2016 International Society for Vaccines

Annual Congress

In collaboration with

Vaccine Renaissance X and DNA Vaccine 2016

Boston Marriott Long Wharf, USA

October 2-4, 2016

www.isv-online.org  www.isvcongress.org
A Welcome from the ISV Congress Co-Chairs

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 all disciplines to the ISV 10th Annual Congress in Boston, MA. The high quality and number of attendees and abstract submissions are a testament to both the consistent superb level of science at the ISV Congress and the important interactions that occur at the Congress amongst the scientists representing different aspects of the vaccine pipeline.

The crucial role of vaccines for ensuring the health of all the world’s people is becoming increasingly obvious with the rapidity of spread of newly emerging diseases, and other challenges such as increasing microbial antibiotic resistance. This ISV Congress continues and strengthens its tradition of presenting cutting edge developments and providing a forum for the best minds and capabilities in the fields of vaccinology and related scientific areas to interact and collaborate.

The ISV Congress is known for its 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. The program is designed to both present the latest scientific findings from groups around the world and to provide a forum for determining and advancing the best means to expedite vaccine development. This year we have a panel discussion addressing how to best deal with emerging diseases such as Zika. Panelists come from key sectors with diverse expertise. The Congress also has a career development panel, a job bank, and will announce a mentoring program to aid the many trainees. 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 and jobs, including onsite interviews. Poster presenters will be able to present short “elevator talks” as a way to hone their speaking skills.

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 designing the program, reviewing abstracts, and agreeing to be speakers and mentors. This is the first time that ISV has handled all the logistical aspects for the Congress, so special thanks are due to Dr. Shan Lu and his team in the Secretariat.

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.

Please join us in welcoming Dr. Victor Dzau, President of the National Academies of Medicine of the US National Academy of Sciences who will be giving the Keynote Lecture on Monday morning, “Biosecurity and winning the war against future epidemics.”

Everyone is encouraged to attend the ISV annual general meeting on Monday afternoon, Oct. 3. 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 Boston! Enjoy the science, the people, the city!

Sincerely,

Margaret A Liu, President ISV, ProTherImmune
Frédéric Tangy, Institut Pasteur
David Weiner, Wistar Institute
Annie De Groot, EpiVax and U. Rhode Island
Co-chairs of 2016 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

Margaret Liu, ProTherimmune, USA
Anne DeGroot, EpiVax, U. Rhode Island, USA
Frédéric Tangy, Institut Pasteur, France
David Weiner, Wistar Institute, USA

SCIENTIFIC COMMITTEE

Brigitte Autran, INSERM, France
Yasmine Belkaid, NIH, USA
John Boslego, Takeda, USA
Manon Cox, Protein Sciences, USA
Hazel Dockrell, LSHTM, UK
Mark Doherty, GSK, USA
Emilio Emini, Gates Foundation, USA
Mark Feinberg, IAVI, USA
Michael Good, Griffith University, Australia
Adrian Hill, University of Oxford, UK
Akira Homma, FioCruz, Brazil
Luis Jodar, Pfizer, USA
Jerome Kim, IVI, South Korea
Hiroshi Kiyono, University Tokyo, Japan
Ousmane Koita, University Bamako, Mali
Adel Mahmoud, Princeton, USA
Yvonne Maldonado, Stanford, USA
Cornelius J.M. Melief, Leiden Univ., Netherlands
Flor Munoz-Rivas, Baylor, USA
Armelle Phalipon, Institut Pasteur, France
Stanley Plotkin, VaxConsult, USA
Edward Rybicki, Univ. Cape Town, South Africa
Allan Saul, GSK Vaccine Inst. Global Health, Italy
Baik Lin Seong, Yonsei University, South Korea
John Shiver, Sanofi, USA
Junzhi Wang, NIFDC, China

CONGRESS SECRETARIAT

Shan Lu, UMASS Medical School, USA
Email: shan.lu@umassmed.edu
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Ted Gibson, UMASS Medical School, USA
Email: edward.gibson@umassmed.edu

Cindi Callaghan, UMASS Medical School, USA
Email: cindi.callaghan@umassmed.edu

CONGRESS VENUE

Boston Marriott Long Wharf, 296 State Street, Boston, MA 02109 USA
Tel: +1-617-227-0800
Website: http://www.marriott.com/hotels/travel/boslw-boston-marriott-long-wharf/

CONGRESS WEBSITE

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

- Sunday, October 2nd  8:00am-7:00pm
- Monday, October 3rd  8:00am-6:00pm
- Tuesday, October 4th  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
Poster presenters should refer to the information board in the registration area to check the location of the board which has been assigned to them.

Posters should be setup between 2:00-6:00pm on Sunday, October 2. Dedicated poster sessions take place on Sunday from 6:15-8:00pm and Monday from 1:00-2:00pm. Poster presenters should ensure that they stand by their posters during the poster viewing sessions.

Posters may remain up for the duration of the conference however all posters must be removed by 4:00pm on October 4th. Please note any materials left after 4PM will be discarded.

WELCOME RECEPTION
A welcome reception will take place on Sunday in the Palm Garden during the poster session. Complimentary passed hors d’oeuvres and beer and wine will be available. You will receive two beverage tickets in your badge holder for beer or wine.

LUNCH
Lunch will be provided in the Harbor View and Commonwealth Ballrooms on Sunday, Monday and Tuesday at no cost for all attendees.
**COFFEE BREAKS**

Coffee breaks will be available in the Foyer of the Grand Ballroom on Sunday, Monday and Tuesday at no cost for all attendees.

**WiFi**

WiFi will be available at the conference free of charge. The login password will be distributed at registration.

**CONFERENCE DINNER**

The Congress Dinner will take place on Monday, October 3 at the New England Aquarium, conveniently located next to the hotel at One Central Wharf. The New England Aquarium is a global leader in ocean exploration and marine conservation. With more than 1.3 million visitors a year, the Aquarium is one of the premier visitor attractions in Boston and a major public education resource for the region.

The Main Exhibit Gallery will open to attendees at 7:00pm and remain open throughout the evening. Begin the night with a cocktail reception while viewing the exhibits; dinner begins at 8:00pm in the Harbor Terrace Tent.

Pre-purchased tickets are available in your badge holder.

A limited number of additional dinner tickets may be available for purchase at the conference registration desk.

**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.
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<th>Time</th>
<th>Event</th>
<th>Details</th>
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<td>Registration</td>
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<tr>
<td>09:30-09:55</td>
<td>Coffee Social (Foyer of Grand Ballroom)</td>
<td><strong>Sponsored by EpiVax, Inc.</strong></td>
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<td>09:55-10:00</td>
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<td>ISV Congress Co-chairs: Margaret Liu, Frédéric Tangy, David Weiner, Anne De Groot</td>
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<tr>
<td>10:00-12:15</td>
<td>Plenary Session One: Vaccine Challenges (Grand Ballroom)</td>
<td>Session Chairs: Shan Lu and Margaret Liu</td>
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<tr>
<td>10:00-10:30</td>
<td>[PL1.1] The Ebola Disaster and How to Prevent it from Happening Again</td>
<td>Stanley Plotkin, VaxConsult</td>
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<td>10:30-11:00</td>
<td>[PL1.2] Development of V920, Live, Attenuated Recombinant Vesicular Stomatitis Vaccine Against Ebola Zaire (rVSV-ZEBOV)</td>
<td>Tom Monath, New Link Genetics</td>
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<td>11:00-11:30</td>
<td>[PL1.3] Chimpanzee Adenoviral Vectors as Vaccines: from Alphaviruses to Zika</td>
<td>Adrian Hill, Oxford University</td>
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</table>
| 11:30-12:15 | [PL1.4] Panel: Challenges of Making Emerging Diseases Vaccines        | Session Chair: Stanly Plotkin, VaxConsult  
Panelists:  
Phil Krause, Food and Drug Administration, Rip Ballou, GSK  
Adrian Hill, Oxford University, Tom Monath, New Link Genetics |
| 12:15-13:30 | Lunch (Harbor View Ballroom and Commonwealth Ballroom)                | **Sponsored by Ichor Medical System, Inc.** |
| 13:30-15:30 | Concurrent Session 1: Mechanisms of Immune Efficacy                  | Session Chairs:  
Nik Petrovsky and Peter Nara  
**Sponsored by:**  
Inovio Pharmaceuticals |
| 13:30-14:00 | [O1.1] Towards the Control of Hepatitis C                              | Michael Houghton, Univ. of Alberta  
**Sponsored by:**  
Eurogentec |
| 13:30-15:00 | Concurrent Session 2: DNA Vaccines                                    | Session Chair: David Weiner  
**Sponsored by:**  
Inovio Pharmaceuticals |
| 13:30-14:15 | [O2.1] Developments in EID                                            | Joel Maslow, GeneOne  
**Sponsored by:**  
Modern Oxford |
| 13:30-14:45 | [O2.2] Developments in RNA Vaccines                                  | Hari Pujar, Moderna Therapeutic  
**Sponsored by:**  
Eurogentec |
| 13:30-14:45 | [O3.1] Immunotherapeutic Approaches for Alzheimer and Prion Diseases  | Thomas Wizniewski, NYU School of Medicine  
**Sponsored by:**  
Eurogentec |
| 13:30-14:45 | [O3.2] Prevention of emerging zoonoses in Central Africa: an overview from a veterinary perspective. | Emmanuel Assana, University of Ngaoundéré  
**Sponsored by:**  
Eurogentec |
| 13:30-14:45 | [O3.3] The enhanced immune responses of pig to PCV-2 vaccine by inoculation with chitosan nanoparticles of recombinant pig interleukin-23 gene. | Yongle Xiao, Sichuan University  
**Sponsored by:**  
Eurogentec |
**Sponsored by:**  
Eurogentec |
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<td>14:45-15:00</td>
<td>[O1.5]</td>
<td>Decoding Immune Evading Mechanisms of pathogens: reordering of immunodominance for new and improved vaccines</td>
<td>Peter Nara, Biological Mimetics</td>
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<td>14:30-15:00</td>
<td>[O2.3]</td>
<td>BEAT-HIV Collaboratory: Path to a Cure. Luis Montaner, Wistar Institute (Continued)</td>
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<td>15:00-15:15</td>
<td>[O1.6]</td>
<td>LAMP-based DNA vaccines suppress IgE production and intestinal anaphylaxis in a murine model of Peanut-induced Food Allergy</td>
<td>Franco Pissani, Immunomics Therapeutic,</td>
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<td>15:15-15:30</td>
<td>[O2.4]</td>
<td>Two doses of a DNA Vaccine Expressing the Glycoprotein Precursor Gene of Lassa Virus Fully Protect Nonhuman Primates from Lassa Fever when Delivered by Dermal Electroporation</td>
<td>Kathleen Cashman, USAMRIID</td>
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<td>15:15-16:00</td>
<td>[O3.5]</td>
<td>Pathologic and Immunologic characteristics of Coxsackievirus A16 infection in rhesus macaques. Ying Zhang, Chinese Academy of Medical Sciences</td>
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<td>15:30-16:00</td>
<td>[O2.5]</td>
<td>Comparative evaluation of electroporation mediated intradermal or intramuscular administration of DNA vaccines against the Venezuelan, eastern, and western encephalitic alphaviruses. Drew Hannaman, Ichor Med. Sys.</td>
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<td>15:30-16:00</td>
<td>[O3.6]</td>
<td>An optimized synthetic DNA vaccine targeting liver stage exported proteins provides sterilizing protection from P. yoelii sporozoite challenge.</td>
<td>Emma Reuschel, Wistar Institute</td>
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<td>15:30-16:00</td>
<td>[O3.7]</td>
<td>Identification and validation of immunodominant antigens as protective prophylactic against Shigella.</td>
<td>Bhrug Yagnik, Sardar Patel University</td>
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<td>16:00-18:00</td>
<td>Plenary Session 2: Insights into the Immunology of Vaccines and Immunotherapy</td>
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<td>16:00-16:30</td>
<td>[PL2.1]</td>
<td>Impact of microbiome on immune responses.</td>
<td>Yasmine Belkaid, NIH</td>
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<td>16:30-17:00</td>
<td>[PL2.2]</td>
<td>A Replication Defective Human Cytomegalovirus Vaccine for Prevention of Congenital Infection</td>
<td>Tong-Ming Fu, Merck</td>
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<td>17:00-17:15</td>
<td>[PL2.3]</td>
<td>Adjuvants play a critical role in universal influenza vaccine design</td>
<td>Nik Petrovsky, Flinders University</td>
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<td>17:15-17:30</td>
<td>[PL2.4]</td>
<td>Side-Stepping Regulatory T Cell Induction to Build Better Vaccines</td>
<td>Anne De Groot, EpiVax, Univ. of Rhode Island</td>
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<td>17:30-17:45</td>
<td>[PL2.5]</td>
<td>An Optimized, Synthetic DNA Vaccine Encoding the Toxin A/Toxin B RBDs of Clostridium difficile Induces Protective Antibody Responses.</td>
<td>Michele Kutzler, Drexel Univ.</td>
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<td>17:45-18:00</td>
<td>[PL2.6]</td>
<td>A subunit protein based therapeutic vaccine design to treat Genital Herpes disease and subclinical infection in a pre-clinical model.</td>
<td>Sita Awasthi, Univ. Penn.</td>
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<td>18:15-20:00</td>
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<td>Concurrent Session 4: Institut Pasteur Special Session</td>
<td>Concurrent Session 5: Vaccines and Biologicals: Production and Efficacy</td>
<td>Concurrent Session 6: JSV/KVS: Adjuvant and Immunomodularity Principles for Mucosal vs. Systemic Immunity</td>
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<td>10:30-12:45</td>
<td>Session Chairs: Christiane Gerke and Shan Lu</td>
<td>Session Chairs: Phil Krause and Britta Wahren</td>
<td>Session Chairs: Hiroshi Kiyono and Baik Lin Seong</td>
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<td>[O6.5] Poly-IC synergizes with OX40 to enhance Ag-specific CD4 T cell response. Paurvi Shinde, U. Conn</td>
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<td>12:15-12:30</td>
<td>[04.5] Synthetic carbohydrate-based conjugates as vaccines: a promising concept illustrated for <em>Shigella</em></td>
<td>Laurence Mulard, Institut Pasteur, Paris, France</td>
<td>Sponsored By VGXI</td>
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<td>[05.7] Electroporation-Mediated DNA Administration as an Antibody Delivery Platform for Passive Immun prophylaxis.</td>
<td>Claire Evans, Ichcr Medical Systems</td>
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<tr>
<td>12:30-12:45</td>
<td>[04.6] A promising vaccine against bubonic and pneumonic plague</td>
<td>Anne Derbise, Institut Pasteur, Paris, France</td>
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<td>12:45-13:15</td>
<td>Lunch <em>(Harbor View Ballroom and Commonwealth Ballroom)</em></td>
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<td>Sponsored By Eurogentec</td>
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<td>13:15-14:15</td>
<td>Poster Session 2 <em>(Palm Garden)</em></td>
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<td>14:15-15:00</td>
<td>ISV Annual Meeting <em>(Grand Ballroom)</em></td>
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<td>15:00-15:30</td>
<td>Coffee Break <em>(Foyer of Grand Ballroom)</em></td>
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<td>Plenary Session 4: Immunization of Individuals in Special Physiological Circumstances</td>
<td>Session Chairs: Sam Katz and Jeffrey Ulmer</td>
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<td>[PL4.2] Maternal Immunization</td>
<td>Flor Munoz, Baylor College of Medicine</td>
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<td>[PL4.3] Group B streptococcus vaccination in pregnant women with or without HIV in Africa</td>
<td>Clare Cutland, Univ. of the Witwatersrand</td>
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<td>[PL4.5] Regression of Advanced Cervical Dysplasia and Elimination of HPV16/18 Infection by VGX-3100 is Statistically Associated with the Presence of Highly Active Peripheral Lytic CD8+ T cells and Cervical Immune Infiltration.</td>
<td>Kimberly Kranyak, Inovio</td>
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<tr>
<td>19:00-22:00</td>
<td>ISV Congress Dinner <em>(New England Aquarium – tickets required)</em></td>
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<td>Tuesday, 4 October 2016</td>
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<td>Plenary Session 5: Insights into Vaccine Targets and Effective Immune Responses</td>
<td>Session Chairs: Flor Munoz and Marie-Paule Kieny</td>
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<td>[PL5.1] Factors Affecting Vaccine Investment by Companies</td>
<td>Luis Jodar, Pfizer</td>
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<td>[PL5.2] Novel Biomarkers for antibody-directed effector function predicting vaccine efficacy.</td>
<td>Galit Alter, Harvard Medical School/MIT</td>
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<td>[PL5.4] Env-specific Rabbit Antibody-Mediated ADCC Activities vis Human Fc-Receptor.</td>
<td>Shixia Wang, UUMS</td>
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<td>[PL5.6] Conformation of a Protein in Virus-like Particles Impacts their Efficacy as Vaccines.</td>
<td>Trudy Morrison, UUMS</td>
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<tr>
<td>10:00-10:30</td>
<td>Coffee Break <em>(Foyer of Grand Ballroom)</em></td>
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<td>10:30-11:00</td>
<td>(O7.1) Can serum vibriocidal antibodies mediate bactericidal activity against <em>Vibrio cholerae</em> O1 in the small intestine of persons recovered from wild type cholera and in recipients of live oral vaccine? Myron Levine, Center for Vaccine Development, Univ. of Maryland</td>
<td>(O8.1) Q Fever; Immune Profiling of Coxiella burnetii Vaccination and Infection by Mass Cytometry Mark Pozansky, Mass. General Hospital, Patrick Reeves, Mass General Hospital</td>
<td>(O9.1) A New Genome-Wide Antigen Discovery Algorithm Identifies Novel In-Vivo Expressed Mycobacterium Tuberculosis (Ive-TB) Antigens Inducing Human T Cell Responses with Classical and Unconventional Cytokine Secretion Profiles. Mariateresa Coppola, Leiden Univ.</td>
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<td>11:00-11:15</td>
<td>(O7.2) A novel RSV vaccine elicits humoral and Treg-cell responses against RSV infection and suppresses vaccine enhanced disease (VED). Bin Wang, Fudan Univ.</td>
<td>(O8.2) Ebola Challenge Study in Non-Human Primates: Role in Supporting the Development of rVSV-EBOV Vaccine. Amy Shurtleff, USAMRIID</td>
<td>(O9.2) Both young and aged effector CD4 T cells that recognize cognate Ag at the &quot;memory checkpoint&quot; differentiate to Tfh and memory cells and promote B cell memory in a vaccine model. Jingya Xia, UMMS</td>
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<td>11:05-11:30</td>
<td>(O7.3) Green tea catechin-inactivated viral vaccine. Baik Lin Seong, Yonsei Univ.</td>
<td>(O8.3) A Phase 1 Study of a DNA Vaccine for Venezuelan Equine Encephalitis Delivered by Intramuscular or Intradermal Electroporation. Lesley Dupuy, USAMRIID</td>
<td>(O9.3) Characterization of Influenza Elicited Humoral Immunity in the domestic Ferret. Greg Kirchenbaum, U. Georgia</td>
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<td>11:30-11:45</td>
<td>(O7.4) Preclinical evaluation of hemagglutinin stalk-based candidate universal influenza vaccines in ferrets. Randy Albrecht, Mount Sinai Med. School</td>
<td>(O8.4) Confirmation of a lethal mouse model for Zika virus infection and development of a novel DNA vaccine that is fully protective against lethal infection in vivo. Trina Racine, Canadian Science Centre for Human and Animal Health</td>
<td>(O9.4) Development of a safe, tolerable and efficacious gene-based immunoprophylaxis delivery strategy to protect against RSV. Trevor Smith, Inovio</td>
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<td>[O8.7] Extreme Polyvalency Induces Potent Cross-Clade Cellular and Humoral Responses in Rabbits and Non-human Primates Megan Wise, UPenn, Wistar Institute</td>
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<td>12:30-12:45</td>
<td>[O7.8] Novel recombinant HIV vaccine candidates based on replication-defective flavivirus vectors demonstrate favorable safety and immunogenicity profile in NHP. Maryann Giel-Moloney, Sanofi-Pasteur</td>
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<td>[O8.8] Vaccines against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) elicit humoral and cellular immune responses in mice Naif Alharbi, Univ. of Oxford, King Abdullah International Medical Research Center</td>
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<tr>
<td>12:45-13:45</td>
<td>Lunch (Harbor View Ballroom and Commonwealth Ballroom)</td>
<td>Sponsored By Invoio Pharmaceuticals</td>
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<tr>
<td>13:00-13:45</td>
<td>Career Development Panel: Laina King, NIH, Shan Lu, UMASS, Annie De Groot, URI/EpiVax, Nicola Lamonica, Johnson &amp; Johnson Innovation (Grand Ballroom-Please bring your lunch from the Harbor View or Commonwealth Ballrooms)</td>
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<td>13:45-14:00</td>
<td>ISV Award Ceremony (Grand Ballroom)</td>
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<td>14:00-16:00</td>
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<td>Session Chairs: Frédéric Tangy and Margaret Liu</td>
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<td>14:00-14:30</td>
<td>[PL6.1] Lessons Learned from Ebola R &amp; D during a Health Emergency</td>
<td>Marie-Paule Kieny, World Health Organization</td>
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<td>14:30-15:00</td>
<td>[PL6.2] Lessons Learned from Sanofi Pasteurs Dengue Vaccine Program and Challenges for Zika</td>
<td>Nick Jackson, Sanofi Pasteur</td>
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<td>15:00-15:15</td>
<td>[PL6.3] ZMAPP, VSV-ZEBO</td>
<td>Gary Kobinger, Laval Univ.</td>
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<td>15:45-16:00</td>
<td>[PL6.6] Discovery of Toxoplasma Gondii Vaccine Candidate Antigens. Mert Doskaya, Ege Univ.</td>
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<td>16:00-16:15</td>
<td>Closing Remarks and Introduction of 2017 ISV Annual Congress (Grand Ballroom)</td>
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Invited Speakers

Galit Alter  Ripley Ballou  Clare Cutland  Victor Dzau  Tong Ming Fu

Michael Good  Thomas Heineman  Adrian Hill  Michael Houghton  Nicholas Jackson

Luis Jodar  Marie-Paule Kieny  Myron Levine  Yvonne Maldonado  Joel Maslow

Thomas Monath  Luis Montaner  Flor Munoz  Albert Osterhaus  Stanley Plotkin

Photos unavailable at time of printing 2016-09-21:

Yasmine Belkaid  Anne Derbise  Xia Jin  Gary Kobinger  Philip Krause  Hari Pujar

Ed Rybicki  Thomas Wisniewski
Galit Alter

Galit Alter received her PhD in experimental medicine from McGill University, and is currently an Associate Professor in Medicine at Harvard Medical School and a faculty member at the Ragon Institute of MGH, MIT, and Harvard. Her research interests lie at the intersection of the innate immune response and the adaptive humoral immune response, with a focus on defining the role of innate immune recruiting antibodies in providing specificity to the innate immune system to kill virally infected cells. In this capacity, Dr. Alter has developed a suite of antibody profiling assays that aim at gaining a deeper appreciation of the correlates of humoral immune activity against HIV and beyond.

Abstract [PL5.2]:

While antibody titers and neutralization are considered the gold-standards for the selection of a successful vaccine, these parameters are often inadequate predictors of protective immunity. Instead, antibodies mediate a diverse array of additional extra-neutralizing Fc-driven activities that may contribute to antiviral control/clearance. Thus, when neutralization fails to predict protection, Fc-mediated antibody activity may underlie protective immunity, requiring the use of more comprehensive antibody profiling tools for the identification of unanticipated humoral correlates of protective immunity and/or for the downselection of protective vaccine antibody profiles. Thus a set of high-throughput, comprehensive assays were developed to capture the remarkable biodiversity of antibody effector functions, linked to multivariate computational tools to begin to probe humoral correlates of protective immunity in an unbiased manner. This analysis now provides a means to not only contrast unique vaccine fingerprints, but also begin to identify unexpected correlates, and perhaps even mechanisms, of immune protection against HIV.

W. Ripley Ballou

W. Ripley Ballou, MD is Vice President and Head, Global Vaccines US R&D Center located in Rockville, Maryland USA. Previously he served as VP & Head Clinical Research & Translational Science for GSK Vaccines in Rixensart, Belgium. Prior to this he served as Deputy Director for Vaccines, Infectious Diseases Development, Global Health at the Bill & Melinda Gates Foundation. Dr. Ballou is an expert in vaccine development and has worked in this field for more than 30 years. Trained in Internal Medicine and Infectious Diseases, he began his work on vaccines at the Walter Reed Army Institute of Research where he led the team that with GlaxoSmithKline co-developed RTS,S, the world’s most advanced malaria vaccine. Dr. Ballou has numerous publications in the field of vaccine development and infectious diseases.

Yasmine Belkaid

Bio and abstract [PL2.1] not available at time of print 2016-09-21

Clare Cutland

Dr. Clare Cutland is a senior research medical officer at the Respiratory and Meningeal Pathogens Research Unit (RMPRU) based at Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa. She qualified with a Bachelor in Science (B.Sc.) and Bachelor of Medicine and Surgery (MBBCh) from The University of the Witwatersrand (Wits), Johannesburg, South Africa. After completing a Diploma in Child Health (DCH), she joined RMPRU as a full time research doctor, under Professor Shabir Madhi. She has been an investigator on numerous phase I, II and III paediatric and maternal vaccine trials and principal investigator on a phase II paediatric measles vaccine trial, a phase III quadrivalent influenza vaccine trial in children and a phase III maternal influenza immunization trial in HIV-positive women.
She was lead trialist on a grant-funded trial to assess the efficacy of chlorhexidine maternal vaginal and newborn skin wipes on reducing neonatal sepsis and vertical transmission of significant pathogens, especially *Streptococcus agalactiae* from mother to infant (2004-2008). The results of this trial were published in *The Lancet* in 2009. She conducted surveillance of pathogens associated with-, and the impact of maternal HIV infection on sepsis in young infants admitted to CHBAH. She has submitted her PhD thesis on aetiology and prevention of sepsis in young infants for examination.

