in preterm infants. He sits on national UK committees concerned with meningitis, Group B streptococcus prevention and immunisation policies in children. He is Chair of the research committee of the European Society of Paediatric Infectious Diseases, Associate Chief Editor of the Pediatric Infectious Diseases Journal and member of the Global Alignment of Immunisation safety Assessment in pregnancy (GAIA) Executive Committee.

**Abstract [O1.2]:**

Streptococcus agalactiae (group B streptococcus (GBS)) is the leading cause of early-onset neonatal infections and neonatal meningitis in many countries. Intrapartum antibiotic strategies have reduced the incidence of early-onset neonatal GBS in a number of countries but have had no impact on late onset GBS infection (LOD). In low/middle income settings, the disease burden remains uncertain although in several countries of Southern Africa appears comparable to or higher than that of high-income countries. As disease may be rapidly fulminating cases can be missed before appropriate samples are obtained and this may lead to underestimation of the true burden. Given the rapid onset and progression within hours of birth as well as the deficiencies in IAP strategies and absence of a solution for preventing LOD, it is clear that administration of a suitable vaccine in pregnancy could provide a better solution in all settings; it should also be cost effective. The current leading vaccine candidates are CPS-protein conjugate vaccines and many trials, including in pregnancy, have been undertaken. Protein-based vaccines are also in development and one candidate is now in clinical trials.

**Peter Hotez**

Peter J. Hotez, M.D., Ph.D. is Dean of the National School of Tropical Medicine and Professor of Pediatrics and Molecular Virology & Microbiology at Baylor College of Medicine where he is also the Director of the Texas Children’s Center for Vaccine Development (CVD) and Texas Children’s Hospital Endowed Chair of Tropical Pediatrics. He is also University Professor at Baylor University, and Fellow in Disease and Poverty at the James A Baker III Institute for Public Policy.

Dr. Hotez is an internationally-recognized physician-scientist in neglected tropical diseases and vaccine development. As head of the Texas Children’s CVD he leads the only product development partnership for developing new vaccines for hookworm infection, schistosomiasis, and Chagas disease, and SARS/MERS, diseases affecting hundreds of millions of children and adults worldwide. In 2006 at the
Clinton Global Initiative he co-founded the Global Network for Neglected Tropical Diseases to provide access to essential medicines for hundreds of millions of people

He obtained his undergraduate degree in molecular biophysics from Yale University in 1980 (phi beta kappa), followed by a Ph.D. degree in biochemistry from Rockefeller University in 1986, and an M.D. from Weil Cornell Medical College in 1987. Dr. Hotez has authored more than 400 original papers and is the author of the acclaimed Forgotten People, Forgotten Diseases (ASM Press) and the recently released Blue Marble Health: An Innovative Plan to Fight Diseases of the Poor amid Wealth (Johns Hopkins University Press).

Dr. Hotez served previously as President of the American Society of Tropical Medicine and Hygiene and he is founding Editor-in-Chief of PLoS Neglected Tropical Diseases. He is an elected member of the National Academy of Medicine, and in 2011 he was awarded the Abraham Horwitz Award for Excellence in Leadership in Inter-American Health by the Pan American Health Organization of the WHO. In 2014-16 he served in the Obama Administration as US Envoy, focusing on vaccine diplomacy initiatives between the US Government and countries in the Middle East and North Africa.

Abstract [PL5.2]:

The neglected tropical diseases represent a group of 20 chronic and debilitating infections recognized as 'NTDs' by the World Health Organization. Most NTDs are caused by eukaryotic parasitic pathogens, which represent complex vaccine targets. Moreover, they are all almost exclusively diseases of extreme poverty without traditional commercial markets, even though many NTDs are re-emerging due to modern 21st century forces including conflict, climate change, human migrations and a shifting poverty paradigm known as 'blue marble health'. For all these reasons, the development of NTD vaccines is extremely complex, further compounded by a rising antivaccine movement, especially in America. Efforts develop NTD vaccines are underway for diseases such as schistosomiasis, hookworm infection, onchocerciasis, leishmaniasis, and Chagas disease are underway at the Texas Children’s CVD. Our approach relies predominantly on recombinant protein technology using low-cost bacterial and yeast expression platforms and 5-20 L scale-up. Both hookworm and schistosomiasis vaccines are in phase 1 clinical trials in the US, Brazil, or Gabon, while the other vaccines are advancing through preclinical development. Each vaccine is being developed through innovative consortia of academic and industrial partners. A unique aspect of our approach is the involvement of partners from the disease endemic countries, with simultaneous capacity building efforts in the area of vaccine biotechnology.

Kathrin Jansen

Kathrin Jansen, Ph.D., is the Senior Vice President and Head of Vaccine Research and Development (VRD) at Pfizer Inc, and a member of Pfizer’s Worldwide Research and Development leadership team. Dr. Jansen leads a fully integrated, global vaccines research and development organization, with responsibilities ranging from discovery to registration and post-marketing commitments and a clinical vaccines portfolio that includes vaccines to prevent or treat diseases of significant unmet medical need such as Staphylococcus aureus, Clostridium difficile, Group B streptococcus and prostate cancer. More recent accomplishments are the global licensures of Prev(e)nar13® to prevent pneumococcal diseases and the development and licensure of Trumenba®, the first vaccine licensed in the United States to prevent invasive disease caused by Neisseria meningitidis serogroup B.

Before the Wyeth acquisition by Pfizer in 2009, Dr. Jansen served as Senior Vice President at Wyeth Pharmaceuticals and on Wyeth’s R&D Executive Committee since 2006 and was responsible for vaccine discovery, early development and clinical testing operations. Dr. Jansen spent 12 years at Merck Research Laboratories where she directed or supported a number of vaccine efforts, including Merck’s novel bacterial vaccine programs and viral vaccine programs (rotavirus, zoster and mumps, measles and rubella). Dr. Jansen initiated and led the development of Gardasil®, the world’s first cervical cancer vaccine.
Dr. Jansen received her doctoral degree in microbiology, biochemistry & genetics from Phillips Universitaet, Marburg, Germany, in 1984. Following completion of her formal training, she continued her postdoctoral training at Cornell University working on the structure and function of the acetylcholine receptor. She then joined the Glaxo Institute for Molecular Biology in Geneva, Switzerland, where she focused on basic studies of a receptor believed to be a drug target to treat allergies.

Dr. Jansen was appointed an Adjunct Professor at the University of Pennsylvania – School of Medicine in 2010. Dr. Jansen has authored and co-authored over 150 publications.

Abstract [PL2.2]:

Antimicrobial resistance (AMR) is an adaptive response mechanism of bacterial pathogens to counteract the globally wide spread but often inappropriate use of life saving antibacterial drugs. Recently, we have been observing an unacceptable increase in AMR characterized by bacterial resistance to not just one but multiple classes of antibiotics coupled with a decrease in R&D efforts to develop new classes of antimicrobial drugs. The consequence of this development is, that infections become more difficult to treat and in some cases impossible to control. In addition, AMR is associated with substantial added economic and societal costs. While renewed R&D efforts to discover the next wave of antibacterial drugs are vital in addition to globally practiced stringent antibiotic stewardship, vaccines are emerging as essential and highly effective tools in the fight against AMR. Prophylactic use of bacterial vaccines prevents bacterial infections in the first place and therefore reduces the need for antibiotic prescriptions, thus minimizing the selective pressure of generating resistant strains. One prominent example of how vaccines can assist in the fight against AMR, are the pneumococcal conjugate vaccines, a success story in not only reducing pneumococcal disease incidence and mortality globally, but also reducing antibiotic use and lowering emergence of antimicrobial resistant strains in vaccinated populations. Another example is the success of H. influenza (Hib) vaccines in reducing β-lactamase mediated resistance to ampicillin and other β-lactam antibiotics. These positive vaccine effects are not just limited to bacterial vaccines. Viral vaccines such as those to prevent influenza can prevent directly the inappropriate prescription of antibiotics for a viral disease and in addition lower the frequency of bacterial superinfections thus furthermore reducing antibiotic use. Apart from pneumococcal conjugate and influenza vaccines, other vaccines currently in development to prevent infections with *Clostridium difficile*, *Staphylococcus aureus*, and Group B streptococcus or respiratory syncytial virus could also play a major role in prevention of AMR, assuming successful development and global use of such vaccines. The presentation will describe the drivers of AMR, make the case how existing vaccines are already participating in combatting AMR, and provide a vision how new vaccines in development may play a major role in reducing AMR in addition of providing direct disease prevention benefit.

Abstract [O4.3]:

Mortality reduction in all ages has added extra years of life to individuals globally. Increasing life spans will likely continue; however, gains may be offset unless accompanied by increasing good health. Ageing individuals and societies demand a comprehensive, systematic methodology to achieve both longer and healthier lives. Such programs must and will include vaccines for the elderly. Individuals never outgrow the need for vaccines. The specific immunizations needed for adults are determined by factors such as age, lifestyle, high-risk conditions, type and locations of travel, and previous immunizations. Some adults incorrectly assume that the vaccines they received as children will protect them through adulthood. However, as we age, we become more susceptible to serious disease caused by common infections (such as flu and pneumococcus). Immunity from childhood vaccines can fade over time and newer vaccines were not available when some adults were children (such as *Streptococcus pneumoniae* and herpes zoster). A vision for adult vaccination will be presented.
Stefan Kaufmann

Founding Director of the Max Planck Institute for Infection Biology in Berlin, where he heads the Department of Immunology. Professor for Microbiology and Immunology, Charité University Clinics Berlin. Studied biology at the Johannes Gutenberg University of Mainz, 1977 PhD (highest degree, summa cum laude). From 1987 to 1991 Professor for Medical Microbiology and Immunology, and from 1991 to 1998 full Professor for Immunology at the University of Ulm. Doctor Honoris Causa from the Aix-Marseille Université. Fellow of the Royal College of Physicians of Edinburgh (FRCP Edin). Honorary Professor of the Universidad Peruana Cayetano Heredia, Lima, Peru; Guest Professor at Tongji University, School of Medicine, Shanghai, China; and Visiting Professor, Peking Union Medical College Beijing, China. Scientific interests: immunity to bacterial pathogens with emphasis on tuberculosis and rational design of vaccines and biosignatures. Developer of a tuberculosis vaccine with successful completion of a phase II clinical trial. Former President and honorary member of the German Society for Immunology. Former President of the European Federation of Immunological Societies and of the International Union of Immunological Societies. Member of the Executive Committee of the Robert Koch Foundation. Chair of the Board of the Schering Foundation. Chair of the Board of the Memento Prize. Board Member of the Global Alliance for Vaccines and Immunisation (GAVI) from 2010 to 2013. From 2009 to 2014 member of the Scientific Board of the Global Alliance for TB Drug Development (TB Alliance). Member of the Strategic Advisory Committee of the European and Developing Countries Clinical Trials Partnership (EDCTP). Initiator of the global Day of Immunology to raise public awareness in immunology. Scientific advisor on “Health” for the G7 summit in Elmau in 2015 as well as for the G20 summit in Hamburg in 2017. Numerous scientific awards. Coordinator of several international and interdisciplinary projects, e.g. Grand Challenge 6 of the Bill and Melinda Gates Foundation. More than 900 publications mostly in high-ranking journals with more than 55,000 citations (Google Scholar); h-index (according to J.E. Hirsch): Google Scholar: 122; Thomson/ISI: 100. Editor or member of editorial boards of more than 20 international scientific journals. Member of numerous professional societies and academies including American Academy of Microbiology, Berlin–Brandenburg Academy of Sciences and Humanities, German National Academy of Sciences Leopoldina, World Innovation Foundation and European Molecular Biology Organization (EMBO).

Abstract [PL1.1]:

Tuberculosis (TB) is a health threat of global dimension with 10.4 million individuals developing active disease and 1.8 million dying in 2015. 2 billion individuals are infected with Mycobacterium tuberculosis (Mtbt), of whom ca. 90% will carry the pathogen lifelong. These individuals remain latently infected with dormant Mtb which is contained by a balanced host response. In 5% to 10% of infected individuals, TB disease emerges due to unbalanced host resistance. Efficacious control of TB requires better vaccines, diagnostics and drugs. The current vaccine, BCG, prevents disseminated childhood TB but fails to protect against pulmonary TB in all age groups. We have genetically modified BCG to improve its protective efficacy and termed the new vaccine VPM1002. It has completed two phase I and one phase IIa trial and is currently assessed in a large phase II trial in HIV-exposed newborn. In 2017, it will start a phase II/III trial in adult TB patients, who had been cured by chemotherapy. In 10% of these individuals, recurrence occurs either due to re-infection or relapse. The capacity of the vaccine to prevent recurrence will be determined in a multi-centric trial in India. Host biomarkers allow differential diagnosis between active TB, healthy individuals including latent TB infection and other diseases. This has been achieved both on the transcriptome and on the metabolome level. More recently, a biosignature which diagnoses subclinical TB and hence prognoses active TB in latently TB-infected healthy individuals has been defined. Such biomarkers not only provide guidelines for preventive drug treatment of TB high risk individuals. They will also allow stratification for high TB risk for clinical vaccine trials with smaller group size and shorter trial length. In addition, biomarker studies have shed light into thus far unknown host mechanisms of protection and pathology as well as resistance and susceptibility.
**Marie-Paule Kieny**

Dr. Marie-Paule Kieny is currently Director of Research at Inserm (Institut national de la santé et de la recherche médicale) in Paris, where she assists the President on international cooperation. Until June 2017, on a leave of absence from Inserm, she served as the Assistant Director-General for Health Systems and Innovation at the World Health Organization. Dr. Kieny also directed the WHO Initiative for Vaccine Research from 2001 to 2010. Key successes under her leadership roles at WHO include the development and licensing of new vaccines against bacterial meningitis; addressing global supply of pandemic influenza vaccine especially in developing countries through technology transfer and manufacturing; vaccines against poverty-related diseases. Such initiatives are continuing priorities of Dr. Kieny.

Before joining WHO, Dr. Kieny held top research positions in the public and private sectors in France which included Assistant Scientific Director of Transgene S.A. from 1981 to 1988 and Director of Research and Head of the Hepatitis C Virus Molecular Virology Group at the Institute of Virology, (INSERM) from 1999 to 2000.

Dr. Kieny received her PhD in Microbiology (1980) and University Diploma in Economics from the University of Montpellier; Diplôme d'Habilitation à Diriger des Recherches from the University of Strasbourg in 1995. She has published over 350 articles and reviews, mainly in the areas of infectious diseases, immunology, vaccinology and health systems.

Dr Kieny has been awarded the title of Chevalier dans l'Ordre National de la Légion d'honneur (Knight in the National Order of the Legion of Honour, France) in 2016; Chevalier de l’Ordre National du Mérite, au titre du Ministère de la Recherche (Knight of the National Order of Merit, under the Ministry of Research, France) in 2000; the Prix Génération 2000-Impact Médecin in 1994 and the Prix de l'Innovation Rhône-Poulenc in 1991.

**Abstract [PL1.3]:**

When the Ebola outbreak in West Africa erupted in the spring of 2014, the global health community was ill prepared to cope. There were no vaccines, no treatments, few diagnostics, and insufficient medical teams and trained responders.

In spite of this lack of R&D preparedness, the Ebola experience demonstrates that, it is possible to compress R&D timelines from a decade or longer, to less than a single year. This is mostly to be credited to an unprecedented collaborative effort building on the availability of a small number of candidate diagnostic tests, drugs and vaccines that could rapidly move into clinical phase evaluation. A series of international consultations and activities – including the organization of a successful Ebola vaccine efficacy trial in Guinea - were led by WHO as a contribution to the unprecedented global efforts to control the Ebola epidemic.

Since September 2015, WHO expert teams, an international scientific advisory board, and partners engaged through global forums are collaborating to articulate a novel R&D model for emerging pathogens likely to cause severe outbreaks in the near future, and for which few or no medical countermeasures exist: the WHO R&D Blueprint. Already, several consultations have been held among national governments and public health agencies, researchers, social scientists and industry. They have selected priority pathogens on which immediate action is warranted, evaluated several promising platform technologies, identified major bottlenecks to international collaboration, agreed upon basic data sharing principles, and explored innovative approaches to conducting clinical trials. New developments on the R&D Blueprint will be presented.
Jerome Kim

Dr. Jerome H. Kim is currently the Director General of the International Vaccine Institute. He retired from the US Army in 2015 where he led the RV144 HIV vaccine trial and the post-trial search for immunologic and virologic correlates of risk. This work was recognized by the Maher Award for Research Excellence from the Uniformed Services University of the Health Sciences and the Department of the Army Research and Development Achievement Award for Technical Excellence. Dr. Kim's research interests include HIV molecular epidemiology, host genetics, and HIV vaccine development. He has authored over 200 publications and received the John Maher Award for Research Excellence from the Uniformed Services University of the Health Sciences in 2013. In 2014 he was recognized as one of the 50 most influential people in vaccines. Dr. Kim graduated with High Honors in History and Highest Honors in Biology from the University of Hawaii and received his MD degree from the Yale University School of Medicine. He is an adjunct Professor of Medicine at USUHS and the Seoul National University Graduate School of Public Health. He is a Fellow of the American College of Physicians and the Infectious Diseases Society of America.

Abstract [PL1.4]:

The coronaviruses infect many species and are often are transmitted (and adapted) between different animals and Man. The severe acute respiratory syndrome (SARS) outbreak of 2003 – 2004 is an example, involving transmission between civets and humans. The recognition of clinical MERS in 2012 quickly revealed a previously unrecognized zoonotic pathogen, now called the MERS CoV. MERS CoV causes minimal disease in its host species (dromedary camels), and many cases of transmission to humans may be asymptomatic. However, in persons with underlying comorbidity the current case fatality rate is 40%. MERS is also frequently transmitted within the household of the index case (often a person who works with camels) and nosocomial spread has also been recognized as a particular feature of this disease. The virus is not efficiently transmitted yet from human to human, so widespread epidemics have not occurred. Treatment is largely supportive. Several animal models have been developed, including transgenic mouse and 2 different non-human primate models. This has permitted testing of vaccines in an animal challenge model, and several vaccines have shown efficacy for disease or infection prevention. To date there has been a single human clinical trial reported, using a DNA vaccine that showed evidence of efficacy in small animal and NHP studies. A number of candidate products could be entering human clinical trials shortly, including both MVA- and chimp adenovirus-based MERS vaccines. WHO has developed an R&D Roadmap for MERS vaccines, and the Coalition for Epidemic Preparedness Innovation (CEPI) will shortly announce the groups selected for funding of MERS CoV vaccines.

Hiroshi Kiyono

Dr. Kiyono obtained his dental degree (D.D.S.) from Nihon University, and Ph.D. from the University of Alabama at Birmingham (UAB). His background as a dentist combined with extensive research experience in the field of Mucosal Immunology at UAB, Max-Planck Institute, Osaka University and at present, the University of Tokyo makes him exceptionally well qualified to discuss the current and future directions of mucosal immunology and mucosal vaccine development. To reflect his scientific contribution, he was listed in ISI Highly Cited Researchers’ List. He is the former President of Society for Mucosal Immunology. He received several prestigious awards such as NIH New Investigator Research Award, NIH Research Career Development Award, The Japanese Society for Vaccinology Takahashi Award and Hideyo Noguchi Memorial Medical Science Award. He has a total of 520 publications in peer review journals and edited a total of 20 books. He served as the Dean of the Institute of Medical Science, The University of Tokyo and is currently Director and Professor for International Research and Development Center for Mucosal Vaccines at the same institute. He is also holding an Adjunct Research Professorship at UAB in USA and Chiba University in Japan.
Abstract [O5.1]:

The aero-digestive mucosal surface is continuously exposed to countless numbers of beneficial (e.g., commensal bacteria) and harmful antigens (e.g., pathogenic microbe), in handling its day-to-day duties. The aero-digestive tract is thus equipped with the mucosal immune system (MIS) offering the first line of surveillance and defense platform against invasion of pathogens. The MIS is equipped with sophisticated immune induction machinery of nasopharyngeal- and gut-associated lymphoid tissues (NACT and GALT) for the generation of antigen-specific humoral and cellular immune responses. Nasal or oral immunization with an appropriate vaccine delivery vehicle thus resulted in the induction of protective immunity in both systemic and mucosal compartments leading to the double layers of protection against pathogens. Our efforts have been aiming at the development of mucosal vaccines against aero-digestive infections. For the respiratory infection, a cationic cholesteryl group-bearing pullulan nanogel (cCHP nanogel) containing pneumococcal surface protein A (cCHP-PspA nanogel) has been shown to a potent adjuvant free nasal vaccine for the induction of PspA-specific protective respiratory IgA and IgG antibodies against pneumococcal infection. For gut pathogen-induced diarrhea, our fusion science among mucosal immunology, agriculture science and plant factory resulted in the creation of rice transgenic (Tg) vaccine, “MucoRice” as a new generation of cold chain-and needle/syringe-free vaccine system which can be harvested under the GMP-closed Hydroponic Production Facility. MucoRice expressing B subunit of cholera toxin (MucoRice-CTB) has been shown to induce antigen-specific IgA-mediated protective immunity against *Vibrio cholera*-induced diarrhea. Both cCHP nanogel and MucoRice are thus attractive mucosal vaccine systems for the control of aero-digestive infectious diseases.

Gary Kobinger

Gary Kobinger is a professor in the Department of Microbiology and Infectious Diseases and the Director of the Research Centre on Infectious Diseases, Faculty of Medicine at Université Laval. He is also an adjunct professor in the Department of Pathology and Laboratory Medicine at the University of Pennsylvania, and an associate professor in the Department of Medical Microbiology at the University of Manitoba. His work focuses on developing and testing new vaccine platforms and immune treatments against emerging and re-emerging viruses of high consequences to public health. Gary's goal as Director is to develop a research framework that can respond rapidly to emerging and re-emerging pathogens.

Abstract [PL3.2]:

The re-emergence of Ebola virus in 2014 was quickly followed by Yellow Fever virus in Angola and DRCongo, as well as the emergence of Zika virus in South America, and each outbreak has had a global impact. These events have further stimulated the support and development of rapid mechanisms to better protect public health, such as malleable therapeutics and novel vaccine platforms. Importantly, some of these promising immunization strategies are capable of progressing to early human evaluation within months of conception. Alternatively, advanced clinical evaluations and the licensure process has highlighted the limitations in the ability of novel technologies to close the gap between generating a safe and immunogenic vaccine and its ability to play a meaningful role in preventing disease in humans in a timely manner. This presentation will use some of the outstanding progresses in vaccine development against Ebola and Zika viruses in the current context of timelines to applicability and discuss the perception of adverse effects in relation to damages from naturally acquired infections.

Wayne Koff

Wayne C. Koff, PhD, is founding President & CEO of the Human Vaccines Project, a public-private partnership with the goal of decoding the human immune system to accelerate development of vaccines and immunotherapies for major infectious diseases and cancers. Prior to joining the Project, he served as CSO & Senior VP, R&D, at the International AIDS Vaccine Initiative; there, Dr. Koff’s accomplishments included: Developing multiple HIV vaccines through clinical trials; Establishing state-of-the art laboratories in the US, Europe, India and Africa; Conducting the first HIV vaccine trials in India, Kenya, and Rwanda;
Establishing the Neutralizing Antibody Consortium identifying new, broad and potent neutralizing antibodies against HIV leading to the discovery of novel targets for vaccine design; and, Established a clinical research network in Africa which conducted seminal studies on HIV pathogenesis, incidence, and acute infection. Dr. Koff has published more than 100 scientific papers and edited eight books on vaccine development.

Abstract [O6.1]:

Vaccines have been one of the most effective public health interventions over the last century, preventing and controlling a broad spectrum of infectious diseases. However, over the last 15 years there have been several failures in late stage vaccine and immunotherapeutic development programs, against viral, bacterial, parasitic and neoplastic diseases. These failures are due in large part to the lack of understanding of the human immune system and reliance on empiric strategies for vaccine development that are unlikely to be effective in the future. The Human Vaccines Project (Project) is a nonprofit public-private partnership that has been established to accelerate development of next generation vaccines and immunotherapies for major global infectious and neoplastic diseases by decoding the human immune system. The Project has created a unique model of engaging multinational pharmaceutical partners with key stakeholders from academia, governments and nongovernmental organizations, into a human immunology-based, milestone driven, global research consortium. The Project aims to decode the human immune system by establishing and following a series of decade long cohorts, varying by age, gender, ethnicity and geography, for deciphering the human “immunome”. This will include the repertoire of B and T cell receptors of the human immune system, and the immune targets recognized by antibodies and T cells for selected infectious and neoplastic diseases to facilitate vaccine discovery. The Project also aims to elucidate the “rules of protective immunity” in humans by conducting strategic, small, iterative exploratory clinical research trials of licensed and experimental vaccines and immunotherapies to systematically solve the key problems impeding next-generation product development. Proposed pilot studies of the Project aimed at assessing the human immunome and immune responses to vaccines across heterogeneous populations will be discussed.

Antonio Lanzavecchia

Antonio Lanzavecchia is known for his work on antigen presentation by B cells and dendritic cells, for his studies on T cell activation, on the cellular basis of immunological memory, and for the development of novel methods to isolate human monoclonal antibodies. Lanzavecchia was born in Italy and obtained a medical degree from the University of Pavia, where he specialized in pediatrics and in infectious diseases. From 1983 to 1999 he worked at the Basel Institute for Immunology and since 2000 is the founding director of the Institute for Research in Biomedicine in Bellinzona, Switzerland. He is Professor Emeritus of Human Immunology at the Swiss Federal Institute of Technology, ETH Zürich. Lanzavecchia received the EMBO Gold Medal, the Cloetta Prize, the Robert Koch Prize and is a member of the EMBO, of the Swiss Academy of Medical Sciences and a foreign associate of the US National Academy of Sciences.

Abstract [O3.1]:

We use cell culture-based high-throughput methods to interrogate human memory B cell and plasma cell repertoires and to isolate antibodies selected on the basis of their neutralizing potency and breadth. Relevant examples are antibodies that neutralize all influenza A viruses or even four paramyxoviruses (Science 2011, 333:850; Nature 2013, 501:439). By targeting conserved structures, these broadly neutralizing antibodies are less prone to select escape mutants and are promising candidates for prophylaxis and therapy of infections, as well as tools for vaccine design. While searching for antibodies that broadly react with malaria variant antigens, we discovered a new mechanism of antibody diversification, which relies on templated insertions of genomic DNA sequences into immunoglobulin genes, followed by somatic mutations (Nature 2015, 529:105). Indeed, human monoclonal antibodies that recognize erythrocytes infected by different P. falciparum isolates acquired broad reactivity through a
novel mechanism of insertion between the V and DJ segments of the entire 98 amino acid collagen-binding domain of LAIR1, an immunoglobulin superfamily inhibitory receptor encoded on chromosome 19. More recently, we studied two large cohorts of individuals living in malaria-endemic regions and found that 5-10% of malaria-exposed individuals, but none of the European blood donors tested, have high levels of LAIR1-containing antibodies that dominate the response to infected erythrocytes (Nature 2017, 548:597). The study also revealed different modalities of LAIR1 insertion that lead to public and dominant antibodies against infected erythrocytes. Sequencing of the switch regions of memory B cells from European blood donors revealed frequent templated inserts originating from transcribed genes that, in rare cases, comprised exons with orientations and frames compatible with expression. Our data suggest that insertion of templated DNA represents an additional mechanism of antibody diversification that can be selected in the immune response against pathogens.

Odile Leroy

Dr Odile Leroy has 30 years of vaccine R&D experience, as scientist in Africa, Corporate Clinical Director at Sanofi Pasteur, and leading 3 European Organisations (EMVI, EDCTP, and EVI).

Abstract [PL6.3]:

EVI, the oldest European Product Development Partnership will celebrate its 20’s Anniversary in 2018. Since 2010 EVI has extended its portfolio from malaria vaccine to diseases of poverty vaccines and to European vaccine research and development infrastructure through EC funded project TRANSVAC. In 2016, EVI has continued to support 20 projects, with 10 vaccine candidates in phase I clinical trials (of which 2 started, 3 continued and 5 completed the clinical assessment) and 5 undergoing pre-clinical development. Concomitantly, EVI is devoting a lot of efforts and resources in harmonisation and standardisation of quality control testing, immuno-assays, animal models, safety assessment, adjuvants and technology platforms. EVI is also coordinating VAC2VAC project aiming on the replacement of animal experiments for assessment of vaccine potency. Since inception EVI has been instrumental in strengthening vaccine research and development and clinical trial capacity in Africa.

Gerald Linette

My primary interest is human cancer immunology. I have been actively involved in the development of new therapeutic approaches for melanoma since 1996, including dendritic cell vaccines, gene therapy, adoptive T cell therapy, and checkpoint inhibitors. My laboratory research has received extramural funding to study human cancer immunology since 2005. As part of a larger clinical multidisciplinary effort, I serve as the principle investigator for numerous cooperative group, industry-sponsored, and investigator-initiated clinical trials. This current translational proposal builds on our longstanding interest in T cell recognition of melanoma antigens coupled with the expertise of our collaborators in technology development in the fields of genomics and proteomics. My scientific interest is identification of HLA class I restricted neoantigens encoded by tumor missense mutations for use in clinical vaccine studies to elicit CD8+ T cells in patients with cancer.

Abstract [O7.1]:

T cell immunity directed against tumor encoded amino acid substitutions has been reported in humans with cancer, thus implicating missense mutations as a source of patient-specific neoantigens. We and others have described a new strategy to identify and validate human tumor neoantigens. Using next generation sequencing technologies with a novel bioinformatics pipeline, HLA class I restricted neoantigens were nominated among expressed melanoma missense mutations. Immunogenicity was evaluated using a novel personalized dendritic cell vaccine matured using 4 stimuli to promote IL-12p70 production. Our vaccination protocol increases the antigenic breadth and clonal diversity of neoantigen-specific CD8+ T cells in all patients treated. The mature DC vaccine is well tolerated without apparent
side effect or autoimmune-related toxicities. Our findings suggest that personalized cancer vaccines targeting private somatic tumor alterations may become feasible in the near future.

**Shabir Madhi**

Professor Shabir Madhi is Executive Director of the National Institute for Communicable Diseases, Professor of Vaccinology and Director of the MRC Respiratory and Meningeal Pathogens Research Unit at University of the Witwatersrand, South Africa.

He is a Vaccinologist who has been involved in researching vaccine-preventable diseases for 20 years. His research which demonstrated a reduction in childhood morbidity through the use of pneumococcal conjugate vaccines (PCVs) and rotavirus vaccines, prompted South Africa to be the first in Africa to introduce these vaccines in national immunisation programs. These studies were also pivotal to the World Health Organization, who then recommended the introduction of these life-saving vaccines into public immunization programs globally. More recently, his research focus has expanded to reducing morbidity and mortality due to infectious causes during early infancy, with a specific focus on maternal immunization as an intervention. He has published over 330 peer reviewed articles, many in leading international journals.

**Abstract [O1.1]:**

Currently death during the neonatal period contributes to 45% of all under-5 deaths globally. Further, a large proportion of deaths in the 1-59 month agegroup occur within 4 months of life, where there is an “immunity” gap against many vaccine preventable diseases. A leading cause of death in these young infants, include lower respiratory tract illness. Protection against some of the leading respiratory pathogens contributing to young infant morbidity and mortality, could be achieved by enhancing transplacental transfer of protective antibody to the fetus, and neonate through breast-milk acquisition. Such passive- protection could be enhanced by boosting epitope specific antibody levels vaccinating pregnant women. In addition to the success of maternal immunization having reduced the burden of neonatal tetanus in low-income countries, more recent data confirm this to be equally effective (92% reduction) in preventing pertussis in infants <3 months age. Furthermore, maternal influenza vaccination is efficacious in prevention of influenza confirmed illness among their infants (50% efficacy until 6 months of age, and up to 80% efficacy up to 8 weeks of age), as well as prevention influenza illness in the women (50% efficacy). It is also possible that influenza vaccination of pregnant women could result in increase birth weight and lower rates of preterm birth. These recent success on maternal immunization, raises optimism on also being able to reduce the burden of RSV in young infants through vaccination of pregnant women. Following on phase II studies demonstrating the safety and immunogenicity of a nanoparticle RSV sub-unit postfusion F protein vaccine in pregnant women, phase III vaccine efficacy studies are currently underway. Additionally, other RSV sub-unit vaccines are also currently in the clinical development pathway in pregnant women, aimed at preventing the leading cause of LRTI hospitalization (25-30% globally) in young infants globally, and preventing at least a 40-50% of the 118,000 RSV deaths which occur annually.

**Jacqueline Miller**

Dr. Jacqueline Miller received a degree in Biological Sciences with Honors from the University of Chicago, and then graduated from Northwestern University’s Feinberg School of Medicine. After completing postgraduate training at the Children’s Hospital of Philadelphia, she joined Merck Research Laboratories as physician conducting clinical trials for novel vaccines. Dr. Miller has worked on the clinical development for over 20 vaccines since joining GlaxoSmithKline 12 years ago. She is currently the Head of Clinical Research and Development and Epidemiology at the GlaxoSmithKline US Vaccine R&D Center, and will provide an overview of the clinical data for HZ/su, GSK’s candidate vaccine against Varicella Zoster Virus, at the meeting.
Abstract [O4.1]:

Herpes zoster (HZ), or shingles, is caused by the reactivation of latent varicella zoster virus (VZV) from the sensory ganglia. The highest risk for HZ is in older and immunocompromised (IC) individuals. HZ may be complicated by post-herpetic neuralgia (PHN), a potentially debilitating chronic pain syndrome. VZV-specific cell-mediated immunity (CMI) and humoral immunity are believed to play a key role in preventing HZ, and the higher HZ incidence in older adults is likely due to declining CMI as a result of immunosenescence. A live viral vaccine is licensed in the US, EU, and other countries, with an efficacy of 70% in those 50-59, 64% in those 60-69, 41% in those 70-79, and 18% in those ≥80 years of age (YOA) [ZOSTAVAX Prescribing Information, United States of America]. This vaccine is also contraindicated in those with IC conditions.

GSK has developed a novel candidate vaccine, Herpes Zoster subunit vaccine (HZ/su) to address the remaining unmet medical need against herpes zoster. HZ/su contains the glycoprotein E antigen, which is the most abundant and immunodominant surface glycoprotein for VZV, as well as the adjuvant, AS01b, containing monophosphoryl lipid A (MPL) and the saponin QS-21. HZ/su activates the innate immune system, which leads to an increased number of Antigen Presenting Cells (APCs) in the draining lymph node. This in turn leads to efficient stimulation of cognate T-cells, which results in memory T-cells and the differentiation of antigen-specific B-cells. In phase III studies, HZ/su was shown to increase CMI by 25-fold and anti-gE antibody concentrations by 42-fold over pre-vaccination values. This translated into vaccine efficacy against HZ of 97.2% in those ≥50 and 91.3% in those ≥70 YOA. This vaccine efficacy was found to be consistent across the age strata studied, even in those ≥80 YOA, who are at increased risk for developing the disease. By preventing HZ, HZ/su also prevented post-herpetic neuralgia (PHN) in 91.2% of those ≥50 and 88.8% in those ≥70 YOA. Consistent with the transient local inflammatory response that is known to be the mechanism of action of AS01b, subjects that received HZ/su reported higher rates of local and general solicited symptoms than subjects who received the saline placebo. The majority of these reactions were mild-to-moderate in severity, and of median duration of 3 days. This did not result in an increase in serious adverse events (SAEs), and 95% of the subjects in both treatment groups returned to receive the second dose. The overall safety profile was acceptable. HZ/su is expected to provide a significant health benefit to people 50 years of age and older. ZOSTAVAX is a registered trademark of Merck Sharpe & Dohme, Corp.

Louis Picker

Dr. Louis J. Picker is currently the Associate Director of the Vaccine and Gene Therapy Institute, a Professor in the Pathobiology and Immunology Division of the Oregon National Primate Research Center, and a Professor in the Oregon Health & Science University’s Departments of Pathology and Molecular Microbiology and Immunology. Dr. Picker was recruited to OHSU in 2000 from the Department of Pathology at the University of Texas Southwestern Medical Center at Dallas where he served as a Principal Investigator, Medical Director of the Flow Cytometry and Clinical Immunology Laboratory, and Co-Director of the Division of Hematopathology and Immunology. He received his medical degree at the University of California, San Francisco in 1982, did an internship, residency, and chief residency in Anatomic and Clinical Pathology at the Beth Israel Hospital and Harvard Medical School in Boston, Massachusetts from 1982-86, and received advanced training in Immunopathology and Experimental Pathology at Stanford University Medical Center in Palo Alto, California from 1986-89. Dr. Picker is known for his work elucidating human/nonhuman primate (NHP) memory T cell biology, T cell mediated mechanisms of protection against persistent pathogens, the immunopathogenesis of AIDS, HIV/AIDS, and TB vaccine development.

Abstract [PL2.3]:

Despite widespread use of the Bacille Calmette-Guérin (BCG) vaccine, tuberculosis (TB) remains the leading cause of global mortality from a single infectious agent (Mycobacterium tuberculosis or Mtb). Previous studies with Rhesus Cytomegalovirus (RhCMV) vectors encoding simian immunodeficiency virus (SIV) inserts have demonstrated the ability of these vectors to elicit and maintain high frequency SIV
antigen (Ag)-specific, effector-memory T cells (TEM) in both lymphoid tissues and extra-lymphoid effector sites and to provide early, stringent control and ultimate clearance of highly pathogenic SIV challenge in ~50% of vaccinated rhesus macaques (RM). Here, in two successive challenge studies, we asked whether Mtb-specific TEM elicited by parenterally administered RhCMV vectors expressing Mtb inserts (RhCMV/TB) could prevent or ameliorate TB in highly TB-susceptible RM. Over both Erdman strain Mtb challenge studies, RhCMV/TB vaccination reduced the overall extent of Mtb infection and disease at necropsy by 68% compared to unvaccinated controls (P=0.0019). In study 2, we found no difference in efficacy between RM vaccinated with 68-1 vs. 68-1.2 RhCMV vector backbones (which differ in CD8+ T cell epitope recognition) or with 68-1 RhCMV/TB vaccines expressing 9 vs. 6 Mtb proteins. In study 1, BCG was not significantly efficacious, and administration of BCG 6 weeks prior to RhCMV/TB vaccination appeared to reduce the efficacy of the latter vaccine. Across both studies, 14 of 34 RM vaccinated with RhCMV/TB alone (41%) showed no granulomatous disease at necropsy (vs. 0 of 17 unvaccinated controls; P=0.0018), despite immunologic evidence of initial Mtb infection after challenge, and 10 of these RM were Mtb culture-negative in all tissues. Whole blood transcriptomic analysis of RhCMV/TB-vaccinated RM prior to challenge revealed that complete protection significantly correlated with expression of a specific set of innate immune/neutrophil genes, suggesting that activated neutrophils might be important co-effectors for RhCMV/TB vaccine-mediated protection. The ability of RhCMV/TB vaccination to completely prevent development of TB disease in more than 40% of vaccinated RM and to provide nearly 70% overall efficacy offers promise that a human CMV/TB vaccine might be effective in preventing pulmonary TB in people.

Félix Rey

Félix Rey is a structural biologist who graduated in theoretical physics in Argentina and obtained his PhD in biochemistry in Orsay, France, in 1988. He then spent 7-years at Harvard university, where he specialized in the structure of viral envelope proteins. In 1995 he returned to France as Junior PI working at CNRS in Gif-sur-Yvette (Paris area). In 2004, he joined Institut Pasteur as Head of the Virology Department until 2012, in parallel to directing the Structural Virology Unit, which he still heads today. Félix Rey is an EMBO member since 2005 and was elected to the French Academy of Sciences in 2010. Among his honors and awards are the CNRS “Médaille d’Argent” in 2004, the Beijerink Virology Prize of the Dutch Royal Academy in 2013, and the Pasteur-Weizmann-Servier award in 2015. His research focus include entry of enveloped viruses by membrane fusion and also the characterization of virus/antibody complexes, providing insight into epitope-focused vaccine design for pathogens such as dengue and Zika viruses.

Abstract [O3.2]:

Zika virus is a member of the flavivirus genus that had not been associated with severe disease in humans until the recent outbreaks, when it was linked to microcephaly in newborns in Brazil and to Guillain-Barré Syndrome in adults in French Polynesia. Zika virus is related to dengue virus, and we discovered that a category of antibodies isolated from dengue patients also potently neutralize Zika virus. We have determined the crystal structures of several of these antibodies in complex with the envelope protein of Zika virus, which reveal the details of a conserved epitope that is also the site of interaction with the precursor prM protein during flavivirus maturation. Comparison of the Zika and dengue virus immunocomplexes (the structures of the latter we had determined previously) provides now a lead for a rational, epitope-focused design of a universal vaccine capable of eliciting potent cross-neutralizing antibodies to protect against Zika and dengue viruses simultaneously. My talk will describe our current attempts to optimize the required immunogens.

Allan Saul

Allan Saul is Institute Director of the GSK Vaccines Institute of Global Health. This Institute is co-located in Siena, Italy, with a major research and development centre of GSK Vaccines. GVGH develops effective and affordable vaccines for neglected diseases of impoverished countries. At GVGH, Dr Saul oversees development of conjugate typhoid and Paratyphi A vaccines, a subunit vaccine for Group A streptococcus
GMMA vaccines for shigella and invasive nontyphoidal salmonella. The GMMA technology, developed at GVGH, which uses genetic engineering to generate high yields of easily purified bacterial outer membrane blebs, offers particular promise for bacterial diseases of low income countries.

Prior to his appointment as Director of GVGH, Dr. Saul had more than 25 years experience working on the development of experimental malaria vaccines, field work in epidemiology and testing control programs for malaria in endemic countries, as well as laboratory based research in antigen identification of potential protective immune responses. He held previous appointments at the National Institutes of Health (NIH), USA, where he was the co-branch chief of the Malaria Vaccine Development Branch and at the Queensland Institute of Medical Research (QIMR) in Brisbane, Australia.

Abstracts [PL 5.1 and PL 6.2] not available at time of print 2017-09-25

**Rafick-Pierre Sékaly**

Dr. Sékaly joined the Pathology Department at Case Western Research University in October 2014. He was awarded the Richard J. Fasenmyer Professorship in Immunopathogenesis in February 2015. Previously, he served as CSO of the Vaccine & Gene Therapy Institute of Florida (VGTI) for five years. Dr Sékaly obtained his Ph.D. in Biochemistry at the Université of Lausanne in 1984 and went on to perform a postdoctoral fellowship, from 1984 to 1987, on immunogenetics and molecular biology of major histocompatibility complex molecules. He received several honours and awards including a Doctorat Honoris Causa from Lyon University (France), the Cinader Award from the Canadian Society for Immunology (2007), the Seacost Healthcare award in 2010. More recently he received the Avant garde award from NIDA for his work on the HIV reservoir. He was the founder and Scientific Director of the National Immune-Monitoring Laboratory (University of Montreal) and the Unit 743 of Inserm (Institut national de la Santé et de la recherche médicale) the second INSERM unit to be awarded outside of France. His extensive expertise in several aspects of HIV disease immune pathogenesis, immune virology and immunotherapies has helped propel his research team generate pioneering findings which highlight the impact of HIV infection on thymic output, T cell memory, mechanisms of CD4 and CD8 T cell dysfunction, and more recently on innate immunity and mechanisms of HIV persistence. His work has resulted in more than 280 peer-reviewed publications. Concurrently, his studies led to 21 patents, including six US patents. The Sékaly group has received several prestigious research grants from the Canadian Government, the National Institutes of Health, and the Bill and Melinda Gates Foundation. Rafick Sékaly’s group was one of the first to identify the role of PD-1 on T cell and monocyte biology in HIV infection. Funding was focused on identifying, using transcriptomics and bioinformatics, predictors of immune responses that lead to protection from viral infections including HIV, SIV and flaviviruses, protection form disease progression and successful immune interventions including adjuvants, licensed preventative and therapeutic vaccines.

Abstract [O6.2]:

Interferons and their downstream transcriptional targets are one of the more complex biological systems as they regulate a large number of biological and immunological processes. Interferons stimulated genes are immediately triggered by viral infections and other perturbations of the host. They include genes with immediate antiviral activity, which can inhibit viral replication and clear virus. On the other hand interferon regulated genes can also trigger the cytokine storm that is a feature of acute progressive viral infections. This exaggerated immune response leads to the upregulation of a negative feedback loop that prevents the development of adaptive immune responses. This presentation will focus on the identification of pre-infection and pre-vaccination gene expression signatures that bias the infection or the vaccine response to a positive or a negative outcome. We will show that the presence of specific interferon signatures prior to infection or prior to vaccination can tilt the balance towards a positive or a negative outcome. This work is supported by BMGF, U19 AI095985 and U19 U19 AI096109
Abstract [O4.2]:

Respiratory syncytial virus (RSV) is an established cause of serious lower respiratory tract disease in infants and older adults. While the unmet medical need for an RSV intervention that targets all infants is clearly established, our understanding of the disease burden in older adults continues to evolve. Several candidate vaccines have been tested for the prevention of RSV disease in older adults and all have failed to meet their primary endpoints in phase 2 proof-of-concept or Phase 3 studies.

The development of MEDI7510, a CHO-cell derived RSV F (120 µg, post-fusion conformation) antigen vaccine formulated with a Toll-like receptor 4 adjuvant, glucopyranosyl lipid A (5 µg), in a 2% stable emulsion (GLA-SE) illustrates the challenges in developing an RSV vaccine for the elderly. Prior to Phase 2b MEDI7510 was shown to protect cotton rats from both lower and upper respiratory tract RSV infection and to boost RSV neutralizing antibodies and elicit mucosal immunity, immune memory and IFNγ secreting F-specific T cells in adults age 60 and older.

A large Phase 2b study of MEDI7510 was conducted enrolling 1,900 subjects who were at least 60 years of age and randomized 1:1 to receive vaccine or saline placebo intramuscularly (IM). All subjects received inactivated influenza vaccine (IIV). The primary objective was to assess the efficacy of a single IM dose of MEDI7510 for the prevention of PCR-confirmed acute RSV-associated respiratory illness (ARA-RI). MEDI7510 failed to meet its primary endpoint, prevention of acute RSV-associated respiratory illness (ARA-RI) in the population studied. There were 15 ARA-RI events in the placebo group for an incidence of 1.6% and 16 in the MEDI7510 group for an incidence of 1.7% with a vaccine efficacy (VE) of -7.1% (90% CI: -106.9%, 44.3%). An analysis of the results, potential reasons for failure, challenges encountered and future considerations for RSV vaccines in the elderly population will be discussed.
Jun Zhang

Professor Jun Zhang got his Bachelor degree on Medicine in 1994 in Shanghai Medical School, and then he received three years’ training on epidemiology and was awarded with master degree in 1997. After then he worked in Xiamen University as faculty until now. In 2005, he and his colleagues founded National Institute of Diagnostics and Vaccine Development in infectious diseases (NIDVD) in Xiamen University. His researches focus on the virology, immunology and epidemiology of many human pathogens. As one of the leading scientists, he is deeply involved in the innovation and translation of the recombinant hepatitis E vaccine, the recombinant HPV 16/18 bivalent vaccine, the recombinant HPV 6/11 bivalent vaccine and many immunologic diagnostic reagents against infectious pathogens. Among them, the Hepatitis E vaccine has been commercially launched in China in 2012; the HPV16/18 vaccine and HPV 6/11 vaccine are under Phase III and Phase I clinical trials in China, respectively; more than 40 kinds of immunodiagnostic kits for infectious disease such as AIDS, tuberculosis, hepatitis, influenza, foot and mouth disease had been marketed in China. He had published > 60 papers in peer reviewed journals.

Abstract [O8.2]:

Hepatitis E has attracted increasing awareness in recent years. Caused by hepatitis E virus, it leads to high mortality in pregnant women and patients with underlying liver disease. Several hepatitis E vaccine candidates showed efficacy in animal models, one of them, Hecolin®, based on E. coli expressed truncated capsid protein HEV239 as antigen, has been licensed and launched in China since 2012 after showing its safety, robust immunogenicity and efficacy in a large scale of phase 3 clinical trial involving more than 100,000 healthy adult participants. The extended observation up to 7.5 years after the first vaccine dose on the phase 3 trial cohort had showed the persistent of vaccine-induced antibody and the sustaining vaccine efficacy against hepatitis E.
Updates on the Development of a Multivalent Group B Streptococcal Vaccine

Group B streptococcus (GBS) infections can have serious consequences for newborn infants, including death and lifelong neurological impairment. Infections can be reduced by screening pregnant women for rectovaginal GBS carriage in the weeks leading up to the delivery. If positive, women are given antibiotic interpartum prophylaxis (AIP). While this approach has reduced GBS early onset disease (EOD), it has had no impact on late onset disease (LOD) which is diagnosed between 1 week and 3 months of life, and infants born to mothers that acquire GBS carriage between screening and delivery. In addition, AIP is very difficult to administer in low and middle income regions.

The mechanism of immunological protection is well understood. Protective maternal IgG antibodies are trans-placentally transferred to the fetus and minimum protective capsular serotype-specific IgG antibody concentrations have been proposed for the most prevalent serotypes Ia, III and V. Serological studies showed that only a small subset of women, however, possess the serotype-specific IgG concentrations able to protect their infants. To that end a prophylactic vaccine delivered during pregnancy that can increase the level of maternal protective antibodies available to the infant is expected to raise the proportion of the population that could naturally protect their infants from infection and provide an alternative approach to GBS screening and AIP. Furthermore, unlike screening and antibiotic prophylaxis, vaccination can be implemented in settings with minimally developed health care infrastructure.

To provide broad protection against GBS, investigational six-valent capsular polysaccharide conjugate vaccine (GBS6) is in development. GBS6 is comprised of capsular polysaccharide conjugates of serotypes Ia, Ib, II, III, IV, and V. Over 98% of current invasive neonatal GBS disease is caused by these six serotypes.

Capsular polysaccharides from each serotype were isolated and individually conjugated to the non-toxic variant of diphtheria toxin, cross-reactive material 197. All six capsular polysaccharide conjugates induced antibodies that facilitated opsonophagocytic killing in vitro. Moreover using a murine maternal protection model, GBS 6 was shown to protect infant pups from type specific GBS disease. GBS6 entered Phase 1 clinical development in June 2017.

Induction of Epithelial Cell-Neutralizing Antibodies against Cytomegalovirus (CMV) in Adults Vaccinated with an Enveloped Virus-like Particle (eVLP) Containing an Optimized Form of Glycoprotein B for Prophylactic Vaccination Against CMV
Joanne Langley, Dalhousie University

A prophylactic vaccine to prevent congenital transmission of human cytomegalovirus (CMV) to newborns and to reduce life-threatening disease in immunosuppressed recipients of CMV-infected solid organ transplants would be highly desirable. Neutralizing antibodies against CMV confer significant protection, and glycoprotein B (gB) is a major target of such neutralizing antibodies. However, one shortcoming of past CMV vaccine candidates may have been a failure to induce durable high titer neutralizing antibody responses that prevent infection of epithelial cells. We have used transient expression of murine leukemia virus Gag protein in HEK 293 cells to construct eVLPs expressing the ectodomain of gB fused to the transmembrane and cytoplasmic domains of vesicular stomatitis virus G protein (gB-G). Electron microscopy analyses of eVLPs expressing native gB vs. gB-G detected different structures, and immunization with eVLPs expressing gB-G were associated with higher neutralizing titers relative to
eVLPs expressing native gB. Based on these preclinical data we initiated a phase I trial in healthy CMV seronegative subjects aged 18-40 evaluating three dose levels (0.5µg, 1µg, and 2µg gB content) of gB-G eVLPs formulated with alum (VBI-1501A), a matched unadjuvanted dose of vaccine (VBI-1501), and a placebo group (n=25 subjects/group). Subjects were immunized at 0 and 2 months, with a final planned immunization at 6 months. We report herein interim safety data through day 84 of the study (1 month after the second immunization). The vaccine was safe and well tolerated at all doses, with no safety signals. The vaccine induced immunity against CMV gB, with clear evidence of dose-dependent boosting of antibody responses after the second vaccination. Formulation of the vaccine with alum clearly enhanced antibody titers. Immunization of subjects with the highest (2µg) dose of VBI-1501A, approximately 10-fold less antigen content that that used in other VLP-based vaccines and past CMV vaccine candidates, induced seroconversion in 100% of subjects and epithelial cell neutralizing antibody titers in 17% of subjects after only two doses of a planned 3 dose regimen. The potency and durability of immunity induced with VBI-1501A after the third vaccination will readout in H1 2018.

The Importance of RSV F Protein Conformation In Stimulation of Neutralizing Antibodies in Animals Previously Infected with RSV

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Respiratory syncytial virus (RSV) is a significant respiratory pathogen but no vaccine is available. RSV vaccine development is complicated by the failure of natural infection to stimulate protective antibody and memory responses to RSV. In addition, most children have been infected with RSV by age five. Immune responses to these infections are poorly protective, however, they could impact negatively the effectiveness of a vaccine. Thus a successful vaccine candidate must stimulate high titers of neutralizing antibody in the face of any preexisting immunity and stimulate protective memory responses. Vaccine studies using naïve animal models may not directly bear on human responses, which will generally be in the context of previous infection.

The goal of this study was to assess the activation of protective immune responses in both mice and cotton rats previously infected with RSV in order to mimic the most common scenario in human populations. Virus-like particle (VLP) vaccine candidates containing different conformational forms of the RSV F protein, a stabilized pre-fusion form of the F protein or a stabilized post-fusion F protein, were used. We report that a single immunization of RSV experienced animals with a pre-fusion F VLP stimulated high titers of neutralizing antibodies while a single injection of a post-fusion F VLP or a second RSV infection only weakly stimulated neutralizing antibody titers. These results suggest that prior RSV infection can induce neutralizing antibody memory responses, which can be activated by pre-F VLPs but poorly by post-F VLPs or a subsequent RSV infection. Consistent with this idea, levels of F specific splenic memory B cells in infected and pre-F VLP immunized mice were significantly higher than those after post-F VLP or RSV immunization. Thus the F protein conformation has a major impact on enhancing production of protective neutralizing antibodies and B cell memory responses in RSV experienced animals. Furthermore, although both VLPs contained the same amounts of RSV G protein, the pre-F VLP stimulated significantly higher titers of total anti-G protein IgG than the post-F VLP in both mice and cotton rats. Thus the F protein conformation also influences the responses against the G protein, which may also impact protective responses.
Epitope-specific antibody responses to HSV-2 glycoprotein D immunization differs depending on the adjuvant

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Herpes simplex virus type 2 (HSV-2) infects nearly one half billion individuals worldwide. Manifestations vary from asymptomatic genital infection to severe, recurrent ulcerative genital disease. HSV-2 poses major health concerns for neonates infected during delivery and is a significant risk factor for the acquisition and transmission of HIV. A vaccine to prevent HSV-2 is greatly needed, yet many challenges exist, including determining the most effective viral antigens to include and the optimal adjuvant. Our laboratory is evaluating the protection provided by a trivalent subunit antigen vaccine consisting of HSV-2 glycoproteins C, D, and E (gC2, gD2, gE2) combined with oligonucleotide CpG and alum as adjuvants. The vaccine is intended to block virus entry mediated by gD2 and immune evasion from complement and antibody mediated by gC2 and gE2, respectively. Here, we evaluate the impact of different adjuvants on inducing antibody responses to epitopes that are crucial for gD2 function. We compared a lipid nanoparticle (LNP) adjuvant, LNP-Adj (made by MRL), with CpG oligonucleotide and alum. We used a novel high throughput biosensor platform to assess whether serum IgG from gD2-immunized mice recognizes crucial functional epitopes on gD2 that are involved in receptor binding, activation of downstream virus entry molecules gH/gL, and cell-to-cell spread. The high throughput platform rapidly assesses whether antibodies produced by immunization compete with a panel of monoclonal antibodies that bind to 7 distinct epitopes on gD2 that are crucial for function. C57BL/6 mice were immunized 3 times at two-week intervals with gD2-LNP or gD2-CpG/alum. Both gD2-LNP and gD2-CpG/alum immunized mice produced antibodies that recognized multiple functional epitopes on gD2; however, the gD2-CpG/alum group recognized 5.8±1.7 gD2 epitopes involved in crucial functions compared with 3.4±1.7 in the gD2-LNP group (p=0.005). The antibody responses in the gD2-CpG/alum immunized mice were also more potent in that the antibodies blocked gD2 binding to crucial epitopes by an average of 46.9%±25.5 compared with 10.8%±10.8 for the gD2-LNP group (p<0.001). Consistent with the epitope-specific responses, we detected higher neutralizing antibody titers in the CpG/alum group (p<0.001), and reduced cell-to-cell spread measured by smaller plaque size (p<0.05). Our results highlight the potency of the high throughput biosensor platform to assess epitope-specific antibody responses. We intend to apply this technology to evaluate antibody responses to crucial epitopes on gC2 and gE2. High throughput biosensor technology represents a major breakthrough in assessing adjuvants to select the one that induces antibodies that block the broadest number of epitopes involved in crucial glycoprotein functions.

Analysis of antibody repertoires reshaped by vaccination with respiratory syncytial virus post fusion protein

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Studying vaccine-induced B cell repertoires is key in antigen (Ag) design for new vaccines. Once activated by binding to cognate Ag, B cells accumulate mutations in the variable regions of immunoglobulins they express. Mutations promoting high-affinity Ag-binding are positively selected for clonal expansion and further diversification of the variable regions. Accordingly, clonally-expanded antibodies are expected to share characteristics, e.g. similar gene usage and heavy chain complementarity determining regions (CDRH3). Studies of such features are critical in designing optimized vaccine Ags to induce higher-affinity and long-lasting neutralizing antibodies.
For this purpose we have studied antibody (Ab) repertoires specific for the fusion protein of respiratory syncytial virus (RSV F protein) which is the leading cause of acute respiratory illness in infants. RSV F is a promising vaccine candidate because it is the target of broadly neutralizing antibodies, some of which bind RSV F in its pre fusion (pre F) and post fusion (post F) conformation. We have sequenced RSV post F-binding Ab repertoires of vaccinees who were immunized with RSV F protein in the context of the observer blind, placebo-controlled, V122 Phase I clinical trial. Subjects of the vaccine-treated group received the vaccine formulated with different adjuvants: MF59, Alum or no adjuvant. The B cell samples were selected based on the vaccinees' individual immune response to the vaccine, i.e. increase in F-specific memory B cell levels, increase in antibody affinities for F protein and fold-change of neutralizing antibody titers. The subjects received two injections one month apart. B cells were isolated at day 0 (pre-vaccination), day 28 (before second administration) and day 181. Since RSV infects all individuals repeatedly throughout lifetime, the study aims to understand how the post F-based vaccine induces B cell clonal expansion and how it reshapes a pre-existing RSV F-specific Ab repertoire.

In this study, we analyze and compare the post F vaccine-induced repertoires with the repertoires at baseline level before vaccination. In order to understand if the vaccine induces antibodies with particular characteristics, we examine clonally-expanded sequences in more detail. In particular, study of CDRH3 lengths, variable gene usage and rate of newly introduced mutations provide information about which sequences out of the pre-existing repertoires are selected for diversification. We further look for sequence motifs shared between clonally-expanded sequences. Eventually, we compare the sequences with RSV F-specific antibodies known to have neutralizing activity and choose some for expression of monoclonal antibodies which will be used for further characterization in neutralization assays and epitope mapping. Considering that the vaccinees likely received different formulations of the vaccine, variations in the vaccine-induced memory B cell repertoires might be linked to different adjuvant formulations.

A novel approach to unravel the effective human antibody response induced by natural infection or vaccination against respiratory syncytial virus (RSV)
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We have implemented a new strategy to isolate and characterize human monoclonal antibodies (humAbs) naturally produced from single sorted antigen-specific memory B cells (MBCs), in which HumAbs are obtained from the B cell culture supernatants in sufficient amount for a qualitative and functional high throughput screening. This approach was applied to profile F protein-specific antibodies from RSV naturally exposed healthy donors and from 10 subjects from phase I clinical study V122_01 (NCT02298179). This is a still blinded placebo controlled study where vaccinated subjects received the Post-fusion antigen (Post F).
Since Abs neutralizing RSV infectivity (nAbs) are mainly triggered by the F protein in its pre-fusion conformation (Pre-F), we used this antigen as a bait to isolate MBCs from naturally exposed subjects. The aim was to obtain a comprehensive profile of the functional Ab repertoire induced by natural infection. On the other hand, as the vaccine used for the clinical study contained the Post-F conformation, we used a Post-F bait to isolate MBCs from vaccinees. The retrieved humAbs were tested for binding versus both conformations and for their neutralizing capability, in order to profile the functional repertoire induced by Post-F vaccination.
Of 1260 Pre-F specific B cell clones isolated from naturally infected donors, 135 produced neutralizing antibodies (nAbs). Paired VH-VL Ig-gene sequences have been obtained for 86 of them to define the VH-VL repertoire. A similar approach was used to interrogate B cells from the phase I clinical study subjects. To date 2174 single F-specific MBCs have been sorted leading to the isolation of 78 nAbs. Sequence analysis allowed us to obtain 35 paired VH-VL sequences. All nAbs isolated from both healthy donors and vaccinated subjects have been assessed in competition assay against known
neutralizing Fabs (D25, motavizumab and 101F) allowing us to predict the antigen region recognized by these nAbs.

As a next step a panel of nAbs will be selected to be expressed as recombinant full length IgG for epitope mapping and further characterization. This strategy can be of great help to understand the impact of RSV vaccination on a pre-existing repertoire and to identify highly functional epitopes supporting the development of new vaccines against RSV.

[O3.5]

**Engineering transient production of HIV-1 broadly neutralizing antibodies by DNA encoded monoclonal antibody technology (dMabs™)**

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The ability of HIV specific broadly neutralizing antibodies (bNabs) to protect or control HIV-1 is well established in animal models. Though these antibodies are powerful and can prevent infection or control viral loads, the cost of production could severely limit the ability to use these antibodies in the field. Additionally, current vaccine approaches have failed to induce similar antibodies. We have developed a novel way to encode and produce bNabs in vivo using synthetic DNA encoded monoclonal antibody (dMab™) platform.

Engineered plasmids encoding the heavy and light chain of numerous bNabs were produced. Initial production levels were low, however using numerous optimization strategies including sequence, formulation, structural modifications and delivery, high levels (up to 100ug/ml) of serum bNabs in mice were achieved. Levels of the antibody are detectable in the serum as early as two days after injection and are sustained for months. Importantly, these antibodies retain functional capabilities including binding to Env and neutralization as measured by the TZM-bl neutralization assay. To lower the possibility of viral escape, using dMabs™, we can encode and express multiple HIV-1 bNabs simultaneously in a single mouse.

dMabs™ represent a novel platform for in vivo antibody production against HIV-1 and possibly other immune targets.

[O3.6]

**Conversion of a DNA-encoded monoclonal antibody (dMAb) into scFv-Fc format improves expression and protects against lethal Zika virus challenge following in vivo gene delivery in a mouse model**

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Zika virus (ZIKV) has emerged as a global health concern due to its rapid spread by mosquitoes and its association with neuropathologies such as microcephaly and Guillain-Barré syndrome. Currently there are no approved vaccines or therapeutics to prevent or treat ZIKV infection. Monoclonal antibodies (mAbs) have the potential to be potent and safe interventions, however, they have a high cost of manufacture, require cold-chain storage, and need repeat dosing to maintain effective levels, features that could limit their use in lower-resource settings where ZIKV is currently endemic. We have previously demonstrated DNA-encoded mAbs (dMAbs) delivered by in vivo electroporation (EP) into skeletal muscle result in sustained, systemic expression of mAbs capable of protecting against pathogens such as Flu and Pseudomonas aeruginosa. Plasmid DNA-based therapeutics are cheaper to manufacture and can be formulated for long-term stability at room temperature. Also, dMAbs are more amenable to screening for structural modifications that improve mAb expression and/or function as these only require changes in the synthetic DNA sequences that can immediately be tested in vivo, rather than extensive protein production
and purification processes that require optimization for each mAb form. Here we describe dMAbs encoding a ZIKV-specific mAb and its various single chain (scFv-Fc) formats. The ZIKV-dMAb was encoded as either a standard IgG molecule, or structurally re-formatted as four alternative scFv-Fc molecules with different heavy chain-light chain orientations and linker sequences. Each of these molecules included the LALA mutation to eliminate the potential of unwanted Fc receptor-mediated antibody-dependent enhancement of viral infection. All four scFv-Fc formats improved ZIKV-dMAb expression both in vitro and in vivo. scFv-Fc linker modifications did not affect ZIKV envelope binding, while changes in heavy-light chain orientations had modest impact on antigen binding. Based on in vivo expression and ex vivo antigen binding results, one scFv-Fc ZIKV-dMAb was selected for efficacy testing in a mouse model of lethal ZIKV challenge. All animals treated with scFv-Fc ZIKV-dMAb survived, while all control animals succumbed to lethal ZIKV infection. These data demonstrate the utility of dMAbs as not just anti-ZIKV agents, but as a platform to easily evaluate multiple mAb modifications for improvements.

[PL2.4]

A recombinant fimbrial prototype vaccine administered by intradermal route protects Aotus nancymae non-human primates from ETEC diarrhea

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Enterotoxigenic Escherichia coli (ETEC) is one of the major causes of bacterial diarrhea in children under five years of age in developing countries and the leading cause of travelers’ diarrhea. Multidrug resistance of ETEC strains, especially to fluoroquinolones, represents a major challenge in current treatment strategies for ETEC diarrhea, as recently highlighted by Begum, et al. (PLos One, 2016). Thus an effective vaccine is required to prevent ETEC diarrhea cases worldwide. The vaccine prototype described here comprises the donor strand complemented (dsc) fusion protein of the minor (CfaE) and major (CfaB) subunits of the CFA/I fimbria (CFaEB) plus the B subunit of the ETEC heat-labile toxin (LTB). A formulation containing a TLR3 agonist adjuvant was down-selected in mice for its ability to induce higher humoral and mucosal immune responses against the vaccinal antigens. This formulation was used to vaccinate Aotus nancymae non-human primates (NHP) by the intradermal (ID) route with or without orogastric (OG) priming with CFaEB plus the single mutant of labile toxin, mLT. Incidence of diarrhea was monitored for 10 days after oral challenge with a CFA/I expressing ETEC strain H10407. In comparison to the buffer control (no protection) or the control group with adjuvant alone [non-significant 14% protection (p=0.31)], the vaccine formulation with an OG/ID regimen demonstrated 84% protection (p=0.02) against challenge, while the same formulation delivered by ID route without mucosal prime yielded 100% protection (p=0.003). These results indicated that an effective vaccine against this intestinal pathogen might be achieved using solely parenteral immunization.
Safety, tolerability and immunogenicity of ExPEC4V (JNJ-63871860) vaccine for prevention of invasive extraintestinal pathogenic Escherichia coli disease: a phase 1, double-blind, placebo-controlled study in healthy Japanese participants

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Background: ExPEC4V (JNJ-63871860) is a 4-valent vaccine consisting of the O-antigen polysaccharides (PS) of the extraintestinal pathogenic Escherichia coli serotypes O1A, O2, O6A, and O25B bioconjugated to the carrier exoprotein A. ExPEC4V (up to 4 µg PS/serotype) demonstrated favourable safety and immunogenicity in a first-in-human study (Huttner A et al. 2017). This study evaluated safety, tolerability and immunogenicity of different doses of ExPEC4V (4 - 16 µg PS/serotype) in healthy Japanese volunteers.

Method: In this Phase 1, double-blind, placebo-controlled study, participants (≥20 years) were stratified into younger (≥20 to <50 years) or older age groups (≥50 years). A total of 24 participants in each group were randomized to a single vaccination with 1 of 3 dose levels of ExPEC4V (4, 8 and 16 µg PS/serotype, n=6/group) or placebo (vaccine buffer, n=2/group) administered intramuscularly in deltoid region in a dose ascending manner. The study consisted of a screening phase (Day -7 to Day 1), vaccination on Day 1, and a follow-up phase to Day 30. Primary objectives were safety and tolerability (solicited adverse events [AEs] were collected in a diary till Day 8). As a secondary objective, immunogenicity (Days 15 and 30) was measured by an enzyme linked immunosorbent assay (ELISA) and an opsonophagocytic killing (OPK) assay.

Results: Of the 48 vaccinated participants, 47 (97.9%) completed; one (2.1%) in placebo group discontinued (family reason). The median age was 41.0 (range 20, 49) for younger group and 56.5 years (range 50, 70) for older group; overall 54.2% were women. A total of 23 (47.9%) participants had ≥1 AE (younger group: n=13 [54.2%]; older group: n=10 [41.7%]). AEs reported in each ExPEC4V dose (µg PS/serotype) were: 4 µg: 16.7%; 8 µg: 50.0%, 16 µg: 66.7%; placebo: 58.3%. Across all treatment groups, solicited AEs were reported in 22 (45.8%) participants (ExPEC4V: n=16 [44.4%]; placebo: n=6 [50.0%]). Pain/tenderness (n=11 [30.6%]) and redness (n=9 [25.0%]) were the most frequently reported solicited local AEs, whereas fatigue (n=4 [11.1%]), headache (n=4 [11.1%]), muscle pain (n=2 [5.6%]), and malaise (n=5 [13.9%]) were the most common solicited systemic AEs in the combined ExPEC4V group. Similarly, unsolicited AEs were reported in 3 (8.3%) participants vaccinated with ExPEC4V and 2 (16.7%) in placebo. No grade 3 AEs, serious AEs, deaths, or discontinuation due to AEs were reported. In Day 30 to Day 1 comparisons, across all doses the proportion of volunteers with ≥2-fold increase in antibody GMT titers ranged from 83.3%-100% (O1A), 100% (O2), 83.3%-100% (O6A) and 75.0%-91.7% (O25B) by ELISA and 66.7%-100% (O1A), 100% (O2), 50.0%-75.3% (O6A) and 50.0%-66.7% (O25B) by OPK. Increased antibody titres observed on Day 15 were similar to those seen on Day 30.

Conclusion: ExPEC4V vaccine at all dose levels (up to 16 µg PS/serotype) was well tolerated and did not show any safety concerns in healthy Japanese participants. All ExPEC4V doses were immunogenic showing a robust increase in antibody titer on Day 15 and Day 30 compared to baseline at each dose measured by both ELISA and OPK. ExPEC4V vaccine is in phase 2 clinical development program.
New promising targets for synthetic Omptin-based peptide vaccine against Gram-negative pathogens

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Omptins, a family of proteases commonly found in various Gram-negative pathogens. These proteins play important role in host-pathogen interaction and have been recognized as key virulence factors highlighting a possibility of developing the Omptin-based broad-spectrum vaccine. The prototypical Omptin, His-tagged recombinant Pla (pro-Omptin) was purified under denaturing conditions and used as a model target antigen. A panel of sera from human volunteers (n=8) vaccinated recently with live plague vaccine (LPV) was tested for antibody response to the Pla antigen. Also, lymphocyte proliferation assay and cytokine profiling (IFN-gamma, TNF-alpha, IL-4, IL-10, and IL-17A) were conducted with the donors' peripheral blood mononuclear cells (PBMCs) in response to in vitro stimulation with recombinant Pla. We used library of 59 overlapping synthetic peptides to map B-cell immune-reactive epitopes by ELISA. We found that all sera from these recently vaccinated individuals were positive for the presence of specific antibodies to pro-Omptin at detectable level (ELISA, immunoblot). In vitro-proliferative response to this antigen revealed the increased level of IL-17A by 14.7-fold while production of IL-4 was reduced. The levels of IFN-gamma, TNF-alpha, and IL-10 were negligible. Eight potential immune-reactive 15-mer linear peptides were identified by library screening that could define host-pathogen interaction. A comparative analysis of amino acid sequences of several Omptin family proteases, such as Pla (Yersinia pestis), PgtE (Salmonella enterica), SopA (Shigella flexneri), OmpT and OmpP (E. coli) confirmed their high cross-homology. The mapped epitopes may represent promising targets for the development of a new generation of effective synthetic Omptin-based peptide vaccine against a number of Gram-negative pathogens.

A Phase 1 clinical trial of a Hantaan virus, Puumala virus, and Hantaan/Puumala virus DNA vaccine delivered by disposable syringe jet injection: preliminary findings

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We have developed hantavirus DNA vaccines expressing the Gn and Gc genes of Hantaan virus (HTNV) and Puumala virus (PUUV). Previously we reported that these vaccines, alone and in combination, were safe and immunogenic when delivered using particle mediated epidermal delivery, or intramuscular electroporation. Here, we have investigated the use of an alternate method of delivering our hantavirus DNA vaccines. Specifically, we conducted a Phase 1 trial evaluating the HTNV, PUUV and HTNV/PUUV combination vaccines delivered using PharmaJet’s Stratis intramuscular disposable syringe jet injection (IM-DSJI) device. The Phase 1 trial consisted of three nine-subject cohorts vaccinated with: 2 mg of the HTNV, or PUUV, or a 2 mg mixture of HTNV and PUUV DNA. Each vaccination was delivered as 1 mg of DNA in a 0.5 mL volume to the right and left deltoid using the IM-DSJI device, for a total dose of 2 mg. Vaccinations were administered on Days 0-28-56-168. There were no study related vaccine or device-related serious adverse events through the active vaccination phase of the trial. Sera were collected at multiple timepoints and neutralizing antibodies were measured using HTNV and PUUV pseudovirion
neutralization assays (PsVNAs). Although the study remains blinded, PsVNA data collected-to-date indicate that this method of vaccine delivery is effective at eliciting HTNV and PUUV neutralizing antibodies in humans. 25 of 27 subjects seroconverted and all of those individuals were still positive through at least Day 252. In some individuals neutralizing antibody titers were very high (e.g., PsVNA50 titers >10,000) and in some subjects responses were detected after a single vaccination. Plaque reduction neutralization tests (PRNT) confirmed the antibodies neutralized authentic HTNV and PUUV. In summary, preliminary data indicate that HTNV, PUUV, and combination DNA vaccine delivered by IM-DSJI are safe and immunogenic in humans. This is among the first reports that the PharmaJet Stratis IM-DSJI device can be used to successfully deliver DNA vaccines to produced neutralizing antibodies in humans.

[PL3.4]

Development of Novel and Safe Single-Dose Vaccines; Preclinical Efficacy Data for Zika, Ebola and Lassa Fever
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GeoVax MVA platform technology is built on a 4th generation MVA vector system that is improved for high expression and stable transgenes during manufacture. It has the advantages of being a live replication-competent vector in avian cells for manufacturing, yet replication-deficient in mammalian cells for vaccination, thus inherently safe. Importantly, MVA vaccines elicit protective T cell as well as antibody responses in animals and humans. The MVA platform can be combined with the potent immunogenicity of VLPs or be used to express proteins in their native conformations enabling vaccines that induce full protection after a single dose. The safety and immunogenicity of the platform was first validated in animal and human studies using DNA and MVA-VLP-HIV vaccines and further expanded for developing vaccines against emerging pathogens or endemic diseases.

In studies for Ebola, Lassa and Zika, a single dose of vaccine fully protected animals against a lethal challenge. Single-dose protection is a favourable characteristic of a vaccine for emerging infectious disease outbreak response, given the speed of spread of pathogens and the impracticality of multi-dose regimens in the under-resourced settings where outbreaks often occurs. Moreover, the MVA-Zika vaccine is based on the NS1 protein which avoids the risk of Antibody Dependent Enhancement of infection, a concern associated with prME-based vaccines. In this talk, the most relevant single dose vaccine efficacy data for vaccines against these 3 different pathogens will be presented as examples for a broad utility of the platform for other indications.

[PL3.5]

A Single-Round Infectious Particle Zika virus vaccine candidate
Karin B. Sundstrom, Wilfried A. Saron, Ashley St John, Eng Eong Ooi

The emergence of Zika virus (ZIKV) has transitioned the virus from a relatively unknown and rare infection to a major health concern. Infection with ZIKV in pregnant mothers is strongly linked to an array of birth defects collectively called Congenital Zika Syndrome (CZS). The exact mechanisms behind CZS are not known, but both in vitro and in vivo studies have shown that ZIKV can infect and replicate in the placenta and can cause fetal damage in animal models. Preventing the spread of ZIKV is thus critical in reducing the health impact of ZIKV, especially the risk of teratogenicity in unborn children in Singapore and globally. In this context, a vaccine that effectively prevents infection and onward transmission would have clear and sustainable advantages. Here we explore a strategy that combines the safety of inactivated/subunit vaccines with the immunogenicity of live attenuated vaccines by using a single-round infectious particle (SRIP) approach.
The ZIKV-SRIP (zSRIP) contains RNA coding only for ZIKV non-structural proteins, packaged inside a Zika virus-like particle (VLP). The VLP packaging ensures uptake of the vaccine by ZIKV susceptible cells. The genome consisting of non-structural genes is self-replicating and expresses the non-structural proteins. Without the structural genes, however, no new infectious particles will be created in the zSRIP infected cells: hence the infection will be limited to a single round in the vaccine recipient, but still be capable of eliciting protective immune responses.

In vitro, zSRIP show similar cell host range as wild-type ZIKV. In addition, infection with zSRIP mimicked infection with wild-type virus with regards of inducing interferon-stimulated genes. Preliminary in vivo results from immunisation of wild-type mice with zSRIP show that zSRIP-immunisation reduces viral load after ZIKV challenge to comparable levels as immunization with wild-type virus. Further feasibility of this single-round infectious particle as a vaccine against ZIKV is currently explored.

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**[PL3.6]**

**Durability and Correlates of Vaccine Protection Against Zika Virus in Rhesus Monkeys**

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An effective Zika virus (ZIKV) vaccine will require long-term durable protection. Several ZIKV vaccine candidates have demonstrated protective efficacy in nonhuman primates, but such studies have typically involved ZIKV challenge shortly following vaccination at peak immunity. In this study, we show that a single immunization with an adenovirus vector-based vaccine, as well as two immunizations with a purified inactivated virus vaccine, afforded robust protection against ZIKV challenge in rhesus monkeys at 1 year following vaccination. In contrast, two immunizations with an optimized DNA vaccine, which provided complete protection at peak immunity, resulted in reduced protective efficacy at 1 year as a result of declining neutralizing antibody titers to sub-protective levels. These data define a microneutralization (MN50) log titer of 2.0-2.1 as the threshold required for durable protection against a high-dose ZIKV challenge in this model. Moreover, our findings demonstrate that protection against ZIKV challenge in rhesus monkeys is possible for at least 1 year with a single-shot vaccine.

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**[PL3.7]**

**A synthetic, consensus DNA vaccine against Zika virus, GLS-5700, is highly immunogenic in humans and induces antibody responses that are protective in a passive transfer mouse challenge model**

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**Background:** Zika virus (ZIKV) is usually a self-limited infection rarely associated with Guillain-Barre syndrome. Infection of pregnant women can lead to severe congenital birth defects. There are no approved therapies or vaccines for ZIKV. We have previously shown that DNA vaccination with CELLECTRA electroporation leads to robust immune responses in mice and non-human primates (NHPs). Immunized mice are protected from lethal ZIKV challenge and from virus induced damage to the
testes. Importantly, passive transfer of immune sera from immunized NHPs also protects mice from lethal challenge.

**Methods:** Zika-001, an open label, Phase I clinical trial (NCT02809443), evaluated the safety and immunogenicity of GLS-5700, a synthetic, consensus DNA vaccine targeting the ZIKV pre-membrane/membrane and envelope proteins (prME). Vaccine doses of 1 or 2 mg were delivered intradermally followed by electroporation with the CELLECTRA-3P device at weeks 0, 4, and 12 to two groups of 20 participants. Cellular and humoral immune responses were assessed at weeks 0, 1, 4, 6, 12, 14, 20, and 36.

**Results:** Immunization with GLS-5700 resulted in robust humoral and cellular immune responses. After the third immunization, all participants had an increase in binding antibody titers compared to baseline. In addition, these antibody responses were demonstrated to be neutralizing: after 3 immunizations, neutralizing antibodies were detected in 60% of participants using a Vero cell neutralization assay. Serum from 77% of participants was able to block 90% of infection in a neuronal cell assay. Importantly, we also completed a passive transfer of immune sera and demonstrated protection against ZIKV infection in an IFNAR-/- mouse challenge model independent of neutralization titer, whereas control mice had 100% mortality. Finally, strong antigen-specific cellular immune responses were detected by IFNγ ELISPOT. Immunization with GLS-5700 was well tolerated and associated with minor local side effects, but not associated with any severe adverse events.

**Conclusions:** The Zika-001 Phase 1 study demonstrated the safety and immunogenicity of the GLS-5700 DNA vaccine for Zika virus in humans. Passive transfer experiments demonstrated that post-immunization human serum was able to protect mice from lethal infection, suggesting that antibodies induced by GLS-5700 vaccination can be protective.

[PL4.3]

**DNA-launched RNA replicon vaccines induce potent anti-Ebola virus immune responses that can be further improved by protein or MVA boosts**

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The 2013-2016 West African outbreak of Ebola virus (EBOV) spread rapidly and resulted in almost 30,000 cases and more than 11,000 deaths. There are currently no licensed therapeutic treatment or preventive vaccines against EBOV disease. However, the devastating outbreak has spurred the development of novel Ebola vaccines. Here, we demonstrate that alphavirus based DNA-launched self-replicating RNA replicon vaccines (DREP) encoding the either the glycoprotein (GP) gene or co-expressing the GP and VP40 genes of either Sudan or Zaire EBOV are immunogenic in mice. Homologous and heterologous prime-boost immunization protocols were employed and evaluated for induction of anti-EBOV-GP binding and neutralizing antibodies, as well as EBOV-specific T cell responses. DREP-GP immunization induced robust and cross-reactive neutralizing antibody responses that could be further boosted by a heterologous booster immunization. An immunization regimen consisting of a DREP-GP prime followed by an MVA-GP boost demonstrated very strong antibody and T cell responses. Moreover, we show that a bivalent approach targeting both Sudan and Zaire EBOV can be employed without significant loss of immunity, despite using a lower dose of the individual vaccine components. This opens for further investigation of a pan-EBOV or even a pan-filovirus vaccine.
**Thermostable plasmid DNA launches a live-attenuated yellow fever vaccine platform that induces protection in vivo**

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**Background:** The mosquito-borne yellow fever virus (YFV) is one of the deadliest viruses and causes high fever, hemorrhage, and devastating liver disease with a case fatality rate of ~34% in the large, recent outbreaks in Angola and Brazil. The live-attenuated YFV vaccine (YFV-17D) that has been introduced more than 80 years ago has mediated efficient protection. Nevertheless, the production using embryonated chicken eggs and the need for a cold chain during storage and transport results in ~300,000 cases and ~80,000 fatalities every year. Overall, more than one billion people are at risk, and outbreaks in urban areas may get out of control due to rapid depletion of the vaccine stockpile.

**Methods:** We here established a YFV-17D-based vaccine platform that can be produced at large scale, does not require a cold chain, and can induce protection against a variety of pathogens. We engineered a stable YFV-17D cDNA that launches replication of our plasmid-launched live-attenuated vaccine (PLLAV) platform. Replication of PLLAV-YFV-17D progeny virus was compared with the commercially available YFV-17D (Stamaril) in vitro and in vivo. In addition, we constructed reporter viruses to track the cellular tropism and dissemination of YFV-17D via flow cytometry and in-vivo luminescence imaging.

**Results:** (1) Intradermal inoculation of (interferon-deficient) AG129 mice with a fluorescent mCherry-YFV-17D reporter revealed that fibroblasts and skin-resident classical dendritic cells (the most efficient inducers of T cell responses) are the initial targets of vaccine replication. (2) Further, intradermal vaccination using a luciferase-expressing PLLAV-YFV-17D reporter led to replication in the inoculated skin and draining lymph nodes after 5-10 days. (3) Vaccination of hamsters with PLLAV-YFV-17D induced neutralizing antibodies at comparable kinetics as the licensed YFV-17D vaccine (Stamaril) and mediated full protection against clinical signs following infection challenge with a virulent YFV strain. (4) Needle-free intradermal delivery of low µg amounts of PLLAV-YFV-17D DNA induced neutralizing serum antibodies in a macaque, as a relevant non-human primate model for human vaccination.

**Conclusion:** PLLAV-YFV-17D is a novel yellow fever vaccine candidate that appears non-inferior to prequalified vaccines, is thermostable, and can be produced in E. coli at large scale and minimal costs. In addition, our PLLAV-YFV17D technology, can be tailored to carry foreign antigen targets for vaccination against various pathogens including Japanese encephalitis, Zika or Rabies virus.

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**A Combination Heroin-HIV Vaccine Abrogates Nociceptive and Locomotive Effects of Heroin and Induces Cross-Reactive Antibodies to Other Abused Prescription Opioids and to the V2-loop of the HIV-1 Envelope Protein**

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Injection drug use is a major factor in the transmission of HIV-1. This study sought to develop a combination heroin-HIV vaccine that blocks the effects of heroin and may be important as a vaccine for HIV-1. The HIV portion of the vaccine utilized a cyclic V2 (cV2) peptide identified as a correlate of prevention of acquisition of HIV in the RV144 phase III clinical trial. RV144 is the only HIV vaccine trial...
that has demonstrated efficacy to date. The heroin portion utilizes a heroin analog (hapten) coupled to a
carrier protein.

**Methods:** cV2 was synthetized and adsorbed to Alhydrogel® (AH). The heroin hapten (6-AmHap) was
synthesized and conjugated to tetanus toxoid (TT). The resulting conjugate was adsorbed to AH-cV2 and
mixed with Army Liposome Formulation (ALF) (liposomes containing monophosphoryl lipid A) as an
adjuvant. Mice were immunized and sera was assessed for antibody titer to heroin and cV2, and affinity
both to heroin and also its metabolites and other opioids. Efficacy against heroin was assessed by
subcutaneous heroin challenge.

**Results:** The TT-6-AmHap vaccine significantly reduced heroin-induced antinociception and locomotion
behavioral changes. The vaccine induced anti-6-AmHap titers of nearly a million. Competition ELISA
demonstrated that 6-AmHap-induced antibodies that cross-reacted to heroin and to its metabolites, 6-
acetylmorphine (6AM), morphine, and other abuse opioids, including oxycodone, hydrocodone and
hydromorphone. The antibodies did not cross-react with the therapies for substance abuse or the
overdose rescue drug naloxone. Antibody titers to cV2 were also in the millions and bound to gp120 and
gp70V1V2.

**Conclusions:** The heroin-HIV vaccine induced protection against heroin challenge and very high titer
cV2 antibodies, a proposed correlate of efficacy in RV144. These findings suggest that an effective
heroin-HIV vaccine is a feasible goal with this type of vaccine formulation.

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**Benefits of High Dose Fluzone® Influenza Vaccine for Anti-Neuraminidase Immune Responses in
the Elderly**

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**Background:** Antibodies against neuraminidase (NA), the second most abundant glycoprotein on the
surface of the influenza virus, contribute to the virus protection and have been correlated with less severe
disease. However, the current vaccine development is mostly aimed toward the dominant protein
hemagglutinin (HA) and the amount of NA is not standardized in current seasonal vaccines nor is the
breadth coverage known.

**Methods:** Young (18-35), adult (35-65) and elderly (65+) people were vaccinated with split, inactivated
quadravalent (QIV) Fluzone® influenza vaccine. Elderly were given a choice of Standard Dose (SD)
(15ug of HA) and High Dose (HD) (60ug of HA) Fluzone formulations. All other age groups were
administered SD QIV vaccine. Sera was collected prior to vaccination and 21 days post-vaccination and
tested for anti-neuraminidase antibodies by enzyme-linked immunosorbent assay (ELISA). Collected
serum samples were also tested for neuraminidase-inhibition (NI) activity by enzyme-linked lectin assay
(ELLA). All ELLA assays were performed with tetrameric-NA protein, therefore by-passing any potential
interference by anti-HA antibodies.

**Results:** Anti-NA antibodies increased against both N1 and N2 NA components in the vaccine between
day 0 pre-vaccination and day 21 post-vaccination in all age groups. In addition, there was an increase in
NI activity in sera collected at day 21 in the young cohort following vaccination with standard dose
dose vaccine and the elderly administered HD vaccine. Elderly administered the SD vaccine did not have a
significant rise in NI titers.

**Conclusion:** Even though split, inactivated influenza vaccine elicits high titer anti-HA antibodies, immune
responses are elicited to other influenza vaccine components following immunization. In this study, QIV
Fluzone® influenza vaccine was administered to individuals between the ages of 18-85 y.o. Both anti-
neuraminidase binding antibodies and NI activity increased in young (18-35 y.o.) administered the SD of
the vaccine, but not in the elderly cohort (65-85 y.o.). There was a statistical increase in NI activity in the
elderly administered the HD QIV Fluzone influenza vaccine. Therefore, administration of the HD vaccine
to elderly has the added benefit of not only enhancing anti-HA HAI activity, but also enhances immune
responses to NA demonstrating another benefit of the HD vaccine immunization for the elderly.
**Zoster Vaccine Effectiveness against Incident Herpes Zoster and Risks of Vaccine Failure in Elderly in the UK**

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**Objectives:** Herpes zoster (HZ) is a painful skin rash that occurs most frequently in older adults and is caused by reactivation of latent varicella zoster virus. The UK introduced zoster vaccination in the national immunization program in 2013. The vaccine is routinely offered to 70 year-olds and, as part of the catch-up, to 79 year-olds. With this study, we assessed the vaccine effectiveness (VE) against HZ in elderly within the total population, immunocompromised (IC) and IC-free people. Additionally, risk factors of vaccine failure were investigated.

**Methods:** This retrospective cohort study included subjects from birth cohorts 1943-1946 (routine) and 1934-1937 (catch-up) in the UK Clinical Practice Research Datalink (CPRD). Vaccinated subjects were compared to unvaccinated subjects for HZ outcomes in primary care using piecewise Cox regression models adjusting for gender, age, chronic obstructive pulmonary disease (COPD), diabetes type 1 and type 2, asthma, IC conditions, smoking, BMI and ethnicity. Ten IC conditions were accounted for, including hematopoietic stem cell transplant (HSCT), solid organ transplant (SOT), chronic kidney disease, several malignancies and autoimmune diseases. Risks of vaccine failure were investigated using quasi-Poisson regression. Potential risk factors of interest were gender, age, chronic obstructive pulmonary disease (COPD), asthma, diabetes type 2, smoking (smoker, ex-smoker, non-smoker), BMI (underweight, normal, overweight, obese), IC and ethnicity (Caucasian, non-Caucasian).

**Results:** For the routine birth cohorts (79280 subjects), we found a VE for HZ of 69.4 % (95% CI: 54.0%-79.7%), 72.4% (95% CI: 42.0%-86.8%) and of 67.9% (95% CI: 47.6%-80.4%) for the first 2 years postvaccination in the total, IC and IC-free population, respectively. For the catch-up birth cohorts (48197 subjects), the VE estimates were comparable. Being diabetic, ex-smoker and underweight are found to be risk factor for HZ vaccine failure.

**Conclusions:** Within the total population, the HZ vaccine provided protection against HZ. Type 1 diabetics, ex-smokers and underweight persons are less protected by vaccination than others.

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**MVA-BN®-RSV Vaccine Boosts Pre-existing RSV Immunity in Young Adult and Elderly Mice**

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Human Respiratory Syncytial Virus (RSV) has been recognized as a cause of lower respiratory tract infection associated with significant morbidity and mortality in infected young children (<2 years) and older adults. The majority of the population is infected with RSV very early in life; however, previous infection does not prevent subsequent re-infection later in life.

Bavarian Nordic has generated an RSV vaccine candidate based on MVA-BN® expressing full-length RSV proteins G, F, N and M2 of an RSV A strain, as well as G protein of RSV B strain. The vaccine vector MVA-BN® (Modified Vaccinia Ankara - Bavarian Nordic) displays high immunogenicity and a favorable safety profile even in immuno-compromised hosts and the elderly, due to its inability to replicate in the vaccinated individuals. Recombinant MVA-BN® vaccine candidates for other infectious diseases and cancer have been shown in clinical trials to retain the safety profile of the vector and to induce balanced B and T cell responses to the transgene(s). Indeed, recombinant MVA-BN expressing RSV antigens was shown to be safe in a repeated dose toxicity study and in a Phase 1 clinical trial MVA-BN®-RSV has been demonstrated to be immunogenic and efficacious in mouse and cotton rat RSV models and is currently being tested in a clinical phase 2 trial in subjects aged 55 years and older. In an effort to mimic vaccination of an RSV experienced population, we investigated in mice (BALB/c and C57BL/6) whether vaccination with MVA-BN-RSV boosts existing RSV-specific immunity. MVA-BN®-RSV administered to mice exhibiting different levels of pre-existing RSV immunity, induced significant increases of RSV-specific antibodies measured in serum and bronchoalveolar lavages and
boosted T cell responses in spleens and lungs. Importantly, MVA-BN-RSV induced similar humoral and cellular responses in young adult mice (2 to 3 months) and aged mice (12 to 20 months), i.e. it was unaffected by potential age-related changes or dysfunction of the immune system.

**Immune responses to intradermal and intramuscular inactivated influenza vaccine among older age group**

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**BACKGROUND:** Influenza viruses cause substantial morbidity across the age spectrum, especially in the older age group. Thus, the older age group is included amongst high priority groups for routine vaccination. However, vaccine-induced immune responses and effectiveness were reported to be relatively low in this group. This study aims to systemically compare the immune response elicited by intramuscular (IM) and intradermal (ID) injection with inactivated seasonal influenza vaccine among older age group above 60 years old in one study.

**METHODS:** It was a prospective, open-label, randomized study. A total of 221 adults aged > 60 years were enrolled and randomized into 2 groups. Group I (n=111) received an intramuscular (IM) inactivated seasonal influenza vaccine while Group II (n=110) received the similar vaccine intradermally (ID). Demographic data and co-morbidity were collected at base line. Safety data was collected 3 days after vaccination by using diary card. Hemagglutination inhibition antibody, neutralizing antibody and neuraminidase inhibition antibody titers were assessed prior to vaccination as well as 30, 45, and 60 days post-vaccination. Data was analyzed using SPSS software version 11.5.

**RESULTS:** Both groups had similar BMI and co-morbidity. Between the ID and IM groups, significantly differences were observed in the seroconversion rate measured using HAI against H1N1 and H3N2 (58/111 vs 44/110 and 68/111 vs 54/110, respectively). However, there were no differences in HI antibody against B/Phuket. The seroconversion rates in both the ID and IM groups were higher in those who were between 60-65 years of age. By giving intradermal vaccination, history of hyperlipidemia and hypertension appeared to be the factors associated with a higher seroconversion rate toward influenza A (p=0.001). The seroconversion rate risk ratio were 1.31 and 1.25 (p<0.05) against A/California/07/09(H1N1) (36.38- 79.14) and A/Songkha/308/13 (H3N2), respectively. Interestingly, the GMT (95% CI) of baseline anti-neuraminidase antibodies among both IM and ID groups were high (53.66). A 4-fold increase after 45 days measured by in neuraminidase inhibiting antibody (NAI) against A/California/07/09 (H1N1) were detected in 23.3% and 24.1% of participants who received ID or IM vaccination, respectively.