The increasing proportion of under-5 year deaths which occur during the neonatal period has encouraged investigation of immunization of pregnant women to protect their newborns.

Dr. Cutland was the clinical lead for the grant-funded maternal seasonal influenza immunization studies which were conducted between 2010 and 2013.

She is currently leading a large grant-funded study to establish a sero-correlate of protection against *Streptococcus agalactiae*, and is involved in maternal influenza and respiratory syncytial virus vaccine trials. She is author or co-author on over 40 peer-reviewed journal articles.

**Abstract [PL4.3]:**

Group B streptococcus (GBS) was identified in the 1970s as the principal cause of early-onset disease in neonates. The incidence (per 1000 live births) of invasive GBS disease globally in infants under 90 days of age is 0.53 (95% confidence incidence: 0.44-0.62), however there is a large variability between regions, with high incidence (1.21; 95% CI 0.50-1.91) in Africa, and low incidence (0.02; 95% CI -0.03-0.07) in South East Asia. Between 65% and 95% of GBS-EOD occurs in the first 24 hours of life, suggesting that sepsis is often established while the foetus is in-utero, therefore highlighting that antenatal or intrapartum interventions are required to prevent GBS-EOD.

Maternal recto-vaginal colonization with GBS is a pre-requisite for early-onset disease (EOD; <7 days old). Identification of GBS-colonized pregnant women in the third trimester, with administration of intrapartum antibiotic prophylaxis during labour has significantly reduced EOD incidence, however this intervention is unobtainable in most low-middle income countries due to limitations in facilities, expertise and funding.

HIV-exposed infants have a 1.69-fold greater risk of GBS-EOD and almost 5-fold greater risk of GBS-late onset disease (7-90 days) than HIV-unexposed infants. The reasons for this increased risk of GBS-related sepsis in HIV-exposed infants is unclear, however it is possibly related to the disturbance of the delicate immunogenic equilibrium normally present between mother and foetus.

Conjugate vaccines (CV) for administration to pregnant women with the specific aim of reducing GBS – disease in young infants are under development or in clinical trials presently, and include the three serotypes (Ia, Ib & III) most commonly associated with GBS-invasive disease. With the shift in serotypes causing GBS-EOD more recently, development of a pentavalent rather than trivalent GBS-CV should be prioritised. An effective GBS vaccine could also be used to probe the role of GBS morbidity and mortality in countries with limited laboratory epidemiological capacity as well as role of GBS in causing stillbirths.

**Anne Derbise**

Bio and abstract [O4.6] not available at time of print 2016-09-21

**Victor Dzau**

Victor J. Dzau is the President of the National Academy of Medicine (NAM), formerly the Institute of Medicine (IOM). In addition, he serves as Chair of the Health and Medicine Division Committee of the
National Academies of Sciences, Engineering, and Medicine, and Vice Chair of the National Research Council.

Dr. Dzau is Chancellor Emeritus and James B. Duke Professor of Medicine at Duke University and the past President and CEO of the Duke University Health System. Previously, Dr. Dzau was the Hersey Professor of Theory and Practice of Medicine and Chairman of Medicine at Harvard Medical School’s Brigham and Women’s Hospital, as well as Chairman of the Department of Medicine at Stanford University.

Dr. Dzau has made a significant impact on medicine through his seminal research in cardiovascular medicine and genetics and his leadership in health care innovation. His important work on the renin angiotensin system (RAS) paved the way for the contemporary understanding of RAS in cardiovascular disease and the development of RAS inhibitors as widely used, lifesaving drugs. In his role as a leader in health care, Dr. Dzau has led efforts in innovation to improve health, including the development of the Duke Translational Medicine Institute, the Duke Global Health Institute, the Duke-National University of Singapore Graduate Medical School, and the Duke Institute for Health Innovation.

As one of the world’s preeminent health leaders, Dr. Dzau advises governments, corporations, and universities worldwide. He has served as a member of the Advisory Committee to the Director of the National Institutes of Health (NIH) and as Chair of the NIH Cardiovascular Disease Advisory Committee. Currently, he is a member of the Board of the Singapore Health System and Hamad Medical Corporation, Qatar. He was on the Board of Health Governors of the World Economic Forum and chaired its Global Agenda Council on Personalized and Precision Medicine.

Since arriving at the National Academies, Dr. Dzau has emphasized Leadership, Innovation, and Impact. He has led important initiatives such as the Global Health Risk Framework for the Future, the Human Gene Editing Initiative, Vital Directions for Health and Healthcare, and Grand Challenges in Health and Medicine. His vision is to advance science and policy to improve health globally. Among his many honors and recognitions are the Gustav Nylin Medal from the Swedish Royal College of Medicine, the Distinguished Scientist Award from the American Heart Association, the Ellis Island Medal of Honor, and the Henry Freisen International Prize. He is a member of the National Academy of Medicine, the American Academy of Arts and Sciences, and the European Academy of Sciences and Arts. He has received nine honorary doctorates.

Abstract [PL3.1]:

Just as the Ebola pandemic is waning, Zika virus is now emerging as a new threat to global health. This highlights the key message contained in the recent report of the National Academy of Medicine’s Commission on a Global Health Risk Framework for the Future: pandemics and infectious disease outbreaks are a continuing threat to human lives, the global economy, and security; and the world is not adequately prepared. There is a real need to strengthen national health systems and improve global and regional responsiveness. Dr. Dzau will discuss the importance of investing in pandemic preparedness, lessons learned from previous outbreaks, and the findings of the report of the National Academy of Medicine’s Commission on a Global Health Risk Framework for the Future.

The Commission makes a compelling case for investing in pandemic preparedness and response as an essential tenet of national security and global economic stability. According to an original analysis commissioned for this report, annualized expected losses from pandemics total $60 billion. The Commission proposes investing just a fraction of that cost—$4.5 billion per year—to make the world much safer from future pandemics. The Commission’s bold and cross-cutting recommendations comprise a three-pronged framework: 1) strengthening public health as the foundation of the health system and the first line of defense; 2) strengthening global and regional coordination and capabilities; and 3) accelerating R&D to counter infectious disease threats.
**Tong Ming Fu**

Dr. Tong-Ming Fu obtained his medical degree at Peking University Health Science Center, formerly Beijing Medical University, and Ph.D. at Pennsylvania State University, Hershey Medical Center. He joined Merck Research Laboratories after his academic trainings in virology and immunology, and has been working at Merck Vaccines Research for over 20-years. He conducted research to support many novel vaccine programs, including Merck HIV-1 and influenza M2 peptide-conjugate vaccines. He also studied immune mechanisms of novel adjuvants and immune modulations by PD-1 blocking mAbs. He is currently the research lead for Merck CMV vaccine.

**Abstract [PL2.2]:**

Congenital CMV infection is one of the leading causes of birth defects in the United States, and developing a prophylactic vaccine is a high priority for public health. Naturally acquired CMV immunity in women prior to conception is effective in preventing CMV transmission to fetus during pregnancy; both humoral and cellular immunity to CMV are likely playing a role in blocking the transmission. However, a live attenuate CMV vaccine is difficult to develop since the CMV is known to establish persistent and life-long infection in host. Aiming to develop an effective and safe CMV vaccine incapable of establishing productive and persistent infection, we first restored expression of viral gH/gL/pUL128-131 pentameric gH complex, and then applied a genetic/chemical switch to two viral proteins essential for replication. This design would allow regulation of viral replication with a small synthetic molecule; the vaccine virus cannot replicate without the chemical in culture. Vaccine is effective in induction of durable neutralizing Abs, mostly due to the restored pentameric gH complex, as well as CD4 and CD8 T-cell responses, in rhesus monkeys. The vaccine is under Phase I evaluation.

**Christiane Gerke**

Christiane Gerke, PhD, is the Head of Vaccine Programs at Institut Pasteur, Paris, France, since July 2015. Her mission in this newly created role at Institut Pasteur is to coordinate the vaccine projects and to build a Center for Vaccinology with a strategic vaccine program with high visibility integrating vaccine research at the Institut Pasteur in Paris and in the Institut Pasteur International Network. Before joining Institut Pasteur, Christiane held appointments at Novartis Vaccines Institute for Global Health (now part of GSK), a small institute with focus on developing effective and affordable vaccines for neglected infectious diseases of impoverished communities. As Shigella Project Leader she led the development of a novel technology, called GMMA, and took the GMMA vaccine candidate against shigellosis from the bench to first in human clinical studies. Christiane then took responsibility as Technology Platform Leader to oversee and coordinate the vaccine projects based on this technology. Christiane received her Ph.D. in Microbial Genetics in 1997 from the University of Tübingen, Germany, and trained as post doc at Stanford University, CA, USA, with focus on host-pathogen interactions.

**Abstract [O4.3]:**

Vaccinology has a strong tradition at Institut Pasteur. It is a strategic topic for institute anchored in Institut Pasteur’s core mission to carry out fundamental research with the aim to improve public health throughout the world. To address challenges in infectious diseases that are still a major cause of death as well as chronic diseases and cancer, Institut Pasteur has recently implemented several programs to further strengthen its vaccine research.

A large-scale horizontal program aims to unite forces at the Institut Pasteur in Paris and in the Institut Pasteur International Network, to create synergies between different disciplines, to stimulate new, innovative, highly competitive research on topics with high social impact, and to accelerate pre-clinical development towards clinical evaluation of vaccine candidates. The program also aims to develop partnerships for the projects with industry or public institutions to bring the results of the research to the people in need.
Special targeted programs are dedicated to Ebola, Zika, Dengue, and malaria. These programs follow a global approach integrating research in epidemiology, diagnostics, therapeutics, and vaccines.

Another major area in vaccinology at Institut Pasteur is the development of vaccine platform technologies. These encompass diverse technologies such as viral vectors, including measles and lentiviral vectors, and synthetic glyco-conjugate technology, which are applied to several of the Institut Pasteur vaccine candidates.

The broad vaccine portfolio contains preventive vaccines for infectious diseases caused by viruses, parasites, bacteria, and fungi, as well as therapeutic vaccines for cancer, HPV, and HIV. An overview of the vaccine programs and selected projects will be presented.

Michael F. Good

Michael Good is a NHMRC Senior Principal Research Fellow at Griffith University. He is the former Director of the Queensland Institute of Medical Research. He graduated MD PhD DSc from the University of Queensland and the Walter and Eliza Hall Institute of Medical Research and undertook postdoctoral training at the NIH.

His research interests are in immunity and vaccine development for malaria and streptococcus.

In 2008 he was made an Officer of the Order of Australia (AO), in 2009 he won the Australian Museum CSIRO Eureka Prize for Leadership in Science and in 2010 was awarded an NHMRC Australia Fellowship. In 2010 he was elected to the Australian Academy of Technological Sciences and Engineering and in 2014 to the Australian Academy of Health and Medical Sciences.

Abstract [PL3.3]:

Many organisms facilitate immune responses by exposing epitopes. It is tempting to think of these epitopes as target vaccine candidates; however this may not be the best strategy as micro-organisms are highly socialist creatures and manipulate immune responses for the benefit of their kin, not themselves. For Streptococcus pyogenes (an organism responsible for more disease types than any other organism and the loss of up to 1,000,000 lives each year), each bacterium expresses a single strain of M protein; however, there are over 200 different M-types. Antibodies readily develop to one type but that still leaves over 200 others untouched. The same applies in malaria where there is extensive polymorphism of merozoite and red cell membrane-expressed surface proteins. Parasitical antibodies to one strain develop but other strains then rapidly fill the void. From a vaccine development perspective this is extremely challenging. The dominant strategy to counter this is to develop polyvalent vaccines, as has been done for pneumococcus. Another approach is to find conserved epitopes; however, if they are conserved and the target of immune responses then why has the organism survived at all? Using peptide scans we identified highly conserved epitopes from the M protein of S. pyogenes. These are not under immune pressure. Indeed they are poorly immunogenic as a result of infection. Curiously, however, vaccination with these cryptic epitopes leads to highly protective immune responses and these are subsequently boosted by natural infection. Thus, epitopes can be cryptic following primary infection, and as such not under immune pressure, but highly visible to a memory immune response. As a result they may be ideal for vaccine development.

Thomas Heineman

Dr. Heineman received his MD and PhD (Virology) from the University of Chicago. After completing his training in Internal Medicine (University of Maryland) and Infectious Diseases (National Institutes of Health), he has spent more than 20 years conducting basic and clinical research in virology with an emphasis on herpesvirus vaccine development. During 11 years at the Saint Louis University Center for Vaccine Development, Dr. Heineman studied the pathogenesis of varicella-zoster virus and led clinical
trials of cytomegalovirus and herpes simplex virus (HSV) vaccine candidates. He subsequently joined GlaxoSmithKline Vaccines where he led the clinical development of their HSV, herpes zoster, cytomegalovirus and Epstein-Barr virus vaccines. In 10 years at GSK, Dr. Heineman and his team conducted over 30 clinical trials with more than 50,000 total subjects culminating in large pivotal efficacy studies of GSK’s HSV and herpes zoster vaccine candidates. Dr. Heineman is now the Vice President of Clinical Development at Genocea Biosciences where he is leading the development of their therapeutic HSV vaccine candidate and other assets.

Abstract [O6.1]:

Herpes zoster (HZ), or shingles, results from the reactivation of latent varicella-zoster virus (VZV) and typically manifests as a painful vesicular rash that can be complicated by chronic neuropathic pain (postherpetic neuralgia). While HZ can occur in anyone previously infected with VZV, the incidence increases in people ≥50 years due to the loss of VZV-specific cellular immunity. A live attenuated VZV vaccine, Zostavax (Merck), is approved for the prevention of HZ in people ≥50 years. However, its efficacy against HZ declines with age from 70% in people 50-59 years to 38% in people ≥70 years presumably due to declining vaccine responsiveness as a consequence of immunosenescence. To counter this effect, GSK is developing an adjuvanted subunit HZ vaccine. This vaccine, HZ/su, contains a single VZV glycoprotein, gE, and the AS01B adjuvant. AS01B contains liposomes and two immunostimulants, monophosphoryl lipid A and the saponin QS21, which transiently stimulate the innate immune system thereby shaping and amplifying VZV-specific adaptive immune responses. Early clinical trials showed that HZ/su induces strong VZV-specific cellular and humoral immune responses in older adults. Two recent clinical trials evaluated the efficacy of HZ/su in adults ≥50 years (ZOE-50 study) and ≥70 years (ZOE-70 study). In these studies, HZ/su was shown to be 97.2% and 91.3% efficacious, respectively, in preventing HZ in people ≥50 and ≥70 years. Remarkably, the efficacy of HZ/su was well preserved even in the oldest age groups. Vaccine efficacy was 91.3% in people 70-79 years and 91.4% in people ≥80 years. These results demonstrate that adjuvanted subunit vaccines can overcome immunosenescence and provide levels of protection in older adults comparable to those in younger people. They also demonstrate that diseases caused by complex pathogens can be prevented by single-antigen vaccines containing adjuvants that modulate and boost antigen-specific immune responses. Finally, these results offer hope that vaccines can be developed for immunosuppressed individuals who may resist the effects of traditional vaccines.

Adrian Hill

Adrian V.S. Hill is Professor of Human Genetics and Director of the Jenner Institute at Oxford University. He leads research programmes in both the genetics of susceptibility to tropical infectious diseases and in vaccine development. The Jenner Institute links human vaccine research at the University of Oxford with veterinary vaccine development at The Pirbright Institute and the UK Animal and Plant Health Agency. The Institute is currently conducting Phase 1/2 trials for malaria, tuberculosis, pandemic influenza, meningitis, HCV, RSV and HIV.

His group has designed and developed candidate vaccines for malaria currently in field trials in endemic countries. His group have undertaken over fifty clinical trials to evaluate new vaccine technologies. In 2014 he led the Oxford-based trials of two candidate Ebola vaccines and rapidly demonstrated their safety and immunogenicity.

He has published about 500 research papers and is a Fellow of the UK Academy of Medical Sciences and of the Royal College of Physicians, a NIHR Senior Investigator and Wellcome Trust Senior Investigator.

Abstract [PL1.3]:

Since their first clinical evaluation in late 2007 simian adenoviruses, particularly those derived from chimpanzees, have been assessed using a wide variety of vaccination approaches against at numerous
infectious diseases and cancer. Target diseases include malaria, TB, HIV, HCV, RSV, Ebola, Marburg, influenza, MERS, meningococcal disease, rabies, dengue, Chikungunya, Zika, Rift Valley fever, human papilloma virus, hepatitis B, melanoma and prostate cancer. Such vaccines are minimally impacted by anti-vector immunity in humans, have a good and growing record of safety in over 5,000 subjects, and a highly efficient manufacturing process on cell lines.

They are being developed for single dose applications against outbreak pathogens, such as Ebola and MERS, exploiting their ability to induce neutralising antibodies with a single dose. For other applications, including liver-stage malaria and HIV vaccines, their capacity to induce CD8+ T cell responses in humans at least as well as any other reported technology has been the focus of many efforts. These responses can be enhanced substantially by heterologous prime boost regimes, typically with poxvirus vector boosting, or with “internal” adjuvants such as fusion with CD74 fragments. A recent development, well-illustrated by liver-stage malaria vaccination, is the ability to enhance protective local tissue resident memory T cell populations by the use of new “prime-target” immunisation approaches.

The recent appreciation that, in addition to Ebola, the world is at risk of outbreaks of at least ten other viral pathogens against which there are no licensed vaccines has led to the development of several initiatives aiming to develop such vaccines with a streamlined and cost-effective approach. At the Jenner Institute in Oxford we have used two simian adenoviral vectors, ChAdOx1 and ChAdOx2, to develop candidate vaccines against twelve such viruses and four of these candidate vaccines are proceeding to clinical evaluation. An update on progress in this area will be presented.

Michael Houghton

Michael Houghton received his PhD in Biochemistry from King’s College, University of London in 1977 and then joined the research divisions of pharmaceutical and biotech companies in the UK and later, in the USA. At G.D. Searle & Co. in the UK, his was among the first labs to identify the structure and properties of the human interferon-beta gene and its encoded protein before going to California to join a start-up biotech company called Chiron. Here, together with George Kuo and Qui-Lim Choo, his lab identified the hepatitis C virus for the first time which has led to the development of blood screens, curative drugs and promising vaccine candidates. He has been offered numerous awards for his work on HCV including the Karl Landsteiner Award, the Robert Koch Medal, the Clinical Lasker award and the International Gairdner Award. He joined the University of Alberta in Edmonton, Canada in 2010 as Canada Excellence in Research Chair (CERC) in Virology where he performs disease-focused research in several areas including the development of a 2nd generation HCV vaccine for clinical testing. In 2013, he assumed the directorship of the newly-formed Li Ka Shing Applied Virology Institute which is funded to develop research innovations from his own CERC lab and from the department of Medical Microbiology & Immunology into the clinic.

Abstract [O1.1]:

The field of viral hepatitis has flourished over the last 50 years progressing from a vague understanding of “infectious hepatitis” and “serum hepatitis” to the eventual identification and characterisation of the diverse hepatitis A, B, C, D & E viruses that together, infect more than 10% of the world’s human population. Being at far lower concentrations than the other hepatitis agents, HCV was refractory to molecular isolation for 15 years until the application of a blind cDNA immunscreening method led to the discovery of its genome in 19891. Blood screening and diagnostics for HCV quickly emerged2 that effectively eradicated post-transfusion hepatitis C from incidences as high as 10% to the current undetectable levels. Drug development progressed far slower, in part due to frequent drug-associated toxicities but during the last 1-2 years, we have seen the emergence of small molecule HCV antivirals that can cure most patients within 1-3 months of therapy thus heralding the first chronic viral infection that can be effectively eradicated. Vaccine development is still at an early stage partly due to an absence of a convenient animal model, the inability to culture HCV in vitro until recently (and thereby provide the ability to detect and measure neutralising antibodies) and the gradual realisation as to the existence of natural immunity. Despite these difficulties, a “T cell HCV vaccine” is currently in phase 2 clinical trials3 and
additional strategies to elicit broadly cross-neutralising antibodies are underway in my and other laboratories. In conclusion, we can be optimistic that a virus that is carried by 170 million people worldwide and which infects several million people every year can be effectively controlled in the future.

Nicholas Jackson

Dr. Nicholas Jackson joined Sanofi Pasteur in 2013 as the Head of Research and Development for the dengue vaccine program. He was responsible for all research and development activities related to the dengue program. Since 2015, he has been appointed VP, Global Head of Research for Sanofi Pasteur.

Dr. Jackson was trained in the United Kingdom and holds a Bachelor of Science degree from Oxford Brookes University, a master of science from the London School of Hygiene & Tropical Medicine, and a doctorate from the University of Warwick in the field of viral immunology.

Subsequently, Dr. Jackson has spent over 18 years in prophylactic and therapeutic vaccine research and development, working on a variety of global vaccine programs (from early research through to Phase 3) in disease areas including Streptococcus, Hepatitis, HSV, HIV-1, RSV, Influenza and Alzheimer’s disease. Prior to joining Sanofi Pasteur, Dr. Jackson served as the head of an immunology platform and also led a platform investigating vaccine candidates in development for a bacterial disease area.

Abstract [PL6.2]:

The recent emergence of Zika virus (ZIKV) as a cause of severe disease has mobilized public health agencies, as well as commercial organizations, to embark on efforts to develop new approaches for combating this infection. ZIKV, a mosquito-borne flavivirus, has only recently been associated with severe disease in humans, including a Congential Zika Syndrome and Guillain-Barré syndrome associated with an unprecedented spread of the virus. These links prompted the World Health Organization to list Zika as a "Public Health Emergency of International Concern" and the US CDC has now declared that ZIKV is a cause of microcephaly and other severe fetal brain defects. As of August 2016, 70 countries and territories have reported evidence of mosquito-borne Zika virus transmission.

As exemplified during the recent Ebola outbreak, international and regional collaborations will be required to advance our understanding of Zika and to accelerate vaccine research and development. Potential challenges for vaccine R&D have been identified, including; limited knowledge about Zika dynamics; unknown incidence rates for clinical complications and the need for diagnostic assays that avoid cross-reactivity with other flaviviruses; and the complexities of symptomatic and asymptomatic infection may lead to the need for clinical trials to demonstrate efficacy against disease/viremia and possibly fetal transmission for registration.

Sanofi Pasteur has dedicated 20 years of research and development for a prophylactic solution for dengue and recently licensed a vaccine in several countries. Lessons learned from dengue are highly pertinent for Zika vaccine considerations and will inform the field accordingly. Sanofi Pasteur has initiated vaccine research and development activities to rapidly proceed through preclinical assessment into clinical evaluation of a Zika vaccine, and is in partnership with the WRAIR to develop their inactivated Zika vaccine candidate. As such, Sanofi Pasteur’s experience in licensed flavivirus vaccines will be leveraged towards Zika vaccine development. Available results and progress will be presented.

Xia Jin

Bio and abstract [O4.4] not available at time of print 2016-09-21
Dr. Luis Jodar is Chief Medical & Scientific Officer for Vaccines Medical Development and Medical/Scientific Affairs at Pfizer Inc. In this capacity, Dr. Jodar heads Pfizer's vaccines medical development, clinical and scientific affairs worldwide comprising around 200 MD, PhD vaccinologists. Dr. Jodar has held different positions at Pfizer including Vice President, Global Vaccines, Medicines Development Group and Scientific Affairs; Head of Vaccines, Medical Affairs for Emerging Markets and Executive Director for Clinical and Scientific Affairs for the Asia Pacific (AP) region at Wyeth. Before joining industry, Dr. Jodar was Deputy Director General at the International Vaccine Institute (IVI) in Seoul, Korea, an international organization established by the United Nations for the development, introduction and use of new vaccines and adjunct professor at Seoul National University, Faculty of Medicine, School of Public Health. At the IVI, he was responsible of directing the IVI's Translational Research Division which conducts programs of interdisciplinary research, including pre-licensure and post-licensure clinical trials, epidemiological research, studies of the economics of vaccine introduction, sociobehavioral and policy research on vaccines against cholera, typhoid fever, shigellosis, rotavirus, Japanese encephalitis, Haemophilus influenzae b, and Streptococcus pneumoniae. Previously, Dr. Jodar worked at the World Health Organization (WHO) leading the global vaccine research and introduction of meningococcal vaccines, activities related to adverse events allegations and quality, safety and correlates of protection of vaccines. Previously he was the focal point of the Task Force for Vaccines and Viral Diseases at the European Commission in Brussels. Dr. Jodar is a neuroimmunologist with doctoral and post-doctoral training in Madrid, Osaka and Nagasaki Universities. Dr. Jodar has worked in vaccine-related programs in more than 50 countries around the world including developing countries in Africa, Asia and Latin America, published in the most accredited peer-review journals (e.g. the Lancet, New England Journal of Medicine, Nature Medicine), and served on a number of advisory groups on vaccines to WHO, the EU, vaccine manufacturers and governments. He teaches at the Institut Pasteur, ADVAC and Columbia University and has a long track-record in launching and coordinating public private vaccine development partnerships to accelerate the development, clinical evaluation, registration, introduction and commercialization of vaccines of public health importance for developing and middle-income countries having received more than $100M in grants. He has also been awarded with several honors including the “cross of officer of the order of civil merit” from the King of Spain for his work in vaccines and vaccination in developing countries.