**CONCLUSIONS:** The seroconversion rates of hemagglutination inhibition antibody, neutralizing antibody and neuraminidase inhibition antibody was modest, especially in those above 65 years of age. The data obtained from this study will benefit the influenza vaccine policy for the elderly population in a developing country such as intradermal route appeared to induced better immune responses especially against influenza A. However, further study is needed in order to explore and improve the vaccination systems used for the older aged population.
Flagellin-based recombinant divalent vaccines induce protective immune responses in a *Porphyromonas gingivalis* and *Fusobacterium nucleatum* mixed infection model in mice
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Chronic periodontitis is caused by interactions between the oral polymicrobial community and host factors. Periodontal diseases are associated with dysbiotic shift in oral microbiota. Vaccination against periodontopathic bacteria could be a fundamental therapeutic to inhibit polymicrobial dental biofilms. Because oral cavity is the site of the bacterial colonization, mucosal vaccines should provide better protection than vaccines administered systemically. We previously reported that a bacterial flagellin, *Vibrio vulnificus* FlaB, is an excellent mucosal adjuvant. Since flagellin is protein-based TLR agonist, recombinant antigen-flagellin fusion protein may serve as a platform for multivalent periodontal vaccine. In this study, we investigated whether intranasal vaccination with flagellin-based recombinant divalent vaccines could induce protective immune responses in a mixed *Porphyromonas gingivalis* and *Fusobacterium nucleatum* oral infection model. We used the Hgp44 polypeptide of *P. gingivalis* and FomA of *F. nucleatum* as fusion protein partners with FlaB. Since the FomA has multiple transmembrane domains, it has been reported that purification yield of the recombinant FomA protein is extremely low. In this study, we successfully prepared FlaB-FomA fusion protein with high purity. Intranasal immunization of the fusion protein-based vaccines induced antigen-specific serum IgG and saliva IgA responses. The divalent antigens did not interfere each other in inducing effective antigen-specific Ab responses. The Hgp44- and FomA-specific serum and saliva inhibited *P. gingivalis* hemagglutination and *F. nucleatum*-induced biofilm formation respectively. Furthermore, mice administered with the recombinant divalent fusion protein vaccines exhibited significant reductions in alveolar bone loss caused by live *P. gingivalis* and *Fusobacterium nucleatum* infections. Taken together, flagellin-based recombinant divalent vaccines was protective against mixed infection periodontitis.

Food grade live oral mucosal vaccine (LacVax™:OmpA) against *Shigella*: Affordable strategies for effective immunisation
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Shigellosis is an acute invasive disease of the lower intestine, which afflicts millions of people worldwide with an estimated one million fatalities per annum. Vaccine is one of the most promising approaches to fight against multidrug resistant *Shigella*. Despite of extensive research during the last two decades, vaccine against *Shigella* is not yet available in the market. To provide safe, effective and broad spectrum vaccine against *Shigella*, we explored food grade bacteria *Lactococcus lactis* (*L. lactis*) for the delivery of conserved antigenic protein/DNA to mucosal sites for effective elicitation of systemic and mucosal immunity. Based on bioinformatics and proteomics analyses, we have found outer membrane proteins (OmpA and OmpC) as conserved immunodominant antigens present in all *Shigella* spp. In order to evaluate the immunogenic potency of selected putative candidates, purified r-OmpA and r-OmpC were administered in Balb/c mice via subcutaneous route. Significant increase in anti-OmpA and anti-OmpC serum IgG and fecal IgA levels confirmed the immunogenic potential of selected candidates. Further, to evaluate the protective efficacy of developed candidate vaccine, we have developed the intraperitoneal *Shigella* murine model which mimics the human. The immunized animals exhibited protection when challenged with *S. flexneri*. Cytokin profiling revealed the shift from Th2 (pre-challenge) to Th1 (post-challenge)
response confirmed the potency of r- OmpA and r-OmpC in generating the cell mediated immune response.

To develop needle free oral mucosal vaccine, OmpA was cloned and expressed in food grade L. Lactis. Construction of r-L. lactis expressing OmpA (LacVax™:OmpA) was confirmed by Western Blot analysis. The immunogenic potential of LacVax™:OmpA was ascertained by administration of LacVax™:OmpA via oral and nasal routes to Balb/C mice. The significant increase in serum IgG, fecal IgA levels and Th1 dominant response revealed successful activation of humoral and cellular immunity. The immunized animals were also protected from shigellosis when challenged with S. flexneri.

Further, the validation of LacVax™:OmpA in higher animals in collaboration with industrial partner is in progress. Moreover, the strength of LacVax™ platform to deliver DNA vaccine and peptide vaccine candidates was evaluated in our lab.

[O5.5]

Efficacy of Novel Epithelial Stem Cell-based AIDS Vaccine to Induce Mucosal Immune Responses and Protect against Repeated Low Dose SIV Challenge
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A vaccine that restricts viral replication at mucosal portal of entry may help controlling HIV infection. We used the epithelial stem cells as permanent source of viral antigens and their differentiated offspring as antigen-producing presenting cells. We developed a SIV single cycle vaccine under the control of the involucrin promoter (pINV-SIVsc), which was tested for its ability to drive SIV expression in terminally differentiated epithelial cells, induce mucosal immune response, and protect against SIV challenge.

Female macaques (n=12) were immunized at week 0 with pINV-SIVsc vaccine and monitored for specific immune responses in blood and various tissues. Animals were challenged at 12 or 24 weeks using repeated low-doses SIVmac239. High levels of mucosal IgA and IgG were detected post-vaccination as well as specific CD8+ T cells expressing a4ß7 homing to the mucosa. Repeated low-dose challenges revealed significant delay and lower viremia with 2-4 logs reduction at peak, 4-6 logs-reduction at set-point, and undetectable viremia by week 16 post-SIV in vaccinated females. Controls had high viremia (log10: 7.4-8.7 viral RNA copies/ml, peak) and significant CD4+ T cells reduction in jejunum. The study demonstrated the efficacy of an epithelial stem cell-based vaccine to serve as antigen delivery system and generate specific humoral and cellular immune responses leading to protection or significant delay and decrease in viremia to undetectable.

[O5.6]

A novel mucosal HIV vaccination regimen to elicit protective HIV-specific immunity
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Human immunodeficiency virus (HIV)-1 has either killed or infected over 76 million people, and infection rates are rising worldwide especially in low- and middle-income countries. Thus, cost-effective vaccines are required to eliminate HIV-1, but to be effective a vaccine will need to elicit robust mucosal immunity given that vast majority of HIV-1 transmissions occur via exposure of the genitoreal mucosa. Herein we report the efficacy of innovative vaccines pioneered in our laboratory to evoke T cell immunity to Gag and non-classical neutralizing antibodies (NAb) to Tat in mucosal and systemic compartments.

The vaccines used in this study include DNA vaccines encoding Gag and a cytolytic protein viz. perforin (PRF) (pVAX-Gag-PRF) or a secreted version of Tat (sTat) and IMX313 (pVAX-sTat-IMX313), and a cocktail of recombinant human rhinoviruses (HRV) encoding Gag and Tat (rHRV). PRF and IMX313 are
strategically encoded in DNA vaccines to act as adjuvants. The expression of PRF results in necrotic cell death, sterile inflammation and exposure of Gag to dendritic cells which can prime (i.e. via cross-presentation) Gag-specific CD8+ T cell immunity. The expression of IMX313 results in oligomerisation (i.e. heptamer formation) of the Tat protein and induction of robust Tat-specific NAb.

Female Balb/c mice (n = 7 per group) in rHRV-DNA regimen were vaccinated with 2 intranasal (IN) doses (2 weeks apart, 5 X10^6 TCID50 per dose) of rHRV and then intradermally (ID) boosted for 2 weeks with a 50 µg cocktail of pVAX-Gag-PRF and pVAX-sTat-IMX313. Subsequently, Gag- and Tat-specific immunity were analysed in mucosal (gut and vagina) and systemic (splenocytes and blood) compartments using various immunological assays. For protective efficacy studies, rHRV-DNA or control vaccinated mice were challenged intraperitoneally with EcoHIV, a surrogate HIV challenge model and viral loads were measured.

ELISpot and intracellular cytokine staining analysis showed that rHRV-DNA vaccinated mice significantly elevated the number of Gag-specific T cells and polyfunctional Gag-specific CD8+ T cells in the mesenteric lymph nodes of the gut and the spleen compared to control vaccinated mice. Splenocytes from vaccinated mice were also stained with H-2Kd-restricted Gag197-205 tetramer, to directly enumerate Gag-specific CD8+ T cells present in vivo. This analysis revealed that indeed the rHRV-DNA regimen significantly elevated Gag-specific CD8+ T cells in vivo. Furthermore, Tat-specific NAb in cervicovaginal lavages and serum were also significantly elevated in rHRV-DNA vaccinated mice compared to control mice. When EcoHIV viral copies were quantified in the peritoneal macrophages, splenocytes and the blood of rHRV-DNA vaccinated mice and compared to the control mice, data showed that rHRV-DNA vaccination resulted in at least a 10-fold reduction in viral load in all these compartments compared to the control.

rHRV-DNA vaccination significantly elevated Gag-specific T cell immunity and Tat-specific NAb in mucosal and systemic compartments which correlated with 10-fold reductions in EcoHIV viral loads in rHRV-DNA vaccinated mice compared to control mice. These data provide the first evidence that a rHRV-DNA vaccination regimen has high potential for eliciting protective HIV-specific immunity and warrants further testing of the rHRV-DNA regimen in higher animal models.

[O5.7]

A novel vaccine against H. pylori-induced gastritis
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BACKGROUND: Diseases arising from infection with the stomach-dwelling bacterium Helicobacter pylori, including gastric cancer and peptic ulcers, result from the development of a severe and chronic inflammation (gastritis). A key feature of this gastritis is a breakdown in the cell-cell junctions between epithelial cells at the stomach mucosal surface that maintains an impermeable barrier between the host tissues and the stomach lumen. In fact, the opening of these junctions is believed a key step in the initial development of gastritis.

It has been previously shown that opening of these epithelial cell junctions is mediated by cleavage of E-cadherin by the H. pylori enzyme HtrA. Opening these gaps allows the bacteria and their components to pass the barrier and enter the stomach tissue, where they can directly interact with the host immune system and drive an inflammatory response.

Despite more than 50% of the world’s population being infected with these bacteria, no vaccine is available that can eradicate this infection or cure gastritis. We therefore explored the potential utility of HtrA as a vaccine antigen aimed at inhibiting the inflammatory response to this infection.

RESULTS: The effectiveness of HtrA as a vaccine antigen against H. pylori-induced gastritis was evaluated in C57BL/6 mice. Vaccination of mice, either before or after infection, was found to be able to completely prevent the development of gastritis. The adaptive immune responses generated by the vaccine include Th1 and Th17 memory immune responses as well as mucosal and systemic antibodies to HtrA. Examination of the mechanism of action has shown, by immunising antibody deficient μMT mice and their sibling littermates as controls, that the inhibition of gastritis induced by this vaccine is mediated
by antibodies. Furthermore, in vitro assays using sera from experimental mice showed that vaccination induces antibodies that prevent HtrA-mediated cleavage of E-cadherin.

**CONCLUSION:** Our findings demonstrate the vaccine-mediated targeting of HtrA as a novel approach for protecting against *H. pylori*-induced gastritis. As *H. pylori*-induced gastritis is the predominant cause of peptic ulcer disease and gastric cancer, an HtrA vaccine provides an ideal approach for preventing *H. pylori*-related gastric malignancies. Moreover, the therapeutic potential of HtrA vaccine makes it suitable for the large proportion of individuals already infected with *H. pylori*.

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**Computational vaccine design for humans and animals using the iVAX Toolkit**

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Computational vaccine design, also known as computational vaccinology, encompasses epitope mapping, antigen selection and immunogen design using computational tools. In silico prediction of immune response to emerging infectious diseases and cancers can accelerate the design of novel and next generation vaccines. The iVAX toolkit is an integrated set of immunoinformatics algorithms that has been in development since 1998. It comprises a suite of immunoinformatics algorithms for triaging candidate antigens, selecting immunogenic and conserved T cell epitopes, eliminating regulatory T cell epitopes, and optimizing antigens for immunogenicity and protection against disease.

iVAX harnesses computing power, genomic data, and advanced immunoinformatics tools to identify T cell epitopes capable of inducing productive T cell immune mechanisms for generation of safe and effective vaccines. Novel algorithms have been implemented to predict peptide binding to human, murine and swine class I and class II MHC alleles. Highly immunogenic peptides conserved across multiple strains of input pathogen sequences are identified using the Conservatrix, EpiMatrix and EpiAssembler algorithms. Potential vaccine candidate epitopes can be aggregated into a string-of-beads design with the VaxCAD algorithm, simultaneously minimizing deleterious junctional epitopes that may be created in the linking process. Two newer tools, discovered and refined in the past few years, include JanusMatrix and iTEM. JanusMatrix is a specially tailored homology analysis tool that examines pathogen/host sequence similarity at the MHC:TCR interface for any given peptide, and predicts potentially cross-reactive epitopes, allowing candidate sequences with potential host cross-reactivity to be preferentially excluded from vaccine constructs. iTEM enables the identification of human-subject unique epitopes, and is ideal for cancer mutanome processing and personalized cancer vaccine design.

Most recently, low immunogenicity H7N9 influenza antigens with high human cross-conservation were engineered to include epitopes more highly cross-conserved with circulating influenza strains, resulting in a 5-fold increase in post-vaccination antibody titers compared to wild type protein [Liu et al. 2015 Human Vaccines & Immunotherapeutics 11:9, 2241-52; Wada et al. 2017 Scientific Reports 7:1, 1283]. The JanusMatrix tool also successfully identified the cross-reactive epitope between the MAGE A3 immunotherapeutic and human titin implicated in two fatalities among affinity-enhanced TCR cancer immunotherapy trial participants. The failure of H7N9 vaccines due to poor immunogenicity illustrates the challenges associated with time-honored approaches to vaccine development, while modern cancer vaccine research has underscored the danger of auto-reactive vaccines and immunotherapeutics. We have applied immunoinformatics tools to develop safe and effective responses to these challenges. The iVAX toolkit has been adapted to accelerate the development of targeted, safe and efficacious vaccines, which will address important global health and biodefense challenges for humans and livestock. [Terry et al. 2015 Expert Rev Vaccines. 14(1):21-35; Moise et al. 2015 Human Vaccines & Immunotherapeutics 11:9, 2312-2321]. Academic and commercial collaborations are welcomed and encouraged.
Computational vaccinology for the design of personalized cancer therapies: from infectious disease to cancer vaccines
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Computational vaccinology has accelerated the pace of vaccine development over the last two decades. T cell epitope prediction tools, historically applied to infectious disease targets, are now being employed to design novel cancer immunotherapies. Coupled with the widespread accessibility of next-generation sequencing, epitope prediction opens the door to production of personalized cancer vaccines targeting gene mutations expressed by tumor cells. However, the majority of epitopes selected by traditional algorithms prove to be non-immunogenic. Poor predictive performance may partially be due to erroneous inclusion of epitopes cross-conserved with self or commensal epitopes recognized by the T cell receptor of regulatory, anergic or deleted T cells. Vaccination with self-like epitopes can lead to weak effector responses, active immune suppression, and toxicity due to immune-mediated adverse effects.

We have developed advanced T cell epitope identification and characterization tools, EpiMatrix and JanusMatrix, that streamline the selection and prioritization of Class I and Class II epitopes. These state-of-the-art tools have been extensively validated in prospective vaccine studies for infectious diseases [Moise et al. 2015 Human Vaccines & Immunotherapeutics 11:9, 2312-2321]. We further validated EpiMatrix’s predictive accuracy using recently published datasets of HLA-bound peptides detected by mass spectrometry, which are independent of training sequence data used in model development. Analysis of sequences from Abelin et al. [2017 Immunity 46, 315-326] shows 96% agreement between EpiMatrix predictions and peptides eluted from commonly expressed Class I HLA alleles, while only 86% of these sequences are accurately recalled by NetMHC and NetMHCpan.

We recently demonstrated the importance of vaccine T cell epitope content by removing a regulatory T cell (Treg)-inducing epitope identified with JanusMatrix from the poorly immunogenic H7N9 influenza hemagglutinin antigen that resulted in significantly increased vaccine immunogenicity [Liu et al. 2015 Human Vaccines & Immunotherapeutics 11:9, 2241-52; Wada et al. 2017 Scientific Reports 7:1, 1283]. Application of these tools in the oncology field may also allow for the prioritization of epitopes exhibiting reduced potential for inducing Tregs, whose activation continues to curtail current cancer therapies. In a retrospective analysis of a cancer immunogenicity study [Strønen et al. 2016 Science 352(6291), 1337-41], EpiMatrix and JanusMatrix differentiate immunogenic and non-immunogenic epitopes with 72% accuracy, as compared to 21% accuracy when using public prediction tools. Increased accuracy is primarily explained by the removal of mutated epitopes that present increased potential for Treg activation.

Results from both the infectious disease and cancer fields demonstrate that EpiMatrix and JanusMatrix may focus epitope candidate selection on higher value sequences than conventional algorithms. Epitopes with low Treg activation potential may then be used to support the development of safer and more effective vaccines.

COBRA HA Induced Hemagglutination-inhibition Antibodies Against a Panel of H3N2 Influenza Antigenic Variants
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Each influenza season, a set of wild-type viruses, representing one H1N1, one H3N2, and 1-2 influenza B isolates, are selected for inclusion in the annual seasonal influenza vaccine. In order to develop broadly reactive subtype specific influenza vaccines, a methodology for designing computationally optimized
broadly reactive antigens (COBRAs) was utilized to produce novel hemagglutinin (HA) vaccine immunogens. COBRA technology was effectively used to design HA immunogens that elicited antibodies that neutralized H5N1 and H1N1 isolates. In this report, the development and characterization of fifteen prototype H3N2 COBRA HA proteins were screened in mice for the elicitation of antibodies with HAI activity against a panel of human seasonal H3N2 viruses that were isolated over the last 48 years. The most effective COBRA HA vaccine regimens elicited antibodies with broader HAI activity against a panel of H3N2 viruses compared to wild-type H3 HA vaccines. The top leading COBRA HA candidates were then tested against a panel of co-circulating variants. Antibodies elicited by the wild-type HA from viruses selected as the vaccine candidates did not efficiently detect these variants. However, the T-11 COBRA HA vaccine elicited antibodies with HAI activity against all co-circulating variants from 2004-2007. This is the first report demonstrating broader breadth of vaccine-induced antibodies against co-circulating H3N2 strains compared to the wild-type HA antigens that were represented in commercial influenza vaccines.

Expanding a web-based programme for identification of invasive M. bovis BCG
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Introduction Mycobacterium bovis BCG is used to vaccinate infants against disseminated TB. Although considered safe, infections have been associated with the vaccine. The vaccine belongs to the Mycobacterium tuberculosis complex and shares similar genetic properties with the virulent strains. Comparative studies have enabled differentiation of BCG from virulent M. bovis and M. tuberculosis. Molecular platforms with bioinformatics solutions are being developed for more accurate characterization of species. Furthermore, web-based programmes for identification of invasive BCG strains are lacking, important tools for detection of pathogens as well as prediction of virulence and or immunogenic genes by analysing the genome.

Aim The aim of this study was to expand a web-based programme for identification of invasive M. bovis BCG strains.

Materials and methods Genomic DNA was extracted from clinical M. bovis BCG isolates and M. bovis BCG Moscow reference strain (the strain currently used for vaccination in South Africa). The clinical strains were obtained in 2014 and are believed to be BCG Danish strain. The sequence reads from the Illumina platform were analysed using CLC genomics workbench 7 and Mauve 2.3.1. The genome sequence data was used to add a level of identifying invasive M. bovis BCG strain to an existing web-based programme.

Results A cladogram comparing the Clinical and the reference genome to 22 M. bovis genomes was generated; the isolates clustered with virulent M. bovis. The web-based program has been released (http://clader.bi.up.ac.za). The program identifies invasive M. bovis BCG strain to an existing web-based programme.

Conclusion The web-based program for identifying invasive M. bovis BCG has been expanded; identification key text file was modified to accommodate M. bovis polymorphic sites. However, further testing of the program using different genomes with longer sequence reads is required. The clinical and the reference strains shared similarities with the invasive M. bovis genome. The similarities shared could attest to BCG as M. bovis descendant. Future studies focusing on the identification of specific, genetic similarities between the clinical BCG and the virulent M. bovis that differs with the BCG reference strains are required to determine virulence factors of the clinical BCG.
First Evaluation in Humans of a Chemically Attenuated P. falciparum Whole Parasite Blood-Stage Vaccine

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Malaria is a leading cause of morbidity and mortality attributable to infectious disease. The possibility of a malaria vaccine was first realized in the 1940s, yet a vaccine capable of inducing long-lasting immunity remains elusive. We have shown that a chemically attenuated whole parasite blood-stage vaccine, consisting of ring-stage malaria parasites attenuated with the cyclopropylpyrroloindole analogue Tafuramycin-A (TF-A), offers profound CD4 T cell-dependent protection against challenge with homologous and heterologous parasites in rodent models. A clinical-grade P. falciparum 7G8 cell bank was used to manufacture and evaluate this vaccine approach in humans. Malaria-naïve volunteers were inoculated with a single dose of $3 \times 10^7$ P. falciparum 7G8 parasitised erythrocytes treated with TF-A, to evaluate the safety and immunogenicity of chemically attenuated P. falciparum blood-stage parasites in humans. They were injected with either ring-stage parasites (n=8) or purified trophozoite-stage parasites (n=3). Parasite-specific antibody and T cell responses were measured. The inoculum was well tolerated. Species and strain transcending CD4 and CD8 Plasmodium-specific T cell responses were induced in recipients whereas Plasmodium-specific IgG was not detected. Production of IFN-γ and TNF was also observed. These studies demonstrate that a chemically attenuated whole parasite P. falciparum blood-stage vaccine can induce Plasmodium-specific T cell responses in malaria-naïve volunteers. These data support evaluating the efficacy of this vaccine approach in humans.

Novel Subunit-Vaccine Approaches Against Tuberculosis

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Bacillus Calmette-Guérin (BCG), the attenuated strain of Mycobacterium bovis, remains the only currently available vaccine against TB since 1921, though BCG is ineffective in adults against pulmonary TB. Also, the protective efficacy of BCG varies from 0 to 85% in different models, prompting an urgent need for the development of an improved and efficient TB vaccine. In this milieu, our laboratory has worked upon two different subunit vaccine approaches- one, a nanoparticle (NP) based vaccine and the other, an adjuvant based TB vaccine. Nanoparticle based vaccine- The immune-modulating properties of Poly- (D, L-lactide-co-glycolide) (PLGA)-NPs encapsulating the H1 chimeric antigen (a fusion of Mtb Ag85B and ESAT-6 proteins) was investigated and their role in protection upon Mtb challenge, was assessed. The H1-PLGA nanoparticles were prepared by water/oil/water solvent evaporation method, with H1 encapsulation efficiency of 86.18±3.2%, size of 246.8±19.4 nm diameter and negatively charged surface (zeta potential = -4.2±0.6 mV). Under physiological conditions, PLGA-NPs degraded slowly and the encapsulated H1 antigen was released over a period of weeks. As a proof-of-concept vaccine candidate, H1-PLGA NPs were efficiently internalized by the THP-1 human macrophage cell line. Six weeks after a single vaccination, compared to H1 alone, mice vaccinated with H1-PLGA NPs showed significant increase in the productions of total serum IgG and its isotypes, with IgG2b being the predominant one, followed by IgG1. A single dose vaccination with H1-PLGA NPs induced a stronger Th1 cellular immune response with an exclusively higher IFN-γ profile than those of H1 alone or blank PLGA nanoparticles in a C57BL/6J mice model. Briefly, the H1-PLGA NPs vaccinated mice displayed -7 and -3 fold increase in the levels of Th1-type IFN-γ and TNF-α cytokines, compared to H1 alone. In protective efficacy studies, H1-PLGA vaccinated mice displayed significant reduction in lung bacillary load (P < 0.05), with a mean survival time (MST) of 177 days, compared to H1 antigen alone vaccinated mice (MST= 83 days). Adjuvant based vaccine- We explored the adjuvant potential of poly-α-l-glutamines (PLG), a lesser-known
component of Mtb CW, that are present in abundance only in pathogenic mycobacteria. Immunomodulatory properties of PLG were evaluated using Mtb ESAT-6 protein as vaccine candidate in C57BL/6J mice model. PLG adjuvant triggered a strong humoral response against ESAT-6 antigen and resulted in significantly elevated levels of total IgG and its isotypes (IgG1, IgG2b and IgG2c). The splenocytes from PLG vaccinated mice upon antigenic stimulation, displayed robust increase in Th1 specific IFN-γ, TNF-α, IL-2 and Th2 specific IL-6 and IL-10 cytokines. Additionally, PLG also activated Th17 response, leading to secretion of significantly high levels of IL-17 cytokine by the splenocytes. The PLG adjuvanted mice recorded 97.4 % and 92.43% reduction in bacterial counts in the lungs and spleens respectively, six weeks after Mtb challenge. The magnitude of reduction was statistically indifferent to BCG vaccinated mice. PLG as adjuvant appears better than DDA-MPL in enhancing protective efficacy of ESAT-6 (P < 0.05). The strong Th1 and Th17 immune response generated by PLG makes it a promising adjuvant candidate for developing effective vaccines against Th1 response dependent diseases like Tuberculosis, Typhoid, Brucellosis, etc.

[PL5.6]

Discovery of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) Vaccine Candidate Antigens by Protein Microarray Scanning and Verification of the Determined Antigens by Recombinant ELISA


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Introduction: Crimean-Congo Hemorrhagic Fever (CCHF) is an acute tick-borne zoonotic disease and caused by a nairovirus of the Bunyaviridae family. The CCHF virus is transmitted by Hyalomma ticks. Since 2002, more than 8000 cases were reported in Turkey, with mortality rate around 5%. The only vaccine against CCHF is inactivated suckling mouse brain vaccine which was developed by former Soviet Union. Overall, development of a safer and protective vaccine against CCHF is required by the community. The aim of this study is to screen sera of CCHF patients with protein microarray to discover antigens that can be used to develop vaccine and diagnostic kits.

Materials and Methods: Initially, novel protein microarray chips containing 24 exons of CCHFV Turkish Kelkit and African Nigerian strains were generated and probed with acute and convalescence CCHF confirmed patient sera (n: 280) collected from the Kelkit region of Turkey. Thereafter, bound antibodies were visualized by confocal laser scanner. Next, the discovered antigens were expressed in E. coli BL21 cells, purified to homogeneity by AKTA-FPLC and used in ELISA to screen anti-CCHFV specific IgM and IgG antibodies.

Results: According to the microarray screening results, S segment both from Kelkit and Nigerian strains encoding viral nucleocapsid protein (NP) and M segment encoding the glycoprotein precursor (GP) gave higher absorbance values with acute and convalescent samples. ELISA using recombinant NP and GP proteins detected IgM and IgG antibodies in acute and convalescent sera.

Discussion and Conclusion: CCHF disease is still a growing public concern in Europe and development a protective and safe vaccine has utmost importance. NP is increasingly regarded as an important target and used in clinical diagnosis because it is the major protein detected during the viral invasion phase. M segment includes mucin like variable domain which has neutralizing antibody epitopes conserved among all strains. Our findings obtained from protein microarray and ELISA probed with sera collected from patients living in Kelkit valley also correlate these previous findings. Overall, NP and GP proteins appear the most antigenic proteins that can be used in development of vaccine and diagnostic kits.

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Clinical and Immunologic Biomarkers for Regression of High Grade Cervical Dysplasia and Clearance of HPV16/18 Infection after Immunotherapy with VGX-3100 in a Phase IIb Clinical Trial

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We have previously demonstrated proof-of-principle of immunotherapy in women with premalignant cervical lesions caused by HPV16 or HPV18. In a Phase IIb study of VGX-3100, which targets HPV16/18 E6 and E7, premalignant lesions underwent complete regression (CR) concomitant with elimination of underlying HPV16/18 infection in 40.0% of VGX-3100-treated patients and in only 14.3% of patients receiving placebo (p=0.002). Here we detail characteristics present that either correlated with or predicted therapeutic benefit with VGX-3100. Combining HPV typing and Pap smear data collected following the third dose (Week 14) was predictive for both histologic regression and the presence of detectable virus at the efficacy assessment twenty two weeks later (predictive value 94%, sensitivity 96%). Patients who had a significant peripheral blood elevation in CD137+ Perforin+ CD8+ T cells specific for the HPV type causing disease (p=0.001) were much more likely to have lesion regression and HPV elimination than women without a detectable peripheral blood response. In comparisons of the intensity of cervical mucosal CD103+ and Perforin+ cell infiltrates pre- and post-vaccination, significant increases were observed in patients displaying exhibiting CR with elimination of HPV infection (p=0.027 and p=0.043, respectively). In sum, we found that quantitative measures associated with an effector response to VGX-3100 antigens were associated with subsequent lesion regression. Consequently, these analyses indicate that certain immunologic responses are associated with successful resolution of HPV-induced premalignancy, with particular emphasis on the upregulation of Perforin in the immunotherapy induced immune response.

Synthetic DNA-encoded monoclonal antibody delivery (DMAb™) of anti-CTLA4 antibodies induces tumor shrinkage in vivo

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Antibody-based immune therapies targeting the T cell checkpoint inhibitors CTLA4 and PD1 have made incredible advances in cancer therapy. In particular, the combination of CTLA4 and PD1 blockade (using ipilimumab and nivolumab, respectively) was recently shown to be the most effective immune therapy for improving progression-free survival in advanced melanoma patients. However, this immune therapy combination strategy is extraordinarily expensive due to high manufacturing costs of monoclonal antibodies, with an estimated annual cost of almost one million dollars per patient. In this study, we focused on the development of a DNA-encoded monoclonal antibody (DMAb™) approach for delivery of anti-CTLA4 monoclonal antibodies in vivo. With this technology, formulated DMAb™ plasmids are injected into the muscle with electroporation, allowing for muscle cells to produce and secrete the DMAb™ for a prolonged period of time without the need for repeated administration. In proof of concept studies in mice, we show that delivery of a DMAb™ plasmid for a monoclonal antibody targeting mouse CTLA4 (clone 9D9) elicits high serum expression (peak expression of 7.9µg/mL in immune-competent C57Bl/6 mice with one injection of DNA). This anti-mouse CTLA4 DMAb™ is capable of inducing tumor regression in A/J mice that were implanted with the immunogenic Sa1N tumor cell line without repeated administration. Furthermore, this anti-mouse CTLA4 DMAb™ is capable of synergizing with a DNA vaccine targeting the tumor antigen TERT in slowing tumor growth for the non-immunogenic TC-1 tumor cell line. We also examined DNA-delivery of the anti-human CTLA4 antibodies ipilimumab and tremelimumab. For these anti-human CTLA4 DMAbs™, we achieved steady-state levels of approximately
75µg/mL and 50µg/mL for ipilimumab and tremelimumab respectively in mice, which is greater than the mean trough levels of ipilimumab achieved in patients (21.8µg/mL serum concentration for 3mg/kg dose). These anti-human CTLA4 DMAbs™ produced in vivo bind to human CTLA4 protein and induce T cell activation in a functional assay ex vivo. These results demonstrate the feasibility of delivering immune checkpoint blockade monoclonal antibodies using DNA for cancer immune therapy. Future studies will explore combination therapies with CTLA4 DMAbs™ and chemotherapy.

[O7.5]

DNA-based cancer vaccines designed by SynCon® technology break tolerance in genetically diverse pre-clinical models
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Cancer vaccines targeting self-antigens have shown limited efficacy in the clinic due to self-tolerance mechanisms preventing or dampening an immune response against them. To overcome self-tolerance, we developed a novel, multi-phase DNA vaccine design strategy (SynCon® technology) that introduces subtle amino acid changes into native cancer antigens. These synthetic immunogens aim to generate cross-reactive T-cells and/or promote epitope spreading. Previously, we have shown in many cases that SynCon® DNA vaccines including the WT1 vaccine had better ability of breaking tolerance and induced robust cellular immune responses when compared to the native self-antigen based vaccines in C57BL/6 or BalB/C single haplotype inbred mice. Here, we went further to ask the question on whether the DNA immunogens designed by SynCon® technology could also break tolerance in genetically diverse pre-clinical models. To address this question, the WT1 vaccine and two additional SynCon® DNA vaccines targeting FAP and TERT were evaluated in two genetically diverse pre-clinical models (CD1 outbred mice and non-human primates). The outbred CD1 mice model was used since these mice are more relevant for inferring immunogenicity in humans due to their genetic diversity. The non-human primate model was also used because it is a highly relevant genetically diverse model for immunotherapeutic vaccine development. Briefly, CD-1 mice were immunized with either SynCon® FAP, a mouse native FAP vaccine, or vector control. Three immunizations were administered two weeks apart with electroporation following each immunization. For the NHP study, rhesus macaques were immunized with SynCon® TERT or WT1 four times, four weeks apart with electroporation following each immunization. Vaccine-induced immune responses were evaluated by IFN-γ ELISpot using the species-specific native peptides. Compared to native mouse FAP, SynCon® FAP was more immunogenic in CD-1 mice (average 407 ELISpot-forming units (SFUs) per 10^6 splenocytes for SynCon vs. 160 SFU/10^6 for native), suggesting that SynCon® FAP was capable of breaking tolerance. Importantly, 14/15 mice in the SynCon® FAP group generated an immune response above 100 SFU/10^6, compared to only 9/15 mice in the native FAP group. In rhesus macaques, both SynCon® TERT and WT1 induced robust cellular immune responses, indicating that the SynCon® TERT and WT1 immunogens was capable of breaking tolerance in NHPs. Taken together, these data demonstrated that SynCon® technology can be utilized to design DNA cancer immunogens that are capable of breaking tolerance in genetically diverse preclinical models. SynCon® DNA immunogens have the potential to break tolerance, induce immune responses and provide clinical benefit for patients with diverse HLA haplotypes. Further investigation of SynCon® immunogens in clinical trials is warranted.
Synthetic Immunogens Drive Potent Antigen Specific Anti-Tumor Immune Responses

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Current treatment modalities for cancers are often accompanied by deleterious side effects and often do not provide long-term control. Immunotherapies that drive potent immune responses are gaining momentum as a promising alternative. Inovio’s SynCon® DNA vaccine platform, delivered with in vivo electroporation, is emerging as a promising approach in the therapeutic vaccine field by demonstrating induction of robust antigen specific responses in the clinic. Recent data from a randomized, placebo controlled, double blind Phase II efficacy study in women with untreated HPV 16/18 associated high grade cervical intraepithelial neoplasia demonstrated that VGX-3100 treatment, a DNA vaccine targeting the E6 and E7 proteins of HPV types 16 and 18, led to the statistically significant regression of CIN 2/3 lesions and viral clearance relative to placebo treated patients. Here, we are looking to apply the same SynCon® DNA vaccine platform to drive potent antigen-specific responses against tumor-associated antigens and break self-tolerance in preclinical models. To this end, SynCon® vaccines were developed to target the self-antigens Wilms’ tumor 1 (WT1) and telomerase reverse transcriptase (TERT). WT-1 is a cancer/testes antigen, which was prioritized first among tumor antigens by the NCI, and is overexpressed by a wide range of solid tumors. TERT is overexpressed in more than 85% of human tumors with little to no expression detected in somatic cells. SynCon® WT1 and SynCon® TERT were highly immunogenic in mice and NHPs. Further, in monovalent and multivalent formulations, SynCon® WT1 and SynCon® TERT significantly decrease tumor burden and increase survival in the TC-1 tumor challenge model. The data also provides evidence for the ability of SynCon® WT1 and SynCon® TERT to break self-tolerance in NHPs. Collectively, these data support further study of this novel approach to immunotherapy of multiple solid tumor types.

Efficacy of consensus DNA-immunization against drug resistance in HIV infection evaluated in a murine tumor model

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An efficient therapeutic vaccine against drug-resistant (DR) HIV applied on the background of ART may help control viral load and prevent development of resistance. Advance of such vaccines is hampered by inability to test their protectivity in small animal models. This can be achieved by challenging animals with syngenic tumor cells stably expressing HIV antigens. Here, we generated murine breast adenocarcinoma cell lines expressing HIV enzymes and tested their applicability for challenge of HIV DNA-immunized mice. We generated HIV-1 FSU_A consensus genes for nonmutated reverse transcriptase/RT_A and RTs with DR-mutations to nucleoside and non-nucleoside RT-inhibitors (RT_An, RT_Ann); nonmutated integrase/IN_A and its variants with two patterns of resistance to raltegravir (IN_AR1; IN_AR2); three protease variants PR_A, PR_A2mut, PR_A3mut, with one, two or three DR-mutations to multiple PR-inhibitors. All had mutations (i) abrogating respective enzymatic activities. Genes for RT with K65R/M184V or K103N/G190S, D64V-inactivated integrase/IN with D64V/E138K/G140S/Q148K, and inactivated protease/PR with D25N/M46I/I54V/V82A were used to generate HIV-enzyme expressing lentiviruses (Evrogen). Lentiviruses were used to transduce 4T1uc2 cells (Perkin Elmer) at multiplicity of
infection 1 to 20. Heterogenic 4Tluc2-based cell lines were obtained with >98% cells expressing HIV-enzymes. On days 1, 28, groups of BALB/c mice (n=5) were intradermally DNA-immunized with: RT_Ain/I; RT_Ainn/II; IN_AiR1/III; IN_AiR2/IV; PR_Ai/V; PR_Ai2mut/VI; PR_Ai3mut/VII; and vector/VIII. Extra mice (n=3/group) were mock-immunized with PBS. Injections were followed by electroporation (BEX). On day 43, mice received two subcutaneous injections of 10^4 cells of lines 4T1Luc2RT_An-10.1 (group I), 4T1Luc2RT_Ainn-10.2 (II), 4T1luc2IN_Ai-1.2 (III), 4T1Luc2-IN_AiR2-1.5 (IV), 4T1luc2-PRi3mut-20.2 (V-VII), or 4T1luc2 (VIII). Tumor growth was monitored by morphologic measurements and bioluminescence imaging/BLI (Spectrum CT; Living Image 4.4 software). After 24 days, mice were sacrificed; tumors, lungs, spleens and livers were excised, placed into 24-well plate with RPMI containing 150 μg/ml luciferin, and monitored by BLI. Number of Luc-expressing cells in organs was calculated using in vitro calibration curves built using 4T1luc2-cells. Directly after, tissues were dehydrated, and paraffin-embedded for histological analysis. Spleens were homogenized, and in vitro immune response of murine splenocytes to HIV-derived peptides was evaluated by IFN-g/IL-2 Fluorospot (Mabtech). Control mice challenged with 4T1luc2-derived cell lines developed tumors sized >100 mm(2). In RT_Ain- and PR_Ai3mut-groups, 80% were protected from establishing tumors expressing respective HIV-enzymes. In IN_AiR1-immunized group, 40% had IN_Ai-expressing tumors albeit 15-times smaller than in mock-immunized mice, 60% were protected. In IN_AiR2-immunized group, 20% had >200 mm(2) tumors; 60%, 3-5-times smaller ones, 20% were protected. Mice DNA-immunized with RT_Ainn, PR_Ai and PR_Ai2mut were not protected. However, in IN- and RT_Ainn-recipients, tumors were smaller than in controls; metastases were infrequent. Protected/partially protected mice exhibited HIV-specific IFN-g/IL-2 response to in vitro stimulation of splenocytes with HIV-peptides. Mice with tumors demonstrated no such response, and low responses to mitogens. Tumorigenic cell lines expressing HIV-1 antigens proved to be effective for assessing protective potential of HIV DNA-vaccines. Immunogenicity of the latter depended on the pattern of DR-mutations. Protection was sequence-specific: DNA-immunogen based on RT with NRTI-resistance mutations and PR with M46I/I54V/V82A mutations induced potent T-cell response that protected mice from challenge with 4T1luc2-cells expressing these enzymes, but not their homologous variants. Our data demonstrate that immune system can be trained to recognize cells harboring DR-HIV antigens and prevent their establishment in the vaccine-recipients. Finding that a single amino acid mutation in the immunogen can abrogate its ability to prevent growth of antigen-expressing tumor cells is crucially important for development of cancer vaccines. Russian Funds for Science 15-15-30039 supported vaccine- and Basic Research 17-04-00583, tumor studies; Swedish Institute 19806/2016-PI and Horizon 2020 project #692293/VACTRAIN, collaborations.

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**[O8.3]**

**DNA and Protein Co-Delivery Vaccines Induce Potent Immune Responses Able to Delay SIV/SHIV Acquisition**

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**Background:** HIV/SIV DNA vaccination induces high and durable T cell and humoral immune responses, which efficiently disseminate into mucosal sites. We developed a method of simultaneous vaccination with DNA and protein resulting in great increase of humoral responses and improved protection against SIV infection in macaques. Further development of the method includes testing of different adjuvants and optimization of vaccine delivery routes.

**Methods:** Macaques were immunized with SIV gag and SIV or HIV env DNA by IM electroporation followed by administration of SIV/HIV gp120 Env protein formulated in TLR-4-agonist based
adjuvants. Vaccine-induced cellular and humoral immune responses were monitored, the animals were challenged via the mucosal route with low dose SIVsmE660 or SHIVCH505.

**Results:** SIV vaccination with TLR-4 agonist based liposomes (TLR4+7 or TLR4+QS21) induced similar robust levels of cellular and humoral responses in RM (12 animals/group), including bAb, Nab, scaffolded V1V2 and cyclic V2 responses to vaccine-matched SIVmac251 and heterologous SIVsmE660. SIV-specific cytotoxic CD4 and CD8 T cells were present at high levels 4 months after the last vaccination, indicating robust long-term memory. The TLR4+7 group showed a trend of increased Nab breath and stronger ADCC activity. Upon heterologous challenge, a trend of delayed viral acquisition was found which was statistically significant in the TLR4+7 group in RM with the TRIM5αααα-resistant genotype. Antibody responses to the heterologous SIVsmE660 (Nab, bAb, V1V2 ab) correlated with delayed virus acquisition. Vaccinees without the TRIM5αααα-resistant genotype showed reduced peak and chronic viremia. Reduction of viremia inversely correlated humoral responses targeting V1/V2 and with cellular responses.

We further examined the importance of delivering the DNA+Protein vaccine by co-immunization in the same muscle versus delivery in separate muscles (20 animals/group). The coimmunization group showed higher vaccine-induced ab and T cell responses. Interestingly, only the coimmunization group showed significant delay in SHIV acquisition. These data indicate that simultaneous recognition of the antigenic determinants present in the two vaccine components (DNA and protein) by the draining LN plays a critical role in the development of protective immunity. The underlying mechanisms are under investigation.

**Conclusions:** Combination of DNA and adjuvanted protein induces high, durable and potent cellular and humoral responses. Coimmunization delivering DNA +Protein in the same muscle is superior in inducing responses able to provide protection against challenge.

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**[O8.4]**

**Preclinical assessment of multivalent vaccine vectors against filoviruses**

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Ebolavirus and Marburg virus are members of the Filovirus family and cause haemorrhagic fevers with high mortality rates in humans. Sporadic outbreaks have been recorded in west and central Africa since 1976, with the largest documented Ebola epidemic occurring in 2014-2015 in Guinea, Liberia and Sierra Leone, claiming over 10,000 lives. Currently, no specific treatment exists besides supportive care, but the recent epidemic has greatly accelerated vaccine development. The two most advanced vaccine platforms that have shown promising immunogenicity against the Ebola Zaire strain (EBOZ) are viral vectors based on vesicular stomatitis virus (VSV) and adenovirus (Ad), both containing the EBOZ glycoprotein as the vaccine antigen. Both platforms induce a protective immune response in nonhuman primate models of infection, involving neutralising antibodies as well as CD8+ T cells. Multivalent vaccines that can protect against several filoviruses at once have clear advantages over monovalent alternatives, as it is difficult to predict which filovirus/strain will cause the next outbreak. In addition, there is considerable geographical overlap in the distribution of filovirus species. We have therefore generated chimpanzee adenoviral vectors which contain the glycoproteins of three filovirus family members: Ebola Zaire, Ebola Sudan and Marburg virus. We have assessed in vitro antigen expression as well as the cellular and humoral immunogenicity of these vectors in mice, and were able to rank the new multivalent vaccines with regard to immunogenicity profiles when compared to relevant control vaccinations (mixtures of monovalent vectors). We have also generated a multivalent poxviral vector (modified vaccinia Ankara, MVA) containing the same filovirus antigens, and show that the immune response elicited by the adenoviral vectors can be boosted with this MVA vector. Taken together, we have successfully developed preclinical vaccine candidates against filoviruses using a strong multivalent prime-boost platform.
Assessment of protective immunity elicited by chimeric hemagglutinin-based universal influenza virus vaccines against pandemic H1N1 infection in preclinical ferret studies

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Due to continuous antigenic drift and periodic antigenic shift of influenza viruses, current licensed seasonal influenza vaccines only confer strain-specific, but not sufficient heterologous and heterosubtypic protective immunity. In order to avoid reformulation and readministration of influenza virus vaccines annually, we developed a novel universal vaccine that induces broad immunity against influenza viruses of multiple subtypes. These novel universal influenza virus vaccines express chimeric hemagglutinins (cHAs), which are composed of a stalk domain derived from a circulating influenza virus strain in combination with an "exotic" head domain. Repeated exposure to such antigens redirects the humoral immune response towards the conserved stalk domain. By using sequential immunization strategies, we compared the protective immunity elicited by one or two doses of live-attenuated influenza vaccine (1x LAIV or 2x LAIV) and a LAIV prime followed by an inactivated influenza virus split-vaccine (IIV) boost (LAIV/IIV). The results from this preclinical study indicated that either 1x LAIV, 2x LAIV or LAIV/IIV regimen could prevent viral replication in the upper (nasal turbinate and olfactory bulb) and lower (lung and trachea) respiratory tract of ferrets infected with a 2009 H1N1 isolate. In particular, the 2x LAIV regimen provided superior immunity that significantly reduced viral shedding in the upper respiratory tract. Notably, the LAIV/IIV and 2x LAIV vaccination regimens induced higher influenza-specific IgA and cross-reactive antibody responses as compared to the other vaccination regimens. Therefore, these preclinical findings suggested that both two doses of LAIV and the combined live-attenuated/split vaccine (LAIV/IIV) immunization strategies confer protection against pandemic H1N1 influenza virus infection in ferrets, supporting the translation of this cHA-based vaccine approach to clinical trials.

A New Dengue Non-Human Primate Protection Model with Improved Translation to Vaccine Clinical Efficacy

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Recent data obtained with the live-attenuated tetravalent dengue CYD-TDV vaccine showed higher protective efficacy against DENV-4 as compared to DENV-2, while results from prior non-human primate experiments predicted comparably high protection against both serotypes. Viral loads observed in naturally-infected humans are generally much higher than those achievable in macaques by subcutaneous or intramuscular inoculation, which may contribute to overestimate vaccine efficacy. More stringent conditions of infection, consisting of intravenous inoculation of 10^7 CCID50 and resulting in about 100-fold increase of the viral load, were established in cynomolgus macaques and subsequently applied to re-assess efficacy of the CYD-TDV vaccine. Complete protection (i.e., undetectable viral RNA) against DENV-4 infection was achieved in 6/6 monkeys, while complete or near complete (transient detection) protection to DENV-2, was observed in only 6/18 animals. DENV-2 RNA was detected for at least 2 consecutive days in all other macaques (12/18), although at titers more than 1log below those of control animals. Viremia parameters were found inversely correlated to pre-challenge homotypic neutralizing antibody titers, emphasizing the key role of these antibodies in controlling DENV infection. Moreover, presence of antibodies to CYD-TDV antigens in all animals after the first immunization dose and post-challenge induction of strong anamnestic responses suggested efficient vaccine priming, which likely contributed to restrict DENV-2 RNAemia. Collectively, these data are in better agreement with CYD-TDV
clinical vaccine efficacy data reported against DENV-2 and DENV-4, and demonstrate better concordance between results in humans and this new dengue NHP protection model.

[O8.7]

Induction of Binding and Functional Antibody Responses to HIV-1 Envelope V2 Peptide with Army Liposome Formulation as the Adjuvant

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The results from the RV144 HIV-1 vaccine trial generated the hypothesis that non-neutralizing antibodies to HIV-1 envelope variable regions 1 and 2 (V1-V2) correlated with reduced risk of infection, with 60.5% efficacy at 12 months (post hoc analysis) that waned to 31.2% efficacy from virus acquisition at 3.5 years. Based on the fact that the V1V2 region exists in different conformations, contains sites of immune pressure, and has regions that bind to α4β7 integrin receptor, we wanted to focus the immune response to this region. To achieve this, we utilized three V2 peptide antigens based on the V2 loop sequence of gp120 92TH023 protein: a synthetic cyclic V2 peptide (CV2), a linear (LV2) and CV2 peptide with palmitic acid (palm-LV2, palm-CV2) at the N-terminus. The latter two peptides were anchored in the membrane of liposomes containing monophosphoryl lipid A [Army Liposome Formulation (ALF)], while the CV2 peptide was adsorbed to Alhydrogel® and then added to lyophilized ALF (ALFA) as the adjuvant. BALB/c mice and New Zealand white rabbits were immunized with palm-LV2-ALF or palm-CV2-ALF or CV2-peptide-ALFA formulations. In mice, both palm-CV2 and palm-LV2, induced a balanced Th1 and Th2 type of response with V2-specific antibody titers over 3 million in the palm-CV2 immunized mice. The anti-CV2 antibodies cross-reacted strongly with A244 gp70-V1V2 (titer 409,600) and gp120 (titer 153,600) proteins. In rabbits immunized with CV2-ALFA, high titers of antibodies were obtained not only against the linear and cyclic homologous V2 peptides (titers 346,667 and 133,333), but also against gp70-V1V2 (titer 533,333) and gp120 (titer 373,333) proteins. The antibodies also cross-reacted with consensus C and Case A2 gp70-V1V2 proteins. Distinctive secondary conformational differences based on CD analysis were observed upon association with liposomes. Palm-CV2 was entirely in a random coil while palm-LV2 was entirely in a β-sheet. The relevance of secondary peptide conformation of V2 as it relates to induction of antibodies with any particular function is unknown. The antibodies did not exhibit neutralization or ADCC activity, but they did demonstrate strong inhibition of binding of CV2 peptide to α4β7 integrin receptor. Recent studies in nonhuman primates with SIV have demonstrated the importance of blocking α4β7 in preventing SIV acquisition. Based on our data and the RV144 results, it may be useful to include V2 peptides along with HIV-1 protein antigen and ALF adjuvants in future HIV-1 vaccines. The induction of robust binding and functional antibodies to V2 might contribute at least partially to protective efficacy through inhibition of viral binding to α4β7 on CD4+T cells in the mucosa. Other regions of the envelope could also collectively contribute to protection through additional mechanisms such as ADCC and neutralization activities.

[O9.1]

Evaluation of ChAdOx1 MERS vaccine in camels in Saudi Arabia

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Middle East Respiratory Syndrome is caused by a novel betacoronavirus (MERS-CoV) that was first isolated in late 2012 in Saudi Arabia. The viral infections have been reported in more than 1900 humans,
ranging from asymptomatic or mild cases to severe pneumonia with a mortality rate of 40%. It is well
documented now that dromedary camels contract the infection and shed the virus without notable
symptoms and such animals had been infected by at least the early 1980s. However, to date, there is no
approved vaccine or antiviral drug to control or treat MERS-CoV. Last year, we reported on
immunogenicity of our MERS-CoV vaccine utilising a Chimpanzee Adenoviral vector by Oxford University
(ChAdOx1), encoding the spike (S) protein of MERS-CoV in mice. Here, we extended our work and report
the vaccine immunogenicity in dromedary camels. Nine juvenile naive camels were used in the study, six
were immunised with a single injection of ChAdOx1 MERS, two with PBS (placebo control), and one with
ChAdOx1 encoding GFP (vector control). Serum samples were collected pre-vaccination, 7, 14, 21, and
28 days post immunisation (d.p.i.). Health and behaviour of the camels were followed daily with no
notable observation. S1-specific antibodies were induced in three out of the six vaccinated camels.
Antibody titres will be determined in the pre and post-vaccination samples. We have also evaluated the
possibility of utilising naturally infected camels as a challenge model for vaccine efficacy in a MERS-
endemic area. Three MERS-CoV infected camels were mixed with five naive (unimmunised) camels in
one barn and were screened by Rapid test (what rapid test – is there a brand name?) and real-time qPCR
for 14 days post mixing. Naïve camels became positive for MERS-CoV starting from 2 days after co-
housing with infected camels and by the tenth day all five camels were positive. We have now started an
extended study in which 10 camels (5 naïve and 5 seropositive) were immunised with ChAdOx1 MERS
and 10 camels (5 naïve and 5 seropositive) were immunised with placebo (n=6) or vector control (n=4).
Each group of these 10 camels will be mixed with infected camels at 28 d.p.i to assess the vaccine
efficacy and the result is expected by end of Sep. 2017.

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**Novel Universal Nanoparticle Vaccines for Bird Flu and Coronavirus Infections in Birds**

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Avian influenza virus (AIV) and infectious bronchitis virus (IBV)-a coronavirus in birds caused serious
diseases in birds resulting in enormous economic losses to the poultry industry. Vaccination is the
cornerstone in controlling AI and IB. However, the development of effective vaccines for these two
diseases is challenging due to the highly mutable nature of these two RNA viruses. We used a so-called
self-assembling protein nanoparticles (SAPNs) to display the conserved influenza (M2e and Helix C) and
IBV (the second coiled-coil sequence of S2 protein and receptor binding domain) antigens in their native
oligomerization states. To make SAPNs adjuvanted, we have added the TLR5 agonist flagellin into the
SAPNs to generate self-adjuvanted SAPNs. Chickens vaccinated with the self-adjuvanted SAPNs induce
significantly higher levels of antibodies than those with unadjuvanted SAPNs and show higher cross-
neutralizing activity compared to a commercial inactivated virus vaccine. AIV SAPN provided protection
against challenge with a highly pathogenic avian influenza (AI) in chickens. The IBV vaccine prototypes
have demonstrated the ability to induce high levels of antibodies, significantly potentiate immune memory,
significantly reduced tracheal virus shedding, protected chickens against post challenge with a IBV M41
strain. These data presented in this work indicate that we have successfully designed and implemented
our self-adjuvanted SAPNs for use as vaccine candidates for AI and infectious bronchitis. It is suggested
that they could be used as a stand-alone – or possibly even better – as an additional component to an
established AI or IB vaccine to broaden the protection of the vaccine.
The application of NHEJ-CRISPR/Cas9 and Cre-Lox system in the generation of multivalent vaccines against avian influenza virus

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Recombinant vector vaccines are effective promising vaccines capable of immunizing against multiple pathogens. Traditional methods for constructing recombinant vaccines involve homologous recombination or bacterial artificial chromosomes but these methods can be time-consuming and labour-intensive. The clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system is a recently developed gene editing technology which has proven beneficial to gene modification and offers an alternative for constructing recombinant vaccines. There are mainly two methods used for gene insertion; error-prone non-homologous end joining (NHEJ) and the high-fidelity homology-directed repair (HDR) pathway. Owing to its high fidelity, most studies focus on using HDR for vaccine development but NHEJ offers some attractive advantages through its high efficiency. In our study, a universal donor system was established by introducing a bait sequence from copGFP targeted by an independent guide RNA. We utilized NHEJ dependent CRISPR/Cas9 to insert a donor influenza HA expression cassette tagged with GFP into a vector duck enteritis virus genome. A Cre-Lox system was later used to remove the GFP. In conclusion, we developed a NHEJ dependent CRISPR/Cas9 system which could be used as a powerful tool for rapidly generating recombinant vector vaccines with high efficiency. This universal donor system can be shared between different vector systems and will be beneficial in resource sharing and developing vaccines against a broad range of pathogens.

Immune Engineered H7N9 Influenza Hemagglutinin Overcomes Poor Vaccine Immunogenicity

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Avian H7N9 influenza poses a threat to human health because of its high case fatality rate consistently observed in annual epidemic waves since its emergence in 2013. The potential for virus adaptations that increase human-to-human transmissibility raises concern for an H7N9 influenza virus pandemic. H7N9 influenza hemagglutinin (HA) elicits weak neutralizing antibody responses in natural infection and vaccination. Based on immunoinformatic analysis and discovery of a Treg-inducing epitope in H7N9 HA, we hypothesized that a stronger CD4+ T cell response to H7-HA would support an improved hemagglutination inhibition (HI) antibody response.

To design an antigenically improved H7-HA (H7-HA-Opt1), we made three amino acid substitutions that delete a previously established Treg-inducing epitope and simultaneously introduces a highly conserved and broadly reactive effector T cell-inducing epitope found in H3-HA. Molecular modeling of H7-HA-Opt1 showed the changes likely stabilize the H7 conformation through a series of hydrogen bonds and ionic interactions with other conserved residues in H7. Recombinantly produced H7-HA-Opt1 demonstrated thermal stability, rosette diameter and size, activity, folding and potency comparable to native H7-HA, suggesting that the sequence modifications did not significantly perturb the native HA structure. In antigenicity studies polyclonal H7 HA-binding IgG antibodies in human sera recognize H7-HA-Opt1 with affinity equivalent to the wild type protein, also suggesting that the modifications did not induce significant structural perturbations.

In immunogenicity studies, a mimetic of the human immune system that uses donor PBMCs to seed peripheral and lymphoid tissue equivalents showed H7-Opt1-HA stimulated a 6-fold increase in activated, effector CD4+ T cells over wild type H7-HA. Immunizations of NOJ immune-deficient mice reconstituted with human PBMCs using non-adjuvanted H7-HA-Opt1, stimulated an average 5-fold greater anti-H7-HA IgG titer and 20-fold greater anti-H7-HA B cell frequency over mice immunized with wild type protein. In a
dose-ranging immunization study using H7N9 VLPs containing either Opt1 or wild-type H7-HA formulated with Alum adjuvant, HLA-DR3 mice that received the Opt1 VLP raised HI antibodies sooner and at lower doses than wild-type vaccine. These studies demonstrate that engineering whole antigens to remove Treg-inducing epitopes and carry memory CD4+ T cell epitopes – without perturbing native antigen structure – supports enhanced antibody development against the native antigen. The Sanofi Pasteur VaxDesign Corporation work was supported internally and in part by contract HHSO100201000035C from the Biomedical Advanced Research and Development Authority (BARDA).

OMV BASED VACCINE FORMULATIONS AGAINST SHIGA-TOXIN PRODUCING ESCHERICHIA COLI STRAINS ARE BOTH PROTECTIVE IN MICE AND IMMUNOGENIC IN CALVES

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INTRODUCTION: Hemolytic Uremic Syndrome (HUS) is a serious human illness principally caused by Shiga-toxin producing *Escherichia coli* (STEC) strains. Massive vaccination against STEC carriage in domestic cattle is considered to be the most effective sanitary-action. In this work we show that Outer membrane vesicles (OMV) based vaccines are not only protective on a murine model of Shiga toxin (Stx) challenge, but also immunogenic in cattle.

MATERIAL AND METHODS: OMV were prepared from STEC O157:H7 strains and designated OMV-O157. The OMV preparation was adsorbed on aluminum hydroxide for vaccine formulation. Protection from a Stx challenge by vaccination was assessed as follows. Mice immunized either with 10µg of an OMV-O157 formulation or with adjuvant (control group) were challenged with a highly concentrated Stx preparation. Signs of distress and mortality were recorded for each group during seven days after challenge.

For immunogenicity testing in calves, three groups of five 6-8 months-old animals were administered subcutaneously three doses, separated by three weeks intervals, of either a 100 µg OMV based formulation, a 50 µg OMV based formulation or adjuvant. Blood samples were collected from the jugular vein and anti-OMV-O157 IgG levels in serum were analyzed by indirect ELISA (OMV-O157 was the antigen in the solid phase). Results were expressed as the relative Optical Density at 450 nm (OD450nm) for each serum with respect to OD450nm from an in-house Positive Control.

Statistical analysis: A linear model was constructed to analyze the immunogenicity of OMV-O157 in calves, based on maximum-likelihood criteria. Response was the dependent variable, formulations (treatment) and days were stated as fixed factors. Error correlation was considered unstructured with heteroscedastic but constant variances for each treatment group. Interaction between days and treatment were evaluated and later contrasted between different treatment groups (p<0,05).

RESULTS: Neither calves nor mice showed any sign of adverse effects after vaccination. While none of the animals in the vaccinated group died during the intraperitoneal challenge assay in mice, there was a 90% lethality in the control group after 7 days of challenge. All OMV based formulations were highly immunogenic in calves, with 50 µg/dose leading to similar responses as 100 µg/dose. This response was significant from 5 days after the initial dose up to 57 days after the final dose, with maximal values 7 days after the third dose. In contrast control group response remained invariable.

CONCLUSIONS: OMV-based formulations are not only protective against Stx lethality in a murine model, but also highly immunogenic in calves. These results, while preliminary in nature, strongly suggest the potential of OMV-based vaccines against STEC carriage in cattle.
Impact of obesity on humoral and cellular immune responses to vaccination against tick borne encephalitis (TBE)

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Obesity has emerged to a worldwide health problem and is meanwhile recognized as a global epidemic by the WHO. Apart from increased co-morbidities, such as diabetes and cardiovascular diseases, obesity has direct effects on the immune system leading to immunosuppression along with increased susceptibility to infectious diseases. Consequently, obese individuals are an important target group for prophylaxis against vaccine preventable diseases, yet only few studies have been undertaken so far to evaluate vaccine efficacy in this risk population.

We therefore performed a single center open label phase IV clinical trial to study the impact of obesity on vaccination to a routine vaccine. Thirty-seven obese individuals (BMI 39) and 36 lean controls (BMI 22), matched for gender and age (mean age 45 y) were booster vaccinated against tick borne encephalitis (TBE, a diseases of high endemicity in Austria).

Obese vaccinees elicited lower neutralizing anti-TBE titers (NTs) than lean controls, though the NTs were in protective range in both groups. The total immunoglobulin levels were equal (IgG) or higher (IgM, IgA) in obese than in lean vaccinees, but natural IgM against pathogens (PC) were significantly reduced in obese, possibly indicative for an increased infection susceptibility. Obese adults showed significantly increased metabolic (leptin, insulin, triglycerides, cholesterol) and proinflammatory parameters and cytokines (CRP, IL-6, IL-1, IL-17) reflecting an overall low grade inflammation. However, TBE-specific cytokines (IL-2, IFN-g) measured in mononuclear cell cultures (PBMCs) did not differ between the groups. Local and systemic side reactions to the TBE vaccine were slightly higher in obese than in lean vaccinees.

Taken together, booster vaccination to TBE in obese individuals was effective at the cellular level along with lower antibody titers, though. The latter might have been also influenced by mechanical factors (e.g. needle length, reduced antigen absorption at injection side). The increased inflammatory state may impact on vaccine reactogenicity and possibly also on long term immunity. Thus, further studies not only on booster but also on primary vaccination with several routine vaccines are necessary to identify optimal vaccination schedules for this risk population.

Vaccine batch to vaccine batch comparison by consistency testing (VAC2VAC)

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VAC2VAC brings together a unique One Health consortium of human and animal health pharmaceutical companies, academia, translational research organisations, Official Medicines Control Laboratories and regulatory bodies with the overall objective to demonstrate proof of concept of the consistency approach for batch release testing of established vaccines. This means that animal-free assays - instead of animal tests - can be used to ensure that each vaccine batch produced is consistent with a batch already proven to be safe and efficacious in registration studies or in clinical use. Hence the name “consistency approach”. It covers vaccine potency, safety and animal welfare. The project aims to promote global understanding and acceptance of these new non-animal methods to facilitate international harmonisation and improved vaccine availability globally.
The three main steps to reach these objectives are:

1) **Development of new or optimisation of existing non-animal methods for consistency testing**
   This is the core activity of the project, with a focus on development and optimisation of physicochemical methods, immunochemical methods, cell-based assays, and multi-parametric assays & bioinformatics.

2) **Pre-validation of selected methods**
   For selected methods developed in VAC2VAC, small-scale multi-centre studies will be set up to assess the transferability and inter-laboratory reproducibility of the methods. Methods that are successful in these pre-validation studies and that are proposed for inclusion in regulatory monographs, will be submitted to the EDQM Biological Standardisation Programme to be considered for further validation studies.

3) **Regulatory acceptance of the consistency approach**
   To maximise the chances of regulatory acceptance and implementation of the consistency approach for batch release, the development of methods in VAC2VAC will involve close cooperation between public partners and industry partners in consultation with the regulatory bodies.

The presentation will outline the project in detail and discuss some early results.

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**[O9.8]**

**Prevention of viral vector aggregation and maintenance of antigenicity and infectivity by means of the amino acid-based SPS® formulation technology platform**

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The functional loss of viral vaccines during processing, shipment, and storage is a significant economic and safety issue. In particular, the shipment of vaccines to countries not providing a complete cold chain is a major challenge and can be addressed by improving the logistics and/or the pharmaceutical stability. Up to 80% of vaccination program costs are due to cold chain issues. Therefore, protecting vaccines from deterioration has been identified by the World Health Organization as one of the most important challenges. By means of our internal data base and Design of Experiment (DoE) approaches, we specifically tailored amino acid-based formulations (Stabilizing and Protecting Solutions; SPS®) that enable increased stability of vaccination relevant viral antigens, viral vectors and virus like particles (VLP). In this study, infectivity of lyophilized adenovirus 5 vectors was fully maintained by SPS® even during storage at 25 °C for 3 weeks and showed only a minor loss < 1 log of titer after 6 weeks. By contrast, infectivity was completely lost already immediately after freeze drying in the original supplier buffer. Electron microscopy and Dynamic Light Scattering (DLS) revealed that SPS® prevented vector aggregation and polydispersity during dry storage up to 4 weeks at a further increased temperature of 40 °C and during liquid storage up to 4 weeks at 37 °C which correlated with maintained infectivity in TCID₅₀ experiments. Interestingly, maximum viral vector stability was observed throughout simulated processing steps, e.g. during freeze-thaw experiments, when SPS® was applied immediately after harvesting of viral vectors from cell culture supernatants. Overall, similar results were obtained with SPS®-formulated virus like particles (VLP) and Modified Vaccinia Ankara (MVA) resulting in improved molecular integrity and/or antigenicity and vaccination efficacy *in vivo*. Taken together, our data showed increased shelf life and improved viral vector-based vaccination efficacy when formulated in SPS®. We therefore hypothesize that SPS® formulations result in higher viral antigen and viral vector yield as well as lower production costs during vaccine manufacturing.
ADVANCE governance framework for public-private collaborations: Towards strengthening vaccine Benefit-Risk monitoring in Europe

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Background: Vaccine Benefit-Risk (BR) monitoring in a collective endeavour between public and private stakeholders can benefit public health. ADVANCE (Accelerated Development of Vaccine beNefit-risk Collaboration in Europe*) is an on-going 5-year Innovative Medicines Initiative project of 47 partners** established to develop a framework for public-private collaborations (PPCs) that could rapidly provide robust data on vaccine BR to support decision-making in Europe.

Objective: One of the main ADVANCE objectives is to develop governance guidance for transparent, ethical and trustable PPCs, where needed, to initiate and implement vaccine BR monitoring, taking into account opportunities and challenges inherent to such collaborations in Europe.

Method: We evaluated existing governance models and guiding documents to identify governance structures applicable to the context of vaccine BR monitoring. Scenarios frequently met by the authors were used to discuss and describe the added value of PPCs and to define functions, roles and responsibilities of the different stakeholders and prerequisites for specific governance bodies. A workshop was organised at the European Medicines Agency with 70 senior experts to gain their experience and advice for the implementation of PPCs in real life settings.

Results: Governance framework and related materials are developed to support scientific experts and organisations who might implement PPC projects for vaccine BR monitoring in Europe. ADVANCE proposes a generic and flexible governance model with different options allowing its adaptation to specific contexts and projects. PPCs leveraging public and private sectors assets may be an added value discussed in the perspective of different research questions at the European level. Stakeholders’ concerns such as scientific independence and public trust are addressed through several recommendations.

Conclusion: Developing a governance framework for PPCs in Europe is a challenging task which could be overcome through a joint action involving key actors in the field of vaccine BR monitoring. It is hoped that the ADVANCE governance guidance will support and facilitate the implementation of PPCs in Europe and allow their sustainability for public health benefit.

* http://www.advance-vaccines.eu/

** European Centre for Disease Prevention and Control, European Medicines Agency, National Public Health Institutes, National Regulatory Authorities, Academic institutions, Clinical Research Organisations, Small and Medium Enterprises and Marketing Authorisation Holders
Both with native and denatured forms of Pd. The FlaB-Pd fusion vaccine preferentially formation in the SDS-PAGE gel, while the antisera induced by Pd only or Pd+FlaB reacted the antisera induced by Pd-FlaB bound only native form of Pd not the denatured confor-
tor 5 (TLR5) dependent, which was abrogated in TLR5-/- mice. Interestingly, we found that the antisera induced by Pd-FlaB bound only native form of Pd not the denatured conformation in the SDS-PAGE gel, while the antisera induced by Pd only or Pd+FlaB reacted both with native and denatured forms of Pd. The FlaB-Pd fusion vaccine preferentially stimulated Pd-specific conformational antibody production. These results suggest that Pd-FlaB fusion protein would serve as an effective anti-norovirus mucosal vaccine, providing protective immune responses in mucosal and systemic compartments neutralizing the native virus.

P1 Recombinant Norovirus P Domain Protein Fused with the Mucosal Adjuvant FlB Induces Protective Conformer Antibody Response
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Noroviruses (NoVs) are a major cause of childhood gastroenteritis and foodborne diseases worldwide. Lack of appropriate animal models or cell-based culture systems makes the development and evaluation of NoV-specific vaccines a daunting task. VP1 is the major capsid protein of the NoVs that acts as a binding motif to human histo-blood-group antigens (HBGAs) through its protruding 2 (P2) domain and can serve as a protective antigen candidate for vaccine development. In the present study, we show that recombinant NoV P domain (Pd) polypeptide formed small particles and induced a robust humoral immune response when administered through intranasal route. Moreover, the mixture of the Pd with the mucosal adjuvant FlB (Pd+FlB) significantly enhanced the antibody response that was further enhanced when Pd was fused with FlB (Pd-FlB) as a fusion protein vaccine. Pd-FlB, as well as Pd+FlB induced a mixed Th1/Th2 type of immune response with a significant induction both of IgG1 and IgG2a antibodies in serum, and also induced strong IgA responses in serum and feces. FlB-mediated antibody responses were toll like rece-

P2 IL-33 is a key regulator of innate and adaptive immunity triggered by particulate vaccine adjuvants
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In recent years polymeric micro- and nano-particles have been increasingly investigated as vaccine adjuvants with the potential to induce cellular immunity and consequently may find application in the development of vaccines against intracellular pathogens and cancer. Since particulate adjuvants are generally not pattern-associated molecular patterns (PAMPs) it is likely that danger-associated molecular patterns (DAMPs) play a key role in particulate driven innate and adaptive immunity. Frequently referred to as a DAMP or alarmin, the cytokine IL-33 was first identified in 2005, as a potent initiator of T helper type 2 polarization. Recent discoveries have extended the range of functions for IL-33 beyond type 2 conditions and its role as an alarmin at barrier sites, with emerging roles for IL-33 in Th1 responses and T cell regulation. Here the role of IL-33 as a regulator of nanopartic-
late adjuvant induced cellular immunity was addressed.

P3 TRANSVAC2 European Vaccine Research and Development Infrastructure
Odile Leroy, TRANSVAC2 Coordinator and the TRANSVAC2 consortium partners*

Strengthening the European vaccine landscape by bridging the translational gap in biomedical research is key to accelerate the development of effective vaccines that are urgently needed to address global health challenge. In order to promote innovation in vaccine development and facilitate access to the knowledge and expertise available in Europe, the collaborative project TRANSVAC2 has recently received funding under the European Commission’s Horizon 2020 Research Programme. In the long term, TRANSVAC2 aims to establish a fully operational and sustainable vaccine Research and Development (R&D) infrastructure in Europe by fostering cooperation between public vaccine R&D institutions of excellence, related initiatives and networks, and industrial partners. The project builds upon the success of its predecessor TRANS-

P4 Development of a novel synthetic consensus DNA vaccine that targets multiple MAGE-A family members for anti-
cancer immune therapy
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Cancer/test (CT) antigens have emerged as attractive targets for cancer immune therapy due to their over-expression in tumor tissues and lack of expression in normal tissues. In particular, several clinical studies have been initiated to target the MAGE-A family of CT antigens for melanoma. These have included recombinant protein immunization and TCR based gene therapy for MAGE-A3. The recombinant protein immunizations resulted in poor CD8+ T cell responses and lack of efficacy thus far in the clinic. TCR based gene therapy induced robust immune responses but unexpected toxicity. There is therefore a need to develop safe and effective therapies targeting the MAGE-A family of proteins for cancer therapy. In this study we performed a thorough analysis of MAGE-A RNA expres-
sion in The Cancer Genome Atlas (TCGA) and demonstrated that a high proportion of patients, in particular patients with melanoma and lung squamous cell carcinoma, exhibit expression of multiple MAGE-A family members simultaneously within the same tumor sample. Based on this information, we designed a consensus MAGE-A DNA vaccine that retains high homology (>65%) to multiple MAGE-A isoforms. Because mice and human MAGE-A family members are poorly conserved, we designed separate consensus constructs for testing in mice and in primates. Upon delivery of this mouse consensus MAGE-
A vaccine intramuscularly followed by electroporation (EP) in C57Bl/6 mice, we detected robust IFN-γ and TNF-α CD8+ T cell responses against multiple MAGE-A isoforms, including MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6 and MAGE-A8 (p<0.005). Furthermore, we detected robust cytotoxic CD8+ T cells in mice immunized with the consensus mouse MAGE-A vaccine. We evaluated the potency and cross-reactivity of this consensus mouse MAGE-A DNA vaccine in genetically diverse, outbred mice. We found that the majority of these mice (14/15) were capable of mounting a cross-reactive immune response and breaking tolerance to multiple MAGE-A isoforms simultaneously. We also demonstrated that this consensus mouse MAGE-A DNA vaccine is capable of allowing tumor growth in a therapeutic tumor challenge using the YUM17 melanoma tumor model and the TC-1 lung tumor model. These results suggest the use of optimized MAGE-A consensus vaccines for cancer immune therapy.

*Partners: Bioaster; Biomedical Primate Research Centre; Commissariat à l’Energie Atomique; European Advanced Translational Research Infrastructure in Medicine; European Clinical Research Infrastructure Network; European Vaccine Initiative; Fraunhofer Institute for Molecular Biology and Applied Ecology; Genibet; Helmholtz Centre for Infection Research; Institut de Recherche et d’Innovation Agroalimentaires; Institut National de la Recherche Agronomique; Institute for Translational Vaccinology; Instituto de Biología Experimental e Tecnológica; Integrated
**P5** Polyoxinidium, a polymer polyelectrolyte adjuvant, potentiated a robust antibody response to chlamydial antigens

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Chlamydia is one of the most successful bacterial pathogens causing sexually transmitted diseases. The development of a prophylactic chlamydial vaccine is still essential to combat the global spread of chlamydial infection. The effective chlamydial vaccine is desired to be formulated in an adjuvant strongly inducing both Th1- and Th2-immune responses to chlamydial antigen(s) with broad cross-serovar coverage. Polyoxinidium (PO) is known as a safe immunopotentiator inducing the enhanced immunity in animals and humans when used with a number of antigens of bacterial and viral pathogens. The PO contains high-polymeric 100 kDa units based on both N-oxide-1,4-ethylenepiperazine and (N-carboxyethyl)-1,4 ethylenepiperazine bromide. This adjuvant has been approved in Russia for a several human licensed viral vaccines. Recently, it was shown that the PO could weaken undesirable harmful immune reaction during vaccination of adults, children and elderly. It is generally recognized that the mechanism of immunomodulation provided by PO is correlated with its strong Th1-inducing adjuvant activity. Nevertheless, little is known whether the PO is capable to markedly elevate the level of antibody response to immunizing antigen potentiating the robust Th2-inducing activity.

The goal of this study was to evaluate whether PO is able to elicit the humoral immune response to chlamydial protein antigens with high protective potency that are promising for the development of novel subunit vaccine. Two antigens were used as model target antigens, such as: (i) the major outer membrane protein (MOMP) which was routinely isolated from the yolk sacs of chick embryos infected with chlamydia; (ii) the 6 kDa His-tagged recombinant protein CdsF, a component of *C. trachomatis* highly conserved type 3 secretion system purified under denaturing conditions. Each antigen was mixed with the PO in 1:1 ratio (v/v) and used for immunization of BALB/c mice. We observed about 15 to 20-fold increase in the level of homologous murine antibody starting on day 12 post-immunization after a single injection in comparison with control groups of mice immunized with either PO or antigen alone. A consequent boosting with each antigen emulsified in the PO elicited high antibody titers at the level up to 1:128,000 (P<0.05). No vaccine-related side effects were registered.

These findings indicate that the PO could be utilized as harmlessness and efficient adjuvant suitable for further testing of chlamydial subunit vaccine candidates.

**P6** Protection against *P. multocida* conferred by an intranasal fowl cholera vaccine in Khaki Campbell ducks

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Fowl Cholera effects the poultry farming includes chickens, turkeys and ducks very severely resulted in large economic losses. The most common cause of fowl cholera is *Pasteurella multocida (P. multocida)*. The duration of infection may range from peracute/acute to chronic infection. The commercial fowl cholera vaccines using parenteral administration are available for protection. Conversely, the pain suffering from injection and restraint can cause of stress, decrease appetite and decrease the egg production in layer chickens and layer ducks. The intranasal vaccination is better alternate method that resembles the natural route of infection. In this study, the recombinant outer membrane protein H (rOmpH) of *P. multocida* strain X-73 was chosen to formulate as the intranasal rOmpH-based fowl cholera vaccine and evaluated the protective efficacy in Khaki Campbell ducks. The key pathway is inhibition the adhesion between the bacterial and host cell. An adhesion inhibition assay was used to compared polyclonal antibody production against the avian *P. multocida* adhesion to duck embryo fibroblast (DEF) cells among parenteral rOmpH-based fowl cholera vaccine immunized group, fowl cholera bacterin vaccine immunized group and the non-immunized groups. The results showed the bacterial cells lost their adhesion ability after treatment with rOmpH-based fowl cholera vaccine. An intranasal fowl cholera vaccine was formulated containing 100 µg rOmpH and 3 µg E. coli enterotoxin B (LTB) as an adjuvant. Ducks were intranasally immunized three times at three-week intervals. Challenge exposure was conducted by inoculation at 3.5 × 10^3 CFU/vial of *P. multocida* strain X-73 at four weeks after the last immunization. The sera IgY and secretory IgA antibody titers were significantly increased (P < 0.05) post immunization. The stimulation index (SI) values of the vaccinated groups were significantly different from the SI values of the non-vaccinated groups (P < 0.05). Protection levels conferred by immunization with an intranasal or bacterin vaccine in ducks against challenge-exposure were 90% and 80%, respectively. There were no significant differences in protection among intranasal and bacterin vaccines. We conclude that the intranasal fowl cholera vaccine protected Khaki Campbell ducks from artificial *P. multocida* infection.

**P7** Structural insights reveal folding similarity of non cross-reactive meningococcal vaccine antigens NadA

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Serogroup B Neisseria meningitidis (MenB) is a major cause of severe sepsis and invasive meningococcal disease, which is associated with 5–15% mortality, and devastating long term sequelae. It can be found on the exposed epithelia of the human upper respiratory tract. Colonization of the epithelial cells is dependent on a two-step mechanism: following the initial pili-dependent attachment bacteria undergo to an intimate adhesion via a specific repertoire of adhesines. Neisserial adhesion A (NadA), a meningococcal trimeric autotransporter adhesin (TAA) that acts in adhesion to, and invasion of, host epithelial cells, is one of three protein antigens in Bexzero: the first genome-derived vaccine against MenB. NadA is present in ~30% of pathogenic isolates and the protein induces high levels of bactericidal antibodies in humans. NadA can be classified in two main groups of genetically-distinct variants. We sought to determine the 3D structure of the main variant of NadA the V3 present in the Bexzero: vaccine by both X-ray crystallography and cryo-electron microscopy, thus providing new insights into the understanding of its biological and immunological functions.

Bexzero is a trademark owned by or licensed to the GSK group of companies.

**P8** Evaluation of lysis methods for adenovirus release from HEK 293 cells

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Non-lytic virus lysis requires methods for the release of virus from the host cell. For lysis of cells, it is common to use detergents for non-enveloped virus and mechanical methods for enveloped virus. The lysis step is important since it will affect the overall yield in a viral vector purification process. The lysis method needs to be selected and optimized to maximize efficiency of virus release with minimal adverse effect on virus stability and no interference with purification. Traditionally, Triton X-100 is used for cell lysis, but is now on the REACH (Registration, Evaluation, Authorization and restriction of CHemicals) list and can only be used in limited amount for research purposes. To find an alternative to Triton X-100, human embryonic kidney (HEK) 293 cells grown in suspension, were infected with recombinant adenovirus, harvested and treated with different concentrations of alternative detergents for different lengths of time or subjected to mechanical lysis by hollow fiber recirculation. Released levels of virus, host cell genomic DNA and total protein were compared with lysis by freeze-thawing and Triton X-100 treatment. Seven candidate detergents were tested in small scale and finally the selected detergent was tested in larger scale. Several detergents showed good lysis performance, however based on the overall results Tween 20 was selected for the lysis step in our adenovirus purification process as replacement for Triton X-100.
**P9** Mycobacterium tuberculosis H37Rv cell wall isolated poly L-glutamates as novel Th1-biased adjuvant

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**Objective:** The immunomodulatory properties of Mtb cell wall (CW) components are well-known and classically highlighted by their use in Freund's adjuvant. Here we aim to explore the adjuvant potential of poly-α-L-glutamates (PLG), a lesser-known component of Mtb CW, that are present in abundance, only in pathogenic mycobacteria.

**Methods:** Immunomodulatory properties of PLG were evaluated using Mtub ESAT-6 protein as a model antigen. Primary parameters were T-cell specific immunity- humoral response, Tu cell development, recall memory etc., were monitored using ELISAs and flow cytometry. The potency of PLG as adjuvant is compared to a known Th1-immunity inducing adjuvant, dimethyl-dioctadecyl ammonium bromide-monophosphoryl lipid A (DDA-MPLA). Effect of PLG in modulating protective efficacy of ESAT-6 in the mice model is examined after challenge with Mtub, by recording clearance/reduction of bacillary load in the lungs and spleen, and long-term survival of the host.

**Results:** PLG adjuvantation triggered a strong humoral response against ESAT-6 antigen and resulted in significantly elevated levels of total IgG and its isotypes (IgG1, IgG2a and IgG2b). The splenocytes from PLG vaccinated mice upon antigenic stimulation, displayed robust increase in Th1 specific IFN-γ, TNF-α, IL-2 and Th2 specific IL-6 and IL-10 cytokines. Additionally, PLG also activated Th17 response, leading to secretion of significantly high levels of IL-17 cytokine by the splenocytes. The PLG adjuvanted mice recorded 97.4 % and 94.3 % reduction in bacterial counts in the lungs and spleens respectively, six weeks after Mtub challenge. The magnitude of reduction is statistically different from BCG vaccinated mice. PLG as adjuvant appears better than DDA-MPLA in enhancing protective efficacy of ESAT-6 (P < 0.05).

**Conclusions:** The strong Th1 and Th17 immune response generated by PLG makes it a promising adjuvant candidate for developing effective vaccines against Th1 response dependent diseases like Tuberculosis, Typhoid, Brucellosis, etc.

**P10** Preliminary immunogenicity assessments of phase 2a clinical trial evaluating a Hantaan virus/Puumala virus HFRS DNA vaccine delivered by intramuscular electroporation

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During the past several years we have conducted research aimed at developing hantaan virus vaccines to prevent hemorrhagic fever with renal syndrome (HFRS). Our lead candidates are DNA vaccines expressing the Gn and Gc genes of Hantaan virus (HTNV) and Puumala virus (PUUV). Previously we reported that these vaccines, alone and in combination, were safe and immunogenic when delivered using particle mediated epidermal delivery, or Chiron Medical Systems intramuscular electroporation (IM-EP) TriGrid Delivery System. Here, we further evaluated the combination vaccine delivered by IM-EP in a Phase 2a study. 120 subjects were randomized into four cohorts. Cohort 1 and 2 received 2 mg of DNA per vaccination and cohorts 3 and 4 received 1 mg. Cohorts 1 and 3 were vaccinated on Days 0-28-56-168. Cohorts 2 and 4 were vaccinated on Days 0-56-168. Sera were collected at multiple timepoints and evaluated for neutralizing antibodies using pseudovirus neutralization assays (PsVNA). Day 84 specimens were also evaluated in plaque reduction neutralization test (PRNTs). All four cohorts demonstrated >80% neutralization. Amongst these, PA based vaccines are most effective for providing immunity against BA, but their low shelf life limits their usage. Previous studies revealed that PA domain IV includes B-cell epitopes designated as ID I, ID II, and ID III; with ID II and ID III possessing more toxin neutralization activity and eliciting high antibody titer than ID I. Moreover, N-terminal region of both LF and EF possessess binding site of PA which are homologous to each other. Here, in this study we have developed and evaluated the protective efficacy and stability of epitope based chimeric vaccine (ID-LFn) comprising ID II ID III region of both LF and EF to find an alternate of unstable PA based vaccines.

**Materials and Methods:** Binding affinities of ID-LFn with anti-Pa/LF/EF antibodies and vice-versa were determined by ELISA and immunoblotting. The stability of chimeric vaccine was assessed using Circular Dichroism Spectroscopy. The vaccine potential of ID-LFn was evaluated by toxin neutralization, lymphocyte proliferation, and cytokine analysis. The protective efficacy was analyzed by challenged immunized mice with virulent strain of BA.

**Results:** Day 56 GMTs were found to be significantly stable as compared to PA. Anti-ID-LFn antibodies recognized PA, LF as well as EF. Though, the total antibody titer, toxin neutralization efficiency was found to be lesser than PA but surprisingly, the T-cell response and hence the protective efficacy of ID-LFn was found almost similar to PA.

**Conclusion:** The ID-LFn vaccine exhibits equal protective efficacy and more stability as compared to PA with the capability of neutralizing PA, LF and EF at the same time. Thus, this can be a reliable vaccine against anthrax with better shelf life.

**P11** Interim results from the HVTN 098 study of PENNVAX-GP® delivered by intradermal or intramuscular electroporation in healthy HIV uninfected adults


Remarkable advances in treatment and prophylaxis of HIV infection have been made in the last two decades. However, the tremendous scientific challenges involved in developing an efficacious HIV vaccine have highlighted by several unsuccessful efficacy trials such as VaxGen, HVTN 505 and the STEP study. Several advances have improved the immunogenicity of DNA vaccines, a safe and flexible platform for vaccination. In the phase 1 HVTN 080 study, a DNA vaccine and adjuvant (plasmasm-12) delivered intramuscularly (IM) in a three-dose regimen with the novel CELLECTRA® electroporation device was safe and well tolerated while eliciting high levels of cellular immune responses to the vaccine immunogens, Gag and Pol but not Env. After extensive optimization of the DNA plasmids and formulation, along with a novel electroporation procedure by intradermal (ID) delivery, a new phase I study, HVTN098 was undertaken. HVTN 098 [sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and conducted by the HIV Vaccine Trials Network (HVTN)] was designed to evaluate the immunogenicity of a four-dose regimen (day 0, months 1, 3, and 6) of PENNVAX-GP® DNA vaccine delivered via ID or IM electroporation, with or without DNA adjuvant, pIL-12. PENNVAX-GP® consists of four highly optimized DNA vaccines encoding clade A and C consensus Envelope (Env) plus multi-clade consensus Gag and Pol. The objectives of HVTN 098 were (1) to determine if the vaccine regimens were safe and tolerable, (2) if lower vaccine doses (1.6 mg) delivered by ID route could elicit immune responses comparable to 8 mg delivered by IM route, and (3) if pIL-12 will improve cellular and/or humoral responses.

The trial included 94 HIV-uninfected participants 18 to 50 years of age. ID/IM electroporation were generally safe and well tolerated. Overall rates of immune responses were 2 weeks post 4th vaccination based on interim data from 76 evaluable participants are as follows: 71 of 76 (93%) participants had cellular responses to at least one of the vaccine antigens (Env A, Env C, Gag, or Pol); 62 of 66 (94%) participants demonstrated binding antibody responses to Env and 43 of 57 (75%) developed neutralizing antibodies to a tier 1 HIV virus (MW9865.26). None of the placebo recipients (0 of 9; 0%) demonstrated either cellular or antibody responses. In the PENNVAX-GP® vaccine/pIL-12/IM route group, 27 of 29 (93%) participants demonstrated a CD4+ response to any of the vaccine antigens, 27 of 28 (96%) demonstrated Env specific binding antibody responses and 21 of 28 (75%) developed neutralizing antibodies to tier 1 HIV. In the PENNVAX-GP® pIL-12/IM route group, 25 of 27 (96%) demonstrated CD4+ responses to any of the vaccine antigens, 19 of 21 (90%) demonstrated an Env specific antibody responses and 13 of 26 (50%) developed neutralizing antibodies to tier 1 HIV. Of note, ID electroporation when compared to IM electroporation was dose sparing in that only a 1/5th of the IM dose was needed to elicit equivalent cellular immune responses. Additional immunological assays are underway to follow up on these promising results seen from the interim analysis.

**P12** Development of a novel anthrax vaccine comprising LF-PA chimera: A demanding alternate to unstable PA based vaccine

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**Introduction:** Bacillus anthracis (BA), the etiological agent of anthrax, secretes protective antigen (PA), lethal factor (LF), and edema factor (EF) as major virulence mediators. Among these, PA based vaccines are most effective for providing immunity against BA, but their low shelf life limits their usage. Previous studies revealed that PA domain IV includes B-cell epitopes designated as ID I, ID II, and ID III; with ID II and ID III possessing more toxin neutralization activity and eliciting high antibody titer than ID I. Moreover, N-terminal region of both LF and EF possesses binding site of PA which are homologous to each other. Here, in this study we have developed and evaluated the protective efficacy and stability of epitope based chimeric vaccine (ID-LFn) comprising ID II ID III region of both LF and EF to find an alternate of unstable PA based vaccines.

**Materials and Methods:** Binding affinities of ID-LFn with anti-Pa/LF/EF antibodies and vice-versa were determined by ELISA and immunoblotting. The stability of chimeric vaccine was assessed using Circular Dichroism Spectroscopy. The vaccine potential of ID-LFn was evaluated by toxin neutralization, lymphocyte proliferation, and cytokine analysis. The protective efficacy was analyzed by challenged immunized mice with virulent strain of BA.

**Results:** Day 56 GMTs were found to be significantly stable as compared to PA. Anti-ID-LFn antibodies recognized PA, LF as well as EF. Though, the total antibody titer, toxin neutralization efficiency was found to be lesser than PA but surprisingly, the T-cell response and hence the protective efficacy of ID-LFn was found almost similar to PA.

**Conclusion:** The ID-LFn vaccine exhibits equal protective efficacy and more stability as compared to PA with the capability of neutralizing PA, LF and EF at the same time. Thus, this can be a reliable vaccine against anthrax with better shelf life.
**P13** Production of recombinant adenovirus in HEK 293 cells using a single-use bioreactor system
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Adenovirus (AdV) based vectors have been widely evaluated as vaccine delivery system in preclinical and clinical studies for various infectious diseases. AdV have also been extensively explored as a viral vector for gene therapy and as an oncolytic virus. The most studied adenovirus vector is the first generation of recombinant AdV serotype 5, making it a suitable system for process development of adenoviral vectors. Manufacturing of safe and efficacious clinical-grade virus relies on a scalable and cost-effective production process. Early-stage studies are often performed using anchorage-dependent cell cultures in roller bottles or cell factories. However, scale-up using these techniques is complicated and limited by the surface area available for the cells to grow on. One attractive alternative for easier scale up using adherent cells is the use of microcarriers. Another solution is to use suspension-adapted cells, which facilitate scale-up but could at the same time lead to lower virus productivity per cell compared to adherent cells. We have established an efficient and scalable process for AdV production using suspension HEK 293 cells cultured in serum-free cell culture medium in a single use stirred-tank bioreactor. Human Ad/5 expressing the green fluorescent protein (GFP) was used for process development. HEK 293 cells adapted to different cell culture media were evaluated for cell growth and virus productivity and a chemically defined serum-free medium was selected for further process development at bioreactor scale. HEK 293 cells were inoculated at a cell density of 0.3 × 10^6 cells/mL. The bioreactor volume was adjusted to 10L and when a cell density of 1 × 10^6 cells/mL was reached, the cells were infected with multiplicity of infection 10. Cell lysis was performed at 42 h post-infection using Tween 20 as an alternative to traditionally used Triton X-100 that is now on the REACH list. Infectious virus titer was determined by automated fluorescent microscopy and by standard TCID50 protocol. Our process generated AdV titers at 10^9 infectious virus/mL in batch mode and demonstrate an efficient and robust process for adenovirus production in a single-use stirred-tank bioreactor. Furthermore, reagents, tools and techniques used in this study can be applied to other suspension cell lines and viral vectors that are currently being evaluated for clinical use.

**P14** Construction and Immunogenicity Analysis of Two Formulas of Nano-immunogenic Heat-Stable Enterotoxin (STa) of Enterotoxigenic Escherichia coli
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The novelities encountered in the field of formulation have provided promising solutions for the problematic vaccine development of haften molecules. In the current study, the novel preparation of a cationic nanoliposomal immunogen of the heat stable enterotoxin (STa) was reported. STa was produced from clinically ETEC isolate of diarrheic neonatal calves and purified using RP-HPLC. STa was loaded into the cationic vesicles which were characterized for their particle size, surface charge, morphology, STa loading, and stability. Results displayed the spherical nature of the STa loaded vesicles, their suitable size and homogeneity represented by a particle size of 228.1 nm and a PDI of 0.202. The surface charge of the STa nanoliposomes was +29.9, demonstrating sufficient stability during refrigeration storage. Additionally, we utilized nanotechnology on the traditional protocol of haften-carrier conjugation and test its efficacy for construction of immunogenic STa. Optimized homogenous PLGA nanoparticles (NP) prepared using the nanoprecipitation technique, were used for conjugating STa using the carbodiimide synthesis. Covalent binding of STa to PLGA NP was confirmed via FT-IR and HNMR analysis. Safety and tolerability of the developed nanoparticulated STa-PLGA conjugate were confirmed by MTT assay on A549 cancer cells. Both formulas, the STa loaded cationic nanoliposome and STa-PLGA NP conjugate, were used for mice immunization and the generation of STa antibody was monitored using ELISA. Both formulas were able to elicit specific STa antibody response and to confer effective protection against STa challenge in mice. The developed system of The STa loaded cationic nanoliposome is a one-step procedure, which overcomes the disadvantage of the complexity of generation of the haften-carrier conjugate. In conclusion, the developed STa-cationic nanoliposomal immunogen is feasible and has the potential to improve effectiveness against ETEC, suggesting its applicability in preventing the harmful effects of ETEC infection in neonatal calves.