Abstract [PL5.1]:

In May 2012, the World Health Assembly approved the Global Vaccine Action Plan (GVAP) to achieve the Decade of Vaccines vision by delivering universal access to immunization and launching two new major vaccines by the end of this decade. In its assessment report from 2014, the GVAP secretariat asked the WHO Scientific Advisory Group of Experts whether conditions were optimal for vaccine research and development to proceed as fast as possible, or were anything other than the inherent scientific challenge standing in the way of progress.

Large pharmaceutical companies are often faced with constrained development budgets and a diverse set of investment opportunities which may include both internal portfolio assets and external business development candidates. Often, these investment options are not limited to vaccines but span across multiple therapeutic areas, geographies, and development phases, adding further complexity and increasing the difficulty of making optimal budget allocation decisions. Given the disparate nature of these investment alternatives, companies often utilize valuation metrics that seek to combine assumptions around key program attributes (revenues, costs, timelines, risk profile, etc.) to quantify the expected value of an opportunity and determine if continued investment is warranted. Valuation metrics typically serve as an initial screening tool to assess the viability of an investment opportunity and, if evaluated properly, can enable some degree of objective comparison to inform investment decisions across a variety of diverse investment options.

In this talk, we will focus on valuation metrics typically used by the pharmaceutical industry to make informed decisions about investment options pertaining to the development of new vaccines.
Marie-Paule Kieny

Dr. Marie-Paule Kieny is Assistant Director General for Health Systems and Innovation at the World Health Organization. Beginning in 2001, she directed the WHO Initiative for Vaccine Research. Major successes under her leadership were the development and licensing of new vaccines against meningitis and against pandemic influenza in developing countries, through pioneering technology transfer. Vaccines against poverty-related diseases and those that disproportionately affect poor and marginalized populations are continuing priorities. Before coming to WHO, she was 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 INSERM from 1999 to 2000. Dr. Kieny received her PhD in microbiology from the University of Montpelier in 1980 and a Diplôme d’Habilitation à Diriger des Recherches from the University of Strasbourg in 1995. She has published over 250 articles and reviews, mainly in the areas of infectious diseases, immunology and vaccinology.

Abstract [PL61]:

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.

Gary Kobinger

Bio and abstract [PL6.3] not available at time of print 2016-09-21

Philip Krause

Bio not available at time of print 2016-09-21

Myron M. Levine

Dr. Myron M. Levine is the Bessie & Simon Grollman Distinguished Professor at the University of Maryland School of Medicine, Associate Dean for Global Health, Vaccinology and Infectious Diseases, and the Founder/Former Director of the Center for Vaccine Development (1974-2014). He is clinically trained in pediatrics and pediatric infectious diseases and in tropical public health and epidemiology. Dr. Levine has extensive experience in design and evaluation of vaccines to prevent bacterial enteric
infections, and has made substantial contributions in basic vaccinology, bacterial pathogenesis, clinical research, field epidemiology and public health. He has 46 years of continual involvement in conducting Phase 1, 2, 3 and 4 clinical trials to evaluate the safety, immunogenicity and efficacy of a wide array of vaccines. He has published over 613 peer reviewed journal articles, is an inventor/co-inventor on numerous patents and is Senior Editor of New Generation Vaccines, 4th ed, a textbook of research vaccinology.

Abstract [O7.1]:

Extensive clinical, epidemiologic and seroepidemiologic data indicate that recovery from wild type *Vibrio cholerae* O1 diarrheal illness is accompanied by substantial resistance against clinical cholera upon subsequent exposure to *V. cholerae* O1 and there is a relationship between serum vibriocidal antibody and incidence of cholera. In a highly cholera-endemic area (Bangladesh), the peak incidence of cholera was seen in young children and progressively diminished with increasing age; in parallel the geometric mean titer (GMT) of serum vibriocidal antibody rose steadily with increasing age. For every two-fold increase in serum vibriocidal titer, the incidence of confirmed cholera fell by half. Three separate clinical studies that tracked the risk of cholera among household contacts of index cholera cases showed that persons with high baseline titers of serum vibriocidal antibody were significantly protected compared to those with low vibriocidal titers.

Clinical studies of experimental cholera in healthy adult U.S. community volunteers (university students and others) showed that an initial episode of experimentally-induced cholera due to wild type Classical biotype *V. cholerae* O1 led to 100% protection against re-challenge 1-2 months later with virulent Classical biotype *V. cholerae* O1 of either serotype (Inaba or Ogawa). Impressively, it was not possible to isolate Classical *V. cholerae* O1 from stool cultures of re-challenged volunteers, suggesting that the initial cholera infection elicited a powerful anti-bacterial immunity that was bactericidal. A remarkable re-challenge study in which four community volunteers were re-challenged ~ three years after their initial episode of Classical biotype cholera allowed an assessment of whether the protection against disease and the powerful antibacterial immunity could persist in a population that does not have repetitive exposure to *V. cholerae* O1 as occurs among persons living in a cholera-endemic area. The U.S. volunteers re-challenged after ~ three years remained solidly protected against cholera and from three of four individuals it was not possible to recover *V. cholerae* O1 in stool cultures (one of four exhibited low level excretion).

Studies with virulent *V. cholerae* O1 of El Tor biotype conferred 90% protection against serotype-homologous or serotype-heterologous re-challenge but ~ one-third of re-challenged of volunteers re-challenged with *V. cholerae* O1 El Tor had positive direct stool cultures. This suggests that the immunity elicited by *V. cholerae* O1 El Tor is somewhat less potent than stimulated by Classical biotype *V. cholerae* O1.

Approximately 90% of the serum vibriocidal antibodies are directed against *V. cholerae* O1 O antigens and the titers in North Americans fall drastically over 3-4 months to approach baseline; nevertheless, they remain above pre-challenge or pre-vaccination (with live vaccine) baseline. Available data will be reviewed to address the question of whether serum vibriocidal antibodies may constitute a mechanistic correlate of protection against cholera that somehow protect the mucosal surface of the human proximal small intestine and that this may be unique to *V. cholerae* O1.

**Yvonne A. Maldonado**

Yvonne (Bonnie) A. Maldonado, MD, is Professor and Chief of the Division of Infectious Diseases, Director of Global Child Health, Department of Pediatrics, and Senior Associate Dean for Faculty Development and Diversity at Stanford University School of Medicine, Stanford, California. Dr. Maldonado attended Stanford University School of Medicine, completed a pediatric residency program and pediatric infectious diseases fellowship at the Department of Pediatrics at Johns Hopkins University. She was an Epidemic Intelligence Service Officer at the Centers for Disease Control and Prevention, prior to joining the faculty at Stanford University. She has led a number of NIH, CDC, Gates Foundation and WHO
funded domestic and international pediatric vaccine studies, as well as studies in prevention and treatment of perinatal HIV infection in the US, Mexico and Africa. She is currently the vice chair of the American Academy of Pediatrics Committee on Infectious Diseases and a member of the HHS National Vaccine Advisory Committee.

She has over 110 peer reviewed publications in scientific journals and is co-editor of the textbook “Infectious Diseases of the Fetus and Newborn Infant” and the American Academy of Pediatrics “Red Book”.

Abstract [PL4.1]:

The global eradication of poliomyelitis caused by wild type polioviruses, a goal set by the World Health Assembly in 1988, may likely be achieved within the next few years. Cases of paralytic poliomyelitis have decreased dramatically in the last three decades, from an estimated 350,000 cases in 1988 to 74 cases in 2015. This achievement has reached not only through significant commitment of resources towards global polio vaccination, but through focused molecular and population epidemiologic research which has led to an understanding of host and viral factors associated with vaccine immunogenicity. However, as global polio eradication efforts continue, knowledge gaps regarding effectiveness of polio vaccines have emerged. A recent outbreak of paralysis associated with wild polioviruses in Nigeria, identified after two years with no detectable wild virus circulation and genetically related to a wild strain last identified in 2011, highlights the impact of “silent” or asymptomatic circulation of wild polioviruses. There are well-known benefits and limitations to the use of both oral (OPV) and inactivated, parenteral (IPV) poliovirus vaccines. For decades, it has been known that canonical point mutations of the three Sabin oral polio vaccine serotypes are associated with development of vaccine-associated paralytic poliomyelitis (VAPP). More recently, the discovery of circulating neurovirulent vaccine derived polioviruses (VDPV), capable of causing paralytic poliomyelitis, threatens eradication. Thus, global cessation of live, oral polio vaccine use as soon as global polio eradication occurs is a high priority. However, understanding the dynamics of persistent circulation of OPV, VDPV, and VAPP strains, and the impact of vaccine poliovirus shedding and transmission on persistent vaccine-derived poliomyelitis is critical for the development of post-eradication vaccination policies. Equally important is the need to identify the determinants and potentiation of the immunogenicity of IPV regimens to assess whether these can be used in lieu of current OPV regimens to control potential post-eradication outbreaks.

Joel Maslow

Dr. Maslow is Chief Medical Officer of GeneOne Life Science Inc. GeneOne specializes in gene based therapies and disease solutions. He has been the medical lead at GeneOne for the past 10 years. Dr. Maslow received his Bachelor of Arts in Biology from LaSalle College, a Masters in Physics from Drexel University, PhD in Theoretical Physics from the University of Virginia, and MD from Jefferson Medical College. After an Internship in Internal Medicine at Thomas Jefferson University Hospital in Philadelphia, he completed residency in Internal Medicine at Abington Memorial Hospital and was Chief Resident also at Abington. Following fellowship in Infectious Diseases at Boston University, he joined the faculty at BU. In 1999 he was recruited to the University of Pennsylvania where he rose to Professor of Medicine and had multiple appointments as Chief of Infectious Diseases, Chairman of Medicine, and Associate Chief of Staff for Research at the Philadelphia VA. He more recently served as Chief of Infectious Diseases also serving as the Director of the HIV and AIDS program and Hospital Epidemiologist at Morristown Medical Center.

Dr. Maslow has been authored or co-authored greater than 75 peer reviewed articles and 15 review articles and book chapters. He is currently working on vaccine development of emerging diseases for Zika virus, MERS CoV, and the Ebola virus. In the past year, GeneOne has successfully had two INDs approved for MERS and Zika in 9 and 6 months, respectively. He serves as clinical lead on the Phase I studies for the three viruses.
Abstract [O2.1]:

Emerging Infectious Diseases (EIDs) pose unique challenges regarding vaccine development. Over the past few years, three epidemics – including two highly lethal illnesses with mortality rates of approximately 40% - have emerged or re-emerged. MERS-CoV, discovered in 2012 remained primarily within the Arabian Peninsula with sporadic cases worldwide and a large outbreak in South Korea in the summer of 2015. Zaire Ebola virus which reached epidemic proportions during 2014 in West Africa was exported across the globe, but with only rare secondary cases outside of the primary countries. Zika virus, however, has become a major pandemic spreading quickly through South and Central America reaching Florida on the US Mainland. Each of these viral pathogens presents both similar obstacles and unique challenges. The desire to introduce vaccine candidates in time to alter the epidemiology of the epidemic is counterbalanced by the time required for discovery and testing. Here we discuss the development of vaccines for EIDs in the context of newly emergent epidemics of MERS and Zika and discuss a roadmap to clinical trial assessment.

Thomas P. Monath

Dr. Monath is currently CSO and COO of BioProtection Systems, the infectious disease subsidiary of NewLink Genetics, where he is leading the development of the most advanced Ebola virus vaccine in partnership with Merck. The vaccine was recently shown to provide 100% efficacy after a single dose in Phase 3. He has over 25 years operating experience in the healthcare biotechnology industry, and is a former partner in the Pandemic and Biodefense Fund at Kleiner Perkins Caufield & Byers. Before joining NewLink, Tom was CMO for Hookipa Biotech, Juvaris and Xcellerex. Between 1992 & 2006, he was CSO and Executive Director of Acambis prior to its acquisition by Sanofi Pasteur, where he directed R & D on vaccines against dengue, Japanese encephalitis, West Nile, yellow fever, Clostridium difficile, and smallpox. The dengue vaccine that he invented, now commercialized by Sanofi (Dengvaxia®), is one of five vaccines he developed that are now licensed or in late Phase III trials. He currently serves on the boards of Vaxxinate, Juvaris, Sentinext, RapidMicro Biosystems, and US Biologic. Before joining industry, Tom served in the uniformed services of the US Army and US Public Health Service for 24 years prior to retiring as a Colonel. He was Director, Division of Vector-Borne Viral Diseases at the CDC and Prevention and Chief of the Virology Division at USAMRIID. Dr. Monath received his undergraduate degree and MD from Harvard. Tom has received many prestigious awards, including the Nathanial A. Young Award (1984), the Richard M. Taylor Award (1996), the Walter Reed Medal (2002) and the James H. Steele Gold-Headed Cane Award (2015). He has served on numerous government and international committees on infectious diseases, biosecurity, WHO expert committees and the National Vaccines Advisory Committee (USA). He has published over 400 scientific papers and 6 books on virology and vaccine development.

Abstract [PL1.2]:

rVSV-ZEBOV is a replication competent virus constructed by reverse genetics so that the Glycoprotein (G) gene of VSV-Indiana is deleted and replaced with the Glycoprotein (GP) of ebolavirus zaire (Kikwit strain). The virus was rescued by transfection of DNA plasmids and plaque purified in Vero cell culture. The biologically cloned virus was amplified to make a master virus seed. Viral progeny were shown to have typical bullet shaped vesiculovirus morphology, envelope spikes composed of ebola GP, and to grow to high titers in Vero cell cultures. Unlike ebolavirus, no soluble GP was produced. Attenuation was shown by absence of clinical signs after parenteral inoculation and of neurovirulence after intracerebral inoculation in mice, hamsters, and nonhuman primates (NHP). Unlike ebolavirus, no soluble GP was produced. Attenuation was shown by absence of clinical signs after parenteral inoculation and of neurovirulence after intracerebral inoculation in mice, hamsters, and nonhuman primates (NHP). Including immunocompromised animals. A single inoculation of the vaccine elicited antibodies and protected mice and NHPs against lethal challenge with ebola zaire. During the 2014 ebola epidemic in West Africa, manufacturing of vaccine batches was intensified to provide doses for clinical trials and emergency control. Between October 2014 and March 2015, seven lots of vaccine totaling approximately 100,000 doses were produced and released, and assays for controlling the vaccine and measuring immune responses were qualified. In 3-4Q 2015, the manufacturing process was scaled up 5-fold at Merck. Phase 1 and 1b clinical trials were initiated in Oct-Dec, 2014 in North America, Europe, and in Gabon and Kenya. Phase 2 and Phase 3 trials were initiated...
in Feb-Apr 2015 in Liberia (sponsored by NIH), Sierra Leone (Sponsored by CDC), Guinea (sponsored by WHO) and the US (sponsored by Merck). Highlights of the results of these clinical trials (which have involved a total of ~17,000 subjects), as well as additional nonclinical data will be presented. In brief, the rVSV-ZEBOV vaccine has been shown to be well-tolerated with no vaccine-related serious adverse events, a generally mild viral syndrome associated with low-level viremia and lymphopenia during the first 1-3 days after inoculation, and a low incidence of virally-mediated oligoarthritis in 3-4% of subjects, which is generally mild and self-limited. A single inoculation provides rapid onset of immunity in humans and protection against challenge in the NHP model. IgG antibodies appear in virtually 100% of subjects by Day 14 after inoculation with a single dose, peak at day 28, and persist for at least 6 months. Neutralizing antibodies have similar early kinetics. Dose response studies showed high antibody responses across a wide range from 3x103 to 1x108 plaque-forming units. A Phase 3 efficacy trial conducted by WHO in Guinea using ring-vaccination around ebola cases demonstrated 100% vaccine efficacy in subjects vaccinated with a single dose at least 10 days previously. V920 vaccine is in late-stage development with the goal of licensure on an accelerated timescale and holds promise as a means of preventing or rapidly controlling future emergences of ebola virus disease.

Luis Montaner

Dr. Luis Montaner is Director of the HIV-1 Immunopathogenesis Laboratory and has enjoyed an active research partnership with community health center Philadelphia FIGHT for more than 20 years. He was recently awarded a $23 million Martin Delaney Collaboratory grant from NIH to advance the cure agenda. He currently serves as Editor-In-Chief of the Journal of Leukocyte Biology with an editorial staff of over 50 investigators. In December 2014, Dr. Montaner was awarded the Herbert Kean, M.D. Family Professorship, a five-year endowment that recognizes high-risk, high-reward research. His research is primarily focused on innate effectors, immune regulation of infection, activation measurements on ART, and translational human immunology-based studies. Dr. Montaner has received numerous distinctions for his work on behalf of people living with HIV/AIDS, including the Jonathan Lax Award from Philadelphia FIGHT, the Founders’ Award from the AIDS Fund of Philadelphia, and a Recognition and Honors Resolution from the Philadelphia City Council. Dr. Montaner and his team are currently enrolling participants in the largest HIV Cure research clinical trial to date.

Abstract [O2.3]:

The inability of antiretroviral therapy (ART) to clear HIV infection, and the observation that a sterilizing cure and/or stable remission can be achieved by a subset of ART-treated persons, has galvanized the interest to advance clinical strategies towards a cure and/or stable remission. Clinical preliminary data from several single agent immunotherapy human trials indicate a potential to inhibit HIV beyond ART. Current HIV cure efforts will seek to develop and test innovative combined immunotherapy strategies to eradicate and/or permanently suppress HIV into remission in the absence of ART.

Laurence Mulard

Laurence A. Mulard graduated from the École Supérieure de Physique et Chimie Industrielles (ESPCI, Paris, France) and the same year completed her MSc at the Université Pierre et Marie Curie (Paris, France). She received her PhD in chemistry from the same University (1991) working in nucleos(t)ide synthesis under the supervision of Prof. Czernecki. After three postdoctoral years at the NIH (Bethesda, MD, USA) studying glycochemistry in Dr Glaudemans’ group, she joined the Institut Pasteur (Paris, France) first as Research Assistant, then as Research Associate. She was appointed Senior Scientist in 2007 and accepted the position of Head of the Unit “Chemistry of Biomolecules” at the Institut Pasteur in 2008. Her research interests are at the interface between Chemistry, Glycoscience and Vaccinology, in the area of carbohydrates and glycoconjugates in relation to disease and biological recognition. Focus is on the development of carbohydrate-based vaccines, diagnostic tools, and on polysaccharide antigen/antibody recognition, which she investigates by use of synthetic chemistry in combination with structural, physicochemical and immunochemical analysis.
Abstract [O4.5]:

Well-defined carbohydrate-based conjugates, encompassing synthetic oligosaccharides specifically designed for their ability to act as functional mimics of "protective" glycan antigens are considered as an attractive alternative to the use of parenterally administered immunogens derived from glycans of biological origin. The concept aims at fulfilling increasing requirements for better defined and safer vaccines, and the use of synthesis might enable greater flexibility in the design and production of vaccine antigens. Herein, the concept is described in the context of bacterial diseases with bacillary dysentery as an example. Indeed, owing to *Shigella* species/serotype diversity and variable geographical distribution, there is an unmet need for a broad coverage vaccine against Shigellosis.

Based on the observation that protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the lipopolysaccharide (LPS), immunogens consisting of synthetic fragments of the putative O-Ag conjugated to a carrier protein have been developed as vaccine candidates against *Shigella flexneri*, the prevalent species in developing countries. Along this line, this contribution will firstly describe the multidisciplinary strategy, which has led to SF2a-TT15, a rationally designed vaccine candidate against *S. flexneri* serotype 2a now entering phase 1 clinical trial. Then, it will highlight ongoing progress in our laboratory toward a synthetic carbohydrate-based *Shigella* vaccine conferring broad species/serotype coverage. Key features include (i) the use of carbohydrate hapten suitable for site-selective attachment to overcome limitations associated to LPS detoxification, random chemical modification and/or low loading, (ii) the control of the carbohydrate chain length, endchain residue and substitution pattern to overcome LPS heterogeneity and confer optimized O-Ag functional mimicry, (iii) the selection of a potent carrier authorized for use in human to allow easy transfer to clinic, and (iv) the display of a carbohydrate loading favoring the induction of a strong humoral immune response following parenteral administration, which is possibly enhanced when the vaccine is adjuvanted.

Flor M. Munoz

Flor M. Munoz, MD, MSc. is Associate Professor of Pediatrics and Molecular Virology and Microbiology at Baylor College of Medicine (BCM) in Houston, Texas. Dr. Munoz is a pediatric Infectious Diseases Specialist interested in the epidemiology and prevention of infections in young infants through pediatric and maternal immunization. She has over 18 years of experience in clinical research, conducting NIH and industry sponsored phase I to IV infant and maternal immunization studies, and clinical trials of antiviral drugs for infants and children with respiratory infections. Dr. Munoz is involved in CDC pandemic preparedness influenza projects that focus on surveillance, vaccine and antiviral effectiveness in hospitalized and ambulatory patients and pregnant women, and in the CDC NVSN ARI Surveillance Network. Dr. Munoz is a member of the Committee of Infectious Diseases of the American Academy of Pediatrics, and COID liaison to the ACIP influenza working group. She is a member of special interest groups on maternal immunization at NIH, CDC, WHO, NVAC, BMGF, and the Brighton Collaboration GAIA Project, which aims to achieve harmonization of definitions for the assessment of safety of vaccines in pregnancy, and IRB Chair at BCM. She is Director of Solid Organ Transplant Infectious Diseases and Infectious Diseases Consultant at Texas Children’s Hospital.

Abstract [PL4.2]:

Immunization of women during pregnancy is an accepted strategy to protect mothers and infants against infectious diseases during a period of high vulnerability. As a public health intervention, maternal immunization has the potential to reduce the morbidity and mortality associated with pathogens that affect the mother, the newborn, or both. Maternal immunization with tetanus, pertussis and influenza vaccines results in direct protection of the mother and the newborn infant. Group B streptococcus and Respiratory Syncytial Virus (RSV) are infections that could be prevented through immunization of pregnant women with safe and effective vaccines that are currently under clinical investigation. The rationale, safety, effectiveness, acceptability and potential impact of vaccination of women during pregnancy will be reviewed in this presentation. Challenges associated with the development and implementation of clinical trials of vaccines administered during pregnancy will be discussed.
Albert Osterhaus

As professor of Virology in Rotterdam and Utrecht, The Netherlands, and in Hannover, Germany, Ab Osterhaus has had a long track record as scientific researcher and PI of numerous major scientific projects.

At Erasmus MC he ran the >40 persons virology diagnostic lab and the > 100 persons virology research lab. His research programme followed a novel integrated “viroscience” concept, bringing together world leading scientists in molecular virology, immunology, epidemiology, pathogenesis, and intervention studies on human and animal virus infections.

After having handed over the chairmanship of the Erasmus MC Viroscience lab in 2014, he is currently establishing new One Health Institutes in Hannover and Utrecht.

Major performances include the discovery of more than 50 new viruses of humans and animals (e.g. human metapneumovirus, human coronaviruses, influenza viruses), elucidation of the pathogenesis of major human and animal virus infections, and development of novel intervention strategies. This has enabled health authorities like WHO, to effectively combat disease outbreaks like SARS and avian influenza. The spin-off, Viroclinics Biosciences BV, is another societally relevant success, allowing effective testing and refining of diagnostic tools and other intervention strategies.

Awards, prizes, guest lecture invitations, (co-)organisership of international meetings and editorships of scientific journals further highlight his international recognition. He has acted as PhD mentor for more than 75 students, holds several key patents and is the author of more than 1100 papers in peer-reviewed journals, together cited more than 50,000 times, and his current H index is 97. He is Editor-in-Chief of two major Elsevier Journals. Most of all, Ab Osterhaus firmly believes scientists have a role to play in translating their knowledge for the benefit and protection of society.

Abstract [PL3.2]:

Complex relationships between humans and animals have created an interface that allowed cross-species transmission, emergence and eventual evolution of a plethora of human pathogens. Until 1900, infectious diseases were the major cause of mortality of humankind, causing an estimated fifty percent of all deaths. In the western world, this decreased to only a few percent, due to the implementation of public health measures and the introduction of vaccines and antimicrobial compounds. This prompted policymakers and scientists to speculate that soon human infectious diseases would be brought under control. Paradoxically, soon thereafter the world was confronted with an ever-increasing number of emerging and re-emerging infectious diseases, like AIDS, Avian flu, SARS, MERS, Ebola, and Zika, spilling over from animal reservoirs. A complex mix of predisposing factors in our globalizing world, linked to major changes in our societal environment and global ecology, collectively created opportunities for viruses and other pathogens to infect and adapt to new animal and/or human hosts. This paved the way for the unprecedented spread of infections in humans and animals with dramatic consequences for public and animal health, animal welfare, food supply, economies, and biodiversity. It is important to realize that due to the complex and largely interactive nature of the predisposing factors, it is virtually impossible to predict what the next pathogen threat will be, from where it will come and when it will strike. However better understanding of the underlying processes may eventually lead to predictions that would improve our preparedness for outbreaks in humans and animals. Investment in a better understanding the human-animal interface will therefore offer a future head start in the never-ending battle against infectious diseases of humans. Importantly, the increased emergence of viral infections is largely paralleled by medical, veterinary, technological, and scientific progress, continuously spurred by our never-ending combat against pathogens. Especially the establishment of vaccine development platforms, widely applicable to both known and unknown viruses will further contribute to an R&D based response preparedness.
Stanley A. Plotkin

Dr. Stanley A. Plotkin is Emeritus Professor of the University of Pennsylvania. Until 1991, he was Professor of Pediatrics and Microbiology at the University of Pennsylvania, Professor of Virology at the Wistar Institute and at the same time, Director of Infectious Diseases and Senior Physician at the Children’s Hospital of Philadelphia. For seven years he was Medical and Scientific Director of Sanofi Pasteur, based at Marnes-la-Coquette, outside Paris. He is now consultant to vaccine manufacturers and non-profit research organizations.