**P15** Efficacy of a Mycobacterium bovis Heat-Inactivated Vaccine against Tuberculosis in Experimentally Challenged Goats
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**Introduction**: Interest in tuberculosis (TB) vaccines for humans and animals is renewed, and small ruminants are suitable models for TB vaccine efficacy assessment. Goats are natural hosts for Mycobacterium bovis and M. caprae, and vaccination may be the best long-term prospect to reduce the prevalence of TB in endemic areas. New vaccines that replace BCG need to improve its efficacy, biosafety or stability, thus a promising strategy is to develop inactivated vaccines. The aim of this study was to evaluate the efficacy a novel heat-inactivated M. bovis (HIMB) vaccine.

**Materials and Methods**: Eight goat kids were parenterally vaccinated with HIMB (10^7 CFU, oil-adjuvanted), 8 were parenterally vaccinated with M. bovis BCG (10^5 CFU) and 8 remained unvaccinated. After 7 weeks, all kids were anesthetized and subsequently challenged with M. caprae (approx. 10^6 CFU) by the endobronchial route. After 9 weeks, all animals were euthanized. Trachea and lungs were carefully removed at necropsy, and respiratory lymph nodes were removed for quantification of TB lesions and bacterial burden. Lungs were fully infiltrated by intratracheal infusion of 10% formalin and were imersed in 10% formalin for 6 weeks. CT scan was then performed to quantify the volume of pulmonary TB lesions. Extrapulmonary TB lesions were fixed in 10% formalin and confirmed by histopathology.

**Results**: HIMB vaccinated group (N=7, one animal was not recovered after anesthesia) showed a significant reduction in the volume of visible lesions in lymph nodes (29 mm³; 95% CI: 22-36; p<0.001), compared to unvaccinated animals (77 mm³; 95% CI: 60-94; P<0.001), being at the same level than BCG vaccinated animals (25 mm³; 95% CI: 14-36). Both vaccinated groups also showed a reduction of bacterial load (HIMB: 4.1 log_{10} CFU, 3.7-4.5 95% CI; BCG: 4.1 log_{10} CFU, 3.6-4.6 95% CI) compared to control animals (4.6 log_{10} CFU, 4.1-5.1, 95% CI), yet not statistically significant. CT analysis of lungs is still ongoing. Only one animal of each vaccinated group and 5 unvaccinated animals showed extrapulmonary TB lesions.

**Conclusion**: Preliminary results indicate that parenteral vaccination of goats with HIMB confers a degree of protection against TB at a similar level than BCG. In view of our results, larger-scale trials in small ruminants should be conducted to further assess the efficacy of parenteral vaccination with HIMB.

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**P16** Towards the conjugation scaling-up of Haemophilus influenzae type b capsular polysaccharide to tetanus toxoid
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Conjugate vaccines have been widely used and its importance is unquestionable. Haemophilus influenzae type b (Hib) is a Gram-negative bacterium that can cause pneumonia and meningitis. The capsular polysaccharide of the Hib is composed by a polymer of ribosylribitol phosphate (PRP) and is the main virulence factor. Therefore, PRP is component of conjugate vaccines. Hib conjugate vaccine is efficient but the high cost of production could avoid its use in low-income countries. Our laboratory has been studying the conjugation of PRP to tetanus toxoid (TT) using DMT-MM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) as activating molecule. The goal of this work is to establish the conjugation process suitable for scaling up.

Three steps composed the conjugation process: 1) PRP (2.2g at 10g/L) was oxidized by NaIO4, quenched with glycerol and purified by tangential ultrafiltration (TUF 5kDa) resulting PRP-Oxi (recovery 93%). 2) PRP-Oxi (2.0g at 9g/L) was mixed with adipic acid dihydrazide (ADH) followed by addition of NaBH4-CN. After 24h NaBH4 was added and the reaction mixture was submitted to TUF (5kDa) resulting PRP-AH (81%). 3) PRP-AH (1.0g at 15g/L) was reacted with TT (1.0g at 15g/L) in the presence of DMT-MM (0.1M). The conjugate product (PRP-TT) was purified by TUF (100kDa) followed by size exclusion chromatography (Sephacryl S-400 XK 50/100, GE Healthcare). The yield of PRP-TT purified conjugate was 35%. The TUF, which is a simple and easy process for scale-up steps, allowed the elimination of no reacted reagents. BALB/c mice were immunized with PRP-TT purified conjugate and produced higher titer of anti-PRP antibodies than the controls groups. The methodology employed in this work seems to be useful and feasible for scaling up.

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There are four serotypes of dengue virus (DENV) which were isolated to the tropics and sub-tropics, but with climate change and increased globalization, are beginning to spread. Since four different types of DENV can be found in the same geographic area, and seeing the serotypes present change over time has introduced a unique challenge for vaccine development. Infection with one serotype of DENV can cause severe flu-like symptoms. However, infection with a second serotype may cause the much more severe dengue hemorrhagic fever or dengue shock syndrome due to the presence of weakly binding non-neutralizing antibodies that act as immune camouflage, allowing increased viral uptake and a much higher viral load to be released into the body. Since incomplete protection can lead to severe dengue, a vaccine must be engineered to raise only a neutralizing antibody response without generating any weakly binding antibodies. The fusion loop (FL), part of Domain II of the viral envelope protein, undergoes a conformational change that fuses the viral membrane with the endosomal membrane of infected cells. This loop is highly conserved across DENV serotypes and it has been shown previously that neutralizing antibodies bind in this area. To avoid non-neutralizing and infection enhancing antibodies, we propose to raise an immune response that generates only neutralizing antibodies. The fusion loop peptide is not stable or immunogenic enough to act as a vaccine antigen on its own, so it must be added to a scaffold, such as the major capsid protein (L1) to human papilloma virus (HPV). Initial expression levels of this chimeric VLP were lower than expected compared to wild type L1. Making such a large insertion may have disrupted the folding of the monomeric L1 or the assembly of the particle. To ameliorate this, we included a “locking” disulfide motif to subsequent designs. Expression of the chimeric proteins increased substantially as seen by Western blotting using an anti-L1 antibody, both in cell lysate and at later stages of maturation into VLPs. Expression can be further improved by the addition of a ratio of wild type L1 to chimeric L1 during transfection. We have shown binding of an anti-DENV mAb to the cyste-bound FL-L1 in ELISA as well, indicating the FL motif is natively folded as part of the chimeric VLP.

Higher yields are necessary for future experiments such as electron microscopy and antibody generation, so we are moving the expression of the chimeric proteins from HEK 293TT cells to S. cerevisiae. Alternative insertion sites in the L1 monomer are being explored as assembly of the VLPs may be hindered by the large inserted peptide near the five-fold axis of symmetry of pentamers. The alternative sites were chosen based on bioinformatic analysis of many L1 serotypes and looking for areas of natural insertions and deletions. Our results show that cysteine “locking” and co-expression of chimeric and wild-type L1 improves efficiency in making chimeric HPV VLPs as carriers of anti-genic peptides.
P21 To have or not to have: What motivates healthcare workers in having the seasonal influenza vaccine?  
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Background: Healthcare workers (HCWs) can play an important role in the transmission of influenza during nosocomial outbreaks, which causes increased morbidity and mortality in patients and residents of nursing homes. Therefore, the Belgian Super ordinance set a target of 80% influenza vaccination coverage in HCWs by 2020. Because the target coverage rate has not yet been reached, we performed a study to gain insight in the motivation of HCWs for influenza vaccination.

Methods: Potential influencing socio-demographic and professional factors, and attitudes towards influenza and influenza vaccination were surveyed in 5141 HCWs in 13 hospitals and 14 nursing homes. Additionally, influenza campaign coordinators of the participating hospitals and nursing homes were interviewed to gain insight in the key factors of success/failure of influenza vaccination campaigns.

Results: The self-reported mean vaccination coverage in the 13 hospitals and 14 nursing homes during the last influenza season was 40.4% and 43.5% respectively. Overall, up to 90% of HCWs find it important not to infect their patients. However, only 20% of non-vaccinated HCWs consider influenza vaccination a duty not to harm the patients they care for. Further, HCWs generally underestimate the likelihood of getting influenza themselves or infecting their patients. Moreover, misconceptions about influenza and its vaccine are still circulating among unvaccinated HCWs. As much as 37.6% of unvaccinated hospital staff and 28.1% of unvaccinated nursing home staff believe that influenza is not dangerous at all. Similarly, 29.7% of unvaccinated hospital staff and 42.5% of unvaccinated nursing home staff believes that vaccination weakens the immune system, and 36.7% of unvaccinated hospital staff and 42.9% of nursing home staff believes they can get influenza after receiving the vaccine. Factors that positively influence vaccination coverage are: encouragement by a supervisor and good practical organization, such as vaccination at a suitable moment, vaccination without the moment, vaccination without the

P22 Harnessing the power of the yellow fever vaccine virus for the development of a therapeutic hepatitis B vaccine  
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HBV is the causative agent of hepatitis B, a liver disease that can evolve to chronicity. An estimated 240 million people, mainly in Asia and Africa, are chronically infected with HBV, with more than 686,000 people dying every year due to complications, such as liver cirrhosis and hepatocellular carcinoma. Current treatments based on nucleos(t)ide analogs control viral replication in most patients, but require life-long therapy. Most patients control an acute infection efficiently without the appearance of any evident clinical symptoms. However, 5-10% of infected adults (and >90% of infected neonates) are unable to clear the virus and develop chronic hepatitis B. In those who clear the virus, vigorous and multi-functional CD4 and CD8 T-cell responses of the Th1 profile (production of IFN-γ) are present. The CD4 T-cell response that is specific for the HBV nucleocapsid protein [also called HBV core antigen (HBc)] is important in HBV control, since this response stimulates the activation of CD8 T-cells. In fact, it has been demonstrated that CD8 T-cells (or CTLs) are the main cellular subset responsible for resolution of the infection, as they clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms. In those patients who do not resolve the infection and develop chronic hepatitis B, the T-cell responses are weakened. Therefore, the restoration of a strong CTL response is the main goal of a therapeutic HBV vaccine, which is urgently awaited to cure millions of people with chronic hepatitis B. To this end, we cloned the sequence of HBc into the capsid gene of the yellow fever vaccine virus (YFV17D), as an efficient vaccine platform. During maturation of the YFV17D polyprotein, HBc is cleaved off and released into the cytoplasm. We have been able to show that this transgenic vaccine virus efficiently drives expression of HBc in addition to the YFV17D proteins upon infection of BK cells. After administration of this recombinant virus to IFN type I and II receptor knock-out mice (AG129), these mice produced HBc-specific antibodies as shown by immunofluorescence. Moreover, we have been able to show that the splenocytes of AG129 mice that have been vaccinated with our HBc-expressing YFV17D produce IFN-γ upon stimulation with immunodominant HBc-derived peptides in an enzyme-linked immunospot assay (ELISpot). Overall, we here present a promising chimeric vaccine candidate for further development and future therapeutic vaccination of chronically infected hepatitis B patients.

P23 Development of a HIV-Vaccine based on the VSV-GP vector  
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Background: Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus, VSV-GP, is a potent vaccine vector, overcoming limitations of wild-type VSV.

Objective: Here, we evaluated the potential of VSV-GP as a vaccine vector for HIV infection.

Methods: We incorporated antigens from HIV or marker genes into the genome of VSV-GP and generated infectious viruses via reverse genetics. These viruses were analyzed for transgene in vitro expression, infectivity, localization and conformation of the antigen. In mice distribution and kinetics of infected cells, antigen-specific and vector-specific immune responses were analyzed. In rabbits HIV neutralizing antibodies were characterized.

Results: Infectious viruses containing antigens from HIV were generated. The addition of the additional antigen did not attenuate VSV-GP replication. HIV envelope variants were expressed in VSV-GP infected cells and incorporated into VSV-GP particles. Crucial epitopes for binding of broadly neutralizing antibodies against HIV such as MPER (membrane-proximal external region), CD4 binding side, V1V2 loop and V3 loop were present on the surface of VSV-GP env particles. After intramuscular immunization of mice, viral replication was limited to the injection side and the draining lymph nodes. No neutralizing antibodies against VSV-GP were induced even after seven boost immunizations. However, high HIV antibody titers were elicited in mice, vectors containing membrane-anchored envelope constructs elicited higher antibody titers than secreted env. In rabbits tier 1A neutralizing antibodies were detectable after prime immunization, which were boosted after a second immunization.

Conclusion: Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector platform.

P24 Next Generation DNA vaccination Approaches – Targeting Alternative Tissues for Electroporation  
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Introduction DNA vaccines are clinically effective when delivered using electroporation (EP). However, the typical intramuscular EP route is associated with transient pain and muscle contraction, and requires invasive electrode needles to be inserted at the treatment site. Here, we discuss two distinct techniques that either specifically targets electroporation to the epidermal region of skin or subcutaneous adipocytes. Both procedures noninvasively transfer the target cells (keratinocytes, dendritic cells, adipocytes) in vivo, with the aim of reducing the invasiveness of DNA vaccination.

Methods Skin - A non-invasive surface only electroporation device was designed which only makes contact with the upper layers of the skin. This device was then used to immunize a plethora of animal models to demonstrate both reporter gene expression and immunogenicity. Adipose - Using an optimized electroporation device design, subcutaneous fat pads of guinea pigs were transfected by performing a subcutaneous injection of plasmid DNA followed by noninvasive electroporation, and reporter construct expression was monitored for 60 days. To demonstrate immunogenicity of this technique, guinea pigs were vaccinated with an influenza DNA construct, using ad pode-EP at administered with different EP parameters and injection techniques.

Results Both devices induced robust reported gene expression in their target tissues of choice. The noninvasive electrode design on both prototype concepts transfected large numbers of keratinocytes, dendritic cells and adipocytes within the targeted treated area. The transfected adipocytes expressed encoded protein for at least 60 days whereas skin expression was observed for approximately 20 days. The optimized ad pode-EP technique generated humoral and cellular immunity comparable to the surface skin-EP. The skin surface electroporation device was additionally used to vaccinate cotton rats which were subsequently protected from challenge with RSV.

Conclusions Large numbers of adipocytes and resident skin cells can be transfected in vivo using electroporation devices designed to concentrate the electric field in the target tissue. These transfected cells elicit a rapid and robust immune response. Because of the potential tolerability and usability advantages, ad pode-EP and skin surface-EP are promising modalities for the application of DNA vaccination in the clinic.
P25 Development of an item pool for a standardized questionnaire measuring parental concerns about pneumococcal vaccination: Results of a literature review and a qualitative study in Germany

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Background and Objectives: Despite a general recommendation for pneumococcal vaccination of children in Germany, vaccination rates are often not completed and recommended timing for vaccination are not met. International studies indicate that different factors influence vaccine hesitancy which are context specific, varying in different settings and with different vaccines. The general aim of our study is the development of a standardized questionnaire to measure parental attitudes and concerns about pneumococcal vaccination in Germany. In a first step, we identified an item pool of relevant questions based on a literature review and a qualitative study.

Methods: International publications of existing instruments on vaccine hesitancy were reviewed and translated into context sensitive and structure to each other. To identify specific aspects of pneumococcal vaccine hesitancy, two semi-structured qualitative focus group interviews were conducted with mothers (2 to 3 participants per group) of incompletely immunized or unvaccinated children aged between 18 to 24 months. Furthermore, five pediatricians and eight other health professionals (clinical experts and medical assistants) were interviewed via telephone in order to determine their experiences with parental attitudes of pneumococcal vaccination.

Candidate items were identified using these approaches.

Results: Eight existing instruments with a total of 156 items on vaccine hesitancy were analysed. After exclusion of thematically irrelevant questions, a pool of 71 items comprising four dimensions, were found. Main dimensions were “knowledge about vaccination,” “beliefs and attitudes,” “sources of information” and “concerns.” Furthermore, content analysis of interview data revealed additional aspects like perceived risk associated with infection or problems in interaction with health professionals mentioned. During the focus groups, mothers mentioned that pneumococcal infections are not a serious health risk in Germany. Moreover, they reported inadequate or lack of medical advice, especially in making an individual choice. Merging results from both approaches resulted in a pool of 48 items consisting of six dimensions relevant for pneumococcal vaccine hesitancy.

Discussion: Using this multi-methods design, we generated an item pool for measuring concerns about pneumococcal vaccination in Germany. Based on this item pool, an immediate development of a standardized questionnaire will be used in a field test to assess feasibility and practicability of the instrument. After further revision, psychometric properties of the questionnaire will be evaluated in a larger (N=100) survey of parents with incompletely immunized or unvaccinated children.

P27 In vivo Expression of Plasmids Encoded IgG for Immune Checkpoint Inhibitors by Synthetic DNA as a New Tool for Cancer Immunotherapy

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Cancers employ various strategies to escape immune surveillance including the exploitation of immune checkpoint inhibitors. Checkpoint inhibitors are receptors found on immune and stromal cells whose function can impact the duration or potency of an immune response. Tumor cells often upregulate ligands for these receptors to protect themselves from the host immune response. Monoclonal antibody (MAb) therapeutics which block checkpoint inhibitor-ligand interactions restore T cell destruction of cancer cells in vivo. MAb's that target the inhibitory T cell signaling mediated by CTLA4 and/or PD1 checkpoint inhibitors have recently gained regulatory approval for the treatment of some cancers based on remarkable clinical outcomes.

Here we have focused on a new method to improve MAb delivery through direct engineering of MAb in the form of synthetic DNA plasmids. This technology would improve many aspects of such a therapy by lowering cost, increasing in vivo expression times and allowing for simple combination formulations in the absence of a host anti-vector immune response, possibly extending use of this groundbreaking therapies to disadvantaged patient populations. We report that “enhanced and optimized” DNA plasmid technology with remarkable clinical outcomes.

P26 Infection-permissive immunity provided by conventional influenza vaccination affects host immune responses upon infection and allows induction of heterosubtypic immunity

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Conventional influenza vaccines aim at the induction of virus-neutralizing antibodies. However due to antigenic drift or shift of the influenza virus, high vaccine efficiencies are not always reported for vaccinees. We investigated to what extent infection-permissive immunity provided by a seasonal trivalent inactivated influenza virus vaccine (TIV) could modulate disease and virus-induced host responses after infection with H1N1 virus that matches the vaccine. More than one TIV vaccination is needed to induce high serum HI titers and prevent rapid mortality upon virus infection in naïve mice. However, single TIV administration correlated with lower viral lung titers and faster recovery after homologous challenge.

Contrary to negative control mice, complete abolishment of virus-specific macrophages, innate immune cells that form the first line of defence against respiratory pathogens, was prevented in TIV-vaccinated animals. Single TIV vaccination allows the induction of cross-reactive NP-specific CD8+ T cells by virus infection as detected in circulation and correlates with protection against subsequent challenge with heterosubtypic H3N2 virus. When focusing on infection-induced mucosal immune responses, TIV vaccination does affect levels of pulmonary B and T cell responses after infection. These results suggest that simple and strategic vaccination with conventional influenza vaccines may still positively modulate disease outcome, thereby allowing induction of heterosubtypic immunity by virus infection.

P28 Immunophenotypes and mediators expression in healthy subjects after seasonal influenza vaccine

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The cumulative morbidity and mortality caused by seasonal influenza is substantial due to the relatively high attack rate. Effective vaccines represent the best approach to prevent pandemic outbreaks of influenza virus infection. Anti-influenza virus host response is a complex process involving both innate and adaptive immunity. CD4+ Foxp3+ Treg cells are a dedicated population of cells that maintain self-tolerance and immune homeostasis. This study was designed to explore the immunophenotypes, immune mediators, and antibody production after seasonal influenza vaccination. The whole blood was collected from healthy subjects before and 10-14 days after seasonal influenza vaccine immunization. The cell surface markers, intracellular staining of Foxp3+ Tregs, and Th1/Th2 cytokines were determined. The antibody titer was measured using the hemagglutination inhibition test. Totally 27 persons (male 10 and female 17) were enrolled, with mean age of 28.5 years. The white blood cell count, absolute neutrophil count, absolute monocyte count and absolute lymphocyte count did not change significantly post vaccination. The expression frequency of CD3+, CD4+, CD16/CD56, CD20+CD25+ and CD20+CD25+ Foxp3+ cells were increased significantly post vaccination. The plasma level of interleukin (IL)-10, IL-17, IL-27, and IFN-γ, was not found to increase significantly after vaccination, in addition to IL-15. All enrollees had seropositive of anti-H3N2 and anti-B/Yamagata, and 92% of anti-H1N1 after vaccination. Tregs are viable targets to enhance immunogenicity of vaccines. These findings also raise the possibility that modulating the Treg expression and function could improve efficacy from vaccination.
P29 Safety and Efficacy of Sulfated Archaeal Glycolipid Archaeosomes as a Vaccine Adjuvant for Induction of Cell-mediated Immunity
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Archaeosomes are liposomal vesicles traditionally comprised of total polar lipids (TPL) or semi-synthetic phospho-glycerolipids of ether-linked isoprenoid phytanyl cores with varied glycol- and amino-head groups. Unlike conventional ester-linked liposomes, archaeo- somes exhibit high pH and thermal stability. When used as adjuvants, archaeosomes can induce robust and long-lasting humoral and cell-mediated immune responses and enhance protection in murine models of infectious disease and cancer. However, traditional TPL archaeosome formulations are relatively complex and semi-synthetic archaeosomes involve many synthetic steps to arrive at the final desired glycolipid composition. We have developed a novel archaeosome formulation comprising a sulfated saccharide group covalently linked to the free sn-1 hydroxy backbone of an archaeal core lipid (sulfated S-lactosylarchaeol, SLA). These semi-synthetic sulfated glycolipid archaeosomes represent a new class of adjuvants that can be synthesized with ease and retain strong immunostimulatory activity for induction of cell-mediated immunity following systemic immunization. Herein, following intramuscular injection to mice we have evaluated: 1) the safety of sulfat- ed archaeal glycolipids; 2) the effect that encapsulation within SLA archaeosomes has on antigen distribution; and 3) the immunostimulatory effects of SLA archaeosomes when used as adjuvant with different antigen types. Overall, we have found that semi-synthetic sulfated glycolipid archaeosomes are a safe and effective novel class of adjuvants capable of inducing strong cell-mediated immune responses against a range of different antigens.

P31 Decreased transmission of Bordetella pertussis and Bordetella parapertussis in mice immunized with a low-LPS whole cell pertussis vaccine
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Background: Bordetella pertussis and B. parapertussis are strict aerobic gram-negative coccobacilli, causing pertussis or whooping cough. For over 40 years whole cell pertussis vaccines (WCP) have been shown to be effective, but are associated with undesirable side effects, causing resistance in population acceptance, which has led to the development of the acellular pertussis vaccines. These vaccines, although less reactogenic, are expensive, making them unavailable for developing countries. The Instituto Butantan, in São Paulo - Brazil, is developing a less reactogenic whole cell pertussis vaccine (Plow), with low-endotoxin content. In this study, we evaluated the humoral immune response against B. pertussis and B. parapertussis induced by Plow and the transmission of these bacteria, from immunized to non-immune animals, after intranasal challenge.

Methods: Using C57BL-6 mice, Plow was compared to a traditional whole cell pertussis vaccine (WCP) and two commercial diphtheria/tetanus/pertussis vaccines with acellular pertussis component (DTaP), Pertacel or Adacel. Results: Our results demonstrated that cell-to-cell SHIV162P3 transmission was less sensi- tive to neutralizing antibodies compared to cell-free SHIV162P3. The triple bNAbs combination showed decreased potency in the TBM-assay than in the PBMC assay. Moreover, inhibition of cell associated transmission was achieved only when 2G12 was present in the bNAbs combination in the TBM-assay, whereas in the PBMC assay the double combination is more potent to neutralize the infection when 2F5 is present. In the TBM-assay, none of individual 2F5, 2G12 and 4E10 can inhibit cell-to-cell transmission while 2F5 can efficiently block it in the PBMC assay. Interestingly the IgG1b12 was very efficient to inhibit cell-to-cell transmission in both assays.

Conclusion: Therefore, our findings support the use of antibody combinations against cell-associated virus in future candidate vaccine/therapeutic regimens.

P30 A Combination of Broadly Neutralizing Antibodies Prevents In Vitro SHIV162P3 Cell-Free Transmission More Efficiently Than Cell-to-Cell Transmission
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Background: HIV transmission from cell-to-cell is a common way for viral dissemination. Indefinitely, it has been suggested that transmission through infected cells is more efficient and more difficult to neutralize than cell-free virus. Nevertheless, most therapeutic and preventive vaccine candidates towards HIV-1 have been evaluated pre-clinically for effica- cy against cell-free viral challenges. We previously reported a protective effect of a combi- nation of three NAb, 2G12, 2F5, and 4E10, against simian/human immunodeficiency virus (SHIV) vaginal transmission in macaques in vivo (Moog C. 2014). However, to date, there is no evidence that strategies that can efficiently prevent ma- caques exposed to cell-free SHIV, like the use of bNAbs, are also able to protect from cell-associated SHIV exposure. Here we aimed to determine in vitro the extent to which bNAbs inhibit cell-free and cell-to-cell SHIV162P3 transmission.

Methods: We developed a cell-to-cell transmission assay and evaluated antibody- mediated virus neutralization using both TBM-bl and human PBMC as target cells and in vivo SHIV162P3-infected spleen cells as donor cells. We used the anti CD4- binding site IgG1b12, the gp120-directed antibody 2G12, and the two gp41-directed antibodies, 2F5 and 4E10 against the two transmission route. The bNAbs were used either alone or in combinations of 3 (2G12+2F5+4E10) and 2 (2G12+4E10, 2G12+2F5, 4E10+2F5).

Results: Our results demonstrated that cell-to-cell SHIV162P3 transmission was less sensi- tive to neutralizing antibodies compared to cell-free SHIV162P3. The triple bNAbs combina- tion showed decreased potency in the TBM-assay than in the PBMC assay. Moreover, inhibition of cell associated transmission was achieved only when 2G12 was present in the bNAbs combination in the TBM-bl, whereas in the PBMC assay the double combination is more potent to neutralize the infection when 2F5 is present. In the TBM-assay, none of individual 2F5, 2G12 and 4E10 can inhibit cell-to-cell transmission while 2F5 can efficiently block it in the PBMC assay. Interestingly the IgG1b12 was very efficient to inhibit cell-to-cell transmission in both assays.

Conclusion: Therefore, our findings support the use of antibody combinations against cell-associated virus in future candidate vaccine/therapeutic regimens.

P32 Comparative investigation of the gene expression profile involved in immune response of the lymphocytes isolated from spleen and lymph nodes and analysis its correlation with lymphoproliferation following ESAT-6 injection in BALB/c mice.
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The ESAT-6 antigen is one of the most prominent antigens expressed by Mycobacterium tuberculosis has been considered as one of the vaccine candidate antigens. Although, ESAT-6 is recognized to modulate host immune responses, the detailed mechanisms are still unknown. One of the most important methods of determination of immune response in monitoring the gene expression involved in the immune system; it is referred to as profiling techniques that provide more details about various immune responses elicited by different vaccines. Profile monitoring is one of the recent research projects that have received increasing interest in research communities. Typically, researchers in order to measure the immune response after vaccine evaluated and interpreted gene expression only in splenocytes. However, the significance of examination of gene expression in lymph node cell that show the irritability of these lymphocytes in subsequent events of the antigen sometimes underestimated because accessibility to lymph nodes is rather complicated. Herein, simultaneously after vaccination, besides, the expression of genes in the four countries Th1, Th2, Th17, and intracellular Signaling molecules involved in immune responses were investigated and compared with the gene expression in the lymph nodes, antibody level was also checked as well. The results obtained from examining the expression of more than 20 genes involved in immune response indicate that some group of genes show different levels of expression in genes extracted from the lymphocytes in the spleen in compared to lymph nodes.

Results of this study suggest that the simultaneous evaluation of gene expression profile in both spleen and lymph nodes is necessary for the assessment process.
**P33 An Optimized DNA encoded monoclonal antibody (DMAB) targeting OspA prevents transmission of Lyme disease**

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A licensed vaccine targeting OspA, an outer surface protein on *Borrelia burgdorferi*, a tick-borne infection which is the causative agent of Lyme disease, was recalled in 2002 due to poor acceptance and additional concerns. While Lyme disease is responsive with antibiotics, treatment of chronic Lyme disease can present obstacles. With no currently licensed Lyme disease vaccine for humans, healthcare workers for Lyme disease are often left with no prevention options for Lyme endemic regions. It has been well established that antibodies are critical mediators in protection against Lyme disease and monoclonal antibody treatment is a promising approach for preventing Lyme infection. However, mAb production has significant limitations including: dose, manufacturing, and cost. DNA encoded monoclonal antibody (DMAB) technology may provide an alternative to passive antibody therapy for generating rapid, antibody-based immunity against vector-borne diseases with the possibility of persistent protection for several months. DMABs are highly optimized DNA plasmids engineered to encode monoclonal antibodies (mAbs) for in vivo DMAB production.

The antigen OspA, which is expressed by *B. burgdorferi* in the tick midgut, is highly conserved in North American strains of the spirochete. OspA presents an interesting protein target as the antibody must bind to OspA on *B. burgdorferi* within the tick to prevent disease. By studying anti-OspA human IgG antibody sequences with variations in the B-cell neutralization capabilities, OspA affinities, and protective efficacies in mice, we engineered and optimized DMAB vectors expressing different DMAB clones. In vitro, we achieved high expression levels for optimized DMAB encoding MAB 319-44, exhibiting significant *B. burgdorferi* neutralization in an in vitro borreliacidal assay. Most notably, in vivo expression of several ug/ml was achieved. Using DMAB 319-44 in a murine Lyme challenge model we were able to observe 60% protection versus the vector control group. Importantly, this is the first demonstration of a transmission blocking DMAB providing protection for the host and suggests additional study of this unique technology as a possible new tool for protection against tick borne infections including Lyme.

**P34 A structure based vaccine by a non-transmissible human parainfluenza virus vector**

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Human parainfluenza virus type 2 (hPIV2) has a negative-sense single-stranded RNA genome and replicates in the cytoplasm. hPIV2 causes acute lower respiratory infection in infants. We successfully obtained a stable packaging cell line expressing hPIV2 F gene and constructed an F gene-defective vector (hPIV2DF) with a non-transmissible property for the delivery of foreign antigens as a vaccine. hPIV2DF can incorporate antigen proteins efficiently and quantitatively on the envelopes, in addition to the trans-gene expression, by fusing a structural or anchoring gene of hPIV2 with an antigenic gene. Enhanced incorporation potentially overcomes a low-level expression of the trans-gene in vivo due to low infectivity or pre-existing antibodies, and also allows the IM, ID and SC injection in addition to IN.

Moreover, treatment of the vector with an extremely low concentration of *b*-propiolactone can efficiently inactivate the genome of hPIV2DF (inactivated hPIV2DF is named as BC-PIV), while keeping the viral fusion properties to target cells intact as well as immunogenicity, similar to those of live viruses.

Therefore, vulnerable antigens such as Zaire Ebola virus (EBOV) GP protein, RSV pre-fusion F protein, and zika virus prM/E protein can possibly be presented on the envelopes of hPIV2DF or BC-PIV as vaccine antigens.

We constructed hPIV2DFs with an EBOV GP gene, an RSV F gene, a zika prM/E gene, and a murine/human malaria CSP gene with/without an hPIV2 anchoring signal by the reverse genetics method. We successfully recovered hPIV2DFs with each antigen gene. The introduced EBOV GP gene has mutations avoiding generation of soluble GP (sgGP) and small soluble GP (ssGP) through RNA transcriptional editing, and GP-mediated entry into a variety of human cell types.

The introduced RSV F gene has mutations resulting in partially stabilized pre-fusion forms or deletion of p27 residues of the furin site. hPIV2DF with mutated EBOV GP expressed only gp34 proteins without sgPssGP proteins and the hPIV2DF/EOBV GP with an additional GFP gene did not propagate in Vero cell in 10 days at a high moi. The virions of the hPIV2DF/EOBV efficiently incorporated the EBOV GP33 proteins. In mice model the hPIV2DF strongly induced EBOV GP33-specific IgG1 and IgG2a antibodies after IM administration. The serum samples had neutralizing activity against the infectivity of VSV-pseudotyped with EBOV GP and showed small activity of antibody-dependent enhancement.

The other results on the vaccine will be shown in the poster.

**P35 Diversity of *Coxiella burnetii* Antigens Recognized by T Cells after Natural Infection in Humans: Identification of Candidate Epitopes for Q-Fever Vaccine Design**

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**Background:** *Coxiella burnetii* (Cb), the causative agent of Q-fever, is a Gram-negative intracellular bacterium transmitted via aerosol. Regulatory approval of an inactivated vaccine used in Australia has been hindered in the US and Europe by reactogenicity in previously exposed individuals. We aimed to discover Cb-specific sequences recognized by human T cells for design of a safe, effective and less reactogenic epitope-based Q-fever vaccine.

**Methods:** Immunoinformatic methods were used to identify HLA class I and class II epitopes conserved across seven sequenced Cb strains from type IV secretion system effector and Cb proteins previously reported to be recognized by antibodies or T cells in human or animal infection. Conserved predictions were validated in HLA binding assays for six class I and eight class II supertype HLA alleles. 143 individuals with and without history of Q fever disease were recruited from the Netherlands site of a Q-fever epidemic in 2007-2011. Subjects were immune profiled and HLA-typed. Exposed subjects were selected for significant cellular reactivity to whole heat-killed Cb in an IFN-γ release assay. HLA class II epitopes were probed for immunoreactivity in cultured IFN-γ ELISpot assays using peripheral blood mononuclear cells from donors with a range of Cb infection outcomes. Epitope predictions were validated in HLA binding assays for epitopes conserved across seven sequenced Cb strains from type IV secretion system effector and Cb proteins previously reported to be recognized by antibodies or T cells in human or animal infection. Conserved predictions were validated in HLA binding assays for six class I and eight class II supertype HLA alleles. 143 individuals with and without history of Q fever disease were recruited from the Netherlands site of a Q-fever epidemic in 2007-2011. Subjects were immune profiled and HLA-typed. Exposed subjects were selected for significant cellular reactivity to whole heat-killed Cb in an IFN-γ release assay. HLA class II epitopes were probed for immunoreactivity in cultured IFN-γ ELISpot assays using peripheral blood mononuclear cells from donors with a range of Cb infection outcomes.

**Results and Discussion:** Candidate epitopes were identified using the HLA class I and class II algorithms identified HLA class I epitopes (10 per supertype allele) and 50 promiscuous HLA class II epitope clusters selected for conservation across sequenced Cb strains, high HLA binding potential, potential to bind multiple HLA alleles, low predicted cross-reactivity with peptides derived from the human proteome or microbiome, and low potential for synthetic production challenges.

No more than two epitopes per source antigen were selected to maximize immunological breadth. HLA binding assays confirmed 89% of class I and 75% of class II predictions (x² p<0.001). T cell responses were identified in the history and seroconversion status in one of four groups: healthy (N=26); exposed, no antibodies (N=39); disease with antibodies (N=39). The proportions of donors responding to HLA class II epitopes and of epitopes recognized increase with increasing immunological and clinical evidence of infection and disease. Analysis of IFNγ responses to HLA class II epitopes revealed that 47/50 peptides stimulated responses in at least one donor. These data demonstrate that a substantial proportion of immunoinformatic-identified epitopes confirmed for HLA binding show immunoreactivity in infection survivors. These epitopes have significant potential to stimulate de novo Cb-specific immune responses in a novel epitope-based Q-fever vaccine.

**P36 Novel-EOBV GP-Specific cellular phenotype responses were observed in non-human primates following Ebola VGX 4201 DNA vaccination delivered ID with IL-12-expressing plasmid**

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Ebola virus (EBOV) causes acute, lethal hemorrhagic fever. The recent 2014 Ebola outbreak was the largest to date with over 28,000 total cases resulting in over 11,000 deaths. Ebola continues to pose a threat as evidenced by the most recent outbreak in the Democratic Republic of Congo just ending in July 2017. Although the fervent research and earliest collaboration surrounding Ebola in the wake of these recent outbreaks have led to fruitful discovery in the field, no vaccines have been licensed to date. Both humoral and cellular components of the immune system may be important for the response against and eradication of EBOV following infection. In our most recent study we interrogated the immune response in non-human primates (NHPs) of our DNA vector vaccine VGX 4201 encoding a consensus sequence of EBOV glycoproteins (GPs) from the EBOV outbreaks leading up to 2014. The humoral and cellular responses were assessed in cynomolgous macaques following intradermal delivery (ID) of a DNA prime-DNA boost regimen of our EBOV vaccine in combination with our DNA-based adjuvant IL-12. As expected following immunization, 100% of animals were seroconverted, and a high frequency of CD4 T cells producing TNF and/or IFN-gamma in response to antigen-specific stimulation was observed. Additionally, we profiled T cells for the expression of activation markers and chemokine receptors, as well as the production of T helper cytokines including IL-4, IL-21 and IL-17. These data combined with our results from our challenge study will further shed light on the potential correlates of protection against EBOV infection and aid in optimizing our vaccine and others.
**P37** Simian adenovirus-vectored vaccines; a platform technology for emerging pathogen vaccine development
Sarah C. Gilbert
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Following recent Ebola outbreaks there has been recognition that a considerable number of other known pathogens have the potential to cause human infections and thereafter to result in further health emergencies. The independent development of vaccines against each of these pathogens would take many years, possibly decades. The cost, which will not be met by commercial vaccine companies since the products are not likely to generate a return on investment, could be so high that only a very few could be funded, and much research effort could be wasted on testing vaccine candidates that are not suitable for development. A different solution is needed to reduce the time and cost taken to develop vaccines against multiple pathogens. Using a platform technology for vaccine development significantly reduces the development time for each individual vaccine since the manufacturing methods and yields, thermostability, safety and immunogenicity of the vaccine are known in advance.

Replication-deficient adenovirus vectored vaccines represent the ideal platform technology for this situation. Encoding one or more antigens from the pathogen within the viral genome, these vaccines result in expression of the antigens within host cells after vaccination leading to strong and protective immune responses mediated by both B and T cells, with no requirement for adjuvant, after a single immunization. Onset of immunity is rapid, and the vaccines can be thermostabilised for ambient temperature storage making them ideal for use in an outbreak situation. Booster doses of the same or a different viral vectored vaccine can be administered in the years following the primary immunisation if long-lasting immunity is required. The vaccines may also be administered by aerosol rather than by using a needle and syringe, and the side effect profile is well-known, short lived and with a low rate of fevers following vaccination. Manufacturing can be completed at small or large scale, with cost of goods decreasing as the scale increases.

Using an appropriate platform technology for development of vaccines against emerging pathogens removes many of the barriers to the availability of vaccines for emergency use to contain and control outbreaks. Alongside these measures need to be taken to allow for rapid vaccine development and deployment. These include the development of suitable animal efficacy models, standardized assays for use in clinical trials, and sharing of reagents and samples during early vaccine development.

**P38** Determining the Effects of GP1 Glycosylation Site Ablation on Guanarito virus GPC cleavage and cell surface expression
Steven Hallam, Sloboda Paessler Ph.D.
Generously supported by the Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX.

Guanarito virus (GTOV) is an emerging New World (NW) arenavirus in the human hemorrhagic fever (HF) group. The virus is recognized as the causative agent of Venezuelan Hemorrhagic Fever (VHF). Recent reports have indicated that the year 2016 contained the 2nd highest incidence of VHF in its endemic area. This may indicate an emergence or re-emergence of this virus. To date, there are no vaccines or vaccine candidates available to prevent GTOV infection. A related NW arenavirus, Junin virus (JUNV), is the only arenavirus with a distributed live-attenuated vaccine, Candid #1 (Can). The arenavirus glycoprotein is translated as a polyprotein (GPC) that is later cleaved into GPC1, GPC2, and the stable signal peptide (SSP). GPC1 contains the receptor binding domain (RBD) and GPC2 is the transmembrane peptide that also functions as the fusion protein. Attenuation studies of Can have shown that a single glycosylation site ablation in the GPC1 subunit of the JUNV GPC polyprotein converts the virus to a live attenuated virus. The effects of ablation at the GPC1 glycosylation sites in JUNV will be studied in this study.

Following recent Ebola outbreaks there has been recognition that a considerable number of other known pathogens have the potential to cause human infections and thereafter to result in further health emergencies. The independent development of vaccines against each of these pathogens would take many years, possibly decades. The cost, which will not be met by commercial vaccine companies since the products are not likely to generate a return on investment, could be so high that only a very few could be funded, and much research effort could be wasted on testing vaccine candidates that are not suitable for development. A different solution is needed to reduce the time and cost taken to develop vaccines against multiple pathogens. Using a platform technology for vaccine development significantly reduces the development time for each individual vaccine since the manufacturing methods and yields, thermostability, safety and immunogenicity of the vaccine are known in advance.

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**P39** Development of a DNA vaccine immunotherapy against Her2 positive breast cancer using TUBO cell line derived animal tumor model
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**Materials and Methods:** DNA vaccines are offering an innovative approach with great therapeutic potential. Cancer immunotherapy by DNA vaccination employs basically active immunotherapy in which immune cells are instructed to actively fight against cancer. In this study, an epitope-driven vaccine was designed targeting Her2 (human epidermal growth factor receptor 2) using immunoinformatics tools. To evaluate the immunotherapeutic efficacy of the final DNA vaccine construct, a murine Her2 tumor model will be used.

**Introduction:** DNA vaccines are offering an innovative approach with great therapeutic potential. Cancer immunotherapy by DNA vaccination employs basically active immunotherapy in which immune cells are instructed to actively fight against cancer. In this study, an epitope-driven vaccine was designed targeting Her2 (human epidermal growth factor receptor 2) using immunoinformatics tools. To evaluate the immunotherapeutic efficacy of the final DNA vaccine construct, a murine Her2 tumor model will be used.

**Results:** We have successfully inserted the multi-epitope Her2 in frame with ScFv and Bcl-xL to the mammalian expression vector pIRES2-EGFP. At the same time TUBO cells were generously supported by the Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX.

**Conclusion:** We have developed a novel DNA vaccine expressing multi-epitope Her2 in frame with ScFv and Bcl-xL in the present study and the animal model to assess the protection conferred by the DNA vaccine. After the vaccination, the immune responses elicited by the multiepitope Her2 DNA vaccine will be determined and the decrease in the tumor size will be associated with the vaccine therapeutic efficiency.

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**P40** Characterization of inter-species reassortment through a Rift Valley fever virus MP-12 vaccine strain
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Rift Valley fever (RVF), a mosquito-borne zoonotic viral disease endemic to Africa and the Arabian Peninsula, is caused by RVF virus (RVFV), belonging to the genus Phlebovirus of the family Bunyaviridae. RVFV is characterized by high rates of abortions in ruminants, and hemorrhagic fever, encephalitis, or blindness in humans. Vaccination of both animals and humans is likely the only effective way to minimize the impact of disease. In the U.S., a live-attenuated MP-12 strain was conditionally licensed as a veterinary vaccine. Toward the development of live-attenuated vaccines derived from MP-12 strain in endemic area, it is important to understand the occurrence of genetic reassortment between RVFV and other members of the Phlebovirus genus. Genetic reassortment was analyzed by a co-infection assay using C6/36 cells. Cells were co-infected with a mixture of recombinant MP-12 (rMP-12) and the genetic variant rMP12-GM50, or with rMP-12 and AMTV. Plaque isolation and genotyping of L-, M-, and S-segments were performed using the surface receptor present at 24 hours post infection. GOLV RNA replication was also evaluated in Vero or PK-15 cells at 28°C with and without co-infection with rMP-12.

No reporter activity was generated from the GOLV M-segment minigenome via RVFV N C with and without co-infection with the RVFV MP-12 vaccine strain. In contrast, with the AMTV M-segment minigenome, GOLV M-segment minigenome mixed with RVFV N and L proteins. GOLV also did not initiate RNA synthesis in Vero or PK-15 cells at 28°C, regardless of co-infection with the RVFV MP-12 vaccine strain. In contrast, with the minigenome assay, AMTV M-segment minigenome showed increased RLuc activities with RVFV N and L proteins, or vice versa. The combination of AMTV N and RVFV L proteins or that of RVFV N and AMTV L, however, did not increase reporter activities. Moreover, the RVFV minigenome, encapsidated with RVFV N and L protein, could not be packaged into AMTV M and L protein, could not be packaged into RVFV G1 and G0 proteins. These demonstrates that the N, L, and G0/G1 proteins are not compatible between RVFV and AMTV. Lastly, co-infection with the rMP-12 strain and AMTV did not form any reassortant strains, although that with rMP-12 and rMP12-GM50 created all combinations of reassortant strains. Taken together, AMTV and GOLV are unlikely to form reassortant strains through RVFV MP-12 strain, due to the incompatibility of the structural proteins.
P4.1 Development of a Plant-made Recombinant Virus-like Particle (VLP) Vaccine Against African Horse Sickness
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African horse sickness is a devastating, infectious, but non-contagious disease that causes great suffering and many deaths among horses in sub-Saharan Africa. The aetiological agent is a dsRNA virus of the same name, African horse sickness virus (AHSV), an orbivirus of the family Reoviridae. The disease has significant economic consequences for the equine industry both in southern Africa, and increasingly further afield as its midge vector spreads with global warming. Live attenuated vaccines have been in use with relative success for more than 5 decades, but there is a risk of reversion to virulence as well as reassortment of the segmented genome between outbreak and vaccine strains. Furthermore, the vaccines lack DIVA capacity, the ability to distinguish between vaccine-induced immunity and that induced by natural infection. Several studies have demonstrated the potential for the use of plant expression systems for the production of VLPs, which are excellent vaccine candidates as they do not contain the virus genetic material and there is no risk of reversion to virulence or re-assortment with wild virus strains. In this study, we investigated the formation of VLPs of two different AHSV serotypes in N. benthamiana by expressing the four structural proteins VP2, VP3, VP5 and VP7 of AHSV - 4 and 5. We successfully expressed the VLPs from recombinant Agrobacterium tumefaciens harbouring the VP genes were co-infiltrated into N. benthamiana and shown to successfully express in plants by SDS-PAGE and western blot analyses. The formation of AHSV VLPs was observed by transmission electron microscopy and large scale purification of these VLPs was carried out by density gradient ultra-centrifugation. The immunogenicity of the particles was first demonstrated in guinea pigs and is currently being assessed in horses which are the main target animals. Our results provide evidence that this system holds excellent potential for the production of a novel AHSV VLP vaccine.

P4.2 Host-viral vector interactions and CCR5 regulation in vector HIV vaccination
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Recombinant viral vectors are an important platform for vaccine development. A number of vectors derived from different viral families (e.g. poxvirus and adenovirus) have been employed for HIV vaccines. However, recent human trials testing HIV vaccines involving different viral vectors have reported some unanticipated and distinct outcomes. In particular, the Step and Phambili trials testing Ad5-vecorted HIV vaccines failed and reported excess HIV infections in some vaccine recipients, suggesting the importance of understanding host-viral vector interactions and potential HIV susceptibility of vaccine (vector)-induced CD4 T cells in HIV vaccination. CCR5 is a co-receptor for transmitting HIV and is a critical factor regulating the susceptibility of target cells to HIV. In our study, we investigated regulation of CCR5 expression on CD4 T cells by different vector HIV vaccines and the associated mechanisms. Mice were prime-boost immunized with ALVAC-HIV or Ad5-HIV vaccine at week 0 and 2, followed by analysis of T-cell response at week 4 in various immune compartments of the immunized mice (spleen, mesenteric lymph nodes, MLN, and Peyer's patch-PP). We found that compared to Ad5-HIV vaccination, ALVAC-HIV vaccination induced markedly lower levels of CCR5+ CD4 T cells in all compartments examined. The frequency of CCR5+ CD4 T cells was highest in PP, followed by in spleen and MLN. Compared to Ad5, ALVAC vaccination induced even more profound decrease in the level of okt5+CCR5+CD4 T cells (~5-fold decrease). Importantly, the levels of total CD4 T cells in different immune compartments were comparable between ALVAC- and Ad5-HIV vaccination. In vitro analyses using human CD4 T cells co-cultured with vector-infected human DCs confirmed the in vivo finding that compared to Ad5 vector, ALVAC induced drastic CCR5 down-regulation on CD4 T cells. Mechanically, we identified that unlike Ad5 vector, ALVAC selectively stimulated strong innate immune activation in DCs, including inflammasome activation and production of innate cytokines and chemokines, via the STING pathway. This innate immune activation by ALVAC mediated CCR5 down-regulation on CD4 T cells. Taken together, our data suggest that in HIV vaccination, selection of viral vectors can significantly impact the quality of T-cell response they induce with regard to CCR5 expression, which is closely associated with their distinct innate immune stimulatory properties. Our study provides new insights into vector design and immune assessment for HIV vaccine development.

P4.3 Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy
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Increased mortality and morbidity occurs in human immunodeficiency virus (HIV)-infected patients who fail to increase CD4+ T cell counts despite achieving viral suppression with antiretroviral therapy (ART). Here we identified an underlying mechanism. Significantly elevated plasma levels of anti-CD4 IgGs were found in aviremic HIV+ immunologic non-responders (CD4+ T cell counts < 350 cells/µl) compared to HIV+ immunologic responders (CD4+ T cell counts ≥ 500 cells/µl) and healthy controls (P < 0.0001, ANOVA). The median plasma levels of anti-CD4 IgG (ng/ml, interquartile range (IQR)) were 14.54 (9.21-24.05), 26.07 (15.92-81.6), and 9.18 (53.19-165.8) for healthy controls, responders and non-responders respectively. Plasma anti-CD8 IgG levels were similar among the three groups (P = 0.13). Notably, plasma level of anti-CD4 IgG was inversely correlated with peripheral blood CD4+ T cell counts (r = -0.53, P < 0.0002) (mean Spearman correlation test) in HIV+ subjects but not in healthy controls (r = 0.21, P = 0.42). Furthermore, antibody-dependent cell-mediated cytotoxicity (ADCC) was performed using purified CD4+ T cells and NK cells from aviremic ART-treated HIV+ subjects co-cultured at a ratio of 1:3 in the presence of purified anti-CD4 IgGs (5 µg/mL) from plasma of HIV+ immunologic non-responders in vitro. Purified total IgGs from non-responders after depleting anti-CD4 IgG were used as a negative control. Anti-CD4 IgG-mediated ADCC activity was measured by NK cytotoxicity, CD4+ T cell viability, and apoptosis by flow cytometry (n = 17). The median percentages of CD107a+ NK cells in ADCC assays were 6.02 (2.9-11.3) and 0.42 (0.21-0.60) induced by anti-CD4 IgGs from non-responders and negative control antibodies respectively (P < 0.0001). The median percentages of CD4+ T cell cytosis in ADCC assays were 6.8 (2.1-16.5) and 0.99 (0.04-1.16) induced by anti-CD4 IgG from non-responders and negative control antibodies respectively (P < 0.0001). These data indicate that autoreactive anti-CD4 IgGs from non-responders have ADCC activity against uninfected CD4+ T cells and may play an important role in the blunted CD4+ T cell reconstitution despite suppressive ART treatment.

P4.4 Effective Therapeutic Vaccine for Hepatitis C virus Infection by Heterologous antigen Prime/Boost Immuno-therapy
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Background: Chronic hepatitis C caused by infection with the hepatitis C virus (HCV) is a global health problem. We aimed to address how HCV expression causes chronic liver diseases and to provide new options for HCV vaccine development. Using a mouse model of hepatitis C, we examined the therapeutic effects of a primobovaccine using a HCV-cDNA vaccine and a recombinant vaccinia virus (vRv) that encodes an HCV protein. Methods: We generated immunocompetent mice expressing HCV structural proteins or whole HCV proteins through the Cre/loxP switching system, mice expressing HCV structural proteins (MxCre/CN2-29) and mice expressing HCV whole proteins (MxCre/RzCN5-15). We constructed the DNA vaccines and attenuated vRv strain LC16m8, which are expressing an HCV structural protein (vRv-CN2), non-structural protein (vRv-N25). Furthermore, we examined homologous-antigen for primobovaccine (N25-cDNA + vRv-N25) or heterologous-antigen prime/boost vaccination (N25-cDNA + vRv-CN2 or CN2-cDNA + vRv-N25).
Results: We found that within 28 days after immunization the HCV core protein levels were significantly lower in mice of the homologous-antigen primobovaccine using DNA vaccine and vRv-N25-treated mice expressing HCV structural proteins (MxCre/CN2-29). We constructed the DNA vaccines and attenuated vRv strain LC16m8, which are expressing an HCV structural protein (vRv-CN2), non-structural protein (vRv-N25). Furthermore, we examined homologous-antigen for primobovaccine (N25-cDNA + vRv-N25) or heterologous-antigen prime/boost vaccination (N25-cDNA + vRv-CN2 or CN2-cDNA + vRv-N25).
Conclusion: We showed that the significant therapeutic effect of heterologous-antigen primobovaccine using DNA vaccine and rVV method. We propose that the heterologous-antigen primobovaccine vaccination (N25-cDNA + vRv-CN2 or CN2-cDNA + vRv-N25) could become an effective therapeutic vaccine.
P45 Early cell recruitment and a Th1 / Th17 cytokine profile induced by a recombinant BCG-LTAK63 strain correlates with enhanced protection against tuberculosis
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Tuberculosis (TB) is responsible for over 9.6 million cases and 1.5 million deaths annually. The only currently available vaccine, Mycobacterium bovis Bacille Calmette-Guérin (BCG), fails to promote pulmonary immunity. The aim of this study was to characterize the innate and long-term immune response induced by a vaccine candidate against TB based on recombinant BCG expressing the non-toxic mutant of E. coli heat labile enterotoxin (rBCG-LTAK63). The recruitment of cells to the peritoneum was evaluated 24 h and 7 days after intraperitoneal administration of BCG or rBCG-LTAK63. We also evaluated the production of reactive oxygen / nitrogen species and inflammatory cytokines. BALB/c mice were immunized subcutaneously to evaluate the long-term immune response. Lung cells were collected and in vitro stimulated to analyze the Th1 / Th17 cytokine profile. The animals receiving BCG or rBCG-LTAK63 showed early cellular infiltration with the presence of neutrophils in the peritoneum at 24 h. The number of lymphocytes was higher in rBCG-LTAK63 as compared to the BCG group at 7 days. An increased nitric oxide production was observed after 24 h and higher hydrogen peroxide concentration after 7 days in the rBCG-LTAK63 group as compared to the BCG group. Higher levels of inflammatory cytokines, TNF-α and INF-γ, were induced in both rBCG-LTAK63 and BCG groups as compared to the controls. A Th1 and Th17 cytokine profile was induced in the lungs of animals 65 days after immunization with rBCG-LTAK63. These results can be correlated with previous data showing superior protection induced by rBCG-LTAK63. Our results demonstrate that rBCG-LTAK63 induces early recruitment of cells with the presence of activated macrophages and lymphocytes. This may be important to develop a Th1 and Th17 immune response and increasing protection against TB. Supported by FAPESP and Fundação Butantan.

P46 Adenoviral type 35 and 26 vectors with a bidirectional expression cassette in the E1 region show an improved genetic stability profile and potent transgene-specific immune response
Authors: Marjia Vujadinovic1 & Kanghui Wu2, Benoit Callendret, Marina Koning, Mark Vermeulen, Barbara Sanders, Esmeralda van der Helm, Adile Gegeci, Dirk Spek, Karin de Boer, Masha Stalnkecht, Jan Serroyn, Maria Grazia Pau, Henneke Sluiter, Roland Zahn, Jerome Custers, Jort Vellinga Presenter: Susann Ludwig
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Genetic vaccines based on replication-competent adenoviral (AdV) vectors are currently in clinical development. Monovalent AdV vectors express one antigen from an expression cassette placed in most cases in the E1 region. For many vaccines, inclusion of several antigens is necessary in order to raise protective immunity and/or target more than one pathogen or pathogen strain. Based on the current technology, a mix of several monovalent vectors can be employed. However, a mix of the standard monovalent AdV may not be optimal with respect to manufacturing costs and the final dose per vector in human. Altogether, the availability of a variety of bivalent recombinant AdV vector approaches is described in the literature. It remains unclear whether all strategies are equally suitable for clinical development while preserving all the beneficial properties of the monovalent AdV (e.g. immunogenicity). Therefore, a thorough assessment of different bivalent AdV strategies was performed in a head-to-head fashion compared to the monovalent benchmark. The vectors were tested for rescue efficiency, genetic stability, transgene expression, and potency to induce transgene-specific immune responses. We report that the vector expressing multiple antigens from a bidirectional expression cassette in E1 shows a better genetic stability profile and a potent transgene-specific immune response compared to the other tested bivalent vectors.

P47 Comparative effectiveness of reminder and recall strategies aimed at improving immunization uptake in practices: A systematic review
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Introduction Immunizations safely and effectively prevent infection and disease. However, targeted vaccination rates have not been achieved for children and adults, resulting in unnecessary vaccine-preventable disease and death. Patient reminder and recall interventions are some of the most effective strategies for raising immunization rates. Reminders are communications to let patients know they are due for vaccination; recalls are communications to let patients know they are late for vaccination. With new technological advances in reminder-recall, it is important to update the evidence of effectiveness to provide clinicians with the best available evidence to improve immunization rates.
Research question To evaluate and compare the effectiveness of various types of patient reminder and recall interventions in improving immunization rates.
Methods We conducted systematic searches within Medline, EMBASE, Cochrane Library, CINAHL, the EPOC Specialized Register, Reference Manager 12, trial registers, and gray literature during 2013 and again in 2017. We included randomized controlled trials (RCT), controlled before and after studies, and qualifying interrupted time series studies written in English. Eligible interventions included immunization-focused patient reminder or recall personal telephone calls, mailed letters, postcards, text messages, automatic electronic telephone calls (autodialer), and combinations of these methods in at least one study arm. We included control groups without interventions. We included receipt of immunizations as eligible outcome measures. Eligible participants included children, adolescents and adults who received immunizations in any setting. We used standard methods published by The Cochrane Collaboration and Effective Practice and Organisation of Care group. Two review authors reviewed lists of titles and abstracts, independently read selected studies, used an abstraction tool to collect study characteristics, intervention descriptions and outcome data, assessed risk of bias, and rated certainty of the evidence using GRADE. We used a formal reconciliation process to resolve disagreements among review authors. We computed risk ratios for individual studies and pooled results for RCTs using the random effects model.
Results We reviewed 5364 studies and included 75 in this review. Studies were conducted in 10 countries in primary care practices and community-based settings. All methods of patient reminder or recall improved immunization rates with participants in the intervention groups being 1.30 (95% confidence interval [CI] 1.23, 1.37) times more likely to have received immunizations compared with control participants. Personal telephone reminder or recall interventions (risk ratio [RR] = 1.75, 95% CI: 1.20, 2.54) were the most effective single method, followed by letter (RR = 1.33, 95% CI: 1.22, 1.45) and text message (RR = 1.28, 95% CI: 1.18, 1.40). Postcard recall was 1.18, 95% CI: 1.08, 1.30) and autodialer interventions were slightly less effective (RR = 1.17; 95% CI: 1.03, 1.32). Patient reminder or recall interventions were effective for childhood, child influenza, adolescent, adult influenza, and other non-travel adult immunizations.
Conclusion Patient reminder or recall interventions are effective at improving immunizations in primary care and community-based settings in developed countries. Newer technological methods, specifically autodialer or text messages, were no more effective than older personal telephone or letter methods, but all work, regardless of patient age.

P48 Oncolytic Virus-Mediated Anti-Cancer Vaccination by atopin encoding oHSV
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Cancer immunotherapy through application of oncolytic viruses (OVs) have shown promise in the treatment of solid tumors. OVs, especially oncolytic Herpes Simplex virus (oHSV) combine advantages of oncolytic therapy and in situ tumor vaccination by local inflammation caused with viral infection associated with oncolysis-formed tumor associated antigens (TAA) to augment immune responses. Although, OVs are capable of breaking the immune tolerance in the highly immunosuppressive tumor microenvironment, but cancer cells often overexpress anti-apoptotic proteins and thus are resistant to apoptosis inducing agents including oHSV. Therefore, we proposed that arming of oHSV with an apoptotic agent might enhance its oncolysis and in situ tumor vaccination potencies. To this end and to ensure selective replication within tumor cells while sparing normal cells, starting with a clinical HSV1 isolate, we first constructed a conditional replication-competent oHSV, in which both copies of viral ICP34.5 and ICP6 gene were inactivated by Blei(Cherry) and Green Fluorescent protein (GFP) reporter gene insertion, respectively (hereafter; HSV-GrDRR). Thereafter, the Blei(Cherry) in both inserted locations of ICP34.5 and ICP6 gene was replaced by Apoptin gene from chicken anemia virus (which induce apoptosis in broad range of cancer cells) to generate the Apoptin-armed oHSV (hereafter; apoptovex); Generation of recombinant viruses was performed with conventional homologous recombination methods. All target genes were located downstream of CMV promoter of pCDNA3.1 during cloning procedures. Application of fluorescent proteins permitted direct observation and viral plaque isolation by fluorescence microscopy (FM). Engineered oHSVs were confirmed by PCR, sequencing and flow cytometry (FC) analyses. Apoptotic potential of the isolated CAV-apoptin gene was confirmed by FC analyses of annexin-V fious and PI stained cells, 48 hours after transfection with Apoptin encoding pcDNA3.1 vectors. Verification of Apoptin expression were confirmed by western blotting in Apoptovex-infected cells. Immunofluorescence of Proliferation rates was determined by staining cells with monoclonal mouse anti-4H10 antibody and LINK4 assay. The majority of oHSVs demonstrated the mean of 2 × 104, 3 × 104 and 6.5 × 104 PFU/ml for HSV-1, HSV-GrDRR and Apoptovex, respectively, indicating less titler for Apoptovex than parental viruses. FC analyses of GFP emission for Apoptovex- and HSV-GrDRR-infected cells (Vero, BHK, T98 GBM, HuH7.5.1, A172 and HepG2) at multiplicity of infection (MOI) of 3.0 indicated high infectivity (>90%) for both constructs but slightly better for the HSV-GrDRR. Accordingly, the cytotoxicity effect of oHSVs in vitro at five different MOIs (0.001, 0.01, 0.1, 1.0, 10) was measured using an in vitro colorimetric assay. Both vectors were determined to have synergy4, 72 hours post-infection, while apoptotic particles were also counted by FM. Results indicated that Apoptovex at MOIs lower than 1 was significantly more effective to induce cell death, whereas HSV-Gr-DRR was not effective at these MOIs, indicating the role of Apoptin insertion for enhanced cytotoxicity. A preliminary study in mouse 4T1 breast tumor model indicated better therapeutic efficacy and increased survival rates for mice immunized with Apoptovex compared to HSV-GrDRR. The mechanism of immunity in immunized mice is currently under investigation to differentiate between type and breath of anti-HSV and anti-tumor T cell immune responses and the immune cells involved.
P49 Combination of independent attenuating mutations in West Nile virus non-structural proteins NS1 and NS4B
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West Nile Virus (WNV) is a mosquito-borne pathogen that first entered the United States (US) through New York in 1999 and quickly spread across the country within 2-3 years. Each year the US can now anticipate in excess of 1000 cases of WNV-induced neurological disease of which 10% will die. Although WNV is now endemic to the US, there is no licensed vaccine available for protection from disease. WNV belongs to the Flavivirus genus along with many other mosquito-borne pathogens. Safe and effective live attenuated vaccines are licensed for the flaviviruses yellow fever and Japanese encephalitis viruses, and a recombinant chimeric live attenuated vaccine has been recently licensed for dengue. Therefore, it is rational that a live attenuated vaccine can be developed for WNV. Using reverse genetics of a WNV NY99 infectious clone (NY99ic), previous work in our lab has generated and screened many WNV mutations in the envelope, NS1, and NS2 genes for attenuated phenotypes. Two of the mutants harboring mutations in NS1-130-132QQA/175A/207A and NS4B-C102S were significantly attenuated for neuroinvasion (intraperitoneal LD90 > 10^3 PFU) and neurovirulence (intracerebral LD90 > 800 PFU) in mice. To eliminate the chances of reversion to a virulent phenotype, it is important to identify multiple different mutations that can be combined to produce a stable vaccine genotype. It is hypothesized that a mutant with attenuating mutations in both NS1 and NS4B will be more attenuated compared to the single gene mutants. To investigate this hypothesis, a new WNV mutant has been generated that combines NS1-130-132QQA/175A/207A+NS4B-C102S mutations. The new mutant has been recovered without additional nucleotide changes in the genome. To investigate multiplication in vitro, multiplication kinetics were completed in triplicate in Vero cells and titers were compared to that of each of the single gene mutants and to wild-type NY99ic. Results indicate that the multiple gene mutant has decreased multiplication from 24-48 hpi, consistent with the NS1-130-132QQA/175A/207A alone, whereas NS4B-C102S alone, and combination of both genes has decreased multiplication from 24-48 hpi, consistent with the NS1-130-132QQA/175A/207A+NS4B-C102S mutations. Results indicate that the multiple gene mutant has attenuated multiplication kinetics were completed in triplicate in Vero cells and titers were compared to that of each of the single gene mutants and to wild-type NY99ic. Results indicate that the multiple gene mutant has decreased multiplication from 24-48 hpi, consistent with the NS1-130-132QQA/175A/207A alone, whereas NS4B-C102S alone, and combination of both genes has decreased multiplication from 24-48 hpi, consistent with the NS1-130-132QQA/175A/207A+NS4B-C102S mutations. Results indicate that the multiple gene mutant has attenuated multiplication from 24-48 hpi, consistent with the NS1-130-132QQA/175A/207A+NS4B-C102S mutations. To evaluate the ability to induce both higher humoral and cellular immunity, and mouse immunization studies were monitored at 7 and 21 days post inoculation (dpi). Consistent with previous studies, the truncated recombinant GRA8 was purified to homogeneity by AKTA-FPLC using His chelating HP column and further by Superdex 200 gel filtration columns. The presence of purified GRA8 protein was demonstrated by SDS-PAGE and Western Blot analyses. Results: The final truncated GRA8 to be expressed by pET101 or by pDNA-3.1 weighted 20.15 kD, included B cell epitope regions and MHC-I epitope regions to which the CTD T-cytolytic lymphocytes will be ligated. Expression in E. coli BL21 Star (DE3) pLysS cells expressed an abundantly expressed GRA8 protein and after processing the cells under high pressure using microfluidizer, recombinant protein purified to homogeneity was obtained as shown by SDS-PAGE and WB images. Discussion and Conclusion: In our previous study wild type GRA8 has shown immunogenicity with sera obtained from animal models infected with T. gondii and patients with toxoplasmosis. In this study, a truncated GRA8 was generated for vaccine development purposes which can be used alone or in combination with other proteins. In addition, this antigenic protein can be used to develop diagnostic tools such as ELISA to assess the presence of acute toxoplasmosis. Acknowledgement: This study was supported by the Higher Education Council Fund of Turkey given to Muhammet Karakavuk.
P53 Structural comparison of human HIV-1 gp120 V3 mAbs of same gene usage induced by vaccine and chronic viral infection
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Understanding of the structural basis of affinity maturation of vaccine induced antibodies (Abs) may help design immunogens that can guide Ab responses in B-cell lineage based HIV vaccine development; comparisons of monoclonal Abs (mAbs) encoded with same VH and VL genes in different stages of maturation can help understand the process. We have identified four human anti-V3 mAbs with the same VH-3*101 and VL-3*101 gene usage, including TAT and TA7. developed from a vaccinee in the HIV vaccine phase I trial, DPP-001 with a polyclonal Env DNA prime-protein boost formulation, and 311-11D and 1334, derived from chronically infected patients. The somatic hypermutation (SHM) rates in VH of the two mAbs from the vaccinee were lower (4.2% and 5.2%) than that of the two from chronically infected patients (8% and 10.1%), while the rates in VL were more comparable. The CDR H3 varied in length from 15 to 22 residues (Katub definition) with very low sequence homologies. We have carried out a structural study of these mAbs by crystalizing their Fab fragments with V3 peptides and our results revealed the atomic details of their antigen-antibody interactions. The structural data showed that these mAbs bind the V3 epitope with a cradling mode, i.e., the V3 beta hairpin laid down along the antigen binding site groove, and their paratopes comprised of residues from both heavy and light chains. Several residues conserved from the germine sequences play a key role in shaping the antigen binding site and in epitope binding while mutated residues created additional contacts. This study confirmed, and clarify how the somatic hypermutation correlating with binding affinity. Interestingly, only residues at the base of the relatively long vertical CDR H3 have contacts with V3, while the rest of the loop does not interact with the epitope, explaining the high sequence diversity of CDR H3 in these mAbs. Our data provide a unique example of germine sequences shaping the antigen binding sites and SHM correlating with affinity maturation of Abs induced by vaccine and by natural HIV infection.

P54 A live-attenuated Zika virus vaccine candidate based on the yellow fever virus comprising the prME of a current epidemic Asian Zika virus strain
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The recent Zika virus (ZIKV) epidemic in the Americas has led to the search for therapeutic vaccines and vaccines to curb or prevent the debilitating disease caused by the virus. A number of replication inhibitors and vaccine candidates have been reported but none has been approved yet. The yellow fever virus (YFV) and ZIKV belong together with the dengue, Japanese encephalitis, West Nile, and tick borne encephalitis viruses to the genus flavivirus. Using our recently developed PLLAV (plasmid-launched live-attenuated vaccine) platform and the live attenuated YFV vaccine strain 17D (YFV-17D) as a vector, we engineered a chimeric vaccine candidate by replacing the aegagropilic surface glycoproteins of YFV-17D with those of a prototypic Asian lineage ZIKV strain isolated from the Yap island in 2007. The chimeric YF-ZIK virus generated appeared over-attenuated and could not be effectively propagated in vitro. In an enforced attempt to propagate the virus in cell culture, we developed a protocol that permitted the replication of the chimera to higher levels for use in experimental animal models and vaccine production. Retrospective analysis revealed that the chimeric virus acquired essential adaptive mutations, which as such could not be successfully selected using the standard/ conventional methods. The chimeric YF-ZIK vaccine candidate generated by our forced adaptation method was shown to gain infectivity for Vero cells and to propagate without the need of further intracellular passage. Our results show that the novel chimeric YF-ZIK is replication competent and highly attenuated when compared to both its parental YFV and ZIKV variants. Safety and immunogenicity studies are currently being carried out using (interferon-deficient) AG129 mice. Protection from wild-type ZIKV infection will be demonstrated in our recently established stringent ZIKV challenge model (Zmurko et al. PLoS NTD 2016). The chimeric YF-ZIK vaccine, which resembles the recently licensed chimeric Japanese encephalitis and dengue vaccines Imovax® and Dengvaxia®, respectively, may be developed as a promising ZIKV vaccine.

P55 Vaccine delivery through the skin harnesses innate cells in remote mucosal tissues that provide cues for CD8 T cell recruitment and protection.
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A remarkable feature of skin vaccination is the generation of protective immunity in remote barrier tissues including the respiratory and female genital tract, yet the mechanism driving recruitment and retention of antigen-specific CD8+ T-cells to these tissues is unclear. To delineate the cellular/molecular changes that instruct CD8 T-cell migration to barrier tissues following skin vaccination, we have used a ‘needle-free’ skin vaccination system using dissolvable ‘microneedle arrays’ (MA) fabricated to contain a replication-defective adenovirus (Ad) vector encoding HIV-1 CNS4 gag (Ad HIV-1 CNS4gag) as an exemplar vaccine within the dissolvable needles for skin delivery in B6 mice. We found that, irrespective of the anatomic skin site used for vaccine delivery, high frequency Db/CNS4 gag tetramer CD8+ T-cells (specific to the DHBV1-1 CNS4 gag polyvalent epitope) tracked to the female genital tract (GT). The recruited CD8+ T-cells were functional, as demonstrated by in vivo killing of peptide pulsed syngeneic targets injected into the vaginal wall, and controlled a vaginal challenge with vaccinia virus expressing the cognate HIV-1 CNS4 gag sequence. In studies to explain how skin primed CD8+ T-cells migrated to the GT, we found Db/CNS4 gag tetramer CD8+ T-cells isolated from the GT expressed a panel of inflammatory chemokine receptors including CXCR3. In a search for possible inflammatory cues that might recruit CD8+ T-cells to the GT, we detected by qPCR a low but consistent copy number of vector DNA bio-distributed to the GT following skin vaccination. Likewise, we found a significant increase in the frequency of innate cells infiltrating the GT in skin vaccinated mice relative to naive controls, notably an early wave of NK cells followed by CD11bLy6Cmonocytes that expressed CXCR9, the cognate ligand for CXCR3. Injection of naive mice intravaginally with a vector dose equivalent to the copy number detected by qPCR after skin delivery in the GT was associated with NK and monocyte recruitment. Moreover, NK cell depletion during vaccination significantly reduced the migration of skin primed CD8+ T-cells to the GT; the NK dependence

Collectively, the data demonstrate a novel mechanism for skin vaccination to induce low-level inflammation in the GT that is sufficient to promote CD8+ T-cell recruitment through a CXCR9 dependent mechanism licenced by NK cells and has important implications for vaccine designs against pathogens transmitted across epithelial barrier tissues.

P56 Study of a novel CpG oligodeoxynucleotide to promote vaccine ability against TB or Plu
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Bacterial or viral infection. In this report, we demonstrate that plasmacytoid dendritic cell (pDCs) were involved in the Th1-type Ab induction by nasal administration with diphtheria toxoid (DT) and G9.1 that is one palindromic CpG-containing phosphodiester (PO) CpG-ODN. Recently, CpG-ODN has been investigated as a therapeutic drug for cancer or allergy, whereas it is attracting public attention as the effective and safe vaccine adjuvant to defend against bacterial or viral infection. In this report, we demonstrate that plasmacytoid dendritic cell (pDCs) were involved in the Th1-type Ab induction by nasal administration with diphtheria toxoid (DT) and G9.1 that is one palindromic CpG-containing phosphodiester (PO) CpG-ODN. With unique polyguanosine-runs, now, we developed tuberculosis booster vaccine and nasal influenza vaccine using G9.1. Activation of Th1 and Th2 immunity was evaluated by cytokine production pattern and T-bet/GATA-3 ratio in human peripheral blood mononuclear cells and mouse bone marrow cells. Adjancutivity was evaluated in mice and guinea pig administered G9.1 with antigen through nasal or parenteral vaccination. G9.1 exhibited stronger IFN-γ-inducing activity and increased T-bet/GATA-3 ratio by enhancing T-bet expression. Nasally administered G9.1 plus DT induced DT-specific mucosal IgA and serum IgG, but not IgE, responses with antibiotics activity in C57BL/6 and BALB/c mice. Induction of Th1-type antibodies, but not Th2-type antibodies depended on pDCs. Enhancement of DTH was observed in the guinea pigs administered with tuberculoss booster vaccine candidates intradermally. Mice given nasal split vaccine plus G9.1 revealed significantly higher levels of influenza HA-specific StgA Ab responses in nasal washes. These results indicate that G9.1 is a promising pDC-dependent PO type Th1 immunity-enhancing CpG ODN as a vaccine adjuvant. These data suggest possible application of G9.1 to development of tuberculosis booster vaccine for BCG-primed populations and nasal influenza vaccine.
P57 Detection of endotoxins in veterinary autogenous vaccines produced in Italy: preliminary data.
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Zooprophylactic Institutes are public companies operating within the National Health Service that guarantee to veterinary services the technical support and scientific collaboration in hygiene and public veterinary health. Since last decades, Zooprophylactic Institutes produce diagnostic reagents, indispensable tools to combat infectious diseases of domestic animals. As it stated in Italian Ministerial Decree, Zooprophylactic Institute Umbria and Marche has the authorization for the production of veterinary autogenous vaccine and auto-vaccines, bovine and avian tuberculin, veterinary diagnostic reagents, antigens and keep vaccines stocks for emergencies.

Endotoxins are high molecular weight complexes associated with Gram-negative bacteria; they are the most significant source of pyrogens in pharmaceutical water, raw materials, parenteral products, and medical devices. It is a real concern for regulatory agencies. According to the Italian Ministerial Decree, for the first time conditions for using Limulus Amebocyte Lysate (LAL) assay as a finished product method were established, but today animals are the gold standard for endotoxins test.

According to Article 47 of Directive 2010/63/EU (3), there is a strong hint to develop and validate alternative methods to determine endotoxins contamination in pharmaceutical products. Being trend to replace animal testing with in-plate methods, we evaluated the amount of endotoxins using LAL assay. Specifically, we used two different techniques as gel clot and chromogenic LAL test in 13 different lots of autogenous veterinary vaccines produced at the Pharmaceutical Unit, Zooprophylactic Institute Umbria and Marche. Furthermore, we analyzed water, adjuvants and different plastic support used routinely in the production chain.

The endotoxins amount found in autogenous veterinary vaccines obtained from Gram-negative bacteria, ranges between 1.49 EU/ml for Moraxella spp. and 13.38 EU/ml for Salmonella abortus oris: 0.081, 0.107 and 0.118 EU/ml for water, aluminum hydroxide 20% and glycerin-phenol 0.04% solution, respectively. Considering that there is still no limits for endotoxins in veterinary autogenous vaccines, in these Italian products the amount is sensible lower compared to the human vaccine limits previously mentioned.

This is the first report of endotoxins evaluation in Italian veterinary autogenous vaccines. Further evaluations will implicate the monocryte activation test (MAT) to detect Gram-positive contamination in different veterinary autogenous vaccines lots, produced from Gram-positive bacteria. These preliminary results represent an interesting point to continue this survey, it could be useful to perform LAL and MAT test as quality control step for vaccines and other diagnostic reagents production in the Pharmaceutical Unit.

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P58 Development of a VLP Based Vaccine for Zika
Darly Manayanip
PaxVax

Zika has resurfaced as a public health emergency and has spread to several countries in the Americas. Zika, a flavivirus transmitted primarily by mosquitoes, can cause serious birth defects during pregnancy, and in adults it leads to increased incidence of Guillain-Barré syndrome. Hence, there is an urgent need for a vaccine. Virus like particles (VLPs) adopt an antigenic conformation similar to infectious virus, yet lacks the viral genome thereby providing a safer alternative for vaccine development. The current study describes the preclinical development of a VLP vaccine for Zika, derived from the pre-membrane (prM) and envelope (E) proteins of the Zika virus. Three PrME sequences were evaluated for VLP expression: Brazilian (SPH 2015), African (MR766) and a chimeric (African prM, Brazilian E ectodomain, African E stem-anchor). The secretion signals evaluated for prM were Interleukin 2 (IL-2), tissue plasminogen activator (TPA) and native Zika secretion signal (ZSS). The constructs were transiently transfected into suspension adapted 293 cells and the extent of protein secretion in the culture supernatant was evaluated. Western analysis indicated that the chimeric construct with the IL-2 secretion signal demonstrated the most VLP expression in 293 culture supernatant. VLP production was scaled-up by transient transfection of suspension 293 cells with chimeric construct in shake flask cultures. Culture harvest from 293 cells were clarified by depth filtration and concentrated by tangential flow filtration. VLPs were purified from the retentate by tandem ion exchange chromatography using Captoheco 700 (GE Healthcare) and Sartobind salt-tolerant membrane chromatography (STIC) (Sartorius) in a flow-through mode. Identity of putative purified VLPs were confirmed by western blot analysis and purity by Coomassie staining. Furthermore western analysis of purified VLP with anti-M antibody indicated that the purified VLPs were predominantly in the mature state. Purified VLPs were quantitated by BCA assay and visualized by negative staining and transmission electron microscopy confirming that the target VLPs were purified by flow-through mode. CB6 F1 hybrid mice were immunized intramuscularly twice with a dose titration of purified Zika VLPs with and without alum adjuvant. Immunogenicity of VLPs were assessed by Zika envelope (E) or VLP-specific ELISA and the neutralizing activity of immune sera was measured using reporter Zika virus particles (Integral molecular). Both the non-adjuvanted and the alum adjuvanted VLPs induced neutralizing antibody. Five to ten fold increase in antibody titer was observed after the booster dose. A dose response was observed with non-adjuvanted VLP but the dose-sparing effect of alum was most apparent at the lower VLP doses. In summary, we present the design, production and characterization of potential VLP based Zika vaccine candidates. The purified VLPs were immunogenic as evidenced by the induction of highly neutralizing antibody. Studies in a mouse challenge model are ongoing to evaluate the protection offered by VLPs following active immunization and after passive transfer of immune sera. Further optimization and scale-up of upstream and down-stream processes for VLP production and purification are also in progress to generate VLPs for non-human primate (NHP) studies.

P59 Promotion of immunity of pig to porcine circovirus-2 vaccine with recombinant Tibetan pig interleukin-12 plasmid in chitosan nanoparticles
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To enhance the immunity of pig against porcine circovirus, a potent adjuvant to cellular immunity to PCV2 vaccine, indicating that VRIL-12-CNP is a potent adjuvant to enhance the immunity of pig against porcine circovirus.

P60 Stability modeling to predict vaccine shelf-life and evaluate impact of temperature excursions from the “cold chain”
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The stability of vaccines is of great interest Industries and government institutions. Accelerated stability studies are designed to determine the rate of vaccine degradation over time as a result of exposure to temperatures higher than those recommended for product storage. However, commonly applied stability predictions based on application of zero- or first order kinetics are very often too simplified for description of the degradation of biological products, which frequently undergo complex and multidegradation reactions. We used an advanced kinetic approach mixing with statistical analysis to fit the forced degradation data (ELISA, NTA...) by computed kinetic parameters, and finally, to predict the long term stability of vaccine. The modeling approach is based on the selection of the most appropriate kinetic equations which fit the degradation rate of compounds subjected to elevated temperatures, accelerating the rate of the reaction.

According to few months data obtained at elevated storage temperatures, “two-step” models were identified to conveniently describe antigenicity of vaccine. We have predicted 2 years antigenicity. The stability modeling procedure was also applied for the prediction of antigenicity during several temperature excursions, thereby demonstrating the accuracy of the kinetic model.

To the best of our knowledge, this is the first procedure mixing a global kinetic approach and modern statistical analyses to accurately determine a vaccine degradation rate able to predict shelf-life of bio-products stored in refrigerated condition and suffered temperature excursions from the “cold chain”. 
P61 Modeling Oral Cytomegalovirus Transmission and Vaccine Efficacy in vitro
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Cytomegalovirus (CMV) causes sensorineural hearing loss and developmental disabilities in newborns when infections are acquired in utero. Pregnant women may acquire CMV from oral exposure to CMV in urine or saliva from young children. Neutralizing antibodies in maternal saliva have the potential to prevent maternal infection and, in turn, fetal infection. To assess the potential for antibodies to disrupt oral acquisition, CMV from culture-positive urine samples (uCMV) was used to study cell tropisms and sensitivity to antibody neutralization. uCMV entered epithelial cells poorly compared with entry into fibroblasts. High concentrations of CMV/perimembrane globulin or monoclonal antibodies targeting highly potent neutralizing epitopes in three viral envelope glycoprotein complexes had little or no impact on entry of uCMV into either fibroblasts or epithelial cells. Addition of complement, prolonged storage at 4 °C, or treatment with trypsin or saliva failed to render uCMV sensitive to antibody neutralization. However, both recombinant antibody neutralization and inefficient epithelial entry were lost after one passage in cultured fibroblasts. Consistent with this, antibodies could effectively limit viral spread within epithelial monolayers if added 24 h after uCMV inoculation. These results suggest that CMV virions that are shed in urine have a different composition from CMV virions that are produced by cells in culture. The underlying mechanisms are not known, but two are likely: (1) uCMV virions could enter cells using the established viral entry glycoproteins but contain factors or modifications that prevent antibodies from accessing neutralizing epitopes within those glycoproteins; (2) uCMV virions could utilize a fundamentally different entry mechanism that does not depend on the established viral entry glycoproteins. These findings have important implications for CMV vaccines. First, the failure of uCMV to enter epithelial cells efficiently calls into question the widely held assumption that CMV targets epithelial cells in the oral mucosa as its initial site of entry into a new host. Second, vaccine-induced antibodies in saliva may be ineffective in preventing maternal oral CMV acquisition; however, once CMV has infected cells in the oral cavity, antibodies in blood or tissues may restrict subsequent viral spread, thereby limiting dissemination and reducing or preventing fetal infection or disease.

P62 Evaluation of the chimeric Japanese encephalitis vaccine (IMOJEV) to protect also against Yellow fever
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Introduction: Yellow fever virus (YFV) 17D is among the most effective vaccines available today, protecting against YFV-induced devastating liver disease. However, the recent urban YFV outbreaks in Angola and Brazil highlight the severe vaccine shortage in an emergency setting. Further, potential spreading of YFV to Asia, where the Aedes aegypti vector mosquitos are abundant, poses a serious future concern. Japanese encephalitis virus (JEV) is a related mosquito-transmitted Flavivirus that is circulating in South-East Asia can cause severe neurological symptoms. The chimeric YFV 17D-derived live-attenuated Japanese encephalitis vaccine (IMOJEV), in which the pre-membrane and envelope genes of YFV 17D are replaced with those of JEV, is approved for human use against JEV.

Methods: Other than protecting against infection with JEV, we here evaluated the additional protective effect of IMOJEV against YFV 17D in a stringent lethal challenge model using AG129 mice. These mice are deficient in the interferon-α/β- and -γ receptors and thus are highly susceptible to YFV 17D infection.

Results: Almost all (n=30/31) IMOJEV-vaccinated animals survived the challenge with a 1000-fold lethal dose-50 of YFV 17D and did not show any signs of disease. In contrast, this dose was 100% lethal in non-vaccinated animals (n=31), with mean days to death of 16 ± 3. Already 7- or 14-days post vaccination, IMOJEV provided 60% or 100% protection (n=5) against the YFV 17D challenge, respectively. IMOJEV vaccination of AG129 mice induced neutralizing antibodies only against the JEV but not against YFV 17D. These results are in line with the fact that IMOJEV encodes for YFV 17D preM and E, mediating virus infection.

Conclusion: IMOJEV protects AG129 mice against YFV 17D independently of neutralizing antibodies. This cross-protection may be mediated via immunity to the YFV 17D non-structural proteins that are encoded by IMOJEV. Consequently, our data suggest that, due to its chimeric nature, IMOJEV may serve as efficient dual vaccine against both, JEV as well as YFV.

P63 Combination therapy of flagellin-adjuvanted cancer vaccine and radiotherapy induces long-term protection in a mouse cervical cancer model
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Accumulating evidence demonstrates that radiotherapy enhances anti-tumor immune responses induced by immunomodulatory therapeutic approaches. Radiotherapy (RT) is widely used cost-effective therapeutics for the various cancers having accompanying adverse effect. Recently it has been reported that flagellin derivatives significantly reduced the severity of radiation-induced side effect and accelerated tissue recovery. We previously demonstrated that flagellin potentiates tumor antigen-specific CD8+ T cell immune responses through TLRS signaling in a TC-1 cancer immunotherapy (IT) model. And intravitreal (IVAg) co-administration of EG/7 peptides with flagellin resulted in tumor suppression indicating flagellin is a potent vaginal adjuvant for a therapeutic peptide cancer vaccine. In this regard, we examined whether flagellin can be used as an adjuvant performing dual role of radioprotection and immunomodulation in an RT/IT combinatorial cervical cancer therapeutic model. When the tumor-bearing mice (5-8 mm in mean diameter) were locally received 20 Gy single dose irradiation, tumor growth was significantly reduced. Additional administration of flagellin-adjuvanted peptide vaccine showed comparable inhibitory effect on tumor growth. Surprisingly the combination therapy of flagellin-adjuvanted cancer vaccine and radiotherapy induced eradication of tumor mass and long-term memory protection against the re-challenge of the same tumor. These results suggest that flagellin is a promising radioprotective adjuvant for RT/IT combination therapeutic modalities against intractable cancers.

P64 Application of Systems Biology approaches for evaluating the vaccine and adjuvant safety at the preclinical and lot release test
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Vaccine is most effective tool for preventing the infectious diseases. Most of vaccine components are derived from pathogens, thus, the safety and consistency in a lot-to-lot basis are needed to ensure in the preclinical phase and after the licensure. Recently, vaccine development platform has been modernized such as introduction of recombinant protein and adjuvant technology to improve immunogenicity. However, assessment methods for vaccine and adjuvant safety has not changed for long time. Here we show that application of systems biology approaches enable to evaluate vaccine and adjuvant safety. We focused on the influenza vaccine as most diverse vaccine platform. We injected the influenza HA vaccine (HAV) and whole particle influenza vaccine (WPV) as a toxicity reference to rat, intraperitoneally and obtain the global gene expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively. We identified 18 genes as biomarkers that can distinguish WPV from HAV and used these biomarkers to quantify expression profile and found that gene signatures in lung clearly differed in each vaccine, respectively.
P65 Innate myeloid responses are highly diverse and strongly differ after the priming and the boosting immunizations

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Innate immune responses to vaccines are critical in the establishment of specific immune memory. A better understanding of these early events may therefore be critical in the design of vaccines to orientate the consecutive adaptive immune response toward a stronger, more efficient protection against pathogens with better safety profile and long term efficacy.

Here, we longitudinally studied the phenotypes and dynamics of blood innate myeloid cells after a first and a second immunization of cynomolgus macaques with the Modified Vaccinia virus Ankara (MVA) attenuated vaccine.

Neutralophils, monocytes and cDCs were transiently impacted in number by vaccination as expected, but without any major difference between the first and the second immunization. The local and systemic inflammation differed between the prime and boost though. Erythe- ma and edema at the site of MVA injection were moderate after the prime, and milder and faster resolved after the boost. Plasma CRP and CXCL10/IP-10 concentrations were increased after both injections but at lower levels after the boost. Mass cytometry allowed to phenotype blood cells mobilized after vaccination in depth. It appeared that some cell subsets actually responded differently after the first and the second vaccine injection, including some being enriched only after the prime or only after the boost. The key features contributing to differences between the innate myeloid cell responses to prime and boost were identified using multivariate analyses. Cells discriminating the best the response to the second injection from the first one were more activated/mature and expressed higher levels of CD45, HLA-DR, Fc receptors CD16 and CD32, integrins CD11a, CD11b, CD11c (some of which form complement receptors), chemokine receptors CCR5 and CXCR4, inflammatory cytokines IL-6, IL-12, CXCL10/IP-10 and anti-inflammatory cytokine IL-10.

Our work revisits innate immunity by demonstrating in vivo that innate myeloid responses differ in quantity, quality and kinetic after one or two vaccine administrations. These new insights constitute the basis for future studies aiming to decipher the differences of innate response after one or two vaccine encounters depend on primary memory responses and conversely how they impact the development of secondary memory responses.

P67 RNAlatter (an ammonium sulfate based solution) is not a suitable inactivating agent for developing attenuated fish viral vaccines

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Ammonium sulfate is a common component of many vaccine formulations. In fish, ammonium sulfate has been examined as an inactivating agent for production of attenuated bacterial vaccine, which resulted in protection of fish when compared to the unvaccinated control group. However, the ability of ammonium sulfate to attenuate fish viruses have not been explored in depth. RNAlatter is one specific formulation of ammonium sulfate that is often used to preserve nucleic acids and proteins, but whether fish viruses are stable and remain infectious after storage in RNAlatter has not been examined. Therefore, the goal of this work is to evaluate the effect of RNAlatter on fish viruses, particularly the ability of fish viruses to maintain infectivity after storage in RNAlatter and their thermal stability in RNAlatter. Viruses from three different families with diverse structural properties were examined; they are; viral hemorrhagic septicemia virus (VHSV) of the Rhabdoviridae family, infectious pancreatic necrosis virus (IPNV) of the Birnaviridae family, and Chum salmon reovirus (CSV) of the Reoviridae family. The viruses were stored in RNAlatter or DPBS control for up to 30 days at either -80 °C, 4 °C, room temperature, or 37 °C. At room temperature and lower, all viruses maintained their infectivity after storage in RNAlatter for at least 30 days. At 37 °C, when stored in RNAlatter, the viruses maintained infectivity longer than in DPBS. VHSV remained infectious at 37 °C for at least 1 day in RNAlatter but was inactivated completely in DPBS over the same period. IPNV remained infectious for at least 30 days in RNAlatter at 37 °C, but in DPBS, it was infectious at day 7 but inactivated by day 30. As with IPNV, similar results were observed with CSV. Therefore, these results suggest that RNAlatter and ammonium sulfate would not serve as a good inactivating agent for making attenuated viral vaccines. RNAlatter does offer some thermal stability to these viruses, which could possibly extend to their individual antigenic components; however, the latter point would require further experiments to confirm.

P66 Live-CMV Vaccines Lacking Viral MHC I Homologs are Highly Attenuated, but Confer Sterilizing Immunity against Congenital CMV Infection in an Animal Vertical Transmission Model

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Background: A vaccine against congenital cytomegalovirus (CMV) is an urgent public health priority. Subunit vaccines designed to elicit antibody responses to viral envelope glycoproteins and/or cell-mediated immune targets have shown some success in clinical trials, but live attenuated vaccines may provide optimal protection, given that such vaccines can induce immune responses to a much wider range of virally encoded proteins.

Potential safety concerns about live virus CMV vaccines, however, have limited enthusiasm for developing this platform. The goal of this work was to generate highly attenuated but efficacious live virus vaccines against congenital CMV infection by targeting viral immune modulation genes for deletion, using a bacterial artificial chromosome (BAC)-based recombineering system.

Methods: CMV's evade NK cells by expressing virally-encoded major histocompatibility class I (MHC I) homologs. Guinea pig cytomegalovirus (GPCMV) encodes three putative MHC I homologs: gp147, gp148, and gp149. A GPCMV lacking all three MHC I homologs was attenuated in vivo but retained immunogenicity and efficacy comparable to parental wild type (WT) virus when used as a live vaccine. Individual targeted knock-outs of each ORF were generated by BAC recombineering. These viruses were characterized and used as preconception vaccines in seronegative guinea pigs. Pregnancy was established and immunogenicity and efficacy of the vaccines compared. A novel INF-γ assay examined cytokine responses in vaccinated and control animals.