He is a member of the Institute of Medicine of the National Academy of Sciences and the French Academy of Medicine. His bibliography includes over 700 articles and he has edited several books including a textbook on vaccines. He developed the rubella vaccine now in standard use throughout the world, is codeveloper of the newly licensed pentavalent rotavirus vaccine, and has worked extensively on the development and application of other vaccines including anthrax, oral polio, rabies, varicella, and cytomegalovirus.

Abstract [PL1.1]:

The epidemic of Ebola killed 11,000 people in West Africa and had severe medical and social impact. However, at least seven candidate vaccines had shown efficacy in primates before the epidemic, but were not carried through human trials and licensure. Therefore, vaccines were not available until the end of the outbreak. To avoid this from happening again with other pathogens we have proposed a global vaccine development fund for emerging pathogens that elicit no vaccine response by pharmaceutical manufacturers because the market is too small. This fund is taking shape under the leadership of the Wellcome Trust, the Norwegian government and other organizations. The idea would be to select candidate vaccines that look effective in animal models through the stages of GMP production, phase 1 and 2 clinical trials and stockpile storage. In emergencies, the stockpile could be used and phase 3 trial and licensure could be pursued.

Hari Pujar

Bio and abstract [O2.2] not available at time of print 2016-09-21

Edward Rybicki

Professor Ed Rybicki received his pre- and postgraduate education at the University of Cape Town, South Africa, where he has worked throughout his career after being appointed as Lecturer in Virology in 1981. He gained molecular biology experience as a visiting scientist at Plant Genetic Systems, Ghent, Belgium in 1984, worked as a Ciba Foundation Fellow at Rothamsted Experiment Station in Harpenden, UK, in 1986, and as a sabbatical scientist at the Boyce Thompson Institute in Cornell University in Ithaca, New York, (1990-1). More recently he visited the Arizona Bodesign Institute, Phoenix, U.S. (2010). He became a full Professor in Microbiology in 2003, was a founder member of the Institute of Infectious Disease and Molecular Medicine in the Faculty of Health Sciences in 2005, and is currently (since 2013) Director of the Biopharming Research Unit in the Molecular & Cell Biology Department at the University of Cape Town. His research originally focused on molecular characterization of plant viruses, which work enabled pioneering discoveries in vaccine-related recombinant gene expression in plants and in insect cells. Prof. Rybicki developed these “biopharming” techniques to produce candidate vaccines against HPV-11 and -16, HIV-1 subtype C, influenza H5N1, and a number of human and animal viruses. He has authored >120 papers and 47 patents. He is a recipient of numerous national awards and honors, including the President’s Award of the South African Foundation for Research Development (1985), the Meiring Naude Medal of the Royal Society of South Africa (1986), and a National Research Foundation of South Africa “A” rating for research achievements.
Abstract [O5.1]:

The CDC has the phrase “The One Health concept recognizes that the health of humans is connected to the health of animals and the environment” on its website — recognizing that the health of people is related to the health of animals, especially in the case of zoonotic diseases. Thus, viral diseases such as rabies, West Nile and Zika and Rift Valley and Crimean-Congo haemorrhagic fevers are all of interest, and there is particular interest in developing low-cost reagents for point-of-care diagnostics that could also be used as vaccines for animals, and possibly also for humans.

A particular problem with many of the diseases of interest to the One Health movement is that they mainly affect people and animals in developing countries, with the result that resources not only to study them, but in particular to develop tools to work with and to combat them, are largely lacking.

A possible answer to at least part of the problem lies in using plants to express proteins of interest. Both transgenic and increasingly transient expression in plants are very well-established technologies for the high-yield, low-cost, highly scalable production of complex biologics, including simple virus-like particles (VLPs) for hepatitis B and human papillomaviruses, 4-component VLPs for rota- and orbiviruses, budded HA-only VLPs for influenza viruses, and even monoclonal antibodies for rabies and Ebola and HI viruses.

Our laboratory has a near 20-year history in this research area, in a relatively low-resource setting and using mainly local African funding. I will describe our successes in making H5N1 HA as a pilot for a pandemic influenza vaccine, the N proteins of Rift Valley fever and Crimean-Congo haemorrhagic fever viruses as reagents for accurate and reliable diagnosis of viral infection, as well as other projects involving human papillomaviruses and non-viral reagents, as models illustrating the potential of plant expression for enabling One Health virology and vaccinology.

Amadou Sall

Amadou A Sall is a virologist with a PhD in Public health. From 2002-2004, Dr Sall has worked in Cambodia as the head of viral hepatitis B at Institut Pasteur Cambodia. In 2010-2011, he works as a Visiting Research Scientist at the Center for Infection and immunity at the Mailman School of Public health at Columbia University of New York on pathogen discovery.

He is currently the Director of Institut Pasteur de Dakar which belongs to the Institut Pasteur International Network. Dr Sall is also director of the WHO collaborating center for arboviruses and viral hemorrhagic fever at IPD. His research focused primarily on diagnostics, ecology and evolution, vaccine and vaccination of arboviruses and viral hemorrhagic fever.

Dr Sall is consultant and member of several expert committees for WHO, OIE and vice chair of the Global Outbreak Alert and Response Network steering committee. He has taught at the University Cheikh Anta Diop Dakar, University of Columbia at New York, Institut Pasteur in Paris, Montevideo, University of Sao Paulo, Hong Kong University, National and Autonomus University of Mexico and Mc Gill university in Canada.

Abstract [O4.1]:

Abstract not available at time of print 2016-09-21

Frédéric Tangy

Frédéric Tangy, PhD, Dr.Sc., Director of Research at CNRS, is the head of the Viral Genomics and Vaccination Research Unit at Institut Pasteur, Paris (CNRS UMR-3569). After his PhD in 1980 and his Dr. Sc. in 1984 at Paris VI University, he made his career as a virologist first at CNRS then at Institut Pasteur. He is director of the international Vaccinology course of Institut Pasteur, vice-president of the Scientific
Over the last two decades a number of new viral diseases such as SARS, MERS or Nipah virus infections have emerged at the animal-human interface, and infections such as Ebola and Lassa viruses, or recently Zika virus, have expanded beyond their usual limited territories. Each time, the global community failed to develop effective interventions in a timely manner. We developed the use of one of the safest and most efficacious vaccines available, the live attenuated measles vaccine, as a platform for the delivery of these new antigens.

Measles vaccination has been used for more than 40 years in over 1 billion children and is 95% efficacious after one or two administration. Measles vaccine is genetically stable and reversion to pathogenicity has never been observed. Taking advantage of these characteristics, we cloned the attenuated measles Schwarz vaccine virus and developed a method to genetically manipulate this negative strand RNA virus into a versatile chimeric or recombinant vector.

Proof of concept in humans for this technology has recently been demonstrated for a measles-Chikungunya vaccine. A Phase I clinical trial showed that the vaccine was well tolerated and induced a robust and functional antibody response after 1 (90%) or 2 immunizations (100%). This trial also demonstrated that pre-existing measles antibodies did not impair the immunogenicity of the heterologous antigen (Ramsauer, 2015, Lancet Inf. Dis.). Thus, pre-immunity to measles due to vaccination or infection will not restrict the use of recombinant measles vector for new vaccines. Many other antigens from HIV, DENV, CHIKV, WN, SARS, H5N1, Ebola, Zika, and Plasmodium have been expressed in this vector and their strong immunogenicity or protective capacity has been established in preclinical animal models, also in the presence of pre-existing measles immunity, highlighting the potential of measles vector as a platform for rapid response to new pathogens.
oligomeric protein conformation, as well as a means to stimulate innate immunity to ameliorate AD pathology (which we are testing in aged squirrel monkeys, a natural model of AD pathology). We have also recently identified novel small, BBB penetrant compounds that specifically bind Aβ oligomers.

Abstract [O3.1]:

Alzheimer’s disease (AD) and the prionoses are both conformational disorders, where self-proteins undergo a conformational change to pathological conformers that are neurotoxic. Neither AD or prion diseases have effective therapies currently; however, a number of immunotherapeutic approaches have shown promise. For AD we have investigated stimulation of innate immunity with TLR9 agonist CpG oligodeoxynucleotide (ODN) and have demonstrated effectiveness at reducing amyloid plaques, vascular amyloid and tau pathology without inducing toxicity in multiple AD mouse models. We have advanced these studies using a well-established non-human primate model of sporadic vascular amyloid, squirrel monkey (Saimiri Boliviensis) and have evidence of cognitive benefits, in association with pathology reductions. We have also developed active and passive immunization approaches for AD. We designed a non-self, oligomeric peptide called pBri, as an immunogen. We tested this approach in APP/PS1, 3xTg and TgSwDI AD models and have documented pBri as an active vaccine reduces amyloid plaques, vascular amyloid deposits and neurofibrillary tangles. We have also used pBri to develop monoclonal antibodies (mAbs). Positive hybridomas were selected by their shared reactivity against Aβ, paired helical filaments and the prion protein. The best mAbs were characterized by blots, surface plasmon resonance and histology in AD tissue. Passive immunization with one such mAb has been tested in 3xTg mice with both tau and Aβ related pathology, where it produced cognitive benefits with pathology amelioration. For prion disease, where infection can occur via a fecal-oral route, we have developed a mucosal vaccination with PrP molecules delivered by an attenuated Salmonella carrier that was successful in preventing transmission and infection of prionoses in susceptible mice. The methodology was scaled up for testing in white tail deer, where we were able to elicit a distinct mucosal and systemic antibody response to prion proteins and achieve a partial protection to an oral challenge with a pathological CWD-prion. This represented the first time a vaccine was shown to be at least partially affective for a naturally occur prion infection, in a normal host animal. A number of immunotherapeutic approaches have potential as therapeutic approaches for both AD and prion diseases.
The generation of an immunogenic second-generation conserved segment HCV T-cell vaccine to target multiple HCV genotypes.

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**Key words:** Hepatitis C Virus, vaccine design, adenovirus

**Background and aims:** HCV genomic variability is a major challenge to the generation of a HCV prophylactic vaccine. We have previously shown that HCV-specific T-cell responses induced by a potent T-cell vaccine encoding a single strain subtype-1b immunogen targets epitopes dominant in natural infection. However, viral regions corresponding to some of these epitopes are highly variable at a population level with a reduction in T-cell reactivity to these variants. We therefore designed and manufactured second generation simian adenovirus vaccines encoding genomic segments that are conserved between viral genotypes (gts) and assessed these for immunogenicity.

**Methods:** We developed a computer algorithm to identify HCV genomic segments from open resource and in-house sequences that were conserved between viral subtypes. Conserved segments below a pre-defined threshold spanning the entire HCV coding genome were combined to create novel immunogens (1000-1500 aminoacids AA) for HCV gts 1 and 3, and 1 to 6 inclusive. Putative artificial epitopes restricted by common HLA super-types in junction regions were abrogated. *In silico* analysis enabled exclusion of potential cross-reactivity with human self-peptides and to identify human HCV T-cell epitopes. Simian adenoviral vaccine vectors encoding the HCV (ChAdOx1-gt1/3 and ChAdOx1-gt1-6) and control immunogens (ChAdOx1-eGFP and ChAdOx1-gt1b-NS) were constructed. Immunogenicity was evaluated in BALB/c, C57BL6 (4/group) and HLA-A2 transgenic mice (5/group) at 108 IU intramuscular dose, using panels of genotype-specific peptide pools in *ex-vivo* IFN-γ ELISpot assays.

**Results:** ChAdOx1 conserved segment HCV vaccines primed high-magnitude, broad, cross-reactive T-cell responses; the mean magnitude of total HCV 1b-specific T-cell responses was 2200 and 989 SFU/106 splenocytes for ChAdOx1-gt1/3 and ChAdOx1-gt1-6 respectively in BALB/c mice targeting multiple genomic regions. BALB/c mice vaccinated with ChAdOx1-gt1-6 assessed using gt 1a, 1b and 3a peptide panels gave mean total HCV-specific T-cell responses of 942, 989 and 2079 SFU/106 splenocytes respectively (Similarly, highly inter-genotypic cross-reactive T-cell responses were seen with ChAdOx1-gt1/3 and in C57BL6 mice). Significant T-cell responses were observed in HLA-A2 transgenic mice after specific A2-restricted peptide sequence stimulation of harvested splenocytes. *In silico* analysis shows that conserved immunogens contain multiple epitopes described in natural HCV infection.

**Conclusions:** Novel pan-genotypic HCV simian adenoviral vectored vaccines encoding conserved segments from all major HCV genotypes contain multiple T-cell epitopes described in human infection and are highly immunogenic in mouse models. These studies pave the way for the assessment of pan-genotypic HCV T-cell vaccines in humans.

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**[O1.3]**

HSV-2 vaccine virus deleted in glycoprotein D (ΔgD-2) elicits high-titer IgG2 antibodies that activate the FcR and protect mice and guinea pigs from skin or vaginal challenge with clinical isolates of HSV-1 and HSV-2, and prevent the establishment of latency.

Betsy C. Herold, Clare Burn, Natalie Ramsey, Brian Weinrick, Kayla Weiss, Chris Petro and William R Jacobs, Jr

HSV prevention is a public health priority. Prior vaccine efforts focused on neutralizing antibodies (Abs) targeting glycoprotein D (gD), but the clinical trial results were disappointing. We engineered a single-cycle HSV-2 strain deleted in gD (ΔgD-2) to test the hypothesis that deletion of this immunodominant glycoprotein might facilitate the induction of a different type of immune response. We tested the vaccine for safety and efficacy in mice using skin scarification and/or vaginal challenge models and analyzed the immune response. Vaccine doses of 10^5 or 10^6 pfu/ml protected 100% of mice (n=180) from lethal challenge using a panel of genetically diverse clinical isolates of HSV-1 and HSV-2 and protected 98% (n=100) of mice from latency as measured in dorsal root ganglia by qPCR for viral DNA or *ex vivo* viral reactivation. Female and male mice were protected and no disease was observed following inoculation of SCID mice. Moreover, the vaccine protected guinea pigs from lethal challenge with a clinical isolate and again no latent virus was detected in DRG by qPCR. HSV-specific Abs (1:800,000), predominantly of the IgG2 subtype, as well as CD4 and CD8 T cell responses were elicited following subcutaneous prime and boost. Passive transfer of immune serum completely protected wild-type, but not FcRγ or Fc neonatal receptor knockout mice, from subsequent lethal challenge. Immunization was associated with rapid recruitment of HSV-specific murine FcγRIV-activating IgG2 Abs into the skin or vaginal mucosa within 48 h of challenge and virus was cleared by day 5 post-challenge. In contrast, a recombinant gD protein vaccine elicited higher neutralizing Abs (1:640) compared to ΔgD (1:5), but little or no FcR activation and failed to protect against latency. These findings challenge the dogma that neutralizing Abs are key correlates of protection and suggest that vaccines that elicit ADCC may provide the greatest protection against HSV.
Immunogenicity and efficacy of a trivalent HSV-2 gC2/gD2/gE2 subunit antigen vaccine in Rhesus macaques and guinea pigs
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We evaluated the immunogenicity of a trivalent HSV-2 subunit vaccine in Rhesus macaques and vaccine efficacy in the guinea pig genital infection model. The vaccine contained HSV-2 glycoproteins C, D and E (gC2, gD2, gE2) to block entry mediated by gD2 and immune evasion mediated by gC2 and gE2. The trivalent vaccine administered with CpG and alum as adjuvants was immunogenic in Rhesus macaques inducing CD4 and CD8 T cell responses, vaginal and plasma neutralizing antibodies, vaginal IgG and IgA antibodies to the immunogens, and plasma antibodies that block immune evasion activities mediated by gC2 and gE2, including C3b binding to gC2 and IgG Fc binding to gE2. After intravaginal challenge, naïve animals developed histopathological evidence of vaginitis, while immunized animals were protected. Additional efficacy studies were performed using the guinea pig vaginal infection model. Guinea pigs were immunized three times at two-week intervals either with the trivalent vaccine, gD2 alone or mock-immunized and challenged intravaginally with 5x10^5 PFU of HSV-2 strain MS. The trivalent vaccine was 99% efficacious in preventing acute and 96% in preventing recurrent genital lesions. The trivalent vaccine outperformed gD2 alone in preventing acute and recurrent genital lesions. Starting 1-2 months post-challenge, vaginal shedding of HSV-2 DNA was detected in 10% of mock-immunized animals, and 5% of gD2 and trivalent immunized animals. On days animals were shedding HSV-2 DNA, infectious virus was isolated from 7/25 (28%) from gD2 immunized, but only 1/27 (4%) from trivalent immunized animals. No infectious virus was isolated from 1000 vaginal swabs on days animals were not shedding HSV-2 DNA. Overall, animals challenged intravaginally after immunization with the trivalent subunit antigen vaccine had acute genital lesions on 1/378 (0.26%) of days, recurrent genital lesions on 14/1158 (1.2%) of days, and shedding of infectious virus on 1/555 (0.18%) of days. The trivalent subunit antigen vaccine is an outstanding candidate for a prophylactic genital herpes vaccine in humans.

Decoding Immune Evading Mechanisms of pathogens: reordering of immunodominance for new and improved vaccines
Peter Lloyd Nara, M.Sc., D.V.M., Ph.D., F.A.A.A.S.

Over the last 250 years, the use of vaccines, a mainstay of preventative medicine and public health has proven to be one of the most successful and cost-effective medical interventions ever discovered. Despite these great advances to human and animal health of the past 5 decades; the basic immunologic principals and technology on which it was created from does not for the most part work against the many remaining pathogens of humans and animals. This is mainly due to evolved immune evading strategies by the pathogens thus making them inherently more resistant due to strain-restricted immunity/antigenic variation/poor memory/disease-enhancement/incomplete immunity and/or shortened forms of immunity and thus represents a major gap in our understanding of the complex evasion mechanisms evolved by the pathogens.

“Deceptive Imprinting” is at the heart of a new understanding of how mutable pathogens create a molecular diversion (decoy) at the level of both the innate and acquired immune host defense systems-- much like how metallic chaff would confuse a radar system trying to locate a missile or plane. On an immunologic level immunodominance and antigenic variation are coupled such that host immune responses are directed to less or non-protective B and T cell epitopes. To circumvent this host evading mechanism we have developed a first generation technology called Immune Refocusing that has been designed specifically to reorder the non-protective immunodominance by identifying/mapping the decoy epitopes and molecularly removing or attenuating it thus redirecting the host immune system to the more protective regions of the microbe.

A discussion for an ongoing paradigm shifting first principals of Deceptive Imprinting, immunology, new insight from querying pathogen genomes through “Pressure Point” Technology and application of the technology of Immune Refocusing will be presented as well as the pre-clinical results for the first in man vaccine for the Human Rhinovirus. These paradigm shifting scientific insights have opened up fresh new approaches to technical advancement and the development of new antigens that can be used for vaccines and deriving new monoclonal antibodies toward inducing improved and broader protective immunity.
LAMP-based DNA vaccines suppress IgE production and intestinal anaphylaxis in a murine model of Peanut-induced Food Allergy.
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Introduction. Peanuts allergies affect over a million Americans and are the most common cause of anaphylaxis. Initial exposure to peanut proteins induces allergen-specific IgE secretion that primes granulocytes to release various allergy mediators upon subsequent allergen re-exposure, triggering potentially lethal anaphylactic reactions. Promising clinical-stage immunotherapy treatments require a prolonged period of desensitizing treatments that gradually induce a state of allergen-specific unresponsiveness. Safer and more effective peanut allergy therapies are urgently needed as peanut immunotherapy has a high risk of inducing severe adverse events, including anaphylaxis. We have previously shown that LAMP-based DNA vaccines suppress allergic IgE production through a Th1/Th2 skewing mechanism. Here we studied whether peanut allergen-encoding DNA vaccines (Ara-LAMP-vax) would abrogate allergic sensitization in mice by oral exposure to whole peanut extract (WPE) and suppress peanut-induced allergic (PIA) Th2 immune responses upon WPE re-exposure (Challenge).

Methods. In a C3H mouse model of food-induced allergy, mice were sensitized to WPE in the presence of cholera toxin by oral gavages once a week for four weeks and later PIA was induced upon peanut re-exposure by challenging the sensitized mice with bigger dose of WPE. Groups of mice were prophylactically treated with Ara-LAMP-vax DNA vaccines or an empty control vector before peanut sensitizations to determine whether Ara-LAMP-vax DNA vaccines may abrogate the initial allergic priming stage and suppress PIA symptoms.

Results. Ara-LAMP-vax immunizations suppressed allergen-specific IgE and IgG1 while enhancing specific IgG2a titers throughout the sensitizations and upon peanut challenge. In addition, Ara-LAMP-vax-immunized mice recovered from PIA symptoms (hypothermia) more rapidly, had fewer activated and IL-4 secreting basophils circulating in peripheral blood as well as fewer peritoneal mast cells.

Conclusion. Ara-LAMP-vax plasmid DNA immunizations significantly suppressed peanut-specific IgE production and priming of allergic responses in a mouse model of PIA, likely through switching the peanut-induced T helper response from a Th2 allergic response to a benign Th1 profile.

Minimally invasive microneedles mediated cutaneous immunotherapy for amelioration of Ovalbumin induced allergy in mice
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Abstract: A novel way to treat Ovalbumin (Ova) induced allergy is demonstrated by use of microneedle (MNs)-based allergen delivery in a mouse Ova allergy model.

Introduction: Allergen specific immunotherapy (ASIT) is clinically approved approach for the treatment of IgE mediated allergies in humans. ASIT involves repeatedly subcutaneous allergen delivery by hypodermic injections with a gradually increasing dose until immune tolerance is achieved. Entire process takes 3-5 years in completion and also associated with side effects. In present study, we demonstrate therapeutic efficacy of MNs coated with Ova and CpG adjuvant (MNs-CIT) to treat Ova allergy and its comparison to conventional subcutaneous approach (SCIT).

Materials and Methods: Balb/c female mice, aged 6-8 weeks were used in experiment. First Ova induced allergy established in mice through ip injection of Ova+alum two doses at one week of interval, followed by three consecutive challenges with high dose Ova. Established Ova allergy treated by Ova coated microneedles and vaccinated mice were further challenged (2nd challenge) to verify the efficacy of immunotherapy (figure 1A). Very next day post challenge, mice were sacrificed, lungs and bronchoalveolar (BAL) fluid were collected for the analysis.

Results and Discussion: Ova is precisely coated on 2D MNs array through automatic dip coater. Coating was uniform and consistent as confirmed by fluorescent microscopy images. After vaccination (AV), MNs-CIT group induced significantly higher anti-Ova IgG response than the MNs (p =0.0001), this response persisted even after challenge. Moreover, MNs-CIT induced superior anti-Ova IgG2a response in comparison to other groups indicate activation of Th1 response which helps in inhibition of allergic response. MNs-CIT considerably suppressed established atopic inflammation similar to the conventional SCIT as indicated by BAL fluid analysis. Infiltration of effector cells in BAL fluid were observed low in MNs-CIT than the MNs (p < 0.0001). Down regulation of Th2 type cytokines IL-4, IL-5 and IL-13 and up regulation of IL-10 further proved therapeutic potential of MNs-CIT. IL-13 expression was also observed low in MNs-CIT than the MNs (p < 0.0001), while expression of IL-10 was considerably high in MNs-CIT (p < 0.0001) than the MNs. Moreover least mucus deposition observed inside wall of lung bronchioles in MNs-CIT than the MNs pointed out the therapeutic efficacy of MNs-CIT.

Conclusions: MNs-CIT successfully treated the established Ova induced allergy in mice, which was comparable to the conventional Sc route, suggesting potential of MNs as a painless and convenient approach for ASIT.

Comparative evaluation of electroporation mediated intradermal or intramuscular administration of a DNA vaccines against the Venezuelan, eastern, and western encephalitis alphaviruses

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Venezuelan, eastern, and western equine encephalitis viruses (VEEV, EEEV, and WEEV respectively) are a mosquito borne alphaviruses that are classified as a CDC/NIH Category B pathogen. Currently, there are no VEEV, EEEV, or WEEV vaccines licensed in the U.S. for use in humans. The development of novel prophylactic interventions for prevention of V/E/WEEV infection in humans is warranted due to the potential for both natural and intentional disease outbreaks. Given the favorable safety profile associated with DNA vaccines and the potential logistical advantages associated with their development and deployment, DNA vaccines represent an appealing approach to the development of novel vaccines against V/E/WEEV. An ongoing program to develop novel V/E/WEEV DNA vaccine candidates has included the use of electroporation (EP) mediated delivery to improve the potency of the candidates. The program has included extensive non-clinical testing of the V/E/WEEV candidates alone in combination for immunogenicity and protection against disease challenge. Results in these studies demonstrated that electroporation mediated delivery of the vaccines either intradermally (ID) or intramuscularly (IM) could elicit potent anti-viral immune responses and protection against viral challenge. These encouraging results prompted the initiation of a randomized, placebo controlled Phase I study designed to compare delivery of the VEEV candidate by intramuscular versus intraderal EP. The study was designed to assess the safety and immunogenicity of the VEEV DNA vaccine candidate as well as the relative tolerability of the two routes of administration. The three arm study (IM VEEV DNA vaccine, ID VEEV DNA vaccine, or placebo) enrolled 41 subjects that received at least one administration. The administration schedule comprised three injections administered at Week 0, 4 and 8. Injections were administered as placebo (saline) or one of two DNA dose levels (0.5 mg/ml or 2.0 mg/ml). The administration procedures were well tolerated with mild injection site reactions comprising the vast majority of adverse events. Although the frequency and severity of injection site reactions was comparable between the two routes of administration, there were differences in the nature of the reactions seen with erythema and induration more common in the ID group and muscle soreness more common in the IM group. There were no vaccine or device related serious adverse events observed and no discernable differences in the nature, frequency, or severity of adverse events between the vaccine and placebo arms of the study. Analysis of serum anti-VEEV neutralization activity demonstrate high rates of seroconversion for both the intradermal and intramuscular routes of delivery and a DNA dose dependent magnitude of response. These data provide initial proof of concept for the vaccine candidate and have set the stage for further clinical development of the V/E/WEEV candidates. The studies have also provided initial insights into the effect of route of administration on the safety, tolerability, and immunogenicity of EP mediated DNA immunization.
Prevention of emerging zoonoses in Central Africa: an overview from a veterinary perspective
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During the last five years significant attention has been given to emerging zoonoses, particularly in geographical
areas with high risk for outbreak of infectious diseases. More specifically zoonotic diseases with high threats have
been targeted for elimination. The remaining diseases could be regarded as neglected tropical zoonoses with serious
public health impact, particularly parasitic zoonotic diseases. We have identified through scoping review methods
more than 40 potential zoonotic diseases in Central Africa including the Congo Basin (Cameroon, Central African
Republic, Congo, Gabon, Democratic Republic of Congo, Equatorial Guinea), one of five areas in the world
considered to be at high risk for emerging infectious diseases. Most of the countries in the area of focus are
implementing global health security agenda programs supported by the international agencies (FAO, OIE, WHO,
USAID, CDC etc). Ongoing activities utilizing a “One Health” approach involving wildlife, environmental health, public
health, and veterinary services are focused on following the potential zoonotic diseases: influenza, Ebola, Marburg
fever, RVF, lassa fever, dengue, yellow fever, Crimea Congo HF, Chikungunya, West Nyle virus, Monkey pox,
coronavirus, rabies and Bas-Congo virus. This global health program is based mostly, in each country, on capacity
building for surveillance, early detection of new threats and response to the emergence of infectious diseases in the
region. Although prevention using vaccination has been described to be one of the most cost effective of all
healthcare interventions in history, for more than 80 percent of zoonotic diseases identified in Central Africa there
is no vaccine available to immunize animals considered to be origins of emerging diseases in humans. Obviously, the
effect of capacity building for surveillance, early detection of threats and response to the emergence of zoonotic
diseases is only a partial solution and will not achieve the long term goal of eliminating the zoonoses in this area. In
light of this limitation, more research is needed on veterinary vaccines for the prevention of diseases at the wildlife-
livestock interface.

Keywords: Zoonoses, infectious diseases, One Health, Prevention, Vaccination, Central Africa.

The enhanced immune responses of pig to PCV-2 vaccine by inoculation with chitosan nanoparticles of
recombinant pig interleukin-23 gene
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Porcine circovirus type 2 (PCV2) is the causative agent of postweaning multisystemic wasting syndrome
characterized by progressive weight loss, respiratory symptoms, jaundice, and has a significant economic impact on
pig industry. Vaccines against PCV2 have been studied intensely and found to be effective in decreasing mortality
and improving growth in swine populations. This study aims to seek for a novel effective way to elevate the immune
response of PCV2 vaccination. The eukaryotic expression vector expressing pig interleukin-23 (IL-23) was
constructed and entrapped with chitosan nanoparticles and then was intramuscularly co-immunized to 3-week-old
piglets with PCV2 vaccine. Before and after vaccination on 0, 1, 2, 4, 8 and 12 weeks, their weights were measured
and blood were collected respectively to test the changes of immune cells, specific antibody and the expression
levels of immune genes by ELISA and quantitative RT-PCR. The results indicate that weight and leukocytes, red
cells, platelet, hemoglobin in blood mounted significantly in the IL-23 co-vaccinated piglets in comparison with the
control animals (P<0.05). The co-vaccinated group recorded higher specific antibody titer and more CD4⁺ and CD8⁺ T
cells up to 12 weeks post vaccination compared to the control (P<0.05). The expression level of IFN-γ, IL-4, IL-15 and
IL-23 genes of the co-vaccinated piglets were markedly higher than the control from post inoculation 0 to 4 weeks
(P<0.05), so were the levels of STAT1, STAT3, STAT4, NF-κB, Bcl-2, Myd88 from 0 to 12 weeks. Overall, the results
demonstrated that expression of IL-23 gene in vivo can enhance the immune response to PCV2 vaccine and has the
potential to be developed into a safe and effective adjuvant to promote the immunity of pig against PCV infection.

Key words: pig, interleukin-23 gene, chitosan nanoparticles, PCV2 vaccine, immune responses
Broadly Protective Influenza Vaccines: Protection against mismatch – bettering standard of care
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Annual vaccination against seasonal Influenza A and B virus subtypes with well-matched inactivated virus (INV) vaccines are highly effective against upper respiratory tract (URT) Influenza infection and induced disease. Protection against infection is thought to be mediated principally by neutralizing antibodies targeting the receptor binding site (RBS) of the hemagglutinin globular head (HA1). Immune pressure on HA1 results in antigenic drift, necessitating worldwide surveillance with subsequent WHO recommendations on strain selection for manufacture of forthcoming seasonal influenza vaccines.

The development of Broadly Protective Influenza Vaccines (BPIV) that can protect against matched as well as drifted or mismatched seasonal and pandemic strains through induction of broadly cross-protective functional antibody responses would provide significant improvement over current standard of care (SOC). BPIV that afford this type of coverage, while unlikely to remove the need for continued worldwide surveillance; will likely reduce the need for frequent vaccine updates. Additionally, a target product profile that also offers longer-lasting immunity would be a substantial advantage of current annual vaccination practices, with potential for year-round manufacture. BPIV that induce both breadth and durability across multiple influenza seasons would be paradigm shifting for the Influenza field and offer significant health care benefits.

As part of our Broadly Protective Influenza Vaccine program, and exploring proof of concept (POC) across several Influenza A subtypes (H1, H3, H5), we have built consensus-based, computationally optimized broadly reactive antigens (COBRAs) on a subtype-specific basis. These prototypes have been demonstrated to fold properly, bind conformation-specific broadly neutralizing mAbs (both HA1 & HA2) as well as agglutinate red blood cells. Prototype HA proteins presented either on virus-like particles (VLPs) or produced using current egg-based manufacturing processes have been tested in-vivo and determined to elicit broadly cross-neutralizing functional antibody responses in multiple species (naive & pre-immune) as well as protect against homologous and heterologous virus challenge. This is the first approach describing the induction of broadly-protective immunity against different Influenza A subtypes using a consensus-based HA strategy focusing on the globular head. The ability to design broadly protective candidate vaccines based on historical sequence information that has the ability to protect against drifted and/or newly emerging variants will be a significant improvement over current licensed approaches.

Pathologic and immunologic characteristics of Coxsackievirus A16 infection in rhesus macaques
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Coxsackievirus A16 (CA16) is a major pathogen of human hand, foot and mouth disease (HFMD) with unclear pathogenesis. Observation of CA16 infection in junior rhesus macaques revealed the presence of characteristic vesicles in the oral mucosa and limbs in combination with viremia and positive viral loads in the tissues, suggesting that this animal is capable of reflecting the pathologic process of the viral infection. The immunologic analysis indicated a defective immune response, which included an undetectable neutralizing antibody and specific memory T cells in macaques infected by CA16, and the existing neutralizing antibody in macaques immunized with the viral antigen was unable to protect against a virus challenge. This immunologic characteristic was presumed to be related to the viral infection identified in the pre-conventional dendritic cell (pre-cDCs) population and might give rise to an unprotected state of infected or immunized macaques against repeated virus challenges. And an inactive CA16 vaccine failed to protect against a virus challenge.

Keywords: Coxsackievirus A16 (CA16), dendritic cell (DC), rhesus macaques, inactivated vaccine
An optimized synthetic DNA vaccine targeting liver stage exported proteins provides sterilizing protection from *P. yoelii* sporozoite challenge

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**Background:** With over 200 million cases of malaria worldwide in 2015, the need for a malaria vaccine is clear. Most vaccine strategies against pre-erythrocytic stages of *Plasmodium* have targeted surface sporozoite antigens like CSP. However, these have shown limited success. Live attenuated sporozoites are the only malaria vaccines that have been shown to be capable of eliciting sterile protection in human and animal models. CD8 T cells, mainly directed against hepatocytes infected with growth arrested liver stage (LS) parasites, are required for this protection in mouse models and correlate with protection in NHP models. We hypothesized that parasite proteins exported to or beyond the parasitophorous vacuolar membrane during LS infection could be displayed on MHC I in the infected hepatocyte and can be targeted by antigen specific CD8 T cells. Therefore, LS exported antigens that are conserved across *Plasmodium* species were chosen as vaccine targets.

**Methods:** Optimized synthetic DNA vaccines encoding 6 LS exported proteins were generated. (ExpoLS1-6). Female Balb/c mice were immunized four times at three week intervals with individual or combinations of ExpoLS antigens with and without the molecular adjuvants IL-12 and IL-33 by IM injection followed by electroporation. Splenocytes and sera were isolated one week after the final immunization and IFNγ ELISpots and flow cytometry were performed to assess the cellular immune response to each antigen. A separate set of mice were immunized with the same vaccine regimen and then challenged 9 weeks after the last immunization with a non-lethal challenge of 250 *P. yoelii* sporozoites injected IV. Mice were then monitored daily for signs of blood stage disease.

**Results:** All ExpoLS constructs induced antigen specific cellular immune responses as measured by IFNγ ELISpot and flow cytometry. A combination vaccine with all 6 antigens with IL-12 or IL-33 was 100% protective against patency upon sporozoite challenge. The 6 antigen combination vaccine was 86% protective when delivered without an adjuvant. Vaccines composed of pairs of LS antigens (ExpoLS1+ExpoLS2, ExpoLS3+ExpoLS4, and ExpoLS5+ExpoLS6) delivered with IL-12 or IL-33 were also 100% protective against patency upon sporozoite challenge. The pairs of LS antigens delivered without an adjuvant ranged from 71-87% protective upon challenge.

**Conclusions:** DNA vaccines expressing the LS antigens ExpoLS1-6 are immunogenic. Immunization with a combination of all these LS antigens and the molecular adjuvants IL-12 or IL-33 provides sterile protection against blood stage disease after sporozoite challenge. Smaller combinations of LS antigens are also protective. Sterilizing protection with the combination of ExpoLS5+ExpLS6 is especially interesting because these antigens are exclusively expressed during LS disease, supporting the hypothesis that CD8 T cells specifically targeting LS exported antigens in infected hepatocytes can provide 100% protection.
Identification and validation of immunodominant antigens as protective prophylactic against Shigella

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Background: Shigellosis, an acute invasive disease of lower intestine, caused by S. flexneri, S. dysenteriae, S. boydii and S. sonnei, afflicting millions of people worldwide with an estimated one million fatalities per annum. Given the magnitude of the public health threat, Shigella vaccine development is considered a public health priority. Further, rapid emergence of multi-drug resistance Shigella spp., demands an effective prophylactic which can provide protective mucosal and systemic immunity against all species of Shigella. Till date, not even a single vaccine candidate providing cross protection against multiple Shigella spp. has been introduced in the public sector despite of constant efforts and extensive research in this field. The advancements in proteomics allow identification of conserved candidates which could be exploited as vaccine candidates. Therefore, the present work aims to develop vaccine candidates capable of eliciting strong protective immune response against majority of Shigella spp. using combinatorial approach of proteomics and molecular biology.

Methods and Results: In the present study, we have selected Outer Membrane Proteins (OMPs) of Shigella as potential immunogens and characterised them with the help of advanced immunoinformatic tools. To experimentally validate the conserved antigens, outer membrane proteins were isolated from all four strains of Shigella i.e. S. flexneri, S. dysenteriae, S. boydii and S. sonnei. Isolated OMPs were then subjected for 2D-PAGE analysis wherein OmpA and OmpC were found to be conserved. To evaluate their immunogenicity, OmpA and OmpC were cloned and purified from recombinant E. coli using affinity chromatography and injected intramuscularly in BALB/c mice at defined regimen. Obtained significantly high serum IgG and fecal IgA titres along with strong cellular responses (cytokine profiling) suggested the immunogenic potential of OmpA and OmpC. Additionally, bactericidal activity using antisera of OmpA and OmpC immunised mice revealed the antibody mediated complement dependent killing of all four strains of Shigella. To evaluate the protective efficacy of OmpA and OmpC as vaccine candidate, the immunized mice challenged with S. flexneri. The immunized mice were protected from Shigella infection and were free from any dysenteriae like symptoms. The obtained data encourage the prospective application of validated candidates for the development of effective vaccine.

Conclusion: Successful identification and validation of immunogenic candidates as protective prophylactic against Shigellosis (Figure 1).

Future Prospects: Selected candidates; OmpA and OmpC will be assessed in clinical trials. Since the vaccine candidates were found to be conserved among other diarrhea causing pathogens, their application as vaccine for diarrhea causing agents will be assessed.

Adjuvants play a critical role in universal influenza vaccine design

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Highly adaptable viruses including influenza continue to circumvent attempts to control them using traditional vaccine approaches. To develop a universal influenza vaccine, there is a need to better characterize the nature of the antigen and of protective immunity. This is particularly true of the many influenza vaccines targeting influenza M2 protein, which have yielded highly variable results. Only when formulated with our novel Advax-SM™ adjuvant did a synthetic vaccine based on a viral capsomere protein that incorporates a key epitope from M2e induce high antibody titers and protect mice against challenge with either H1 and H3 influenza strains. The Advax-SM™ adjuvanted vaccine also protected pregnant dams and their vaccine-naïve pups against a lethal influenza challenge and similarly protected neonates immunized as early as 7 and 11 days of age. Vaccine protection was lost in B-cell deficient µMT mice, or wildtype mice immunized with antigen alone in the absence of Advax-SM™ adjuvant confirming that protection is not only antibody dependent, but requires high titers only achievable with a potent adjuvant such as Advax-SM™. These results provide hope that a more broadly protective influenza vaccine may be achievable providing a suitably optimized antigen and adjuvant are combine.

[O3.7]

[PL2.3]
A common strategy used by viruses and bacteria to evade immune responses uses regulatory T cell (Treg) induction to inhibit effector immune responses. A rational vaccine strategy would aim to avoid Treg induction in order to enhance immune recall mechanisms that protect against disease. We hypothesized that protein antigens may contain Treg-inducing epitopes enabling pathogen persistence and reduced vaccine efficacy.

To discover pathogen-sourced epitopes capable of recognizing Tregs trained on human proteins in tolerance development, we designed an immunoinformatic algorithm, JanusMatrix, that parses a T cell epitope sequence into its two faces at the antigen presenting cell/T cell interface, one directed toward the major histocompatibility complex (MHC) molecule and the other toward the T cell receptor (TCR). For a pathogen-sourced epitope, the algorithm searches the human proteome for the same TCR face in 9-mers capable of binding the same MHC allele.

Using JanusMatrix, we conducted informatic and experimental studies to explore our hypothesis. We computationally screened a wide range of human-host viruses and bacteria for TCR-face similarity to self and discovered that chronic viruses and bacteria generally appear more human-like than those that cause acute infection (He, BMC Bioinformatics 15:S1, 2014; He, Vaccine 33:6922, 2015).

In one experimental study, we discovered a promiscuous class II epitope located within non-structural hepatitis C virus (HCV) protein p7 that exhibits homology with hundreds of human sequences. The epitope induces an increase in CD4+CD25+FoxP3+ Treg number and function in peripheral blood leukocyte cultures derived from an HLA-diverse cohort of HCV-infected patients, but not in cultures derived from patients who spontaneously cleared HCV or from non-infected individuals (Losikoff, J Hepatol. 62:48, 2015).

In an experimental study of avian H7N9 influenza, we discovered a human-like epitope in hemagglutinin (HA) that expands CD4+CD25+FoxP3+CD39+ Tregs and reduces IFNγ secretion when co-incubated with non-human-like H7N9 epitopes in peripheral blood leukocyte cultures (Liu, Hum Vaccin Immunother. 11:2241, 2015). We applied this finding to design an antigenically improved H7-HA by introducing three modifications to recombinant HA (rHA) that delete a highly conserved Treg activating epitope. Monoclonal antibodies raised against wild type H7-HA recognized engineered rHA (Opt1 rH7-HA) with affinity equivalent to the wild type protein, suggesting that modifications did not induce significant structural perturbations. Vaccination of immunodeficient mice reconstituted with human PBMCs (N=8) using nonadjuvanted Opt1 rH7-HA stimulated higher anti-H7-HA IgG titers and higher frequencies of anti-H7-HA plasma cells than mice immunized with wild-type protein. Vaccination of HLA-DR3 transgenic mice with Alum-formulated H7N9 virus-like particles containing Opt1 H7-HA stimulated protective level of hemagglutinin inhibition (HAI) antibodies, suggesting that modifications of H7-HA preserved neutralizing epitopes. The Opt1 H7N9 VLP vaccine raised HAI antibodies sooner and at lower doses than wild-type H7-HA VLP vaccine.

In conclusion, homology with the human proteome represents a novel means by which viruses and bacteria that seek to establish chronic infections escape human immunity and ensure their survival. Epitope-driven approaches to vaccine design that carefully consider T cell subsets primed in immunization hold promise for enhanced vaccine efficacy against pathogens that adopt this immune evasion strategy.
An Optimized, Synthetic DNA Vaccine Encoding the Toxin A/Toxin B RBDs of Clostridium difficile Induces Protective Antibody Responses
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Clostridium difficile–associated disease (CDAD) constitutes a large majority of nosocomial diarrhea cases in industrialized nations and is mediated by the effects of two secreted toxins, toxin A (TcdA) and toxin B (TcdB). Key toxin-neutralizing epitopes have been discovered within the carboxy-terminal receptor-binding domain (RBD) of the toxins, which has generated interest in developing the RBD as a vaccine target. While numerous platforms have been studied, very few data describe the potential of DNA vaccination against CDAD. Therefore, we created highly optimized plasmids encoding the RBD from TcdA and TcdB and immunized C57BL/6 mice and rhesus macaques intramuscularly followed by in vivo electroporation. In these animal models, vaccination induced significant levels of anti-RBD antibodies within the serum and feces that could neutralize toxins in an in vitro cytotoxicity assay. Moreover, mice that were actively immunized with the DNA vaccine or given passive transfer of immunized non-human primate sera were protected from a lethal intraperitoneal challenge of purified C. difficile toxins.  Finally, immunized mice were significantly protected following orogastric challenge with strains of C. difficile spores that were homologous (VPI 10463; n=10/10) and heterologous (hypervirulent UK1; n=4/8) strains to our vaccine antigens. These data demonstrate the robust immunogenicity and efficacy of a TcdA/B RBD-based DNA vaccine in preclinical models of acute toxin-associated disease. This work is funded by a Department of Defense Congressionally Directed Medical Research Grant W81XWH0910382 (http://cdmrp.army.mil) and a MARS aging endowment to MK.

A subunit protein based therapeutic vaccine design to treat Genital Herpes disease and subclinical infection in a pre-clinical model.
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Genital herpes infection increases the risk of HIV acquisition and transmission by 3- to 4-fold. A successful genital herpes vaccine will help control two overlapping epidemics. We are pursuing a protein subunit-based therapeutic vaccine that will treat individuals already infected with herpes simplex virus type 2 (HSV-2). The goals of a therapeutic vaccine are to reduce recurrent genital lesions (disease) and subclinical infection, as measured by genital shedding of HSV-2 DNA and infectious virus. Subclinical infection is an important target as it accounts for much of the sexual transmission of genital herpes. We evaluated a trivalent subunit antigen vaccine consisting of HSV-2 glycoproteins C, D and E (gC2, gD2, gE2) with the intent of blocking virus entry mediated by gD2; blocking virus attachment to cells, and inhibiting innate immunity mediated by complement by gC2; and blocking cell-to-cell spread and immune evasion from antibody mediated by gE2. We administered gC2/gD2/gE2 with CpG, a TLR-9 agonist that enhances CD8 T cell responses, and alum, to stimulate antibody production. The vaccine was administered 3 times at two-week intervals to guinea pigs that were previously infected intravaginally with HSV-2. The vaccine significantly boosted gC2, gD2 and gE2 antibody levels as well as enhanced neutralizing antibody titers. The mock-immunized animals had recurrences on 48 of 624 days (7.7%) compared with 29 on 738 days (3.9%) in the trivalent group, which represents 49% fewer recurrent lesion days. The impact of the vaccine was apparent from the time of the first immunization. In addition, a significant reduction in vaginal shedding of HSV-2 DNA 52% (P=0.039) was noted compared to mock vaccinated animals, which did not become apparent until the third immunization. We evaluated the recovery of infectious virus from vaginal swabs following the third immunization, as we consider infectious virus a relevant marker for risk of transmission. Infectious virus was isolated on 5/26 (19.2%) days when mock-immunized animals were shedding HSV-2 DNA compared with 1/11 (9.1%) days when the trivalent-immunized animals were shedding HSV-2 DNA. Although this difference was not statistically significant, the results raise the interesting possibility that immunized animals are less likely to infect sexual partners even when shedding HSV-2 DNA. We conclude that therapeutic immunization reduces the number of days animals have genital lesions and genital shedding of HSV-2 DNA, and that on days with vaginal shedding of HSV-2 DNA, immunized animals are less likely to be shedding infectious virus compared to mock-immunized animals.
Clinical Development of Novel Conserved Element HIV pDNA Vaccine Able to Maximize Breadth and Magnitude of Immune Responses

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Protection against HIV has been difficult to obtain, in part because the high viral mutation rate generates a broad repertoire of viable alternatives to the epitopes targeted by the immune system of the host, thereby eluding the immune system. In addition, potential “decoy” epitopes may inhibit protective response. We addressed these issues by the development of a pDNA vaccine targeting highly conserved elements (CE) of HIV-1 selected on the basis of stringent conservation, functional importance, broad HLA-coverage, and association with viral control. Two immunogens were engineered consisting of 7 collinearly arranged CE from p24 differing by 7 AA (1 AA per CE) to cover >98% of group M sequences and were expressed from a single vector. All CE pDNA-vaccinated macaques developed robust CE-specific memory responses with a significant fraction of cytotoxic T cells. In contrast, vaccination of macaques with HIV full-length gag DNA was very inefficient in inducing CE responses (50% responders; fewer CE recognized). Different pDNA prime-boost regimens consisting of CE prime followed by a boost with pDNA expressing either full-length Gag or a combination of CE+full-length Gag were compared. The gag DNA booster vaccination significantly increased the preexisting CE responses. Interestingly, the combination of CE+gag pDNA as booster vaccination efficiently increased also the breadth of preexisting CE responses, indicating a significant change in epitope hierarchy. pDNA-induced T cell responses rapidly disseminate into secondary lymphoid organs and effector mucosal sites, the portal of entry of HIV. CE responses were long-lasting and were maintained for >2 years. A single booster vaccination with CE pDNA resulted in rapid increase of pre-existing responses reaching up to ~7% of total T cells, indicating the persistence of potent memory CE-specific T cells. Similar data were obtained using an analogous SIV CE pDNA prime-boost vaccine regimen.

In conclusion, priming with CE DNA is critical to induce broad responses to vulnerable sites of the virus while avoiding variable or decoy targets that may divert effective T cell responses towards less protective viral determinants. Combination of CE and full-length immunogens provides a novel strategy to increase the breadth and magnitude of cellular and humoral immunity. This strategy allows for the development of robust T cell responses targeting a broad number of epitopes, including subdominant conserved viral epitopes. The expanded breadth of the responses could provide an advantage in restricting viral propagation. This vaccine regimen is now moving into a phase I clinical trial (HVTN 119).
Unitizing MVA-VLP platform for development of single dose vaccines: Tetravalent vaccine against hemorrhagic fever and Zika viruses
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Both Ebola and Zika viruses have been known for many years; yet, in recent epidemics, their emergence has caught public health officials off-guard, with no vaccines or drugs available to stem their global spread. Ebola (EBOV, formerly Zaire ebolavirus) as well as Sudan (SUDV) and Marburg viruses (MARV) are currently the most virulent species in the Filoviridae family. They can cause up to a 90% case fatality rate in humans and are re-emerging in Central and West Africa, with multiple outbreaks since 1970. Lassa fever virus (LASV), a member of the Arenaviridae family, is epizootic in an overlapping region with EBOV and causes severe and often fatal haemorrhagic illnesses resulting in 67,000 deaths annually. Zika is also a rapidly spreading, emerging infectious disease transmitted by mosquitoes carrying the Zika virus (ZIKV), which causes infant microcephaly, Guillain Barre Syndrome (GBS), and other neurological disorders in a subset of infected individuals.