Results: We confirmed gene expression of these MHC I genes by detection of mRNAs encoding gp147, gp148, or gp149 during viral infection. Gp147 and gp149 were glycosylated when transiently expressed as epitope-tagged proteins. Viruses with individual gene knock-outs Δ147, Δ148, and Δ149 were generated by BAC recombineering and evaluated in vivo. Δ148 had no discernable phenotype. Δ147 and Δ149 were cleared from blood 7 days earlier than WT and failed to induce transient elevation of blood IFN-γ mRNA that occurred following WT infection (p<0.01). Neutralizing and GPCMV-binding antibodies induced by Δ147 and Δ149 were lower on day 14, but indistinguishable from WT on days 28 and 70 post-inoculation. In a pre-conception vaccination mid-gestation challenge model, efficacy of vaccination with Δ147 or Δ149 was comparable to WT. Maternal viremia occurred in naïve dams at 5.3x105 copies/ml in naïve controls to <100 copies/ml in vaccinated animals, and fetal infection declined from 35% in naïve animals to 0% in all three vaccine groups (p < 0.01). Average pup weight improved from 65 grams for pups born to naïve dams to 89, 92, or 90 grams for pups born to Δ147-, Δ149-, or WT-immunized dams, respectively (p<0.001). Pup mortality declined from 83% in naïve dams to 0, 10, and 21% in Δ147-, Δ149-, or WT-vaccinated dams, respectively (p<0.0001).

Conclusions: Live virus CMV vaccines can be attenuated, enhancing safety, yet retain immunogenicity and demonstrate protective efficacy against congenital CMV infection.

These results support the hypothesis that gp147 and gp149 function as NK evasins. Deletion of MHC I homologs could enhance the safety and immunogenicity of a live human cytomegalovirus vaccine.

P68 The Impact of Ten-Valent Pneumococcal Conjugate Vaccine (PCV10) on Streptococcus Pneumoniae Nasopharyngeal carriage Rates: Phenotypic and Genetic Diversity of Isolates from Addis Ababa, Ethiopia

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Background: Ten-valent Pneumococcal conjugate vaccine (PCV10) was introduced in Ethiopia in October 2011, but its efficacy and impact on disease require large prospective studies. This study was done to determine the impact of PCV10 vaccine by evaluating nasopharyngeal carriage, and analyze the phenotypic and genetic diversity of pneumococcal isolates.

Material and methods: A total of 789 newborn babies were enrolled at the age of 6 weeks at the time of their first PCV10 vaccine. Two hundred six and 201 babies were re-sampled at the age of nine months and two years after completion of PCV10 respectively. Nasopharyngeal swabs were taken for bacteriological analysis before vaccination at the age of nine months and two years after completion of PCV10 respectively. Nasopharyngeal swabs were taken for bacteriological analysis before vaccination at the age of six weeks and after completion of vaccine at the age of 9 months and 2 years. A total of 325 pneumococcal isolates were serotyped and characterized by Pulsed Field Gel Electrophoresis (PFGE) and 12 isolates analyzed by multilocus sequence typing (MLST).

The carriage rate of Streptococcus pneumoniae at the age of 6 weeks, 9 months and 2 years were 26.6%, 56.8% and 47.6% respectively. A total of 61 serotypes of Streptococcus pneumoniae were identified from 325 isolates, and 6A, 11A, 15B, 23F, 15A and 19F dominated in decreasing order. Twenty percent and 11.1% from six weeks and nine months isolates respectively were covered by PCV10 vaccine. Molecular typing also showed the diversity of isolates.

This study highlights the presence of very diverse serotypes in the country, and PFGE and MLST result indicates case of a possible capsular switching event.
P69 DNA immunization with drug resistant HIV protease gene protects mice from the establishment of syngenic tumors expressing this, but not the homologous HIV proteases
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The development of therapeutic HIV vaccines needs the models to prove the efficacy of the vaccine prototypes in small laboratory animals. The suitable approach can be challenging of immunized animals with syngenic tumorigenic cell lines stably expressing HIV antigens. Protease sequences from treatment-naive patients infected with the FSU-A HIV-1 strain, predominant in the former territory of USSR were selected, aligned and used to create a consensus PR (PR_A). Consensus humanized gene was synthesized (Evronog, Russia). An inactivation mutation D25N was introduced into PR_A to increase expression (PR_Ai). PR_Ai-coding sequence was cloned into pVax1 for eukaryotic expression. Primary drug resistance mutation pattern conferring resistance to the inhibitors NFV, SQV, IDV, RVR, ATV and LPV, likely to occur in the FSU-A strain (M46I, IS4V, V82A) was selected based on data available in the Stanford HIV drug resistance database. Mutations were introduced into the pVaxPR_Ai by site-directed mutagenesis to generate inactivated drug-resistant PR variants with mutations M46I/IS4V (PR_Ai2mut) and with M46I/IS4V/V82A (PR_Ai3mut). PR_A genes were cloned into lentiviral vector RRL.SIN.PKG(Evrogen), viable lentivirus was obtained only in case of PR_Ai3mut. The latter were used to transduce murine mammary adenocarcinoma cell line expressing luciferase (4T1UC2, Perkin Elmer) at multiplicity of infection of 20; heterogenic cell line 4T1UC2 PR_Ai3mut was obtained with >98% cells expressing drug-resistant inactivated HIV protease as was confirmed by genomic DNA and total mRNA analysis.

P69 (continued)

BALT/c mice were primed and after one month boosted by 2x20ug i.d injections of pVax-PR_A (Group I, nn 1-5), pVax-PR_Ai2mut (II, nn 1-5), pVax-PR_Ai3mut (III, nn 1-5), or pVax1 (IV, nn 1-5) followed by electroporation (BEX Ltd, Japan). Mice 6-8 in each group with mock-immunized with PBS. Forty days post-prime animals were challenged by sc injection of 10^4 4T1uc2-PR_Ai3mut cells. Tumor growth was monitored by in vivo bioluminescence imaging (BLI, Spectrum CT). After 24 days, mice were sacrificed, primary tumors, lungs, livers and spleens were excised for immunohistochemical analysis. All mock-, vector-, pVaxPR_Ai and pVax-PR_Ai2mut-immunized mice developed palpable tumors. Five pVaxPR_Ai3mut-immunized mice, only one developed single solid tumor which was 15-times smaller than tumors in other mice; none had metastasis in either lungs, liver or spleen. In vitro immune response of murine splenocytes to PR-derived peptides was evaluated by dual IFN-γ/IL-2 Fluorospot (Mabtech, Sweden). Only DNA-immunization with PR_Ai3mut induced a sustained immune response which inhibited growth of PR_Ai3mut-expressing tumor cells, other immunizations were not protective. Thus, immunization with plasmid encoding drug-resistant HIV-1 enzyme provided close to sterilizing immunity against tumor cell expressing this, but not the homologous protein variants. This is a proof of concept that DNA-immunization can induce cytolytic immune response specifically recognizing regions of drug resistance mutations. Finding that a single amino acid mutation in the immunogen can abrogate its ability to prevent growth of antigen-expressing tumor cells is crucially important for the development of cancer vaccines. Supported by Russian Science Fund 15-15-30039 and Russian Fund for Basic Research 17-04-00583. Mobility and method acquisition was supported by PI project 19806/2016TP of the Swedish Institute, Horizon 2020 Tuckering project VACTRAIN 692295.

P70 Induction of genotype-cross reactive, hepatitis C virus-specific cell mediated immunity in DNA-vaccinated mice.
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A major aim of any hepatitis C virus (HCV) vaccine is to generate multiantigenic, multi-genotypic responses, more likely to protect against challenge with the range of genotypes and subtypes circulating in the community. The use of cocktail vaccination regimens and vaccines encoding consensus HCV sequences are attractive approaches to achieve this goal. We previously reported that a cytoplasmic DNA vaccine (PRF-DNA) encoding perforin (PRF) significantly augments anti-viral T cell immunity as PRF-DNA vaccine more efficiently and durability activates dendritic cells to present antigens to CD8+ T cells, compared to canonical DNA. Here, in a series of mouse studies we compared the efficacy of the PRF-DNA vaccine encoding a consensus HCV non-structural (NS) SB protein containing gt1b and gt3a T cell epitopes with that of a cocktail encoding the respective gt1b and gt3a proteins. In another study, the efficacy of a multiantigenic cocktail regimen encoding gt1b and gt3a HCV NS3/NS4A/ NS4B/NS5B was compared with the responses elicited by DNA vaccines encoding their genotype-specific counterparts. As a PRF-DNA vaccine encoding multiple NS proteins (NS3/4A/4B/5B) from gt3a elicits responses to each of the encoded proteins, we examined cross-reactivity against NS proteins from gt1b and vice versa, and compared this with the cross reactivity elicited by a gt1b/gt3a cocktail vaccine using the same regimen. In addition to ELISPOT we exploited a novel fluorescent target array (FTA) to evaluate cytotoxic T lymphocyte (CTL) and T helper (Th) cell responses against numerous HCV epitopes-pulsed targets from gt1b and gt3a in vivo.

P70 (continued)

Following immunisation with PRF-NSSB vaccine, CMI responses showed that the cocktail NSSB (gt1b + gt3a) regimen induced higher responses than the consensus NSSB. Studies with the gt1a vaccine (gt1a) demonstrated that not all the epitopes encoded in this vaccine were targeted. This is consistent with previous reports that the resistance of HCV has not been considered as a major limitation for vaccine development. The results of this study suggest that the breadth of the T cell responses to HCV epitopes is reduced when consensus sequences are used. This study revealed that the cocktail elicited in vivo CTL and Th cell responses to proteins from both genotypes while the breadth of these multi-antigenic, multi-genotypic T cell responses was greater than those observed following vaccination with DNA encoding the genotype-specific proteins. This represents an attractive translational strategy for development of universal HCV vaccines capable of inducing broad HCV-specific T cell responses against multiple genotypes to prevent persistent infection.
**P71 Major neutralizing epitopes of influenza A virus H5 hemagglutinin: Templates for a broad vaccine design**

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**Objectives:** Highly pathogenic avian influenza (HPAI) H5 subtype viruses cause serious outbreaks amongst chickens resulting in major economic losses in the poultry industry. Vaccination remains to be the best method to prevent and control influenza. However, rapid emergence of antigenically drifted H5N1 viruses, timeline for vaccine strain selection, poor growth properties of selected vaccine strains and the need for high-level biocontainment facilities in vaccine manufacturing are major drawbacks of current influenza vaccine development. Our study thus focuses on generating broadly protective monovalent vaccine against H5N1 by modifying major neutralizing epitopes of HA.

**Methods:** We developed an epitope modified non-pathogenic H5N3 (A/duck/Singapore/97) vaccine for broad protection against influenza H5 subtype. H5N3/HA mutant reassortant viruses with PR8 backbone and baculovirus surface-displayed HA mutants (BacHAm) were generated by mutating amino acids at the 140th loop and 190th α-helix of hemagglutinin. In this study, we modified the antigenic site by mutating amino acids in the 140th loop and the 190th α-helix of the HA of H5N3 (A/duck/Singapore/97) vaccine strain, which are key neutralizing epitopes of H5N1. These modified HA were used to generate H5N3/PR8 reassortant virus and BacHAm, mutants which were then evaluated for their immunogenicity and cross-neutralizing efficacy against H5N1 viruses in a mouse model.

**Results:** The results showed that mice immunized with RG-H5N3/HA mutant-2 induced HAI and cross-neutralizing antibody titers against most H5N1 clade 2.2.1.3, 2.2.1, 2.2.3, and 2.3.2 viruses as compared to mice immunized with either RG-H5N3mutant-1 or RG-H5N3. Also, the cross-neutralizing efficacy of RG-H5N3/HA mutant vaccines were confirmed by testing reactivity with reference chicken anti-H5N1 clade 2 virus sera.

**Conclusion:** Our findings suggest that the use of non-pathogenic H5 viruses antigenically related to HPAI-H5N1 allows for the development of broadly protective vaccines and reduces the need for BSL3 containment facilities.

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**P72 Plant-made vaccines to papillomaviruses: the Cape Town story**

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Attempts to make prophylactic vaccines for human and animal papillomaviruses (PVs) using plants as a means of production date back nearly twenty years; however the first proof that this was even possible was only provided in the early 2000s, with the production at low yield in transgenic plants of L1 major capsid protein-based virus-like particles (VLPs) for HPV types 11 and 16, and for cottontail rabbit PV. The rationale for using plants was that they could provide a high-yielding eukaryotic production host that could produce vaccine protein much more cheaply than conventional cell culture-based approaches. However, the first feasible prophylactic HPV-16 L1 VLP vaccine candidate was only made in our laboratory in 2007, via Agrobacterium-mediated transient expression at high yield of a human codon-use optimized HPV-16 L1 gene in tobacco. This produced stable VLPs that elicited high-titre neutralising antibodies in mice, for a successful proof of concept. Since then, our group has gone on to successfully express L1-based VLPs for two low-risk (6 & 11) and seven more high-risk HPV types – one more than the Merck Gardasil-9 vaccine – to provide evidence for the feasibility of a multivalent plant-made HPV prophylactic vaccine. We have further produced VLPs from co-expressed HPV L1+L2 proteins, and bovine PV type 1 L1+L2.

Plant-made prophylactic vaccines to treat HPV infections and HPV-caused cervical cancer have a similar history, with expression of candidate vaccines coming from first generic and then transient expression. The E7 oncprotein of HPV-16 was a popular target; proofs of concept in animal models of human cancers showed the viability of the approach. Our group used a transiently-produced artificial E7-based fusion protein that accumulates in plants as ~10μm diameter protein bodies: these are high-yielding, easy to purify, and are self-adjuvanting, and are protective and therapeutic in a mouse C3 HPV-16-specific tumor model. Moreover, they are also effective in the form of a DNA vaccine.

We recently succeeded in making HPV-16 pseudovirions (PsVs) – L1+L2 particles containing a 5-8 kb genome-like circular dsDNA – in plants, by co-expressing the two proteins in the presence of a modified ssDNA plant virus-derived replicating vector. These PsVs could deliver DNA encoding a reporter gene under a mammalian promoter to mammalian cells, and could be used in a pseudovirus neutralisation assay with immune sera or MAbs. We have extended the range of PsVs to include HPV-18 and -35, and BPV-1, and are presently testing cellular delivery of a therapeutic DNA vaccine candidate based on our previous E7 protein body construct. If successful, this will pave the way for plant-made prophylactic vaccines against PVs, and of a PV PsV-based plant-made DNA vaccine.

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**P73 Mapping of the B-cell Epitope Breadth Elicited by Vaccination with H1N1 COBRA influenza virus**

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We previously described the design and characterization of computationally optimized broadly reactive influenza hemagglutinin (HA) antigens (COBRA) expressed on the surface of viral-like particles (VLP) vaccines. These COBRA HA expressing VLPs elicited a broadly reactive humoral immune response against divergent H1N1 viral strains. In order to dissect and characterize the COBRA elicited antibody responses, a panel of monoclonal antibodies (mAbs) was generated following COBRA HA immunization and characterized for their ability to bind to HA antigens belonging to different H1N1 isolates. In brief, different groups of BALB/c mice were intranasally, intraperitoneally or intramuscularly primed with COBRA HA viruses (named P-1, X-3 and X-6) as well as with a pandemic H1N1 strain (ca09) and then intraperitoneally or intramuscularly boosted 23 days post-infection (p.i.). Sera were collected at different time points (0, 21, and 37-59 days p.i.) and their biological activity evaluated by ELISA for HA binding and hemagglutination inhibition (HAI) activity. Sera derived from COBRA-immunized groups exhibited a broad binding and HAI activity against a panel of seasonal and pandemic H1N1 HA and viruses, while sera from CA09-immunized mice showed a very restricted binding and HAI activity profile. In order to better characterize the humoral immune response, splenocytes from COBRA P-1 immunized mice were collected and fused with SP2/0 myeloma cells for hybridomas generation. A panel of mAbs endowed with different binding specificities and biological characteristics was obtained. An extensive characterization of this mAb panel yielded a better understanding of the antibody affinity for specific HA epitopes, as well as the mechanism of the elicited antibody breadth by COBRA HA antigens. These mAbs were used to generate escape mutants in viruses with wild-type HA proteins to identify specific HA epitopes associated with broadly-reactive HAI activity and virus neutralization. Overall, the molecular dissection of the elicited immune response, is useful to analyze and understand the mechanism(s) of the antibody breadth, such as blocking of the receptor binding site or antibody dependent cytotoxicity (ADCC), elicited by COBRA HA proteins as prophylactic vaccines, as well as antigenic tools, for the design and generation of broadly-effective entry inhibitors.

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**P74 Vaccine-induced pre-existing immunity modulates both innate and adaptive immune components following influenza virus infection**

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In this work we investigated how pre-existing immunity provided by influenza vaccination can affect the outcome of sequential infections with influenza viruses of different subtypes. We took advantage of different efficiencies of intramuscular vaccination with trivalent inactivated vaccine virus (TIV). TIV vaccination in the quadriceps muscles of Baltic mice results in low HA-specific humoral antibody levels as assessed by ELISA and inhibition of hemagglutination. It can also modulate disease following sublethal infection, which correlated with protection of alveolar macrophages and control of virus replication. Host immune responses like cross-reactive T cell responses and pulmonary infiltration of myeloid cells following virus infection are still allowed and correlate with protection against reinfection with a heterotypic virus. Vaccination with the same TIV in the hamstring muscles is more efficient in induction of HA-specific humoral immunity, which correlates with better but not full protection during subsequent infection. TIV vaccination allowed but affected establishment of germinal center formation in both lung and draining lymph nodes and interfered with establishment of lung resident mucosal CD8+ T cells after vaccine-matched H1N1 infection. This restriction of cellular immune responses at the lung mucosal sites resulted in reduced protection during subsequent infection with a vaccine-mismatched H3N2 influenza infection. Vaccine-induced humoral immune responses are boosted by H1N1 infection and provide protection during reinfection with H3N2 virus in the absence of overt detectable lung resident CD8+ T cells and reduced germinal center B cells. Vaccine-induced influenza-specific CD8+ T cells in the periphery are recruited to lungs immediately following infection, but fail to turn into long term mucosal tissue resident T cells as observed in non-vaccinated animals. Finally we show that an innate stimulus delivered at the mucosal surface can delay onset of morbidity during reinfection but relies on pre-existing immunity in order to be protective.
P75 Murine adenocarcinoma cells stably expressing HIV antigens as a surrogate model of HIV infection for testing the protective efficacy of DNA vaccine prototypes in mice
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Tumorigenic cell lines stably expressing HIV antigens can be used to challenge laboratory animals immunized with HIV vaccine prototypes, to evaluate the protective potential of the immune response. This approach was tested here in mice DNA-immunized with variants of drug-resistant HIV integrase. Full-length sequences of 34 integrase genes of HIV-1 clade A prevalent in the territory of the former Soviet Union were translated and aligned, and the amino acid consensus was created. An inactive form of consensus clade A integrase (IN_Ai) was created by mutating the first residue of the integrase catalytic triad motif D64 to V. Inactive IN_Ai was further supplemented with mutations caused resistance against HAART drugs mutations of resistance to RAL: L74M, E32Q, V15I, N159H, G163R (IN_R1), and E138K, G140S and Q148K (IN_R2) (Shadrina O et al, 2014). The consensus humanized genes were synthesized and cloned into pVax1. Murine breast adenocarcinoma cell line expressing firefly luciferase 4T1Luc2 (Perkin Elmer) was subjected for lentiviral transduction at multiplicity of infection (MOI) of 1, and cell lines stably expressing IN_Ai (4T1Luc2, IN_R1,2) and IN_AiR2 (4T1Luc2, IN_R1,5) were generated (Evrogen, Russia). Cell lines with higher MOI, or expressing active IN could not be obtained due to IN toxicity. Mice were immunized and month later boosted by ID injection of 2x20 mg of plasmid pVaxIN_AR1 (Group I, n=1-5, pVaxIN_AR2 (II, n=1-5), or pVax1 (III, n=1-5) followed by electroporation (CUY2Etditl, Bex, Japan). Mice nn 6-8 in each group were mock-immunized with PBS. Forty days after prime group I was challenged with 104 4T1Luc2 IN_R1,2; II, with 105 4T1Luc2 IN_R1,5; III, with 4T1Luc2 cells injected subcutaneously. Tumor growth was monitored by in vivo bioluminescence imaging (BLI; Spectrum CT). After 40 days, mice were sacrificed, primary tumors, lungs, livers and spleens were excised. Tumor lumen was assayed for metastasis by ex vivo BLI. All mock- and vector-immunized mice challenged with 4T1Luc2 IN_R1,2, or 4T1Luc2 IN_R1,5, or parental 4T1Luc2 cells developed tumors sized >100 mm2. Among IN_AiR1-immunized mice, 2/5 had IN_Ai tumors 15-times smaller than in mock-immunized animals, and 3/5 were tumor-free. Among IN_AR2-immunized mice, 1/5 had tumors comparable to mock-immunized mice, 3/5 showed 3-5 reduction in tumor size, and one mouse was protected. On contrary to mock- or vector-immunized mice, IN-gene immunized mice had no metastasis in either liver, lungs or spleen. Protected and partially protected mice showed weak IN-specific cellular response manifested by IFN-, IL-2 and dual IFN-/IL-2 production in response to in vitro stimulation of splenocytes by peptides representing immunodominant CD4+ T-cell epitopes of IN. Mice with tumors demonstrated no IN-specific cellular response, and low responses to mitogens. This demonstrated that DNA-immunization with drug-resistant INs confers partial resistance to establishment of tumors expressing the immunogen and homologous IN variants. Tumorigenic cell lines expressing HIV-1 antigens are, thus, effective tools for identifying drug-resistant HIV vaccines in small laboratory animals. Experiments were supported by Russian Science Fund 15-15-30039 and Russian Fund for Basic Research 17-04-00583. Mobility and method acquisition were supported by Swedish institute PI project 19806/2016TP, and Horizon 2020 project VACTRAIN#692293.

P76 Biomimetically engineered demi-bacteria potentiate vaccination against cancer
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Introduction: Failure in enhancing antigen immunogenicity has limited the development of cancer vaccine. Inspired by effective immune responses towards microorganisms, we engineered demi-bacteria (DB) as a carrier for cancer vaccines.

Methods: DB were transformed from Bacillus via optimised hydrothermal treatment. Their performances on dendritic cell activation, antigen cross-presentation, T lymphocyte proliferation were investigated in vitro and ex vivo. Using ovalbumin as a model antigen, we also systematically verified the feasibility of the as-designed biomimetic vaccine in vivo for cancer therapy and prophylaxis.

Results: These DB with typical pathogen morphology exhibit intrinsic mannosse receptor agonism and an ideal hollow/porous structure to accommodate antigen and adjuvant, such as CpG. Therefore, multiple immunostimulatory elements integrate in a pattern closely resembling that of bacterial pathogens. Such pathogen mimicry greatly enhances antigen uptake and cross-presentation, resulting in stronger immune activation suitable for cancer vaccines. Indeed, DB-based biomimetic vaccination in mice induced synergistic cellular and humoral immune responses, achieving potent therapeutic and preventive effects against cancer.

Discussion: Application of microorganism-sourced materials thus presents new opportunities for potent cancer therapy. In the future, we will prepare DB from varying microorganisms using the current strategy and will screen for other candidates with high performance. Considering the facile guest component loading, we will also test the universality of the biomimetic vaccine formulation by developing defined tumor-associated antigens and other pathogen-derived immunomodulatory agents, promoting a further step toward clinical application.

P78 Enhanced gene expression and elicitation of host immune responses after optimization of pDNA formulation and delivery systems
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DNA-encoded monoclonal antibody (DMAb) and DNA vaccine strategies are currently being employed to target a myriad of diseases. The recent successes of this platform have been attributed to major advancements in the efficiency of the in vivo transfer of the transgene. The combination of novel pDNA formulations with electroporation has significantly enhanced in vivo gene expression, resulting in the elicitation of enhanced immune responses to DNA vaccines and increased systemic levels of DMAb. Furthermore, formulations have been developed which significantly increase the shelf-life of DNA drugs, permitting clinical DNA vaccine preparations to be stored at 4°C for at least one year. Here we will outline all these advances and provide specific examples of how they have been applied to advance the pDNA-based immunoprophylactic and immunotherapeutic platform.

P77 Flagellin as an adjuvant for mucosal vaccines
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The toll-like receptor 5 (TLR5) agonist flagellin is an effective adjuvant for vaccination. Recently, we demonstrated that the adaptive responses stimulated by intranasal administration of flagellin and antigen were linked to TLR5 signaling in the lung epithelium. The present study sought to identify the antigen presenting cells involved in this adjuvant activity. We first found that the lung dendritic cells captured antigen-bound and activated conventional dendritic cells (both CD11b+ and CD103+) involved in this adjuvant activity. We first found that the lung dendritic cells captured antigen-bound and activated conventional dendritic cells (both CD11b+ and CD103+) very efficiently in a process independent of TLR5. However, TLR5-mediated signaling was involved in this adjuvant activity. We first found that the lung dendritic cells captured antigen-bound and activated conventional dendritic cells (both CD11b+ and CD103+) involved in this adjuvant activity. We first found that the lung dendritic cells captured antigen-bound and activated conventional dendritic cells (both CD11b+ and CD103+) involved in this adjuvant activity.
P79 Virus-like particles coupled to magnetic microspheres as sensitive antibody detection reagents

Charles Shoemaker1,*, Keersten Ricks1,‡, Les Dupuy1,‡, Olivier Flusin1,‡, Matt Voorhees2,², Carolyn Six2,³, Catherine Badger2,³, Conner Schmaljohn1,‡, Randal Schoepf1,²,¢  1corresponding authors 1Authors contributed equally. "Virology Division, "Virology and Immunology Special Studies Group, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA; Enveloped viruses comprise the vast majority of pathogenic viral diseases affecting human populations. Viral glycoproteins present on the surface of these viruses are frequently the targets of vaccination strategies, as well as therapeutics. Measurement of antibody titer against these antigens is a common method of assessing vaccine response in human individuals and populations, as well as animal models. These antibody responses are usually evaluated via direct immunosassays (i.e., ELISA), using recombinant protein or inactivated viral materials, which can come with limitations due to antigenic fidelity, safety, and/or sustainability. For analytical approaches solely dependent on reactivity, virus-like particles (VLPs), which present the glycoproteins in a membrane-anchored conformation, are a desirable reagent because of their ease of manufacture, homogeneity, and lack of safety concern. Herein, we outline the design and implementation of a novel diagnostic reagent, which pairs the sustainability of a VLP with the sensitivity of magnetic immunosassays to serve as a versatile tool for detection of humoral response in a serum sample. As a proof of concept, Venezuelan equine encephalitis virus (VEEV) E1/E2 glycoproteins were incorporated onto a retrotiral core VLP. After characterization, the VEEV VLPs were conjugated to fluorescent, magnetic microspheres to create VLP-coupled microspheres (VCMs). These VCMs were shown to detect both IgG and IgM in non-human primate and clinical human serum samples, in two hours, with 100-fold greater sensitivity than traditional cell lysate-based direct ELISA. The VCMs were assembled to lymphoproliferation and storage post-lyophilization at 4°C, RT, and 37°C for at least one month. We also extended the VCM platform to other priority viral pathogens including Lassa virus (LASV), Ebola virus (EBOV), Marburg virus (MARV), and Crimean-Congo Hemorhagic Fever Virus (CHFV) to create an IgG-detection multiplex with similar assay sensitivities as singleplex assays. This VCM platform will serve as a powerful tool for assessing seroconversion in individuals or populations both pre- and post-vaccination.

P80 Recombinant modified vaccinia virus Ankara generating Ebola virus-like particles

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There are currently no approved therapeutics or vaccines to treat or protect against the severe hemorrhagic fever and death caused by Ebola virus (EBOV). Ebola virus-like particles (EBOV-VLPs) consisting of the matrix protein VP40, the glycoprotein (GP), and the nucleoprotein (NP) are highly immunogenic and protective in non-human primates against Ebola virus disease (EVD). We have constructed a modified vaccinia virus Ankara (VVA)-based recombinant co-expressing VPA40 and glycoprotein (GP) of EBOV Mayinga and the nucleoprotein (NP) of Tai Forest virus (TAFV) (MVA-BN-EBOV-VLP) to launch non-infectious EBOV-VLPs as a second vaccine modality in the MVA-BN-EBOV-VLP-vaccinated organism. Human cells infected with either MVA-BN-EBOV-VLP or MVA-BN-EBOV-GP showed comparable GP expression levels and transport of complex N-glycosylated GP to the cell surface. Human cells infected with MVA-BN-EBOV-VLP produced large amounts of EBOV-VLPs that were decorated with GP spikes but excluded the poxviral membrane protein B5, thus resembling authentic EBOV particles. The heterologous TAFV-NP enhanced EBOV-VP40-driven VLP formation with comparable efficiency as the homologous EBOV-NP in a transient expression assay, and both NP's were incorporated into EBOV-VLPs. The levels of EBOV-GP-specific neutralizing and binding antibodies induced by MVA-BN-EBOV-VLP and MVA-BN-EBOV-GP in mice were similar, raising the question whether the quality rather than the quantity of the GP-specific antibody response might be altered by an EBOV-VLP-generating MVA recombinant.

P81 Immune Profiling of Coxiella burnetii Vaccination and Infection by Mass Cytometry

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Background: Coxiella burnetii (Cb), a highly infectious and resilient intracellular bacterial pathogen, is the cause of Q fever, which can require months of antibiotic treatment. The current Cb vaccine is approved in Australia alone and reactogenicity can occur in persons previously exposed to Cb. An ongoing study will characterize the immune responses from additional donors. The Q-fever Cb vaccine is comprised of Cb intracellular (CI)-based direct ELISA. The CI-VLPs were stable to lyophilization and storage post-lyophilization at 4°C, RT, and 37°C for at least one month. We also extended the CVM platform to other priority viral pathogens including Lassa virus (LASV), Ebola virus (EBOV), Marburg virus (MARV), and Crimean-Congo Hemorhagic Fever Virus (CHFV) to create an IgG-detection multiplex with similar assay sensitivities as singleplex assays. This CVM platform will serve as a powerful tool for assessing seroconversion in individuals or populations both pre- and post-vaccination.

P82 INTRODUCING A NOVEL AND RELIABLE COMBINATORIAL THERAPY AGAINST ANTHRAX

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Bacillus anthracis (BA), the causative agent of anthrax, is a potential bioterrorism threat, for animals as well as humans. Mortality in the patients occurs due to bacteremia as well as toxemia. Protective antigen (PA), lethal factor (LF) and edema factor (EF) are the major virulence factors of BA. Lack of any worldwide licensed vaccine against this deadly disease needs a reliable vaccine or therapeutics which can target bacteria as well as its toxins. Till now, antibiotics are given to treat the disease by blocking the growth of BA but none of the strategy target secreted toxins (PA/LF/EF). Therefore, a combinatorial therapy involving toxin inhibitors together with antibiotics may prove a novel reliable treatment strategy against anthrax.

In order to search for potent anti-PA/LF candidates, high throughput virtual screening was performed. Two docking platforms - Glide high throughput virtual screening (HTVS) and Extra precision (XP) were used with their default settings for performing screening of a few databases targeting protective antigen (PA) and lethal factor (LF). The databases screened against the target proteins include Natural remedies, Sigma Myria compounds and a database of synthetic compounds. The study suggested TGG, QD (natural) and OP12, OP10 (synthetic) as the potential inhibitory molecules of PA-LF binding. Native-PAGE confirmed the specificity of these inhibitors towards oligomerization site of PA. Interaction of the inhibitors with PA/LF/EF was studied through Surface Plasmon Resonance. These inhibitors were found to have remarkable toxin neutralization potential in vitro. The efficacy of these inhibitors was analyzed by toxin neutralization assay. In vivo challenge studies in mice with virulent strain of BA showed significant protection level when administered along with antibiotics. Thus, these inhibitors can be the potential anti-anthrax compounds which with antibiotics can provide a best way to protect the host from rapidly spreading infection of BA.
In previous studies we showed that DNA vaccines expressing the codon-optimized GP genes of Ebola (EBOV) or Marburg (MARV) viruses protect both mice and nonhuman primates from viral challenge when delivered by intramuscular electroporation (IM-EP). To determine if we could achieve equivalent immunogenicity and protective efficacy in the absence of electroporation by using improved DNA vaccines, we tested co-expression of the EBOV GP DNA vaccine and eliciting EBOV-specific immune responses to obtain the best results. The molecular adjuvant genes evaluated included those for the Th1-inducing cytokine IL-12 and the granulocyte growth factor GM-CSF, both of which have demonstrated significant adjuvant effect when included in clinical DNA vaccine formulations. Additionally, we tested enhancement of IFN-γ/CpG production by a plasmid encoding the cytosolic RNA innate immune sensor retinoic acid-inducible gene 1 (RIG-I), which has been shown to be required for clearance of flavivirus infections. We also compared EBO GP DNA vaccine constructed in minimalized plasmids (Nanoplasmid™ vectors, Nature Technologies, Corp), which are smaller than traditional DNA vaccine plasmids and have been shown to have improved uptake, persist longer in transfected cells leading to increased transgene expression, and result in enhanced immune responses and improvements in immunological memory. Nanoplasmids expressing EBO GP were designed with or without a RIG-I agonist and CpG motifs. Additionally, we have used the nanoplasmid platform to develop and evaluate DNA-launched virus-like particle (VLP) vaccines that will generate VLPs endogenously within the host, eliciting EBOV-specific immune responses. Studies in mice demonstrated that intramuscular injection of the standard EBO GP DNA vaccine along with molecular adjuvants enhanced immunogenicity when compared to responses obtained with the EBO GP DNA vaccine alone. We also found that mice vaccinated by IM injection of the nanoplasmid DNA vaccines developed increased virus-specific antibody and cellular immune responses as compared to mice vaccinated with the standard EBO GP DNA vaccine. Furthermore, nanoplasmid vaccinated mice survived challenge with a lethal dose of ma-EBOV, suggesting that we may achieve protection equal to that obtained previously using EP delivery.

This study explored several prime-boost strategies in rhesus macaques using various novel hepatitis B virus (HBV) vaccines that showed promise as prophylactic and therapeutic approaches in our previous study using a mouse model. The tested vaccines included an HBV particle subunit (HBSS1) vaccine and the recombinant vaccinia (fVJS1,RVJC1) or DNA vaccines(pVRC-S1, pVRC-CS1),in which S1 containing S (1-232aa) and PreS1 (21-47aa),S1 containing Core (1-144aa) and PreS1 (1-42aa). The profiles and maintenance of humoral activity (IgG and neutralizing antibodies) and cellular immunity (interferon-γ production assessed by IFN-γ enzyme-linked immunosorbent spot (ELISpot) assay) against individual target(S, PreS1,Core) of HBV were investigated in a longitudinal study following various vaccination protocols until 98 weeks post-1st vaccination. We found that the anti-preS1 antibody was rapidly elicited after the first priming with pVRC-S1 and pVRC-CS1, while the anti-HBs or anti-HBc were not detectable until 2 weeks after the second or third DNA immunization. However, poor CMI against S, PreS1, and Core were detected after three immunizations with the DNA immunogen. Recombinant vaccine virus RVJS1 and RVJC1 boosting significantly enhance HBV specific humoral and cell-mediated immunity against all target antigens(S, PreS1, and Core), especially CMI against Core was most robust and maintained detectable until 98 weeks post-1st vaccination. Furthermore, the HBV S, preS1 and C specific antibody and CMI were enhanced after re-boostered with HBSS1 at 50 weeks. In addition, robust neutralizing activity was also detected among serum of the vaccinated rhesus macaques at week 52. In conclusion, the anti-preS1 antibody was rapidly elicited than anti-S or anti-Core antibody in rhesus macaques after vaccination. Furthermore, DNA/RVJ heterologous prime-boost elicits similar strong humoral immunity against all targets(S, PreS1, and Core) but more robust and lasting cellular immunity (CMI) against Core than S and preS1. The immunity against HBV S, PreS1 and C could be further boosted with HBSS1. Therefore, our data pave a way for optimization of HBV vaccine candidates and application in human beings, especially for therapeutic application.

The persistent public health threat of infection with the Middle East respiratory syndrome coronavirus (MERS-CoV) highlights the need for an effective MERS-CoV vaccine. In this study, we prepared and vaccinated mice with either a Spike protein or an inactivated MERS-CoV vaccine (IAV). To prepare the IAV, quantified MERS-CoV was inactivated with 0.4% formaldehyde and dissolved in phosphate-buffered saline (PBS) after purification. The S or N protein-specific IgG antibody responses and neutralizing antibody was induced by vaccine with combined adjuvant (Alum plus CpG), which significantly increased S-specific IgG2a, IgG2b, and IgG3 but not IgG1 at 2 weeks postimmunization. None antigen-specific T-cell immunity was detected after vaccination based IFN-γ ELISpot assay. Nine days after the last immunization with vaccine regimes, mice were transduced intranasally with 2.5×10^10 PFU of Ad5-hDPP4, then the transduced mice were infected intranasally with MERS-CoV (1×10^4 pfu). Three days post infections (dpi) with MERS-CoV, the lungs of mice were harvested for virological and pathological detection. Lung viral titers were detected in vaccinated mice after challenge. Compared with the control group, immunized mice showed slightly lower pathologic damage to the trachea. None hypersensitive-type lung pathology occurred when mice were given spike protein or an IAV and challenged with infectious virus. Protection against eosiophilic immunopathology by vaccines containing combined adjuvant (Alum plus CpG) correlated better with enhanced antigen-specific immunity, supporting the strategy in this study as a promising approach for MERS-CoV vaccine development. In addition, our data highlights the critical importance for development of effective and safe coronavirus vaccines of selection of inactivated strategy and adjuvant.
P87 Self-replicating RNA as a generic platform for vaccines against emerging infectious diseases
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Immune responses after nucleic acid immunization can be significantly boosted by using alphavirus replicons, which can be delivered either as self-replicating RNA (RREP) or DNA-launched RNA (DREP). The intrinsic immunostimulatory properties of alphavirus replicons as well as their ability to stimulate cellular and humoral immune responses make them well-suited for use as vaccines. While DREP has been extensively studied and showed its efficacy in a number of preclinical trials, its weakness is the fact that all DNA vaccines needs to be delivered into the nucleus of the transfected cell for initial transcription. As a consequence, only dividing cells will express the antigen thus limiting the efficacy of the vaccine. In contrast, an RNA vaccine would only need to be delivered into the cytoplasm of any cell to be able to express the antigen of interest. This makes RREP the platform of choice for the future and modern RNA technology has now reached the stage of development that allows clinical implementation. The rationale for this study was to compare head-to-head the immunogenicity and efficacy of RREP and DREP platforms. In doing so, we have chosen to use two models. The first one builds on the HIV EVA EU project and supports the continued development of these vaccines for HIV clinical trials. As a parallel model, we have chosen to express Chikungunya virus (CHIKV) antigens from the vectors, an infection model we have established in our laboratory, further clinical development of RREP as a generic vaccination platform.

P88 An online metadata profile catalogue and privacy and ethics recommendations for supranational vaccine pharmacoepidemiological research in Europe: an ADVANCE initiative
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Background The Innovative Medicines Initiative (IMI) project Accelerated Development of Vaccine benefit-risk Collaboration in Europe (ADVANCE) started in 2013, with the mission to establish a prototype for a sustainable, robust system that will rapidly provide best available scientific evidence on post-licensure vaccination benefits and risks for well-informed decisions by all stakeholders. Analyses of large population databases increase the ability to detect rare adverse events (AE) and conduct of supranational studies allow a better interpretation of data on vaccine benefits among EU member states. Clinical data in electronic health record systems (EHR) are a rich source for such studies. In order to rapidly launch pharmacoepidemiological studies using EHR, ADVANCE has created a platform of metadata from available data sources in Europe and has developed recommendations regarding data access, privacy and ethical requirements for cross-border data sharing.

Methodology and Results Based on a variety of existing surveys, a specific ADVANCE international research readiness (AIRR) survey was developed to collect information on data sources that might provide data to vaccine benefit-risk studies; supported by sophisticated metadata to facilitate rapid identification of suitable study sites. The survey was disseminated among the consortium and professional networks. The information was collected using a survey and has established a catalogue of metadata profiles on the European Medicinal Information Framework (EMIF) platform in order to provide ready access to researchers. Using a specific privacy and ethics guidance (PE-tool), technical safeguards and ethical clearance procedures were collected during a proof of concept study and reviewed. Finally, the new EU General Data Protection Regulation (GDPR) that will come into effect in 2018 and its impact on large-scale pharmacoepidemiological studies was assessed. The principle of data protection by design, the requirement of the conduct of a data protection and/or privacy impact assessment and the mandatory appointment of a data protection officer are critical components of the new EU GDPR.

Conclusions In ADVANCE, a European system is developed and tested for the conduct of rapid vaccine benefit-risk studies and pooling of data. In order to facilitate implementation of such systems, member states, facilitated by the European Commission, need to seek agreement on efficient interpretation, implementation and enforcement of the new General Data Protection Regulation and should be prepared to meet the new EU GDPR requirements.

P89 Elucidation of mechanism undergoing elimination of HPAI H5N1 virus in mice vaccinated with recombinant H5 HA virus based on vaccinia virus vector
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Background: Highly pathogenic avian influenza virus subtype A/H5N1 (HPAI H5N1 virus) has been considered a potentially serious pandemic threat. Therefore, the development of safe and effective vaccines is urgently required. So far, we had generated recombinant H5 HA vaccine based on highly attenuated vaccinia virus DIs strain (rDIs-H5 HA), and investigated the preventive effect of rDIs-H5 HA against lethal infection of HPAI H5N1 virus in both mice and cynomolgus macaques. In this study, we attempted to identify the precise mechanism by which rDIs-H5 HA protect animals efficiently from lethal HPAI H5N1 virus infection.

Methods: BALB/c mice were inoculated intradermally with rDIs-H5 HA at 1×107 PFU. At 4 weeks after the vaccination, these mice were infected with lethal dose of HPAI H5N1 virus [A/whooper swan/Hokkaido/1/08]. To clarify the role of immune cell subsets in the preventive effect of rDIs-H5 HA, we depleted either CD4+ cells or CD8+ cells from vaccinated mice using corresponding specific antibody. We further investigated the role of antibodies binding specifically for H5N1 virus in preventive effect of rDIs-H5 HA using Fc receptor-gamma deficient mice (FcRγ−/− mice).

Results: At 1 day after HPAI H5N1 virus infection, the pulmonary viral titers in vaccinated mice were approximately one-tenth compared to those in unvaccinated mice. We focused on the effect of binding antibodies to lower the viral titer at 1 day post-infection (dpi) in vaccinated mice, because our previous study demonstrated that neutralizing antibodies against HPAI H5N1 virus were not induced sufficiently in vaccinated mice before the challenge infection of HPAI H5N1 virus. In FcRγ−/− mice inoculated with rDIs-H5 HA, the initial viral titer was approximately 10-times higher than that in vaccinated-wild type mice. These results suggested that the interaction of FcRγ-bearing cells with binding antibodies specific for H5N1 virus contributes to lower the viral titer at 1 dpi in vaccinated mice. In addition, the increase of pulmonary viral titer 3 days and 6 days after HPAI H5N1 virus infection was observed in CD4-depleted mice that had been inoculated with rDIs-H5 HA. Furthermore, vaccinated mice could eliminate HPAI H5N1 by 9 dpi even though either CD4+ cells or CD8+ cells were depleted from them. Therefore, we have studied to clarify the cell subsets that play a critical role in eliminating the virus during late phase of HPAI H5N1 virus infection.

Conclusion: The preventive effect of rDIs-H5 HA is independent of antibody neutralization activities against HPAI H5N1 virus infection. Further study to elucidate the mechanism of the preventive effect of rDIs-H5 HA would propose a novel vaccination strategy.
Development of Tier 2 neutralization responses to an HIV-1 heterologous prime-boost vaccine regimen can be promoted by pox virus but not DNA priming

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With a HIV-1 burden of around 6 million people in South Africa, there is enormous incentive to locally develop a HIV-1 vaccine against the main prevalent subtype in sub-Saharan Africa (subtype C). In this study we aimed to generate a heterologous prime-boost inoculation regimen that elicits high-titre antibody responses to the HIV-1 subtype C envelope protein (Env) by comparing DNA or MVA priming vaccines followed by Env protein boosters. Furthermore, we investigated whether the formation of virus like particles (VLPs) induced by the inclusion of a HIV-1 subtype C mosaic Gag gene in the prime would increase immunogenicity. The HIV-1 subtype C Env gene used was derived from the super-infecting virus from donor CAP256 who later developed broadly cross neutralizing antibodies.

A HIV-1 Env gene construct was made which contains a flexible linker replacing the furin cleavage site to circumvent processing difficulties while maintaining structure. This was truncated to gp150 to increase stability in DNA and MVA vaccines. For MVA vaccines, gp150 was targeted into both MVA wildtype (MVAC5) and MVA mosaic Gag (MVAGC5) vectors, in the I8R-G1L locus. VLP formation from Gag-encoding MVA and DNA vaccines was confirmed by electron microscopy and gradient centrifugation. A gp140 version was expressed in HEK293 cells and shown to produce soluble trimeric Env. This was purified by lectin affinity chromatography and size exclusion chromatography. For immunogenicity studies, rabbits were primed at weeks 0 and 4 with 1*108 PFU of MVA or 100μg of DNA gp150 +/- 100μg mosaic Gag. All four groups were boosted with ~40μg of soluble gp140 in Alhydrogel® (1:1 v/v) at week 12 and 20. MVA groups received an additional boost at week 28. Serum was taken every 4 weeks and analysed using matching CAP256 Env binding ELISAs and viral neutralization assays.

In contrast to DNA priming, MVA primes elicited Env binding antibodies at all time points before protein boosting. However, peak binding antibody titres appeared to be the same for all four groups, with no effect of the Gag gene in the prime. Again, only MVA priming induced viral neutralization responses to subtype C Tier1A HIV-1 MW965.26 and Tier 1B 6644 starting at week 8. Most encouragingly, Env protein boosts of MVA primed animals resulted in autologous Tier 2 neutralization (5/9 rabbits), with IC50 neutralization values ranging from 1:28 to 333. When Gag was included in the prime, this was observed after the first protein boost (2/5 rabbits). Furthermore, there was a correlation between Tier 2 neutralization and lower Env antibody binding titres (p<0.01).

These results suggest that MVA priming should be further explored for an HIV-1 vaccine, and are planning to investigate different adjuvants.
HIV-1 CN54 gag 308–318 peptide (or the control targets). Phenotypic analysis revealed that in vivo killing was observed in the vaginal epithelium, as determined by recovery and flow immunodominant HIV-1 CN54 gag epitope were demonstrated. Consistent with this, robust retention for at least one year in the genital and respiratory tract. Such cells also co-

Stabilization of virus protein structure and nucleic acid integrity is challenging yet essential to preserve the transcriptional competence of live recombinant viral vaccine vectors in the absence of a cold chain. When coupled with needle-free skin delivery, such a platform would address an unmet need in global vaccine coverage against HIV and other global pathogens. It has been widely assumed that local vaccine delivery to the mucosae is necessary to functional memory CD8+ T-cell responses at the epithelial barriers. Here we investigated the immunising properties of a simple, fast dissolving microneedle array (MA) skin delivery system using a live recombinant human adenovirus vector (Ad) encoding HIV-1 CN54 gag in a biodegradable polymer matrix containing a disaccharide as preservative. Dried Ad MA immunisation in B6 mice induced CD8+ T-cell expansion and multifunctional cytokine responses in the spleen and skin draining lymph nodes that were equipotent with conventional injectable routes of immunisation. Moreover, this mode of antigen delivery to the skin via Ad MA immunisation also induced primary CD8+ T-cell responses in mucosal tissues. Within the female genital tract, high frequency tetramer specific CD8+ T-cells were detected. The droplets were shown to endow the adsorbed antigens with lateral mobility and force-dependent curvature changes during APC contact, which offered larger contact area to facilitate the co-internalization of the antigens and the droplets. Moreover, PPAS induced robust T-cell-mediated responses compared with other groups, which demonstrated simultaneous stimulation on Th1 and Th2 responses, with the strongest activation of genital centre in the draining lymph nodes, the highest OVA-specific antibody titers, the largest proportion of OVA-specific cytotoxic lymphocytes and central memory T cells in the splenocytes.


discussion: The special traits of the droplets (lateral mobility and elasticity) may have major implications on the robust T-cell-mediated responses, which may offer alternative inspiration for the rational design of elastic partuculate adjuvant systems.