To speed the development of vaccines, GeoVax is leveraging its 4th generation Modified Vaccinia Virus Ankara-Virus Like Particle (MVA-VLP) technology, improved for transgene stability and designed to produce VLPs in the vaccinated individual, for the construction of vaccines against hemorrhagic fever (HF) as well as Zika. The platform has been validated in non-human primates (NHP) as well as in 5 clinical trials for its MVA-HIV VLP vaccine. The tetravalent (TV) vaccine against HF viruses is composed of four recombinant MVA vectors that express VLPs displaying the native glycoproteins (GPs) of the four target HF viruses. For the Zika vaccine, two MVA-VLP vaccine candidates are being developed from ZIKV strain Suriname 2015 sequences: one expressing pre-Membrane and Envelope (prME) genes, and the other expressing prME + Non-Structural protein 1 (NS1) genes. These antigens were chosen based on documented evidence that flavivirus prME and NS1 proteins are sufficient to elicit protective immune responses.

Initial Proof of Concept studies included efficacy studies in rodents and non-human primates (NHPs) with MVA-VLP EBOV (Makona). This vaccine candidate demonstrated 100% protection against EBOV in guinea pigs, Syrian golden hamsters, and NHPs (as a single dose or 2 dose regimen). This is the first report, to our knowledge, that an MVA replication deficient vector can confer full protection. The next step is testing the MVA-VLP-TV vaccine in rodents and NHPs. The production of ZIKV prME VLPs was demonstrated by EM in transient infections of DF1 cells with MVA shuttle plasmids encoding either ZIKV prME or NS1 sequences. ZIKV-specific antibodies detected both E (54kD) and NS1 (40kD) proteins in cell lysate and supernatant (only E) of infected cells. Animal efficacy experiments using two different mouse models developed at the CDC and the University of Georgia are targeted to start in the near future.
Background: Over the past years, Janssen Vaccines*, has developed an Adenovirus production platform (AdVac®) that allows for highly intensified manufacturing of adenoviral vector vaccines, by the increased productivity of the PER.C6® technology infection process combined with the optimized vaccine purification process. Leveraging this platform know-how, highly accelerated timelines for development and manufacturing are possible. This has been exemplified in the recent past during the Ebola outbreak in West-Africa. As a response to this public health crisis, Janssen Vaccines accelerated the development of an Ebola vaccine regimen using a prime-boost combination of two different components, namely Ad26.ZEBOV and MVA-BN-Filo, that are based on Janssen’s AdVac® technology and the MVA-BN® technology from Bavarian Nordic A/S, respectively.

As part of this commitment, consistency in production and high quality standards are required. Here we examine the level of consistency and quality of the Ad26.ZEBOV component during the manufacturing campaign by assessing a set of parameters that constitute the product profile (including product integrity and purity).

Methods: The Ad26.ZEBOV Drug Substance manufacturing process comprises an upstream process (PER.C6® cell expansion and vaccine production) followed by a downstream purification process. The development of virus seed and drug substance manufacture was accelerated by applying platform approaches. For demonstration of consistency, various key process performance parameters were collected during a large manufacturing campaign. Parameters included for example cell population doubling levels and cell viability and the ratio of virus particles concentration:infection units of the product. For all analyses, 99% $\beta$-expectation tolerance intervals were calculated.

Impurities clearance was demonstrated.

Results: FIH was reached within 4 months after start of GMP manufacture. In the production campaign over 2 MM dose equivalents of Ad26.ZEBOV DS were produced at two sites: Bern, Switzerland (n=9) and Leiden, Netherlands (n=9). In the early phase of the campaign some process changes were implemented to accommodate the specific behaviour of the Ad26.ZEBOV in the platform process and to facilitate rapid production. Data was pooled whenever possible.

During intensified cell production, cell population doubling values and viability values were consistent. In the downstream process, the viral particles were purified while maintaining the integrity of the virus particles. Impurities in all batches were effectively cleared with residual concentration below or around the detection limits of for HCP and for HC-DNA. All release criteria were met and process performance was consistent between batches and across sites. On average the yield per manufacturing process was 130k dose equivalents.

Future outlook: As a consequence of the large dataset collected as part of the manufacturing campaign, consistency has been shown at the anticipated final manufacturing scale and these data will be used to support the licensing process. This retrospective validation of the manufacturing process should be considered to support emergency use of vaccines. Furthermore, these data demonstrate how the AdVac® platform technology can be efficiently leveraged as a response to future public health crises.

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*Janssen Vaccines is a part of Janssen Vaccines & Prevention B.V., one of the Janssen Pharmaceutical Companies of Johnson & Johnson
Expanding Global Vaccine Availability Through Supported Technology Transfer to DCVM- The IVI Experience

Julia Lynch, Viliam Pavliak, Sudeep Kothari, Sushant Sahstraabuddhe, Inkyu Yoon, Laura Digilio, Sonia Pagliusi, Jerome Kim

The production capacity of current manufacturers is insufficient to meet the global demand and achieve universal vaccination with existing products, and new vaccines are in the pipeline holding the promise of further reducing the burden of infectious disease. The global vaccine supply is disproportionately directed to higher priced markets in wealthier countries, often neglecting the poorest markets, where vaccines are most needed. Developing countries vaccine manufacturers (DCVMs), with their willingness to accept public-sector prices are essential partners to ensure a sustainable, affordable, supply of vaccines to meet the world’s projected needs. The International Vaccine Institute (IVI, Korea), a not-for-profit dedicated to discovery, development and delivery of safe, effective and affordable vaccines for global public health, has accomplished numerous technology transfer and development assistance activities with multiple DCVMs over the last decade.

With the goal of making an affordable oral cholera vaccine (OCV), IVI worked in cooperation with VaBiotech (Vietnam) under funding from the Bill and Melinda Gates Foundation to reformulate their ORCVAX beginning in 2004. To expand availability of OCV, IVI transferred the new OCV technology first to Shantha Biologics (India), and their vaccine Shanchol obtained licensure in 2009 and WHO pre-qualification in 2011. With the GAVI announcement to launch a 20 million dose global stockpile by 2018, further production increases became a global priority and IVI transferred the OCV technology to EuBiologics Co., Ltd. (Korea) in 2010 with the additional support of the Global Health Investment Fund. Euvichol obtained WHO prequalification in 2015. Most recently, IVI initiated technology transfer of OCV to Incepta Vaccine Ltd. (Bangladesh) in 2014 with licensure in Bangladesh expected in 2017.

Expanding beyond cholera, IVI developed a new typhoid vaccine using conjugation technology initially developed at NIH that consists of Vi-polysaccharide conjugated to diphtheria toxoid (Vi-DT). The new vaccine is anticipated to confer greater durability as well as protection of infants. IVI Vi-DT technology was transferred to Shantha Biologics in 2009, BioFarma (Indonesia) and SK Chemicals Co Ltd (Korea) in 2013, and Incepta Vaccine Ltd in 2014. IVI will continue working with these partners on vaccine development and WHO prequalification.

IVI is the lead organization for the Dengue Vaccine Initiative (DVI) consortium. Through a grant from the German government, DVI has assisted the development of dengue vaccines based on a US NIH technology licensed to Instituto Butantan (Brazil) and Vabiotech (Vietnam) since 2013. As with other IVI partnerships, the collaborative support to the DCVMs has focused on providing technical support for process development, project management, regulatory issues and the design and conduct of clinical trials.

Among IVI’s newest initiatives is an agreement with Incepta Vaccines Ltd in June 2016 to co-develop Typhoid-Pneumococcal conjugate vaccine comprised of Vi-polysaccharide conjugated to pneumococcal surface antigen PspA. And, most recently, IVI announced a partnership with Xiamen Innovax Biotech Co., Ltd. (China) to assess the suitability of their Hepatitis E vaccine, Hecolin, for prequalification by the WHO.

Through alliances which have matched developing countries’ needs and partner capability, and with the generous support of third party funders, IVI has facilitated access to technology and capability building with DCVMs across the research and development spectrum, enabling significant enhancement in global availability of life-saving vaccines. These DCVMs are demonstrating success in producing high-quality, affordable vaccines, changing the landscape of vaccine access for the world’s most vulnerable populations.
[O5.4]

Boosting vaccine development for infectious diseases of low and middle income countries
Allan Saul, GSK

Despite spectacular advances over the past century in the development of vaccines, antibiotics and public health measures, infectious diseases still exact a huge toll with more than 11 million deaths per year. People living in low and middle income countries bear the brunt of this with over 90% of the burden measured either as deaths or DALYs occurring in these countries. Despite advances in medical science, over 60% of the burden of infectious disease is caused by organisms for which there are no registered vaccines, and a substantial part of the remaining 40% of infectious disease (e.g. tuberculosis), the existing licensed vaccines are not well suited for use in low and middle income countries. Thus, there is a major opportunity for making a substantial impact with development of new vaccines.

However, available resources are seriously limiting. GFINDER 2015 estimates that there was a total of $1.2 billion spent globally on “neglected diseases” with a total of only $92 million for vaccines for 21 diseases excluding development of new vaccines for HIV, TB, malaria and Ebola and improved vaccines or vaccine deployment for pneumococcus, meningitis and rotavirus. With an estimated cost on average of $1 billion to develop a vaccine, three things need to be done to accelerate vaccines for “neglected diseases”

1. More funding
2. Rational prioritization of vaccine development on a global scale to better use existing resources
3. Faster/less expensive ways of developing and deploying vaccines.

In this presentation, I will review efforts underway for all three, but concentrate on a proposal for more rational global prioritization by considering separately three classes of vaccines: 1. For diseases with an ongoing high burden (e.g. diarrheal disease); 2. As an “insurance policy” for preventing future disease outbreaks (e.g. Ebola, SARS, etc.); and 3. For disease eradication (e.g. Polio).

[O5.5]

ChimeriVax-based Zika vaccine, a live-attenuated approach.
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Zika virus is a mosquito-borne flavivirus that has been circulating in Africa and Asia for over 60 years, human infections range from asymptomatic to acute febrile illness. Recently, an unprecedented explosive outbreak in French Polynesia and the Americas has linked Zika infection to microcephaly in infants of mothers infected during pregnancy and other birth defects and neurological disorders, mostly Guillain-Barré syndrome. Due to the potential global health risk, it is imperative to develop an effective vaccine to prevent infections. The ChimeriVax (CV) platform is a well-established technology that has been successfully used to develop licensed vaccines against multiple flavivirus targets including West Nile (WN), Japanese Encephalitis (JE) and Dengue. Sanofi Pasteur’s experience in licensed flavivirus vaccines will be leveraged against Zika vaccine development. Available results and progress will be presented.
DNA-delivery of monospecific and bispecific monoclonal antibodies targeting *Pseudomonas aeruginosa* expresses functional antibodies that protect mice in a lethal model of pneumonia

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Each year, over 2 million Americans acquire antibiotic-resistant infections that are becoming increasingly more difficult and costly to treat. *Pseudomonas aeruginosa* is a gram negative bacteria naturally found on the skin and in the environment but can present as an opportunistic pathogen in burns/wounds, pneumonia, and on medical implants. Monoclonal antibodies (mAbs) have been isolated that target the *Pseudomonas* PcrV type III secretion system (V2L2MD) and a bispecific candidate, MEDI3902, has also been engineered to display additional specificity for the Psl exopolysaccharide (Science Trans. Med. 2014, MedImmune, AstraZeneca). Although these mAbs can clear infection *in vivo*, significant manufacturing costs are associated with production for routine delivery. Alternative delivery strategies that could significantly expand the availability of mAb therapy are urgently needed. DNA vectors can be utilized to deliver a foreign transgene directly into skeletal muscle, employing the cells as biological factories to produce and secrete a desired protein. We designed an optimized platform for DNA-delivery of monoclonal antibodies (DMAb) that encode an antibody heavy and light chain and express full length human IgG1. We developed DMAb-V2L2MD and DMAb-MEDI3902 candidates, evaluated expression *in vitro*, and delivered the DNA constructs *in vivo* by intramuscular injection, followed by electroporation (IM-EP). *In vivo* antibody expression was monitored in Nude and BALB/c mice and functionality assayed by opsonophagocytosis and cytotoxicity assays. Groups of BALB/c mice (n=8/group) were administered either DMAb-V2L2MD, DMAb-MEDI3902, an unrelated DMAb, or purified MEDI3902 at 5 days before lethal pneumonia challenge with an aggressive *Pseudomonas aeruginosa* strain. 100% protection was observed with the bispecific DMAb-MEDI3902 and 90% protection with the monospecific DMAb-V2L2MD. Together the data suggests that DMAbs targeting *Pseudomonas* can be delivered prophylactically to prevent infection. Importantly, we demonstrate DNA-delivery of a functional bispecific antibody *in vivo* that is protective against lethal infection. This approach expands the accessibility of monoclonal antibody therapy for treatment of antimicrobial-resistant infections as well as other infectious diseases.
Electroporation-Mediated DNA Administration as an Antibody Delivery Platform for Passive Immunoprophylaxis
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Many potent neutralizing monoclonal antibodies (mAbs) against a broad range of pathogens have been identified. However, only two recombinant monoclonal antibody products have been licensed for use in the prevention or treatment of infection. The U.S. Food and Drug Administration first licensed the respiratory syncytial virus-specific mAb, palivizumab, in 1998 for use in pediatric patients at high risk of severe disease. The anthrax toxin-specific mAb, raxibacumab, was licensed in the U.S. in 2012 for treatment or prevention of infection with the bacterium, bacillus anthracis. Over the past 18 years, >20 mAbs for infectious disease have been evaluated in clinical trials worldwide, yet no others have progressed in product development through licensure. In addition to efficacy, significant factors limiting the use of parenteral administration of mAbs for prevention of infectious diseases include logistics and cost-effectiveness. Sustained protection from infection is likely to require relatively frequent administration of antibodies that are expensive to produce and purify. The issues of compliance with frequent clinic visits and cost-effectiveness are significant hurdles to the deployment of mAbs as anti-infectives. One promising approach to address these limitations is the delivery of nucleic acid-based biologics encoding mAbs to the somatic cells of the recipient. By offering the prospect for sustained endogenous production of mAbs following a single administration, this approach has the potential to address the key limitations of direct parenteral administration of the mAbs themselves. We have established the basic technical feasibility of a DNA-based platform for endogenous expression of mAbs through the development of rodent and non-human primate (NHP) models for evaluating electroporation-based delivery of mAb-encoding plasmids. Sustained (>3 months) mAb expression was achieved in mice, rats, and NHP following a single administration of plasmid DNA to muscle using the TDS-IM electroporation system. No adverse effects associated with muscle-based mAb expression were noted in our studies. In addition, we demonstrated that plasmid-based delivery can be used to express multiple antibodies within the same animal, suggesting that an oligoclonal platform may be feasible in humans. As is often observed with administration of recombinant mAbs to humans, some NHP developed host anti-mAb responses, especially upon readministration of mAb-encoding plasmid DNA. This finding suggests that there is the potential for immune responses to influence the kinetics of mAb detection/activity in serum. It also indicates that approaches are needed for reducing the likelihood of anti-mAb responses, such as selection of mAbs with low intrinsic immunogenicity. Overall, our studies have established that DNA-based delivery of mAbs through the administration of plasmid DNA with electroporation is a promising approach for immunoprophylaxis.

Yeast-produced recombinant virus-like particles of coxsackievirus A6 elicited protective antibodies in mice
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Coxsackievirus A6 (CA6) has recently emerged as the predominant pathogen of hand, foot and mouth disease (HFMD), causing significant morbidity in children and adults, resulting in the typical HFMD symptoms and also atypical manifestations. Moreover, CA6-associated HFMD can also develop to severe meningitis and encephalitis. The increasing prevalence of CA6 infection and its associated disease burden underscore the need for effective CA6 vaccines. However, CA6 grows poorly in cultured cells, making it difficult to develop inactivated whole-virus or live attenuated vaccines. Here we report the development of a recombinant virus-like particle (VLP) based CA6 vaccine. CA6 VLPs were produced in Pichia pastoris yeast transformed with a vector encoding both P1 and 3CD proteins of CA6. Analysis indicated that the cleavage of P1 protein resulted in three subunit proteins VP0, VP1, and VP3, which compose the VLP structure. Immunization with CA6 VLPs elicited high level CA6-specific antibodies (geometric mean titer of 17,959). Passive transfer of anti-VLP antisera protected recipient mice against lethal CA6 challenge; on the contrary, the mice receiving control antisera developed severe clinical symptoms and none of the mice survived during the whole period. Collectively, these results demonstrate that CA6 VLPs represent a viable CA6 vaccine candidate which warrants further preclinical and clinical development.

Keywords: Coxsackievirus A6, virus-like particle, vaccine, Pichia pastoris
Flagellin as a versatile adjuvant for mucosal vaccines
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TLR ligands are considered attractive adjuvants for vaccines and immunotherapy. Flagellin is the cognate ligand for Toll-like receptor 5 (TLR5) of host cells. TLR stimulation leads to activation of innate immunity and subsequently modulates adaptive immune responses. In this presentation, we show that flagellin has a unique immunomodulating activity in the mucosal immune compartment. (1) Flagellin could be used as an adjuvant for mucosal vaccines. Mucosal co-administration of a *V. vulnificus* flagellin (FlaB) with microbial antigens induced significantly enhanced antigen-specific IgA responses in both mucosal and systemic compartments and IgG responses in the systemic compartment. Intranasally administered FlaB colocalized with CD11c as patches in DCs and caused an increase in the number of TLR5 expressing cells in draining lymph nodes. Further, we tested whether FlaB could serve as an effective mucosal adjuvant for an inactivated trivalent influenza vaccine (TIV), *Streptococcus pneumonia* antigen PspA, and Norovirus P domain antigens. In those vaccine formulations, flagellin exerted excellent adjuvanticity in combination with antigens. (2) Flagellin could serve an efficacious adjuvant for vaccines and immunotherapies against noninfectious intractable diseases in the mucosal compartments such as allergic asthma and cervical cancer. We found that therapeutic doses of flagellin together with allergens suppress allergic asthma by inhibiting pathogenic T\(^{n17}\)/T\(^{n12}\)/T\(^{n17}\) responses while generating regulatory DCs (DC\(_{\text{reg}}\)) and T\(_{\text{reg}}\) cells. Adoptive transfer of FlaB/allergen mixture-induced DCs effectively inhibited asthma. In the peripheral blood from allergic asthma patients, FlaB treatment induced DC\(_{\text{reg}}\), which subsequently induced allergen-specific Foxp3\(^+\) T\(_{\text{reg}}\) cells in a lymphocyte co-culture while inhibiting T\(_{\text{n17}}\)/T\(_{\text{n12}}\) responses in an IL-10-dependent manner. For cervical cancer, we examined whether flagellin can be used as an adjuvant for topical therapeutic cancer vaccine in a genital cancer model. Intravaginal co-administration of E6/E7 peptides with flagellin resulted in tumor suppression and long-term survival of the tumor bearing mice. IVAG immunization of E6/E7 peptide with flagellin induced accumulation of CD4\(^+\) or CD8\(^+\) cells and T cell activation in draining genital lymph nodes (gLN). The co-administered flagellin elicited antigen-specific IFN-\(\gamma\) production in gLN and spleen. The IVAG administered flagellin co-localized with CD11c\(^+\) cells in the gLN T cell areas and enhanced TLR5 expression.
A system vaccinological approach to vaccine and adjuvant safety: establishment of a novel safety evaluating system for adjuvant

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Vaccines are administered to healthy humans, including infants, so the safety and efficacy must be very high. Therefore, evaluating vaccine safety in preclinical and clinical studies, according to World Health Organization guidelines, is crucial for vaccine development and clinical use. Several adjuvants have also been developed and approved for use in vaccines. However, the addition of adjuvants to vaccines may cause unwanted immune responses. Here we show that the systems biological approaches in safety evaluation of vaccines insight of influenza vaccines. We selected 18 genes from analyzed microarray data that differently clustering between REV (toxicity reference whole virion inactivated influenza vaccine), PDV (pandemic whole virion inactivated influenza vaccine), HAv (hemagglutinin-split influenza vaccine), and SA (saline)-treated rats in lung. These 18 genes could evaluate any dosing route for vaccination: intraperitoneal, intramuscular and intranasal vaccination. The bodyweight changes, leukocyte reduction and these marker genes expression levels after the vaccination were applied into logistic regression analysis. Logistic regression equation was calculated in each of factors to evaluate the probability of being classified into either the HAv and REV in test vaccine. The 16-gene biomarker combination yielded a sensitivity of >90% and a specificity of >90%. To examine whether these marker genes can evaluate adjuvanted vaccines, we assessed the 4 type of adjuvant, which was combined into influenza vaccine. Although the expressions of the marker genes was varied by adjuvant, higher expression in poly:IC-adjuvanted vaccine, which is known to have toxic, was obtained. We applied these genes expression data into the logistic regression equation, which was developed in above resulting. The results showed that the marker genes expression levels can clearly separated into REV or Poly I:C adjuvanted vaccine and HAv, aluminium adjuvanted vaccines or other vaccines. Our results indicate that our marker genes panel can be used as a novel vaccine safety evaluation system. Taken to this knowledge, we will be able to provide a more complete mechanism of vaccine- or adjuvant-induced toxicity and these can help guide the development of the next generation of vaccines. Funding: This study (Adjuvant Data Base Project) was supported by Grants from the The Ministry of Health, Labour and Welfare (MHLW) and the Research on Development of New Drugs, the Japan Agency for Medical Research and Development (AMED).

Adjuvant Formulations and Delivery Platforms Influence the Antibody Responses to Trimeric gp145 and gp120 HIV-1 Envelope Proteins

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It is being increasingly recognized that adjuvants and delivery platforms influence the type of immune response generated. We examined whether immunization with HIV-1 envelope proteins along with Army Liposome Formulation (ALF) comprising liposomes containing monophosphoryl lipid A, previously known as L(MPLA), could induce binding and functional antibodies including V2-specific antibodies. Antibodies to V1V2 region of gp120 protein were identified as one of the correlates of lowered HIV-1 infection risk in the RV144 phase III HIV-1 vaccine trial. New Zealand White Rabbits were immunized with JR-FL HIV-1 gp145 protein trimerized with foldon or with SF162-gp120 utilizing a combination of different adjuvant formulations, ALF and ALFA (antigen adsorbed to aluminum hydroxide and then added to ALF) or a prime-boost strategy using different adjuvants for the prime and the boost. ALF was used for priming and transcutaneous immunization (administration of the protein on the surface of the skin) together with heat labile enterotoxin as the adjuvant was used as the boost. Serum samples were analyzed for antigen-specific IgG antibodies. Sera and purified IgG from pre-immune and immune sera were assessed for neutralization of primary HIV-1 in a macrophage system, inhibition of viral entry into A3R5 cells assessed by quantitative RT-PCR, ADCC, and inhibition of binding to the integrin receptor α4β7. All of the adjuvant formulations tested induced antibodies against gp120, scaffolded gp70 V1V2 proteins, and V2 and V3 peptides. However, the V2-specific antibodies were significantly higher with the prime-boost adjuvant group and more potent in neutralizing primary HIV-1 in the macrophage system. This study highlights the importance of the adjuvants and the delivery platforms for inducing high titer binding and functional antibodies. The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.
Poly-IC synergizes with OX40 to enhance Ag-specific CD4 T cell responses
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Adjuvants have been traditionally used in vaccines to increase the magnitude of adaptive immune response (B or T cell) but challenges still remain. Poly-IC, an analog of dsRNA, is an adjuvant known to promote Th1 responses either through activation of TLR3 on dendritic cells (DC’s) or via induction of type I IFN and can act together with costimulation to enhance immunity. OX40 costimulation in itself is known to promote Th1 response by enhancing Ag-specific CD4 T cell expansion, effector differentiation, and survival. Thus our aim is to understand, if Poly-IC and OX40 can synergize together to promote Ag-specific CD4 T cell expansion and Th1 differentiation. To study this we use a model Ag, Ea 52-68 peptide that activates TCR transgenic CD4 T cells with Vα2 Vβ6 TCR that also express Thy 1.1 marker. T cells were adoptively transferred into C57BL/6 mice followed by immunization with different combinations of Ea, poly-IC and anti-OX40 to test the efficiency of OX40 costimulation with Poly-IC. Our results show that, Ea peptide when administered together with Poly-IC and OX40 synergizes to promote heightened Ag-specific CD4 T cell expansion compared to either alone in spleen and liver on day 6 or 8. The expansion is reduced with IFNaR1 blockade, suggesting that Type I IFN is essential for this T cell expansion. We next studied if OX40 expression on Ag-specific CD4 T cells is essential for their expansion. For this we first co-transferred WT (Thy1.1+ 1.2+) and OX40-/- (Thy1.1+ 1.2-) cells mixed in 1:1 ratio to CD45.1 mice and tracked their expansion after Ea+poly-IC+anti-OX40 or Ea+anti-OX40 immunization on day 8. In liver, WT T cells expanded significantly more that OX40-/- T cells with both of the immunizations, whereas in spleen the presence of poly-IC in addition to anti- OX40 helped OX40-/- T cells expand equally well to WT T cells suggesting that poly-IC can compensate for OX40 signaling. IFNaR1 blockade did not impact Ag-specific CD4 T cell expansion with either immunization in this competition model, suggesting a mechanism other than Type I IFN signaling. Our second strategy employed transferring WT or OX40-/- T cells separately in C57BL/6 mice followed by immunization with Ea+anti-OX40 to understand the individual role of OX40 in Ag-specific CD4 T cell expansion. Our results show that, on day 8 WT T cells expanded significantly more than OX40-/- T cells in spleen, while in liver they tend to expand more perhaps not as significantly, suggesting that OX40 expression on CD4 T cell is essential for expansion. Moreover IFNaR1 blockade reduced WT T cell expansion in spleen, for which we are performing more experiments to understand the crosstalk between OX40 and Type I IFN signaling. Finally, we also demonstrate that Type I IFN is essential for Th1 differentiation even during OX40 costimulation. Collectively, the results from our study can benefit the development of vaccines for certain infectious diseases and cancer, where Th1 responses are crucial.
Amyloid-inspired Synthetic Peptide Hydrogels as Vaccine Adjuvants
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A crucial challenge in vaccine development is to balance safety with immunogenicity, and subunit vaccines that incorporate protein or peptide antigens are safer than traditional live attenuated or killed vaccines. However, subunit vaccines require additives called ‘adjuvants’, to amplify the immune response to the antigen. Due to the chemical heterogeneity and toxicity associated with most plant- or pathogen-derived adjuvants currently under development, those clinically approved in the U.S. are limited to alum (aluminum hydroxide) and AS04 (alum mixed with a synthetic TLR4 agonist). Therefore there is a compelling clinical need for new vaccine adjuvants that not only potentiate strong immune responses, but also are chemically defined in composition and minimally toxic.

In recent years, successful applications of nanotechnology and bioengineering in the field of vaccine development have enabled the production of novel adjuvant technologies. In this work, we investigated amyloid-inspired synthetic peptide hydrogels as vaccine adjuvants using the immunoprotective envelope protein domain III (EIII) of West Nile virus as an immunogen in mice. Naïve mice or mice vaccinated with WNV EIII in alum adjuvant or a commercial veterinary equine vaccine (West Nile-Innovator®, Fort Dodge Animal Health) were used as controls. Data indicated significantly higher anti-EIII antibody titers in mice vaccinated with EIII in hydrogel adjuvant compared to Alum-EIII or the veterinary vaccine. Plaque reduction neutralization testing of pooled pre-challenge sera showed detectable activity in the Hydrogel-EIII group (PRNT 50 titer of 80) but not the Alum-EIII group (PRNT 50 <20). PRNT50 titers for sera from the veterinary vaccine group were 160-640. Following challenge with 100 pfu of WNV (i.p), mice vaccinated with Alum-EIII had similar levels of weight loss and mortality compared to naïve mice (80%), whereas mice vaccinated with Hydrogel-EIII exhibited lower weight loss and mortality (40%). These findings suggest that the hydrogel adjuvant was more efficient at inducing anti-EIII responses following only two doses of antigens compared with alum. As expected, mice vaccinated with the veterinary vaccine were fully protected against WNV infection with no significant weight loss or mortality.

In conclusion, there is a clinical need for potent yet safe vaccine adjuvants that can induce strong antibody responses against subunit antigens and have better safety profiles compared to live attenuated formulations. Synthetic peptide hydrogel adjuvants described here may present an attractive alternative for the rapid development and formulation of subunit vaccines against emerging infectious diseases.

Sulfated Archaeal Glycolipids as a Safe & Effective Vaccine Adjuvant for Induction of Cell-mediated Immunity
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Archaeosomes are liposomal vesicles composed of ether glycolipids unique to the domain of Archaea. Unlike conventional ester-linked liposomes, archaeosomes exhibit high thermal and extreme pH stability, low proton permeability and possess strong adjuvant and immunostimulatory properties making them an attractive vaccine delivery vehicle. Traditionally comprised of total polar lipids (TPL) or semi-synthetic glycerolipids of ether-linked isoprenoid phytanyl cores with varied glycol- and amino-head groups, when used as adjuvants archaeosomes can induce robust and long-lasting humoral and cell-mediated immune responses against multiple antigens and enhance protection in murine models of infectious disease and cancer. However, traditional TPL archaeosome formulations are relatively complex comprising several lipid species. Semi-synthetic archaeosomes can be derived, but typically require a combination of several phospho-glycolipids (negative and neutral charged) to produce a stable, uniform-sized liposome formulation. Moreover, they involve many synthetic steps to arrive at the final desired glycolipid composition. Herein, we present a novel adjuvant formulation comprising a sulfated saccharide group covalently linked to the free sn-1 hydroxyl backbone of a archaeal core lipid (sulfated S-lactosylarchaeol, SLA). SLA individually or mixed with uncharged glycolipid (lactosylarchaeol, LA) constitutes vesicles that are less complex than traditional archaeosomes and when used with entrapped antigen (ovalbumin, melanoma associated tyrosinase-related protein [TRP] or glycoprotein 100 [gp100]) resulted in strong cell-mediated responses in mice and protection against subsequent B16 melanoma tumor challenge. Thus, semi-synthetic sulfated glycolipid archaeosomes represent a new class of adjuvants that will potentially ease manufacturing and scale-up, while retaining immunostimulatory activity.
Regression of Advanced Cervical Dysplasia and Elimination of HPV16/18 Infection by VGX-3100 is Statistically Associated with the Presence of Highly Active Peripheral Lytic CD8+ T cells and Cervical Immune Infiltration

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Infection of cervical tissue by a High Risk variant of the Human Papillomavirus (HR-HPV) such as HPV16 or HPV18 may cause a number of histological alterations, ranging from benign changes to pre-malignancies such as cervical intraepithelial neoplasia (CIN) to invasive adenocarcinoma. Current clinical guidelines for treatment of cervical disease indicate intervention of a surgical nature upon confirmation of CIN grades 2 or 3, such as cold-knife conization or a loop electrosurgical procedure (LEEP). To date, there is no approved non-surgical option for treatment of CIN2/3 driven by infection of HPV16 or HPV18. We have previously reported on the clinical efficacy of VGX-3100; a candidate plasmid-based immunotherapy delivered by in vivo electroporation using the Cellectra device for the treatment of HPV16 or HPV18 induced CIN2/3. In that Phase Ib double blind placebo controlled study, we demonstrated that treatment with VGX-3100 was efficacious as defined by our primary endpoint of a significant increase in the frequency of CIN2/3 lesion regression and our secondary endpoint of lesion regression with concomitant elimination of HPV16/18 infection. We have further undertaken post-hoc analysis of the immune system of patients enrolled in the trial in regards to both peripheral and cervical cellular immune responses. Assessment of peripheral T cell responses revealed that patients in the VGX-3100 treatment cohort had a significant increase in the frequency of highly activated VGX-3100 specific CD8+ T cells as gauged by the expression of CD137 (p<0.0001) that was not present in the placebo cohort. Additional analysis revealed that these cells co-expressed numerous markers of lytic capability, including Granzyme A, Granzyme B, Granulysin and Perforin. The frequency of CD8+ T cells exhibiting expression of these four lytic markers concomitant with CD137 was significantly elevated only in VGX-3100 treated patients who achieved the primary endpoint (p=0.0002) or the secondary endpoint (p=0.0001) of the trial. Moreover, analysis of cervical tissue including both healthy epithelium and stroma revealed a significant increase in the presence of CD8 positive immune infiltrates only in treated patients who achieved primary (p=0.002) or secondary endpoints (p=0.001). Further analysis of these tissues demonstrated significant increases in cells staining for the tissue resident memory T cell marker CD103 in these same patient populations (p=0.017 for primary endpoint, p=0.016 for secondary endpoint), suggesting the establishment of immunological memory in these tissues. Taken together these data confirm that the presence of highly activated and lytic capable CD8+ T cells in the periphery and CD8 positive as well as CD103 positive immune infiltrates in cervical tissue constitute immune signatures that are statistically associated with CIN2/3 regression and elimination of HPV16/18 infection after treatment with VGX-3100.
A Novel Heroin Conjugate Vaccine Abrogates Nociceptive and Behavioral Effects of Heroin through the Induction of High Titer and High Affinity Antibodies to Heroin and Its Degradation Products

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Background: An effective vaccine against heroin will need to induce high levels of antibodies that bind heroin and its metabolites with high affinity. Antibody-bound heroin will not cross the blood-brain, thereby, blocking the effects of heroin. We developed a novel heroin conjugate vaccine that elicits high titer antibodies that had significantly higher binding to heroin than previously reported haptens. This new vaccine protected rodents from intravenous heroin challenge.

Methods: The heroin hapten (6-AmHap) was synthesized and conjugated to tetanus toxoid (TT) for comparison with a morphine-like hapten (TT-MorHap). The conjugates were mixed with Army Liposome Formulation (ALF) (liposomes containing monophosphoryl lipid A) as an adjuvant and studied in mouse and rat models for antibody titer and affinity to heroin, its metabolites and other opioids. Efficacy was assessed by subcutaneous and intravenous heroin challenge.

Results: The TT-6-AmHap vaccine significantly reduced heroin-induced antinociception and locomotion behavioral changes following subcutaneous and repeated intravenous heroin challenges in mice and rats. The vaccine elicited very high IgG levels of ~1.2 mg/mL. Competition ELISA demonstrated that 6-AmHap-induced antibodies had significantly higher affinities than TT-MorHap-induced antibodies to heroin and its metabolites, 6-acetylmorphine (6AM), morphine, morphine-3-β-glucuronide and morphine-6-β-glucuronide. Using competition equilibrium dialysis with UPLC-MS/MS quantification, the K_d values of the 6-AmHap-induced antibodies to 6AM and morphine were ≤0.5 nM and the heroin bound was ≥90%, while MorHap-induced antibodies had 10-fold higher K_d and had low heroin binding. In addition, 6-AmHap antibodies crossreacted with abused prescription opioids like hydrocodone, hydromorphone, oxymorphone, codeine and levorphanol.

Conclusions: TT-6-AmHap is an improved vaccine candidate that may be developed into a therapeutic for heroin and opioid abuse.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of the Army, Department of Defense, or NIH, or the U.S. Government.
Different Strains of Recombinant *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) Expressing HIV Gag Prime Different Types of Immune Responses When Boosted with SAAVI MVA-C

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BCG is widely used as a tuberculosis vaccine with an accepted safety profile in babies. Recombinant BCG (rBCG) based vaccines expressing codon optimized HIV-1 subtype C Gag, were constructed using three novel BCG strains and a standard strain (kindly supplied by AERAS): the Danish wild type strain of BCG (rBCG WT), a pantothenate auxotrophic strain of BCG Danish (rBCG ΔpanCD), the Danish strain expressing perfringolysin O (rBCG pfo) and a pantothenate auxotrophic Danish strain expressing perfringolysin O (rBCG ΔpanCpfo). This study investigated modified BCG strains as a recombinant HIV vaccine as a prime for a vaccine based on modified vaccinia Ankara named SAAVI MVA-C boost in a mouse model. SAAVI MVA-C expresses gag, pol, RT, tat and nef as a polyprotein as well as gp150 from HIV subtype C. Mice vaccinated with ΔpanCD and ΔpanCpfo (gag/control) presented with significantly fewer liver granulomas (as measured by CD3+ staining) and less active granulomas (as measured by iNos) as compared to mice vaccinated with the WT and pfo strains. Mice vaccinated with rBCG ΔpanCpfo (gag/control) as well as rBCG ΔpanCpfo (gag/control) were able to clear the mycobacteria by day 7 post-infection. In mice ΔpanCD well as ΔpanCpfo strains of rBCG in induced less pathology than the WT Danish and the pfo strains. The magnitude and quality of vaccine induced HIV specific T cell immune responses following ex vivo stimulation of splenocytes was assessed in mice primed with rBCG and boosted with SAAVI MVA-C. Responses to stimulation with two HIV-specific CD4+ and one CD8+ peptide were measured by IFNγ ELISPOT assay, cytokine bead array (CBA) and multi-parameter flow cytometry. All rBCG (gag) vaccines were able to prime for significant vaccine specific boost responses. The response to the two CD4+ peptides tested differed according to the rBCG used to prime the MVA response. Whilst rBCG pfo (gag) and rBCG ΔpanCD (gag) primed for significant CD4+ (13) and CD4+ (17) boost responses respectively, rBCG ΔpanCpfo primed for a SAAVI MVA-C boost of both CD4+ epitopes. Multi-parameter flow cytometry indicated that the rBCG modifications can work synergistically to induce the greatest collective HIV specific bi- and multi-functional CD4+ and CD8+ cells as seen when priming with rBCG ΔpanCpfo (gag). Lastly, mice primed with rBCG pfo (gag) and rBCG ΔpanCpfo (gag) induced greater CD8+ T EM and T CM responses as compared to mice primed with rBCG WT (gag) and rBCG ΔpanCD (gag). In conclusion priming with either rBCG ΔpanCD (gag) or rBCG ΔpanCpfo (gag) leads to less pathology and enhanced T cell responses as compared the wild type strain following a SAAVI MVA-C boost.

Env-specific Rabbit Antibody Mediated ADCC Activities via Human Fc-Receptor

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**Background:** Effective Antibody Dependent Cell-mediated Cytotoxicity (ADCC) is an important antiviral effector function for HIV vaccine development. In preclinical studies, rabbit models have been used widely for studying vaccine-induced antibody (Ab) responses, however, direct measurement of rabbit ADCC using human effector cells is lacking due to the knowledge gap on the interactions between human Fc receptors (FcγRs) and rabbit antibody Fc. Recently we have developed a panel of HIV-1 Env specific rabbit monoclonal antibodies (RmAbs) and further analyzed their potential ADCC activities using human effector cells.

**Methods:** The immune rabbit sera generated by Env DNA prime-protein boost and Env-specific RmAbs were produced as previously reported. Chimeric Human Fc/RmAbs (H/RmAbs) were generated by replacing the rabbit Fc portion with human IgG1 Fc. A novel Rapid Fluorimetric ADCC (RFADCC) assay was used to measure the lysis of target cells coated with recombinant gp120 by human PBMCs effector cells in the presence of RmAbs or H/RmAbs. Rabbit antibody binding to human FcyRs was evaluated by ELISA.

**Results:** Chimeric H/RmAbs retained the gp120 protein binding properties of the wholly RmAbs from which the chimeric H/RmAbs were derived, and mediated potent ADCC. Interestingly, the original RmAbs also mediated ADCC activities of the same magnitude as the H/RmAbs. The immune rabbit sera collected from the same rabbit of RmAb being generated also displayed ADCC activities. Furthermore, ADCC activities for both RmAbs and H/RmAbs are epitope specific, as one mAb specific for V3 loop mediated strong ADCC but the one mAb specific for a V2 epitope did not, although both anti-V2 and anti-V3 antibodies bound to the antigen comparably.

**Conclusion:** Our results demonstrated that rabbit antibody can be directly used to evaluate the ADCC activities with human effector cells, which further highlights the value of rabbit models to study more comprehensive antibody functions. NIH Grant Support: AI-087191, AI-065250, AI-082274, and AI-082676. Bill and Melinda Gates Foundation Grant OPP1033112
Elimination of HIV from infected cells by IgG-conjugated enfuvirtide

Enfuvirtide (T20) is the only marketed HIV fusion inhibitor, requiring a twice-daily subcutaneous injection. We have site-specifically tethered a dimerized T20 at each of the carboxyl termini of either the heavy or light chain of a humanized IgG, resulting in a class of novel anti-HIV agents with two-four molecules of T20 that potently neutralize primary isolates (both R5-tropic and X4-tropic), as well as T-cell-adapted strains of HIV-1 in vitro, with EC50 values in the subnanomolar range, which are 10- to 100-fold lower than enfuvirtide and attainable whether or not the constitutive antibody targets HIV-1. The potential of such conjugates to purge latently infected cells was also demonstrated in a cell-to-cell viral inhibition assay following viral activation. Thus, attaching multiple copies of T20 on an IgG should overcome the limitations of enfuvirtide and may lead to better avidities of the anti-HIV effects. In recent studies we exploited the immunogenicity of the T20 peptide which harbors epitopes for broadly HIV neutralizing and ADCC antibodies in variant forms.

Conformation of a Protein in Virus-like Particles Impacts their Efficacy as Vaccines
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We have explored the potential of virus-like particles (VLPs) as RSV vaccine candidates. As a vaccine platform, we used VLPs composed of the Newcastle disease virus (NDV) core proteins, the nucleocapsid and membrane proteins. Assembled into this VLP were two chimera proteins containing the ectodomains of RSV F and G proteins fused to the transmembrane (TM) and cytoplasmic (CT) domains of NDV F and HN proteins, generating F/F and HN/G chimera proteins respectively. The NDV CT and TM domains in the chimera proteins facilitate efficient assembly of the RSV glycoprotein ectodomains into the VLPs. Immunization of mice with a single dose these F/F and HN/G containing VLPs, without adjuvant, effectively stimulated anti-RSV F and G protein antibodies.

The paramyxovirus F protein is folded into a metastable pre-fusion conformation and, upon fusion activation, refolds, through a series of conformational intermediates, into the post fusion conformation, which is structurally very different from the pre-fusion form. To explore the impact of F protein conformation in the VLP anti-RSV immune responses, VLPs were constructed to express F protein ectodomains stabilized in pre-fusion (Pre-F/F) or post-fusion (Post-F/F) configurations. Following immunization of mice, without adjuvant, Pre-F/F containing VLPs induced dramatically higher neutralizing antibody titers than the Post-F/F containing VLPs or the wild type F/F containing VLP. Immune responses to the Pre-F/F containing VLP were also far superior to those induced after RSV infection. Cotton rats, which are more permissive to RSV infections than mice, are an accepted standard animal model for RSV. Positive results in this model with vaccine candidates, anti-viral drugs, or antibodies have led directly to human trials. Thus we immunized cotton rats with VLPs containing the wild type F/F chimera protein or with VLPs containing the Pre-F/F chimera protein and compared immune responses in these animals with RSV infection. We found that Pre-F/F VLPs, without the use of adjuvant, stimulated high titers of neutralizing antibodies, titers far higher than those stimulate by VLPs containing the wild type F/F chimera protein and compared immune responses in these animals with RSV infection. We found that Pre-F/F VLPs, without the use of adjuvant, stimulated high titers of neutralizing antibodies, titers far higher than those stimulate by VLPs containing the wild type F/F chimera protein and compared immune responses in these animals with RSV infection. Furthermore, Pre-F/F VLP immunization of these animals protected them from virus replication in the lungs and in nasal passages upon RSV challenge. Thus assembling a stabilized pre-fusion form of the RSV F protein into VLPs resulted in a far superior vaccine candidate. This result suggests that manipulation of the conformation of VLP associated antigens can improve the effectiveness VLPs as a vaccine.
A novel RSV vaccine elicits humoral and Treg-cell responses against RSV infection and suppresses vaccine enhanced disease (VED)

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Background: Respiratory syncytial virus (RSV) infection is a major cause of respiratory tract disease in children under 5 years old. It leads to 64 million cases of bronchiolitis and viral pneumonia and causes approximately 200,000 deaths annually. Moreover, there are direct correlations between RSV infection in childhood and development of asthma in adulthood. The infections have become a heavy medical and economic burden around the world, particularly in developing countries, yet no vaccine for the prevention of RSV in early life is available. The effectiveness and particularly safety issues of RSV vaccines have puzzled researchers for more than 50 years, since the first formalin-inactivated RSV vaccine (FI-RSV) caused recipient children severe lung injuries and two infants died in a phenomenon that was then called vaccine-enhanced disease (VED) in the 1960s. A recent study has demonstrated that FI-RSV-induced VED was due to expulsion of Treg cells from lung since the Treg cells are important immunoregulatory cells to control inflamed reactions and minimize tissue damage.

Methods: We developed a novel strategy of immunizing animals with a recombinant RSV G protein together with cyclosporine A (CSA) twice subcutaneously. Levels of neutralizing antibodies, IL-10 producing Treg cells and lung tissue pathological assessments were analyzed and evaluated after RSV challenge.

Results: This novel vaccine induced not only a higher level of NAb against RSV infection, but most importantly, also significantly higher levels of Treg cells that suppressed VED in the lung after RSV infection. The induced responses provided protection against RSV challenge with no sign of pneumonia or bronchitis. Treg cell production of IL-10 was one of the key factors to suppress VED.

Conclusion: The study suggests that this new approach could be a promising vaccine candidate against RSV infection in children and older people. This is exciting not just for RSV vaccine development, but for infectious diseases with a similar problem, as well as non-infectious diseases such as allergy, asthma and autoimmune diseases.

Green tea catechin-inactivated viral vaccine

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Traditionally, chemical agents such as formalin or β-propiolactone have been widely used as inactivating agents for preparing inactivated viral vaccines. Concerns have been raised that formalin inactivation may hamper proper presentation of epitopes in antigen presenting cells, lowering the vaccine efficacy, or contributing to vaccine-associated enhanced respiratory disease (VAERD). The polyphenolic catechins present in the green tea (GT) have been confirmed to have a broad-spectrum antiviral activity against important human infectious pathogens including influenza viruses. Here we show that catechins from green tea could be used as effective inactivating agents for preparing inactivated influenza vaccines with desired level of immunogenicity and protective efficacy. The treatment with green tea extract (GTE) results in a complete and irreversible inactivation of influenza viruses in a dose-dependent manner. When the GT-inactivated influenza viruses are inoculated in naïve mice, robust systemic anti-influenza antibody response were elicited, providing the mice with complete protection from lethal infection with wild type viruses. LCMS/MS analysis showed that, in contrast to formalin that chemically modifies ε-amino group of lysine, a major anchor residue in MHC class II interaction essential for epitope presentation, catechin-mediated viral inactivation involves crosslinking with cysteine residue of antigen, preserving major epitopes contributing to vaccine efficacy and safety. With well-known safety, tolerability, and immune stimulation, green tea catechins have great potential for improving traditional vaccines and developing new vaccines for emerging viral diseases.
Preclinical evaluation of hemagglutinin stalk-based candidate universal influenza vaccines in ferrets
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Introduction: Influenza virus vaccines licensed for immunization in the US reduce the impact of influenza; however, these vaccines predominantly induce immune responses that specifically neutralize influenza viruses that are antigenically highly related to the vaccine strain. In order to develop a universal influenza virus vaccine which protects against antigenically divergent strains we designed vaccine constructs aimed at inducing an immune response against the conserved stalk domain of the hemagglutinin. We utilized the ferret model of influenza respiratory disease in preclinical studies to evaluate the efficacy of stalk-based universal influenza virus vaccine strategies to confer protection against influenza virus infection.

Methods: We developed live attenuated influenza virus (LAIV) vaccines and inactivated influenza virus (IIV) vaccines expressing chimeric hemagglutinins. Ferrets were immunized with prime/boost immunization regimens that included LAIV and/or IIV in an attempt to focus immune responses toward the conserved stalk domain of the H1 hemagglutinin. Seroconversion was assessed by measuring hemagglutinin antibody responses by enzyme-linked immunosorbent assay (ELISA). Following immunization, ferrets were directly challenged by intranasal infection with an H1N1 influenza virus.

Results: Intranasal immunization of ferrets with LAIV vaccines expressing chimeric hemagglutinin constructs resulted in the absence of clinical signs of disease, and low or undetectable virus titers. Whereas a LAIV prime/boost immunization regimen significantly reduced viral titers from respiratory tract and nasal wash samples, the IIV prime/boost immunization regimen only modestly reduced virus titers. Importantly, we were able to correlate reductions in virus titers with levels of hemagglutinin stimulated stalk-specific antibody responses and showed superiority over the current standard of care.

Discussion: In summary, a preclinical evaluation of influenza virus vaccines expressing chimeric hemagglutinin constructs was performed with the ferret model of influenza. Live attenuated influenza virus vaccines expressing chimeric hemagglutinin constructs exhibited a desirable safety profile in ferrets. Our stalk-based universal influenza virus vaccine regimens induced stalk-specific antibody responses in ferrets that reduced viral loads after influenza virus challenge. The novelty and significance of these findings support the translation from preclinical evaluation of vaccines stimulating stalk-specific antibody responses to clinical trials.
Influenza viruses remain a major threat to a wide range of animal species, causing acute respiratory illness of variable degree of severity in humans, swine and avian species. Influenza vaccination is the most effective measure for disease prevention. However, the ever-changing nature of influenza viruses and the co-circulation of multiple antigenically distinct lineages in some animal host populations represent major hurdles to current vaccines due to limited cross-protection and the risk of vaccine-associated enhanced respiratory disease (VAERD). Live attenuated influenza vaccines (LAIVs) closely mimic natural infection and have been shown to provide improved cross-protective immune responses compared to inactivated vaccines by eliciting local mucosal immunity and systemic B cell and T cell-mediated responses. Ascertainment of safety and stability of attenuated viruses remain a crucial step during the development of LAIV candidates. Here we describe the development and characterization of an alternative platform to generate live attenuated influenza virus vaccines for human and agricultural use. The incorporation of genetic modifications by design in the polymerase complex of A/quinea fowl/Hong Kong/WF10/1999 (H9N2), A/turkey/Ohio/313053/2004 (H3N2) and B/Brisbane/60/2008 virus strains rendered these viruses attenuated while retaining immunogenicity and constitute the base of our vaccine platform. These master donor viruses (MDV) were extensively characterized in vitro, displaying impaired polymerase activity and restricted replication at elevated temperatures. Genetic stability of incorporated genetic modifications was demonstrated following successive passages of MDVs in both tissue culture and embryonated eggs. Vaccine safety and efficacy studies were carried out in a number of relevant animal models and confirmed the superior performance of our vaccine platform compared to current available vaccines. MDVs exhibited diminished or inexistent replication in the lower respiratory tract, induced robust IgA and IgG response following intranasal immunization, provided homologous and heterologous protection by blocking or reducing virus replication while promoting rapid viral clearance and prevented the development of VAERD in pigs when animals were challenged with an antigenic mismatched strain. These findings highlight the safety and efficacy of our vaccine platform and its potential and versatility as master donor virus strains for the generation of live attenuated vaccines for humans and agricultural use.