Methods: PPAS were prepared by self-assembly of PLGA nanoparticles and antigens on the squaene droplets. And T-cell-mediated responses in mice were evaluated by the activation of germinal centre (GC) in the draining lymph nodes, serum antigen-specific antibody titers, proportion of antigen-specific cytotoxic lymphocytes and central memory T cells.

Results: PPAS were prepared with uniform sizes and dense arrays of OVA on the surface. And the droplets were shown to endow the adsorbed antigens with lateral mobility and force-dependent curvature changes during APC contact, which offered larger contact area to facilitate the co-internalization of the antigens and the droplets. Moreover, PPAS induced robust T-cell-mediated responses compared with other groups, which demonstrated simultaneous stimulation on Th1 and Th2 responses, with the strongest activation of germinal centre in the draining lymph nodes, the highest OVA-specific antibody titers, the largest proportion of OVA-specific cytotoxic lymphocytes and central memory T cells in the splenocytes.

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P97 Immunization with drug-resistant HIV RT gene protects mice from challenge with RT-expressing tumor cells

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The success of antiretroviral therapy turns HIV-1 infection into chronic controllable disease. However, drug resistance (DR) evolves which may render the therapy ineffective. This motivates the development of immunotherapies which would prevent or thwart resistance development. Here, DNA-immunogens against DR HIV were developed based on reverse transcriptase (RT) and their immunogenicity was tested in the tumor challenge model. For this, consensus HIV-1 RT clade A FSU_A strain (RT_A) was designed by multiple alignment of RT amino acid sequences from untreated HIV-1 patients using the MUSCLE engine, respective humanized gene was synthesized (Evoengine) and cloned into pVax1. RT_A was supplemented with primary mutations of resistance to NRTI (RT_A_N; K65R, M184V) or NNRTI (RT_A_NN; K103N, G190S) abundant in the Russian Federation. RT_A was supplemented with primary mutations of resistance to NRTI (RT_A_N; K65R, M184V) or NNRTI (RT_A_NN; K103N, G190S) abundant in the Russian Federation (Bobkova M et al, 2013) and inactivation mutations (in; D185N, D186N, E478Q) yielding pVaxRT_Ain, pVaxRT_Ain_N, and pVaxRT_Ain_NN. Expression of RTs in eukaryotic cells was confirmed by Western blotting. RT genes were cloned into lentiviral vector, the latter was used to transduce murine mammary adenocarcinoma cells expressing luciferase (4T1luc2, Perkin Elmer) at MOI of 10. Presence of RT-gene inserts in genomic DNA of daughter 4T1luc2 RT-bearing clones was confirmed by PCR, and RT gene expression, by detecting respective mRNA, and by Western blotting. Resulting clones preserved high tumorigenicity in BALB/c mice. We inquired if RT-DNA immunization can protect mice from establishment of RT-expressing 4T1luc2-tumors. For this, mice (5 per group) were immunized with 2x20 g RT-DNA delivered intradermally on days 0 and 28; injections were followed by electroporation (CUV21EditII, BEX Ltd). Forty days after prime mice were challenged with 104 4T1luc2 cells expressing RT variant with pattern of DR mutations corresponding to immunogen. Tumor growth was monitored for 19 days after implantation using in vivo bioluminescence imaging (BLI; Spectrum CT).

P98 Construction of a tri-specific antibody (tsAb) encoding IL-15 for CD16-mediated, ex vivo stimulation of NK cells via coupling to hemagglutinin neuraminidase (HN) of Newcastle Disease Virus (NDV)-infected tumors vaccines for cancer immunotherapy

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Positive effects on long-term survival of cancer patients are reported upon postoperative “antimumor vaccination”. However, although human tumor cells express tumor associated antigens (TAAs), immune responses against them are usually weak compared to responses against foreign microorganisms. Autologous tumor vaccines (TC-NDV) generated by patient-derived tumor cells (TC) modified through infection by NDV provides TAAs associated with proper danger signals via the viral cell surface-expressed HN protein. To further enhance the immunogenicity of the CT-NDV for ex vivo stimulation of natural killer (NK) cells in cancer immunotherapy, herein we report construction of an IL-15-encoding tsAb molecule that specifically bind to HN molecules and CD16 on the surface of NDV-infected TC and NK cells, respectively. The constructed immunoligand synapse triggers the activation of NK effector function for induction of cytokine such as interferon (IFN-γ) and tumor necrosis factor (TNF-α) and target cell death. IL15 as a signal for NK cell development, proliferation, and activation is supposed to further enhance the effect of CD16-HN bi-specific (bs) Ab. To this end, the pcDNA3.1 plasmid encoding for anti-CD16-anti NDV-HN tsAb (gifted by Dr. Momburg, Germany) was used for insertion of the synthetic human IL15 cDNA. The final construct was used to stably transfect the Human Embryonic Kidney (HEK) cells under G418 pressure. Alternatively, following the insertion of IL-15 sequence, the Fc part of the antibody was deleted and the remaining part of tsAb was subcloned from pcDNA3.1 plasmid into the bacterial expression vector pET22b and the final recombinant construct was transformed into E. coli. Restriction analyses and sequencing results indicated the proper insertion of the IL-15 sequence (378 bp) and construction of both tsAb encoding plasmids.

P98 (Continued)

Secreted Abs from stably-transfected HEK cells were purified from media by protein A-columns. The E.coli-derived tsAb, following IPTG-induction (1mM), was purified by nickel affinity chromatography in native form via 6xHis-tag of the expressed protein. Integrity and purity of both HEK- and E.coli-derived tsAb proteins were characterized by SDS-PAGE and Western Blot analyses (using anti-human IgG-HRP and anti-His HRP conjugates against HEK- and E.coli-derived tsAbs, respectively) which indicated the expected the expected the expected the expected 98 kD and 68 kDa proteins corresponding to the HEK- and E.coli-derived tsAbs, respectively. Analyses by flow cytometry using CD16 and NDV-HN ligands indicated proper and almost similar binding specificities for both HEK- and E.coli-derived tsAbs. This result implied the possibility for application of E.coli-derived tsAb to replace for the low-yield HEK system. To evaluate the functionality of the constructed tsAb for NK activation, peripheral blood mononuclear cells (PBMC) were isolated from blood by centrifugation using a Histopaque gradient (Sigma-Aldrich), and NK cells were enriched via magnetic beads (Miltenyi-Biotec). Isolated NK cells induced higher amounts of IFN-γ (measured by ELISA) and demonstrated higher cytolytic activity (measured by Cayman-LDH Cytotoxicity Assay Kit) in presence of tsAb and TC-NDVs compared to that of bsAb indicating the positive role of IL-15 insertion. These promising in vitro results might warrant further in vivo studies on the constructed tsAb for ex vivo stimulation of NK cells for cancer immunotherapy.
P99 Analyses of anti-BAG1 and anti-GRA1 specific IgG1/IgG2a polarity in murine model infected with Toxoplasma gondii tissue cysts and oocysts
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Introduction: IgG2a/IgG1 polarity in murine sera samples is an indication that the cellular immune response is towards Th1 or Th2 response. Protection against Toxoplasma gondii is mainly conferred by Th1 cellular immune response. T. gondii has three forms in its life cycle which are sporozoites and tissue cysts which convert to bradyzoites as the infection becomes chronic. BAG1 is a bradyzoite specific cytoplasmic antigen which has similarities to small heat shock proteins. The expression of BAG1 is upregulated in bradyzoites, facilitates the tissue cyst formation and takes important roles in the formation of immune response against toxoplasmosis. GRA1 is located in the electron dense secretory organelles and shown to be up regulated in sporozoites. Although GRA1 is being expressed at the beginning of conversion from tachyzoites to bradyzoites, its expression is progressively repressed after the second day of conversion process. In the present study, we aimed to analyze the anti-BAG1 and anti-GRA1 specific IgG1/IgG2a polarity in murine model infected with Toxoplasma gondii tissue cysts and oocysts to determine BAG1 and GRA1 roles in cellular immune response and finally if they can be valuable vaccine candidate antigens.

Material and Methods: Initially, two groups of Swiss mice administered with sporulated oocysts (contains sporozoites) and tissue cyst. Serum samples were collected from each mouse prior to infection and 1, 2, 3, 6, 10, 15, and 40 days after infection. Then, we expressed and purified recombinant BAG1 and GRA1 proteins and developed a Rec-ELISA that detects anti-BAG1 and anti-GRA1 specific lgG1/lgG2a responses.

Results: In tissue cyst infected mice, anti-BAG1 antibody response increased at day 40 and the polarity was significantly towards IgG2a response (P<0.05). anti-GRA1 antibody response increased at day 15 and continued to increase at day 40 and polarity was also significantly towards IgG2a (P<0.05). In oocyst infected mice, anti-BAG1 antibody response increased at day 40 and the polarity was a little towards IgG1 response (P=0.472), anti-GRA1 antibody response increased at day 40 and the polarity was a little towards IgG2a (P= 0.584).

Discussion and Conclusion: The results showed that in tissue cyst infected mice a predominant IgG2a response indicating Th1 response occurs as the infection becomes chronic. In oocyst infected mice, anti-BAG1 IgG1 and anti-GRA1 IgG2a are a little higher. Overall, as the infection becomes chronic, a significant IgG2a response is observed in tissue cyst infected mice and an IgG1/IgG2a balance occurs in oocyst infected mice. In sum, BAG1 and GRA1 induce strong IgG2a responses and can be part of a multivalent vaccine against toxoplasmosis.

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P100 Dectin-1 sensing is required for chitosan-induced cGAS-STING-dependent type I interferon production by dendritic cells
Moran HB, Carroll EC, Mor A, Taylor P, Lavelle EC

The introduction of vaccines is regarded as one of the most successful medical interventions to date. However there is a clear need for the development of new vaccines for diseases including HIV, TB and malaria and for cancer which require the induction of a potent cellular immune response. Advancements in the field of vaccine research have resulted in a move away from the use of whole organisms and towards the use of subunit vaccines which consist of highly purified antigens with an improved safety profile. Adjuvants are immunostimulatory components that are included in subunit vaccine formulations to help direct and amplify adaptive immune responses. Chitosan is a cationic polysaccharide that has been examined in an adjuvant setting due to its biocompatible and biodegradable nature. The polysaccharide has been shown to have the capacity to induce Th1 to help direct and amplify adaptive immune responses. Chitosan is a cationic polysaccharide, which has been shown to have the capacity to induce Th1 type interferon (IFNs) and enhances antigen-specific Th1 responses in a type I IFN receptor-dependent manner. Induction of type I IFNs and DC maturation by chitosan requires the cytoplasmic DNA sensor cGAS and the adapter protein STING. To date, no specific receptors have been identified as mediators of the interaction between chitosan and DCs. However several receptors have been associated with the recognition of chitin, chitosan’s naturally occurring precursor, including the C-type lectin receptor (CLR), Dectin-1. Thus a potential role for Dectin-1 in the recognition of chitosan by DCs was investigated. While chitosan uptake by DCs was not dependent on Dectin-1, the induction of type 1 interferons was compromised in the absence of the CLR. Furthermore the upregulation of DC activation markers by chitosan was abrogated in the absence of dectin-1, providing further evidence of a role for dectin-1 signalling in chitosan adjuvancy. The activation of the cGAS-STING pathway by chitosan is induced by mitochondrial DNA release. Chitosan induces both mitochondrial depolarisation and mitochondrial fission, potentially contributing to the subsequent release of DNA. Overall these results indicate that chitosan induces cGAS-STING dependent DC activation through engagement with the CLR Dectin-1.

P101 A production study on influenza H5N1 clade II vaccine development using novel single-use bioreactors
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Since the 2003 WHO Bulletin, more than 700 human cases infected by influenza H5N1 virus and more than 60% mortality rate were reported. The most effective method to prevent such outbreak of the highly pathogenic avian influenza (HPAI) H5N1 virus is vaccination. Therefore, the vaccine production should have a rapid process for the preparation of pandemic influenza viruses. The cell-based technology for vaccine production has several advantages such as short production time, closed environment of handling highly pathogenic viruses, and easy scaling-up process. In the recent report, the new HPAI H5N1 strain which is the second genotype of H5N1 virus (clade II) started to spread over many countries. The WHO has generated several H5N1 clade II candidate vaccine viruses which were ready to manufacturers for vaccine production. In this study, two novel disposable bioreactors, BelloCell and TideCell were used to produce HPAI H5N1 clade II virus vaccine candidates NIBRG6 (clade 2.3.4) and NIBRG30 (clade 2.3.2.1). The conditions and parameters of upstream bioprocess in BelloCell system for MDCK and Vero cells line were established for process scaling-up. The results compared with the spinner-flask culture, the average MDCK cell doubling time in BelloCell was decreased from 32 to 25 hours. The two cell lines obtained higher cell density in the BelloCell was also achieved. In addition, the total HA viral titer and cell-specific virus yield were similar between BelloCell and spinner-flask culture systems. The 10 L TideCell002 reactor, a larger scale of BelloCell system, was also used to produce influenza H5N1 virus vaccine strains. The results showed that higher concentration of HA antigen and virus yield we could have achieved in the 10L TideCell002. The study demonstrated that BelloCell and TideCell systems could be a manufacturing platform for the H5N1 influenza vaccine production.
**P102** Determination of immunogenicity and protection conferred by a 6-valent recombinant protein vaccine adjuvanted with Montanide against lethal challenge with *Toxoplasma gondii*

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**Introduction**: *Toxoplasma gondii* is an obligate intracellular parasite that infects all warm-blooded animals and humans. Toxoplasmosis is asymptomatic in healthy individuals whereas may lead to death in immune compromised patients such as organ transplant and cancer patient or AIDS patients. Congenital toxoplasmosis can cause lethal malformations in the fetus. *T. gondii* also is responsible for outbreaks caused by oocyst tainted water supplies. Drugs effective for *T. gondii* are teratogenic for the fetus. Due to these reasons, a safe and protective vaccine against toxoplasmosis is required by the community. In our previous study, we have screened more than > 2,870 candidate exon products by sera FPLC and developed a 6-valent recombinant protein adjuvanted with Montanide [6-Valant (+) Montanide]. Mice were vaccinated two times at three week intervals and then humoral and cellular responses in goats.

**Results**: In this study, we aimed to develop a multivalent recombinant protein vaccine using these prioritized proteins.

**Material and Methods**: We expressed all the wild type prioritized proteins in small scale and selected the most abundantly expressed 6 proteins based on the SDS-PAGE and WB results. Thereafter, we expressed them in bioreactor, purified to homogeneity with AKTA-FPLC and developed a 6-valent recombinant protein adjuvanted with Montanide [6-Valant (+) Montanide]. Mice were vaccinated two times at three week intervals and then humoral immune response was analyzed by Western blot and Rec-ELISA, cellular immune response was determined by flow cytometry and cytokine ELISA. Mice were infected intraperitoneally with a lethal dose of the *T. gondii* Ankara strain tachyzoites to determine protection conferred by vaccination. 6-Valant (+) Montanide vaccine induced strong total IgG response compared to controls (P<0.0001). Analysis of IgG1 and IgG2a responses displayed that polarization protection conferred by vaccination. Vaccine induced strong protective TH1 immune response. The survival time of the vaccinated mice was increased to 8.38 ± 2.13 days which was significantly higher than controls (P<0.01).

**Discussion and Conclusion**: In this study, we generated a multivalent recombinant protein vaccine containing 6 wild type recombinant proteins and tested its efficacy against lethal toxoplasmosis for the first time. Overall, the results designated that the 6-Valant (+) Montanide induces a strong protective immune response and increases protection against toxoplasmosis.

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**P103** Respiratory and systemic immune responses to Aujeszky’s disease virus (ADV) in early mucosally vaccinated piglets

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**Introduction**: Aujeszky’s disease (ADV) is a porcine herpervirus 1, which infects the respiratory tract cells and is lethal during the first weeks of age. ADV transmission is mediated by respiratory and genital secretions. Current intramuscular (IM) vaccination protocols, usually applied at weaning, induce poor mucosal immunity, frequently failing to prevent and control the disease. Thus, we studied a systemic/mucosal route of early (before weaning) vaccination using an inactivated ADV vaccine in two and fourteen day-old groups of SPF miniature Vietnamese pigs (n=6 each) with their respective age controls, measuring the specific anti ADV antibody (ELISA) and mRNA cytokine (qPCR) responses in systemic and mucosal samples. Our results showed that the systemic (serum) antigen-specific IgG and IgA responses were similar between both age groups and evident one week after the mucosal immunization. However, the IgG responses in bronchialalveolar lavage, nasal secretion and saliva, in the immunized piglets, were lower and heterogeneous when compared to the IgA responses which showed higher and sustained levels after an intranasal boost. Moreover, PBMC and lungs cells, from immunized piglets, showed significant fold increases of IFN-γ, IL-4 and IL-10 mRNA cytokines after in vitro stimulation with ADV, regardless of age of immunization. Our data suggest that early mucosal immunization induced similar systemic and mucosal immune responses to older pigs; therefore, early protection against ADV in virus susceptible animals, could be induced using this early vaccination protocol.

**Acknowledgments**: This work was partially supported by the ICyTDF (267/09) Mexico City and CONACYT (60941) Mexico. We thank DMV, MSC Laura Zapata, Antonia Cobá (INIFAP-SAGARPA, México) and MVD Enrique Chávez Castañeda for technical assistance.

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**P104** REVACCINATION OF GOATS WITH *MYCOBACTERIUM BOVIS* BCG SHOWED ENHANCED ANTIGEN-SPECIFIC INTERFERON GAMMA PRODUCTION BY MEMORY T CELLS

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**Introduction**: New vaccines or vaccination strategies that confer better protection against tuberculosis (TB) than the single-dose vaccination with *Mycobacterium bovis* BCG are required. The aim this study was to assess the duration of BCG protective immunity as well as the effects of BCG revaccination (homologous prime-boost strategy) on the immunological responses in goats.

**Materials and Methods**: Twenty-two goat kids from were vaccinated with BCG and other 11 remained unvaccinated. Eleven out of the 22 vaccinated kids were revaccinated with BCG 56 weeks later. The ex vivo interferon-gamma (IFN-g) release after stimulation with whole blood with *M. bovis* tuberculin (PPD-B) was measured prior and after each vaccination by ELISA (ID Screen® Ruminant IFN-g, ID, vet). At week 59 (3 weeks after revaccination), a trichromatic flow cytometric staining with monoclonal antibodies to CD4, CD45RO and IFN-g was used to investigate the frequency of PPD-B-specific T cell subsets and intracellular IFN-g production in the 3 groups.

**Results**: The analyses revealed that peripheral ex vivo antigen-specific IFN-g detected at 8 weeks after prime vaccination was undetectable one year later, while homologous boosting of BCG not only showed detectable ex vivo antigen-specific IFN-g released, but also increased the proportion of IFN-g-producing memory T CD4+ and CD4- cells compared to single BCG vaccinated (P < 0.01 and P < 0.05, respectively) and unvaccinated (P < 0.05) groups.

**Conclusion**: BCG revaccination 56 weeks after prime vaccination increases the proportion of IFN-g-producing memory T-cell subsets, thus potentially contributing to the mechanisms of long-term protection against TB infection.

**Acknowledgments**: This work was partially funded by a grant from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), reference number RTA2015-00043-C02-01 (FEDER co-funded) and by IRTA’s own resources. IRTA is supported by CERCA Programme / Generalitat de Catalunya.

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**P105** Pre-clinical efficacy and safety of therapeutic vaccines for treatment of heroin and prescription opioids abuse and toxicity

Raleigh MD, Laidenbach M, Baruffaldi F, Peterson SJ, Winston S, Pentel PR, and Pravetoni M* presenter

In the US, 2.6 million people are dependent on heroin and prescription opioids such as oxycodone, which led to over 50,000 opioid-related overdose deaths in 2016. To address the opioid abuse epidemic, our group has developed a series of vaccines against heroin and prescription opioids. These vaccines containing opioid-based haptons conjugated to keyhole limpet hemocyanin (KLH) elicit high concentration of high affinity opioid-specific IgG antibodies that block opioid distribution to the brain, and reduce opioid-induced behavioral effects in mice and rats. These vaccines do not interfere with the endogenous opioid system or prevent the use of opioid-based medications. To manufacture and formulate these vaccines for clinical testing, we have focused on optimizing hapten synthesis and bioconjugation chemistry, purification at scale-up conditions, and characterization of immunogens for protein content, stability, size, hapten:protein ratio, aggregation, and residuals. To test the efficacy of the re-formulated opioid vaccines, rats were immunized with the morphine hapten conjugated to KLH (M-KLH) or the oxycodone vaccine OXY-KLH and challenged with a range of opioid doses that model human abuse. Vaccination was effective in dose-dependent blockade of heroin and oxycodone distribution to the brain. To test vaccine impact on opioid toxicity, rats immunized with oxycodone vaccines were compared with regard to oxycodone-induced hotplate analgesia and oxycodone-induced respiratory depression and hypoxemia. Vaccination shifted the dose-response curves to the right, representing protection, for each of these endpoints. These data indicate that vaccines may provide some protection against opioid overdose. Naltrexone was effective in immunized rats, providing complete and rapid reversal of respiratory depression. These studies also provided evidence that opioid vaccines can be combined with the opioid antagonist naltrexone. To address potential safety concerns, we showed that antibody-bound opioids do not activate mu opioid receptors. Finally, these vaccines can be formulated using clinically-approved adjuvants, including alum and MF59. To summarize, pre-clinical efficacy and safety data support clinical evaluation of therapeutic vaccines as a strategy to treat opioid abuse and toxicity.
**P107** Comparison of Safety of a HSV-1 Gene Mutant M3 strain with Varicella Live Littenuated Vaccine Oka Strain
Lei Liu, Xingli Xu and Qihan Li*
Institute of Medical Biology, Chinese Academy of Medical Sciences, Peking Union Medical College

HSV1 (Herpes simplex virus I) is the pathogen causing the herpes disease with a prevalence of more than 40% in the population, there is no effective vaccine for the application, but the herpes virus family α-subgroup members —the attenuated live vaccine (Oka strain) of varicella zoster virus (VZV) has been successfully applied. At present, the strain of HSV1 M3 strain with missing UL7, UL41 and LAT gene was constructed in our laboratory. In this study, we compared the changes of pathological characteristics after BALB/C mice infected with different infection modes of Oka and different doses (103,104,105 pfu) of M3, thus the safety of HSV1 mutant M3 was analyzed. Oka strain and M3 strain infected mice showed no significant acute reaction after 7 days infection, there were no difference comparing with control mice; 10 days of weight change in mice of each dose of M3 strains compared with the Oka strain and control group were no significant difference; the M3 strain also showed low viral load after 1month or 3months infection, which was not significantly different from the Oka strain; M3 strains infected BALB/C mice in addition to individual slight inflammatory cell infiltration, local hemorrhage, glial cell, compared with Oka strain and control group, no other significant pathological damage; taking nerve tissue of BALB/C mice after 1month or 3months virus infection in vero cells to see whether they can cause viral re-activation, for seven consecutive days without Cytopathic effect(CPE) reaction, and the cells were in good condition. Compared with the Oka strain of varicella live attenuated vaccine, the BALB/C mice showed reasonable safety after being infected with M3 strain, It can provide the preliminary basis for whether HSV1 mutants could be used as a candidate vaccine for attenuated live vaccines.

**P108** Yellow Fever Vaccination Centre : An Experience sharing from India - free of disease
Dr Vikas Bhatia (presenting author)
Dean, Professor and Head Dept of Community and Medicine AIIMS Bhubaneswar, India
Dr Swayam Pragya Parida Astt Professor, Deptt of Community and Family Medicine AIIMS Bhubaneswar, India

Background: Worldwide over 200,000 cases and 30,000 deaths of Yellow Fever (YF) are reported per year. YF is endemic in Africa and Latin America with combined population of 900 million. India despite having all favourable conditions has never reported any case.

Methodology: Govt of India has laid down a strict policy on vaccination. Advocacy and policy influencing with Ministry of Health and Family (MOHFW) was undertaken for strengthening of vaccination centres facilities.

Results: Until 2014, there were 27 authorized vaccination centres in the country for a population of over 1.25 billion. AIIMS Bhubaneswar is a premier tertiary level medical Institute which started it’s hospital in 2014. State of Odisha with population of 400 million is located in the eastern part but did not have any centre. The YF vaccination centre became operational in May, 2015 and is providing services to people. In a short span of another one year, 12 more centres were established in other parts of the country. Vaccine is procured from Russia and France by MOHFW. Between May 2015 to April 2017, 1555 persons were vaccinated of which 81.9% were males. Over three-fourth got vaccinated who were travelling to YF endemic countries for professional reasons. No adverse reaction has been reported till date. Logistically, it has reduced the long travel time for the purpose of vaccination, has become financially viable to the people of state. Other newer centres have also extended the benefit. Awareness about YF vaccination centre was also assessing.

Conclusion: Enhancement of YF vaccination centre facilities has further strengthened the efforts to keep India free of this dreaded disease in addition to making logistically beneficial to the public.

**P109** Ecological Model for immunizations as health promotion and health behavior
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Background: Ecological models of health promotion have been widely used in many health promotions and prevention such as the Colorectal Cancer Control Program (CRCGP) by the Centers for Disease Control and Prevention (CDC). However, there is no established ecological model applied for health promotion known in Japan. The author decided to create and propose an ecological model for immunization from insights obtained through two conducted questionnaire studies according to six constructs of the model. Two studies were to investigate 1) factors which influence seasonal flu immunization coverage among older adults 65 years and older (Study I), 2) factors which influence mothers’ decision to take mumps vaccination for their children (Study II), Method: The questionnaire asked their age, immunization status, working status, subjective life standards, regions where they live, living with family or not, medical history and Health Belief Model (HBM) factors such as the perceived efficacy of vaccinations, perceived severity of diseases, cues to the action, perceived barriers such as fear of harmful side effects, being busy, offered at inconvenience in geographic location, the vaccine is expensive and others. Open-ended questions about vaccinations were included. Outcome measures were defined as “non-vaccinated,” and we conducted qualitative and quantitative analysis. Results: According to six ecological model constructs, following factors were identified to influence vaccinations. 1) Interspersal factors of demographics such as age (Study I and II), income and cost (Study II), family situations such as living abroad (Study I), biological factors such as disease histories (Study II); psychological factors such as fear of side effects (Study I and II), self-rated health (Study II), perceived severity (Study I and II), perceived vulnerability (Study II) and perceived efficacy (Study I and II). 2) The social and cultural environment of the society and culture such as immunization program withdrawal histories (Study I and II) and side effect incidence history (Study I). 3) Interspersal processes and primary groups of social support such as social networks, family, friends (Study I), counseling from medical professionals (Study I and II), exercise and healthy life style (Study II). 4) Institutional factors of health insurance, health care services, health care facilities for immunizations that can facilitate traveling and access immunizations (Study I and II). 5) Community factors of living places and workplace environments (Study I and II), community based organizations that can support mothers (Study I), information environment and counseling services that provide (Study I and II), media that can provide correct information (Study II) and local government communication including subsidy information (Study II). 6) Public policies of implementing national and local laws of immunizations such as the national immunization program (Study I), subsidies for immunization (Study I and II), safety review and evaluation (Study I and II), guideline provisions, safety database streamlining and publishing (Study I and II). The author aligned these resource factors and constituents in an ecological model.
Teleost comprise one of the most primitive groups of vertebrates what also implies an immune system that differs from mammals’ one in many aspects. One of the most remarkable differences is that their innate immune system plays a more important role, what is translated in a higher diversity of molecules involved in comparison to mammalian species. In fact, it is of primary importance in combating infections. These differences need to be understood to complete the knowledge of fish immune system and it seems to lack the key piece that allow us to understand this matter. Fish red blood cells (RBCs), which unlike mammal ones, are nucleated, could be part of this strategies because, just recently, a set of biological processes relevant to immunity have been described in the nucleated RBCs of a diverse group of organisms: pathogen recognition, pathogen binding and clearance and cytokines production.

So far, one of the best strategies to controlling and preventing viral diseases is DNA immunization. At the present time, only the DNA vaccine based on the rhabdoviral glycoprotein G (gpG) gene has been shown to be effective for all fish rhabdovirus tested, an important family of virus that cause millionaires of losses every year in aquaculture. However, it is necessary more knowledge about the immune response that DNA immunization is triggering in order to search new and more effective strategies.

Therefore, in order to investigate the implication of fish RBCs in the immune response induced by a DNA vaccine, we show for the first time that trout RBCs are able to express the glycoprotein G of Viral Haemorrhagic Septicaemia Virus (VHSV) (GVHSV) in vitro and modulate the expression of genes when they are transfected with a DNA vaccine. Then, the transcriptome of a culture of transfected RBCs was analysed and its profile resulted in changes in genes involved in the immune response, proteasome components, complement system, oxidative stress and metabolism. High expression levels of GVHSV were also detected. The differential expression analysis revealed genes implicated in the innate immune system which include antiviral proteins, interferon and cytokine signalling.

In summary, we suggest that RBCs could be immune cell mediators playing an active role in regulating the molecule expression and thus acting as part of the immune stimulation induced by a DNA vaccine.

P1110 RNA-SEQ OF RAINBOW TROUT RBCs TRANSFECTED WITH A DNA VACCINE IN VITRO SHOWS A POTENTIAL ROLE OF TROUT RBCs AS MEDIATORS IN THE IMMUNE RESPONSE INDUCED BY DNA VACCINES IN FISH.
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The dynamics of Immunoglobulin V-gene usage and clonotype expansion in mice after prime and boost immunizations as analyzed by NGS
Diego J. Farfán-Arribas, Shuying Liu, Shixia Wang and Shan Lu
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Traditionally, the humoral responses elicited by vaccines are mainly monitored by the levels and quality of polyclonal antibodies in serum and other specific tissues at various time points after immunization. The introduction of monoclonal antibody (mAb) technology enabled examining the quality of individual antibody components in an immune serum including the immunoglobulin (Ig) germline gene usage and sequence. However the process of isolating mAbs is selective and does not necessarily reflect the full picture of antibody responses. Next generation sequencing (NGS) technology with its high-throughput analysis on a large pool of genes at the same time, provides a powerful tool to investigate the immune repertoire with unprecedented efficiency and depth. In the current study, an improved NGS approach was developed to study the B-cell repertoire evolution in a simple mouse immunization model including only two DNA immunizations. The combination of 5’RACE and Ion Torrent long reads enabled unbiased immunoglobulin repertoire analysis even from small amounts of peripheral mouse blood. The B-cell population expanded by the vaccine displayed a relatively strong clonality. Upon priming with the first DNA immunization, a consistent pattern of V-gene and CDR3 usage, indicative of “public clonotypes” was observed. This pattern diversified after the subsequent boost, and thus the major clonotypes in each individual were different to those of other mice in spite of having received the same vaccine regimen. Nevertheless, several highly ranked V-genes and CDR3s were still shared among different mice. These results established a new platform valuable to perform longitudinal analyses of the Ig germline gene usage and clonotype evolution throughout an immunization regimen in a commonly used animal model.

P1120 Packing of Sephacryl™ S-500 in ReadyToProcess™ columns and evaluation of Ulf Segerbo1, Christer Larsson1, Tim Kellogg2, Nigel Pheasey2
1) GE Healthcare, Uppsala, Sweden, 2) Amgen Woburn, MA, USA

Vaccine purification processes often involve processing through a soft chromatography resin operation based on size exclusion. Sephacryl S-500 HR is an example of a size exclusion resin that is used for vaccine processing. To prevent addition of bioburden, the process is kept closed without exposure to the outside. This also protects operators from being exposed to situations where the target is harmful. Closed, disposable products enable a better control of bioburden and remove the need to open and clean surfaces that have been exposed to harmful substances. An example of this is the repacking of a chromatographic column in a process with an active virus. The requirement for cleaning validation is also removed. This poster presents the results from the development of a packing method that generates a stable bed in disposable ReadyToProcess column, and confirmation of column performance in a vaccine process. To generate a column bed height suitable for size exclusion chromatography, two, 20 cm disposable columns were connected in series.
**P113 A NEW PROTOCOL FOR BOVINE PAPILLOMAVIRUS AUTOLOGOUS VACCINE AND RELATED EFFECTIVE ASSESSMENT**

Authors: M. Pellegrini1, S. Corbelli1, S. Cardaioli1, A. Di Paolo1, M. Bugatti1, K. Forlì1, L. Anzalone1, M. Cigolati1, G. Severi1Affiliation: 1 Istituto Zoonrofilito Sperimentale dell’Umbria e delle Marche (IZSUM), Via G. Salvemini 1, 06126 Perugia, Italy

**Introduction** Papillomavirus (PV) belongs to the Papillomaviridae family: DNA-oncogenic viruses that can infect animal’s epithelium and mucosa causing benign or tumorous proliferative lesions. PV is species-specific and shows high resistance to common detergents and organic solvents, also it is stable at low pH values and high temperatures.

Papillomavirus can affect animals and humans, generating lesions at head, neck, joints, limbs, nipples, genital organs, enteric mucosa and bladder. The disease causes severe economic losses in livestock breeds, i.e. mastitis and milking difficulty due to lesions in mammary glands. Since many years, the bovine anti-PV auto-vaccine produced at Zooprophylactic Institute of Umbria and Marche has always been effective in preventing the disease spread; however, the protocol needs new technologically advanced procedures to obtain a better immunization, in line with the most modern pharmaceutical production and control protocols. The aim is to improve and to standardize the auto-vaccine production and evaluate its immunogenicity and effectiveness in bovine livestocks.

**Materials and Methods**

One hundred thirty five bovines belonging to Frisona and Charolaise breeds of Central and Northern Italy farms, of which 78 with symptomatology and clinical signs and 52 healthy subjects (control) were selected and inoculated with 3 subcutaneous doses (5 ml each) at 15-days intervals. All animals have always been monitored through biomolecular and histopathological methods, also by electron microscopy. The experimental vaccine, inactivated overnight at +4 °C, has been obtained by biopsy homogenisation, centrifugation and increasingly tight filtrations. As quality check, virus presence was evaluated by electron microscopy; in addition stability and abnormal toxicity were assessed by animal testing in the laboratory. **Results and Conclusion** Visible results of clinical remission were observed after the third and last inoculation. The remission had a gradual course: at first, only a color variation in the tumor mass is reported (grayish tonal tendency to black) before decreasing in size and completely regressing. In all cases, total regression of PV lesions was visible 25-30 days after the end of vaccination cycle. Until now, the PV vaccine was an auto-vaccine produced exclusively for the animal which had the Papilloma removed.

With this new method, we validated a protocol able to improve immunization starting from “the farm virus”, capable of protecting bovines in the same breeding, thereby reducing both the number of surgical interventions in the herd both cases of infection within the farm itself. The results showed a 97% efficacy of the vaccine with a total regression of PV lesions. The 100% of clinical remission was observed for the first appearance of PV lesions during the post-vaccination period, even though they shared the spaces with the infected animals. In conclusion, we can demonstrate state that the new autogenous vaccine production and administration protocol allows both to effectively counteract the clinical forms of papillomatosi and to prevent the spread of the virus within the breeding, demonstrating in both cases a high degree of effectiveness.

**P114 Recombinant vaccinia virus expressing fused protein TB10.4-HspX of Mycobacterium tuberculosis boosts BCG efficacy against tuberculosis**

Eunyung Shin, Hye-Sook Jeong Yong-Ran Lee, Hye-Ran Cha, Sang-Won Lee, and Jung Sik Yoo* Division of Vaccine Research, Center for Infectious Diseases Research, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Osong, Republic of Korea

BCG vaccine has been widely used in many countries but it shows limited protective effect for adults and latent infection against M. tuberculosis. Therefore, effective novel TB vaccines and vaccination strategies are urgently required. In this study, fused protein of TB10.4-HspX was selected as antigens for vaccine candidates. TB 10.4 belongs to secretory protein ESAT-6 family in early stage of infection, which induced immune response. HspX is known as dominant antigen related with long term survival during latent stage. Vaccinia virus as recombinant vectors and delivery models has several advantages that carry many foreign genes and stimulate production of high level cell mediated immunity. In this study, we constructed recombinant vaccinia virus expressing fused protein TB10.4-HspX (rVV/TB10.4-HspX) of M. tuberculosis using the attenuated vaccinia virus. Boosting BCG-immunized mice with rVV/TB10.4-HspX induced the strongest antigen-specific T-cell responses, including polyfunctional CD4+ or CD8+ T-cells expressing the three cytokines of interferon-gamma (IFN-g), tumor necrosis factor-alpha (TNF-a), and interleukin-2 (IL-2). The significant reduction in bacterial load and histopathology of lung was obtained in mice vaccinated with BCG prime and rVV/TB10.4-HspX booster when compared with BCG and rVV/TB10.4-HspX alone.

The results suggest that rVV/TB10.4-HspX may be an efficient booster vaccine against TB with a strong ability to enhance prior BCG immunity.

**P115 Immunogenicity and protective efficacy against tuberculosis of recombinant vaccinia virus expressing antigen 85B of Mycobacterium tuberculosis**

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There is licensed BCG vaccine but the protective effect against infectious M. tuberculosis is limited in adults, so novel development of tuberculosis (TB) vaccines is urgently required. Antigen 85 complex of M. tuberculosis is located in cell wall, thus it was known as one of targets for development of anti-mycobacterial drugs and novel vaccine candidates. Vaccinia virus as recombinant vectors and delivery modes has several advantages, which can carry many foreign genes and stimulate production of high level cell mediated immunity. In this study, we constructed recombinant vaccinia virus expressing Ag85B (rVV/85B) of M. tuberculosis using the attenuated vaccinia virus KVAR103. We immunized C57BL/6J subcon-taneously with BCG or rVV/85B, then boosted with rVV/85B. As results, rVV/85B induced immune responses of antigen specific IgG and production of polyfunctional T cell secreting 3 types of cytokine (IFN-γ, IL-2 and TNF-α) in both prime and boost vaccination. However, the level of IFN-γ from lung and spleen after boost vaccination was not significant. To determine the protective efficacy of rVV/85B as prime or boosters of BCG, the mice were challenged 10 weeks with virulent M. tuberculosis H37Rv after the last vaccination. The reduction in bacterial load and histopathology of lung was obtained in rVV/85B-primed mice. However, boosting BCG-primed mice with rVV/85B showed slightly reduction than the BCG alone vaccinated mice. Our results showed the feasibility for development of more effective TB vaccines for improved control of TB.

**P116 Parasite antigenic diversity and host genetic factors suggest two mechanisms that may reduce the efficacy of RTS,S malaria vaccine**

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**Introduction** RTS, S/AS01 vaccine is the leading malaria vaccine candidate regarding clinical development stages and has raised the hopes of becoming the first licensed malaria vaccine to be available for endemic areas. However, the latest data from a phase III trial conducted in 11 sites in 7 sub-Saharan countries have shown that the vaccine efficacy against clinical malaria was only 28.3% in the older study group, and lower in the younger study group, and there were significant variations in vaccine efficacy across the study sites. The aim of our study is to address the genetic variability in the host and the parasite using immunoinformatic tools to establish their role in the variability of the vaccine’s efficacy in trials. **Methods** Key antigens in the RTS,S vaccine were evaluated using the iVAX suite of immunoinformatics tools to predict T cell epitopes restricted by class I and II HLA, and compared those predictions to a cohort of Malawian subjects whose HLA haplotype and infection strains of malaria is known. Using the JanusMatrix algorithm, we compared predominant T-cell epitope clusters in each Malawian variant CSP sequence for cross-reactivity with human sequences and with other CSP variants. Finally, we used a novel informatics algorithm called EpICC (Epitope Content Comparison) to quantify the likelihood that a candidate vaccine strain will induce protection against other strains, based on the nature and similarity of their T cell epitopes. **Results** The prevalence of HLA-DR associated with dominant T cell epitopes in the vaccine was inversely correlated with the prevalence of the epitopes in circulating strains of malaria. For example, T-cell epitopes that are found in malaria strains circulating in Malawi were more often restricted to rare HLA-DR alleles. This suggests that the parasite has evolved to escape HLA DR-restricted immune responses for survival purposes. In addition, our JanusMatrix analysis for conservation of the TCR facing residues of common T cell epitopes show that T-cell epitopes that are highly conserved across the malarial variant strains had TCR-facing residues that were highly conserved in the human genome. EpICC analysis showed that the CSP component of the RTS,S vaccine had a low degree of T-cell epitope relatedness compared to the circulating variants. **Conclusions** Variations in observed vaccine efficacy of the RTS,S vaccine may be impacted by the low immunogenic content, poor coverage of pathogenic variants, the potential for human-cross-reactivity, and limited conservation to prevalent circulating strains.
Synthetic nucleic acid antibody prophylaxis (DMAb) + DNA vaccine confers rapid and durable protective immunity against Zika virus challenge

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Objectives: Significant concern has been raised over the past two years from the increased global spread of the mosquito-borne flavivirus, Zika (ZIKV). Accompanying this spread has been an increase in cases of the devastating birth defect microcephaly as well as cases of Guillain-Barre syndrome in adults in many affected countries. Currently there is no vaccine or therapy for this infection, however we sought to develop a combination approach that provides more rapid protection and durable protection than traditional vaccination.

Methods: A novel immune-based prophylaxis/therapy strategy entailing the facilitated delivery of a synthetic DNA consensus prME antigen vaccine along with designed DNA encoded biologically active anti-Zika envelope monoclonal antibodies was developed and evaluated for antiviral efficacy, as well as for the ability to overcome shortcomings inherent with conventional active vaccination or passive immunotherapy.

Results: A collection of DMAbs were developed which were potent against ZIKA in vitro and could be expressed in serum within 24-48 hours of administration. The prME vaccine was also potent after adaptive immunity was developed, protecting against infection, brain and testes pathology in relevant mouse challenge models and in NHP challenge. IM delivery of potent DMAbs protected mice from viral challenge within days of delivery. Combined injection of DMAb and the ZIKV-DNA vaccine afforded rapid and long-lived protection in these same challenge models, providing an important demonstration of the advantage of this synergistic approach to pandemic outbreaks.

Conclusion: We show that simple DNA formulations by enhanced EP (Celletra®) delivery provide improved protection in relevant ZIKA challenges. This novel approach has advantages in EID and pandemic vaccine strategies including Zika.
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<td>(CIS Building)</td>
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<td>Margaret Liu, ISV President</td>
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<tr>
<td>10:30-12:00</td>
<td>Plenary Session One: Innovative Clinical Designs and Human Challenge Models to Accelerate Efficacy Testing</td>
<td>(CIS Building)</td>
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<td></td>
<td>Session Chairs: Denise Doolan, James Cook University; and Frédéric Tangy, Institut Pasteur</td>
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<tr>
<td>13:30-15:30</td>
<td>Concurrent Session 1 (CIS Building) Maternal and Neonate Immunization Session Chairs: Kathrin Jansen, Pfizer; and Shabir Madhi, University of Witwatersrand</td>
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<td>Concurrent Session 2: (Duclaux Building) Institut Pasteur - Bringing Vaccinology to the World Session Chairs: Armelle Phalipon and Frédéric Tangy, Institut Pasteur</td>
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<td>Concurrent Session 3: (François Jacob Building) Structural Vaccinology and Protective Monoclonal Antibodies Session Chairs: Florian Krammer, Icahn School of Medicine at Mount Sinai; and Joon Haeng Rhee, Chonnam National University Medical School</td>
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<tr>
<td>16:00-18:00</td>
<td>Plenary Session 2: Vaccines as Solutions to AMR Threats Session Chairs: Danilo Casimiro, Aeras</td>
<td>(CIS Building)</td>
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<tr>
<td>18:00-19:00</td>
<td>Poster Session # 1 Sponsored by (CIS Building)</td>
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<tr>
<td>18:30-20:00</td>
<td>Welcome Reception Sponsored by (CIS Building)</td>
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### Friday, 6 October 2017

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<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Location</th>
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<tbody>
<tr>
<td>08:00-10:15</td>
<td>Plenary Session 3: Emerging Infectious Diseases Session Chairs: Shan Lu, UMass Medical School; and Marie-Paule Kieny, Inserm</td>
<td>(CIS Building)</td>
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<tr>
<td>10:45-12:30</td>
<td>Plenary Session 4: Novel Vaccine Concepts / Emerging Technologies Session Chairs: Jeffrey Ulmer, GSK; and Connie Schmaljohn, USAMRIID</td>
<td>(CIS Building)</td>
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<tr>
<td>13:30-14:45</td>
<td>Poster Session # 2 Sponsored by (CIS Building)</td>
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<tr>
<td>14:45-15:45</td>
<td>ISV Annual General Meeting Sponsored by (CIS Building)</td>
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<td>16:00-18:15</td>
<td>Concurrent Session 4: Vaccines for the Elderly (CIS Building) Session Chairs: Tonya Villafana, MedImmune; and Britta Wahren, Karolinska Institute</td>
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<td>Concurrent Session 5: Mucosal Vaccination (Duclaux Building) Session Chairs: Linda Klavinsks, King's College London; and Hiroshi Kiyono, University of Tokyo</td>
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<td>Concurrent Session 6: Systems Vaccinology and Computational Vaccinology (François Jacob Building) Session Chairs: Annie De Groot, EpiVax Inc.</td>
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### Saturday, 7 October 2017

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<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Location</th>
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<tbody>
<tr>
<td>08:00-10:00</td>
<td>Plenary Session 5: Neglected and LMIC Diseases Session Chairs: Allan Saul, GSK; and Sarah Gilbert, University of Oxford</td>
<td>(CIS Building)</td>
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<tr>
<td>10:30-12:35</td>
<td>Concurrent Session 7: Cancer Vaccines and Immunotherapy (CIS Building) Session Chairs: David Weiner, The Wistar Institute and Joon Haeng Rhee, Chonnam National University Medical School</td>
<td>(CIS Building)</td>
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<td>Concurrent Session 8: Viral Vaccines (Duclaux Building) Session Chairs: Christiane Gerke, Institut Pasteur; and Ted Ross, University of Georgia for Vaccines and Immunology</td>
<td>(CIS Building)</td>
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<td>Concurrent Session 9: One Health (François Jacob Building) Session Chairs: Ed Rybicki, University of Cape Town; and Nathalie Garçon, Biaster</td>
<td>(CIS Building)</td>
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<tr>
<td>12:45-14:00</td>
<td>Lunch (Building &quot;Social-Modules&quot;) Sponsored By: Pfizer</td>
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<td>13:15-14:00</td>
<td>Career Development Panel and “Meet the ISV Fellows” Sponsored by (CIS Building)</td>
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<td>14:00-14:30</td>
<td>ISV Award Ceremony Sponsored by (CIS Building)</td>
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<tr>
<td>14:30-15:30</td>
<td>Plenary Session 6: Public Private Partnerships Session Chairs: Margaret Liu, ProTherImmune; and David Weiner, The Wistar Institute</td>
<td>(CIS Building)</td>
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<td>15:10-15:50</td>
<td>OPEN DISCUSSION WITH ALL PARTICIPANTS Sponsored by (CIS Building)</td>
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<tr>
<td>15:50-16:00</td>
<td>Closing Remarks and Introduction of 2018 ISV Annual Congress Sponsored by (CIS Building)</td>
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