Ebola virus (EBOV) is one of the most virulent pathogens known to human. Neutralizing antibodies play a major role in protection against EBOV infections. Thus, an EBOV vaccine capable of inducing a long-lasting neutralizing antibody response is highly desirable. Current vectored vaccine candidates are limited to single boosting injection due to anti-vector immunity. We report here that a heterologous prime-boost regimen can elicit durable EBOV-neutralizing antibody response in mice. A chimpanzee serotype 7 adenovirus expressing EBOV GP (denoted AdC7-GP) was generated and used for priming. A truncated version of EBOV GP1 protein (denoted GP1t) was produced at high levels in Drosophila S2 cells and used as the boosting antigen. Immunization studies showed that the AdC7-GP prime/GP1t boost vaccine regimen was more potent in eliciting neutralizing antibodies in mice than either the AdC7-GP or GP1t alone. Neutralizing antibodies induced by the heterologous prime-boost regimen sustained at high levels for at least 18 weeks after immunization. In a previous study, we developed an EBOV-like particle (EBOVLP) based luciferase reporter system that enables the evaluation of anti-EBOV agents in vivo outside the biosafety level four (BSL-4) facilities. Inoculation of mice with the reporter EBOVLP led to the delivery of Fluc protein into target cells and rapid generation of intense bioluminescence signals that can be blocked by the administration of EBOV neutralizing mAbs (manuscript in submission). Herein, in vivo challenge studies revealed that the entry of reporter EBOV-like particles was prevented in the mice receiving the heterologous prime-boost regimen even at 120 days after the final dose. These results indicate that this novel AdC7-GP prime/GP1t boost vaccine regimen represents an EBOV vaccine approach capable of establishing long-term protection, and therefore warrants further development.
Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality. The primary risk factors for developing HCC are cirrhosis (independent of its etiology) and chronic infection with hepatitis B virus and/or hepatitis C virus. HCC incidence continues to rise: the incidence and mortality rates for HCC have tripled in the USA within the past thirty years. In the European Union HCC incidence constitutes 8.29 cases per 100,000 with a shift of the primary cause in Europe towards alcoholic and non-alcoholic fatty liver disease, as well as metabolic disorders like diabetes mellitus and overweight as significant risk factors for HCC development. Current treatment options for HCC are insufficient and/or are limited to very early stages of the malignant disease and cannot prevent recurrence. There is only one approved drug, sorafenib, a multikinase inhibitor shown to increase survival of patients with HCC for nearly 3 months. There is a continuing need for innovative, alternative therapies for HCC.

In our previous studies we have developed orthotopic pre-malignant and malignant models of liver disease (Kang* & Yevasa*, et al, *Nature*, 2011; Dauch, et al, *Nature Medicine*, 2016). Using pre-malignant liver disease model we showed that immunosurveillance plays an important role in sustained elimination of pre-cancerous hepatocytes and that particularly antigen-specific CD4 Th1 immune response is indispensable for precancerous hepatocytes’ clearance (Kang* & Yevasa* et al, *Nature*, 2011). Thus, induction of Th1 immune response confers protection against liver cancer and we therefore hypothesized that *Listeria monocytogenes*-based immunotherapy that has been shown by numerous studies being able to promote CD4 Th1 responses towards Listeria-associated antigens could be used as a possible immunotherapeutic approach in HCC settings.

In present study, taking the advantage of transposon-based Ovalbumin-expressing orthotopic murine HCC model, we have tested attenuated *Listeria monocytogenes* carrying Ovalbumin (Ova) as vaccine candidates for prevention- as well as for treatment of developed HCC. The first proof-of-principle experiments confirmed that attenuated Listeria successfully deliver tumor-associated antigens (Ova). Attenuated strain of *Listeria monocytogenes* has been shown in our experiments as a promising vaccine candidate that confers protection against liver carcinomas as well as prolongs the survival of mice harboring aggressive Ova-expressing HCC. The protection seems to be dependent on the induction of antigen-specific CD4 and CD8 T cells, whereas antibody production is not correlating with protection.

In conclusion, using orthotopic clinically-relevant HCC murine models we were able to show that vaccination strategy using attenuated Listeria strains for the delivery of tumor-associated antigens has strong perspectives to prevent- as well as to treat and/or prolong survival of patients with liver cancer. Attenuated Listeria-based immunotherapy is able to elicit nontoxic, systemic anti-tumor response (cancer immunosurveillance), that makes it particularly well-suited for use in the setting of HCC.
Novel recombinant HIV vaccine candidates based on replication-defective flavivirus vectors demonstrate favorable safety and immunogenicity profile in NHP

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The results of HIV vaccine efficacy trial RV-144 (NCT00223080) provided the first evidence that a HIV vaccine is possible; with 60% and 31.2% efficacy, at 12 months and 3.5 years respectively. The Pox-protein public private partnership (P5) recently announced meeting the Go-decision to proceed to a pivotal efficacy study using a regionally adapted RV144-like vaccine regimen in South Africa. Furthermore, to support the iterative approach of vaccine development, we are evaluating an alternative vector, RepliVax® (RV) for delivery of HIV antigens. RV vectors are based on flavivirus genomes and have been demonstrated to be highly attenuated and immunogenic, eliciting durable immunity in various animal models including NHP against TBE and rabies. West Nile (WN) virus based RV vectors were engineered to express HIV clade c Gag or Env immunogens in place of the C-prM-E deletion. High titer stocks (8 logs) of RV-Gag and RV-Env candidates were generated in helper Vero cells expressing the WNV C-prM-E proteins in trans. High stability of both Gag and Env (gp120TM) gene inserts was demonstrated using RT-PCR, sequencing and immunostaining of infected cells. Robust protein expression and correct maturation and transport were observed for Gag and Env. In -vitro studies have demonstrated that RV-HIV constructs stimulate the innate immune signatures in human PBMCs similarly to fully infectious flaviviruses. The RV-HIV candidates elicited immune responses in mice against Gag and Env and demonstrated to be highly attenuated in sensitive 2-3 day old suckling mouse neurovirulence studies. The immunization schedule in the NHP model assessed immunogenicity of heterologous vectors as compared to DNA priming followed by vector boosting which was used as a benchmark. All vaccines appeared to be safe and well tolerated in the animals. All groups elicited comparable magnitude of neutralizing antibody titers and ADCC responses throughout the course of the study. The level of IgG responses appeared to be similar between groups for the Env gp120, Env gp140, V3 and V1V2 proteins at all-time points. Collectively, the data indicated the RepliVax® vectors can be used in combination with other vectors to elicit HIV specific cellular and humoral immune responses as a potentially effective vaccination strategy.
[O8.1]

**Immune Profiling of Coxiella burnetii Vaccination and Infection by Mass Cytometry**
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*Coxiella burnetii (Cb)* is a highly infectious and stable bacteria that causes Q-fever in humans. Treatment for Q-fever can require months of antibiotic administration; therefore, vaccination is considered critical to infection control. The currently available vaccine may induce severe reactogenicity in previously exposed patients. The Q-VaxCelerate consortium project aims to develop a safer, effective, and less reactogenic Q-fever vaccine.

To characterize the immune response to *Cb* infection or vaccination in both human and mouse samples we developed an approach utilizing Cytometry by Time Of Flight mass spectrometry (CyTOF) to measure >35 immune-parameters simultaneously. Using CyTOF we measured a broad array of cell populations, activation makers, and cytokines in both human and murine blood samples. Donors were recruited from a region that experienced an outbreak of Q-fever (2007-2011) and characterized for pre-existing immunity against *Cb* by measuring anti- *Cb* antibody titres and IFN production. Blood from characterized donors was stimulated with killed *Cb* and analyzed by flow cytometry and CyTOF. *Cb*-exposed patients exhibited increased IFN, TNF, and IL-1 expression in T-cells, NK cells, and macrophages. We continue to recruit and characterize human donors, working to assess immune responses across a diversity of HLA types and clinical responses. Transgenic HLA-DR3 C57/Bl6 mice were infected with *Cb* and blood samples collected 2 days prior or 2, 10, 24, and 35 days post infection. CyTOF analysis of murine samples indicated early increases of innate immune cell numbers and activation, including increased expression of TGF. During late stage infection, day 24 and 35, CD8 T-cells increased expression of IL-2, IL-6, and IL-10. Together these data reveal novel hallmarks of the immune response to *Cb* during and following infection. Ongoing animal studies will characterize the immune response from vaccination through challenge with *Cb* in both wild-type and HLA-DR3 C57/Bl6 mice.

Analyses of immune cell sub-types (e.g. Tregs, macrophage subsets) from both human and mouse samples are also underway. The data from these studies will inform the definition of immune signatures of *Cb* infection to facilitate the testing and evaluation of candidate vaccines for *Cb* and may lead to insights on the diversity of acute and chronic clinical sequelae of *Cb* infection.

[O8.2]

**Ebola Challenge Study in Non-Human Primates: Role in Supporting the Development of rVSV-EBOV Vaccine**
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A saline-controlled, blinded vaccination study was designed to evaluate the protective efficacy of a recombinant vesicular stomatitis virus vaccine expressing EBOV glycoprotein (rVSV-ZEBOV-GP) against lethal Ebola virus infection in the non-human primate (NHP, cynomolgus macaques) Ebola virus infection model. Animals were administered saline, or various decreasing doses of rVSV-ZEBOV-GP by intramuscular injection, at one administration. The primary objective of the study was to measure the anti-EBOV GP specific IgG response and assess the anti-rVSV-ZEBOV-GP neutralizing antibody response in serum of vaccinated NHP, and evaluate the effect of vaccination at various decreasing doses on protection post-EBOV challenge. The safety objectives of this study were to evaluate vaccinated animals daily for overall well-being and health parameters, as well as determine vaccinemia in vaccinated NHP on days immediately following vaccine administration. The study was performed to assess immune responses in the vaccinated animals and to determine what level of response correlates with protection. Once a threshold for protection in the non-human primate model is established, formal immunobridging to humans will be conducted by applying the appropriate immune correlate threshold collected from these NHP samples, and samples from other future studies, to the human immune responses in current phase II/III studies of V920 in Sierra Leone, Guinea and Liberia. Taken together, these data support the development of this vaccine and may support future licensure.
A Phase 1 Study of a DNA Vaccine for Venezuelan Equine Encephalitis Delivered by Intramuscular or Intradermal Electroporation
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There remains a need for FDA-licensed vaccines to protect against human infections caused by the biological threat agents Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV). Previously, we demonstrated that a DNA vaccine expressing codon-optimized envelope glycoprotein genes of VEEV (pWRG/VEE) delivered by intramuscular (IM) electroporation (EP) using the Ichor Medical Systems TriGrid™ Delivery System (TDS) elicited potent immune responses, to include high levels of virus-neutralizing antibodies, in multiple animal species and provided protection against aerosol VEEV challenge in mice and nonhuman primates. Subsequently, we performed a Phase 1 study to assess the safety, reactogenicity, tolerability, and immunogenicity of this DNA vaccine candidate when administered by IM or intradermal (ID) EP using the Ichor TDS to healthy adults. Subjects in the IM EP groups received 0.5 mg (N=8) or 2.0 mg (N=9) of pWRG/VEE or a saline placebo (N=4) in a 1.0 ml injection. Subjects in ID EP groups received 0.08 mg (N=8) or 0.3 mg (N=8) of DNA or a saline placebo (N=4) in a 0.15 ml injection. Subjects were monitored for a total period of 360 days. No vaccine- or device-related serious adverse events were reported. Based on the results of a subject questionnaire, the IM and ID EP procedures were both considered to be generally acceptable for prophylactic vaccine administration, with the acute tolerability of ID EP delivery judged to be greater than that of IM EP delivery. All subjects (100%) in the high and low dose IM EP groups developed detectable VEEV-neutralizing antibodies after two or three administrations of pWRG/VEE, respectively. VEEV-neutralizing antibody responses were detected in seven of eight subjects (87.5%) in the high dose and five of eight subjects (62.5%) in the low dose ID EP groups after three vaccine administrations. There was a correlation between the DNA dose and the magnitude of the resulting VEEV-neutralizing antibody responses for both IM and ID EP delivery. These results indicate that pWRG/VEE delivered by either IM or ID EP is safe, tolerable, and immunogenic in humans at the evaluated dose levels. Consequently, we are currently completing pre-clinical studies required to allow for Phase 1 testing of similar DNA vaccines for EEEV and WEEV as well as a combined formulation of the VEEV, EEEV, and WEEV DNA vaccines delivered by IM and ID EP.

Confirmation of a lethal mouse model for Zika virus infection and development of a novel DNA vaccine that is fully protective against lethal infection in vivo.
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Zika virus, a positive strand RNA virus belonging to the family Faliviridae, was initially identified in 1947 in the Zika forest of Uganda in a sentinel non-human primate who displayed a mild fever. Initial work performed in the 1950’s with this virus demonstrated that it required several serial intracranial passages within immunocompetent mice in order to become lethal. Since then, very little advancement on the understanding of the pathogenesis of Zika virus and development of adequate animal models has occurred. The current Zika virus outbreak in South and Central America, which has been linked to increased incidences of Guillian-Barré syndrome and microcephaly in newborns, has brought to the fore front how little is known regarding this virus and highlighted the need for effective vaccines and therapies. To this end, a DNA-based vaccine expressing a consensus sequence of the Zika virus pre-membrane and envelope proteins (prME) was designed using genome sequences of viruses isolated between 1952 and 2015. In order to test this novel DNA vaccine, a lethal model of Zika virus infection was developed using mice lacking IFN α/β receptors (IFNAR). Here, 6-week old IFNAR mice were challenged with 1 x 10^6 plaque forming units (PFU) of a strain of Zika virus originating from Puerto Rico (PR209) via various routes of infection. All routes resulted in significant weight loss and in paralysis and were thus uniformly lethal. Once this lethal model of infection was established, the protective efficacy of the prME DNA vaccine was tested through the immunisation of IFNAR mice. IFNAR mice were either immunised with one or two doses of prME vaccine or the control vector pVAX1, and subsequently infected with either 1 x 10^6 PFU or 2 x 10^5 PFU of PR209. All vaccinated animals survived the challenge, regardless of the number of immunisations they received, while 70-90% of the control animals succumbed to the infection. In addition to the clinical outcome of these studies, the laboratory analysis of samples collected throughout the experiments will also be presented and discussed.
Identification of dengue-specific and cross-reactive B cells using labeled viruses following natural infection and vaccination.
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Methods to directly identify and characterize memory B cells ex vivo have been challenging since the majority of B cells see conformational epitopes. Therefore, developing reagents that are recognized by the B cell receptor is more complicated compared to peptide-MHC tetramers used to characterize antigen-specific T cells. We recently established an antigen labeling method that allows ex vivo identification of dengue virus-specific memory B cells using multiparametric flow cytometry. We used multiple serotypes of fluorescently labeled dengue viruses (DENV) together to identify serotype-specific and cross-reactive B cells in PBMC obtained during natural infection and after vaccination. Brightly labeled DENV-specific and cross-reactive B cells were identified in all donors with up to 8% of all class switched memory B cells being antigen-specific. Furthermore, we found that many subsets of antigen-specific B cells (naïve B cells, plasmablasts and reactivated memory B cells) are present in the circulation during acute dengue infection. Labeled DENV are useful reagents that reveal changes in the phenotype of DENV serotype-specific and cross-reactive B cells. With clinical trials of tetravalent dengue vaccines ongoing, understanding how subsets of B-cells respond to multiple DENV serotypes at one time will provide important insights into the humoral immune response.

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A novel tetravalent formulation combining the four aggregated domain III-capsid proteins from dengue viruses induces a functional immune response in mice and monkeys
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Dengue virus (DENV) is the most prevalent mosquito-borne virus affecting humans. Infection with any of the four dengue serotypes can cause a range of clinical manifestations that goes from self-limiting illness to potentially lethal severe dengue disease. ChimeriVax™, a dengue vaccine developed by Sanofi Pasteur, has recently been registered in a number of countries. However, this vaccine has exhibited low protective capacity against DENV-1 and DENV-2 during efficacy trials conducted in endemic areas, despite the induction of neutralizing antibodies. The Cuban vaccine candidate is based on recombinant proteins that contain two viral regions to induce B and T cells: the domain III of the envelope protein and the capsid protein. The chimeric protein from DENV-2 (domain III-capsid (DIIIIC-2)), when presented as aggregated incorporating oligodeoxynucleotides, induced antiviral and neutralizing antibodies, cellular immune response, and conferred significant protection to mice and monkeys. After this proof of concept the remaining constructs were obtained and characterized using mice and human DENV-positive sera. Later, the immunogenicity, in terms of humoral and cell-mediated immunity, and the protective capacity of each protein were assessed in mice. Significant protection was afforded as measured by the limited viral load in the mouse encephalitis model. The assessment of the tetravalent formulation in non-human primates also demonstrated that the formulation induced neutralizing antibodies and memory cell-mediated immune response with IFN-γ-secreting and cytotoxic capacity, regardless the route of immunization used. Taken together, it can be asserted that the tetravalent formulation of DIIIIC proteins constitutes a promising vaccine candidate against dengue virus.

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Extreme Polyvalency Induces Potent Cross-Clade Cellular and Humoral Responses in Rabbits and Non-human Primates

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Due to the extreme diversity of HIV, there is a pressing need to determine possible unique vaccine combinations of Envelopes (Envs) which induce enhanced breadth and functional antibody responses. Since DNA vaccines are easy to manufacture and formulate, we sought to determine if increased breadth within a vaccine correlates with robust and broad vaccine induced responses.

We have developed over 40 different DNA plasmids expressing consensus as well as primary HIV Envs. We have shown that all of these optimized plasmids are able to induce both cellular and humoral responses in mice. Different combinations of Envs were testing in rabbits to further characterize the humoral responses and explore neutralization. Rabbits immunized with clusters of clade A transmitted founder (TF) gp160 DNA induced cross-clade binding titers with limited neutralization. Including TF Envs from different clades increased binding titers as well as neutralization breadth and potency. Formulating the gp160s to be administered to the same site induced faster seroconversion than delivering the Envs at separate sites. The most potent combination was moved forward into non-human primates, which were immunized with clusters of gp160 DNAs (14 different Envs in total) at weeks 0, 4, 8, 12 and boosted at weeks 48 and 85. The vaccine induced cross-clade cellular and humoral responses after two immunizations. These responses increased after each immunization and were maintained into memory. In addition to binding, the vaccine also induced tier 1A and 1B neutralization titers and antibody dependent cellular cytotoxicity against both homologous and heterologous targets. Boosting at week 48 and 85 further increased both responses. We show that DNA plasmids encoding consensus and TF Envs are expressed and induce a potent immune response. We observed for the first time that exposure of the immune system to multiple Envs at one time can dramatically change the immune phenotype by inducing broader breadth of responses which has significant implications for HIV vaccine development.

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Vaccines against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) elicit humoral and cellular immune responses in mice

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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 1700 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, up to date, there is no approved vaccine or antiviral drug to control or treat MERS-CoV. Here, we report our MERS-CoV vaccine development programme, utilising two viral vectors: Modified Vaccinia virus Ankara (MVA) and Chimpanzee Adenoviral vector by Oxford University (ChAdOx1), each encoding the spike (S) protein of MERS-CoV, which resulted in ChAdOx MERS and MVA MERS vaccines. Two groups of BALB/c mice were immunised with a single injection of ChAdOx MERS or with ChAdOx MERS prime and MVA MERS boost. A third group of mice were immunised with a homologous prime-boost MVA MERS vaccination. All groups developed high titres of S-specific antibodies that were also able to neutralise the infection of either MERS-CoV or MERS-CoV pseudotyped viral particles, in microneutralisation assay or pseudotyped viral particles neutralisation assay, respectively. In addition, all three groups developed IFN-γ-secreting CD8+ T cells in spleens, these cells also produced TNF-α and IL-17. We concluded that a single vaccination with ChAdOx MERS induced a significantly high titre of neutralising antibodies. Therefore, we are planning to vaccinate dromedary camels in Saudi Arabia with a single injection of ChAdOx MERS, in the coming months. The vaccine is also scheduled for a phase I clinical trail in collaboration between University of Oxford, UK and KAIMRC, Saudi Arabia.
A New Genome-Wide Antigen Discovery Algorithm Identifies Novel In-Vivo Expressed Mycobacterium Tuberculosis (IVE-TB) Antigens Inducing Human T Cell Responses with Classical and Unconventional Cytokine Secretion Profiles.

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New strategies are urgently needed to develop better tools to control TB, including identification of more promising antigens for improved diagnostics and more effective vaccination. A requirement for such Mtb antigens, particularly in the context of vaccination, is that they are expressed in vivo during Mtb infection in the major target organ, the lung. A second requisite is that such antigens can elicit immunity in the human host. Using genome-wide transcriptomics of Mtb infected lungs of highly TB-susceptible (C3HeB/FeJ) mice, we have developed data sets and methods to identify the most promising IVE-TB (in vivo expressed Mtb) antigens expressed in the lung. We isolated Mtb RNA from the lung of highly susceptible (C3HeB/FeJ) and resistant (C57BL/6J) mice at early and late time points after aerosol Mtb (Erdman) infection. Quantitative expression analysis of 2,068 Mtb genes from the predicted first operons of Mtb allowed identification of the most upregulated IVE-TB genes during early and late phase infection. By combining analysis of high-level conservation throughout a set of whole-genome sequenced Mtb-complex strains (n=219) with the presence of predicted HLA-class Ia and II binding peptides as well as homology with other pathogenic mycobacteria, we selected the most promising IVE-TB candidate antigens. Several Mtb IVE-TB recombinant proteins were well recognized by T-cells from in vitro Mtb purified protein derivative (PPD) and Mtb ESAT6/CFP10-positive donors detected by proliferation and multi-cytokine production. This was validated in an independent cohort of latently Mtb-infected individuals. Significant T-cell responses were observed in the absence of clear IFN-γ-production. Collectively, the results underscore the power of our novel antigen discovery approach in identifying Mtb antigens, including those that induce unconventional T-cell responses, which may provide important novel tools for TB vaccination and biomarker profiling studies. Importantly, the generic approach we describe can be applied to any other infectious disease.
Both young and aged effector CD4 T cells that recognize cognate Ag at the “memory checkpoint” differentiate to Tfh and memory cells and promote B cell memory in a vaccine model.

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Annual influenza epidemics cause 3-5 million cases of severe illness and up to half a million deaths worldwide. Vaccines are the most efficient approach to prevent infection and transmission, but they are inefficient in the elderly. Age-associated functional defects in naive CD4 T cells lead to poor help for B cells and reduced long-lived Ab and memory CD4 T cells. Therefore, a vaccination strategy that would enhance the response of aged CD4 T cells could promote both B and T cell memory generation. We recently showed that CD4 effectors generated by influenza A virus (IAV) made very few memory cells if they could not make autocrine IL-2 and that such IL-2 production depended on cognate Ag recognition at the effector stage, defining what we call the ‘memory checkpoint’. Full differentiation of Tfh also requires such Ag recognition after 6 days post infection.

Here we test whether providing adult or aged CD4 T cells with Ag/APC at the ‘memory check-point’ can augment the CD4 and Ab response to inactivated IAV vaccine. We use a reductionist in vivo model with an irrelevant TcR transgenic host, where we can add the IAV-specific naïve CD4 T cells (young or aged from TcR transgenic donor specific for IAV) and vaccinate with inactivated IAV as the vaccine. We then introduce the CD4 T cell Ag, as viral peptide-pulsed recognized by the donor T cells on APC at 6 days, corresponding to the memory checkpoint. We follow the donor CD4 T cell effector and memory generation to cognate Ag and function and the B cell Ab response to other determinants. Our results show that when with either young adult or aged CD4+ T cells again recognize cognate Ag at the ‘memory checkpoint’ their better differentiate into Tfh and memory cells with a more differentiated memory phenotype suggesting a better quality of memory. Interestingly, this also induced increased IAV-specific IgG memory B cells (MBCs) as well as IgG-secreting long-lived plasma cells (LLPCs) in both spleen and bone marrow. Thus providing CD4 effector cognate Ag recognition at the ‘memory checkpoint’ promoted both CD4 T and B cell response to inactivated viral vaccine and enhanced memory immunity, suggesting novel strategies may improve vaccine efficacy.

Characterization of Influenza Elicited Humoral Immunity in the Domestic Ferret (Mustela putorius furo)

Kirchenbaum GA, Barber JP, Penrose T, Chan R, Bebin AG and Ross TM

The ferret is considered the gold standard for modeling human influenza infection and transmission. Despite the importance of this animal model, characterization of the immune response by flow cytometry (FCM) is severely hampered due to the limited number of commercially available reagents. To address this unmet need, we screened 418 monoclonal antibodies (mAb) with defined specificity for surface staining of ferret splenocytes by FCM. We also identified a mAb with reactivity with the nuclear antigen Ki-67, enabling identification of proliferating cells. To improve existing tools, and to enhance our study of the B cell response elicited by influenza infection or vaccination, we generated a panel of novel mAb with specificity for ferret immunoglobulin (Ig). These mAb were subsequently screened for reactivity with ferret peripheral blood mononuclear cells by FCM and demonstrate specificity for CD79b+ B cells. Moreover, several of these mAb exhibited a staining pattern consistent with specificity for the light chain of surface B cell receptor, and enable segregation of kappa and lambda. Additionally, we identified a mAb with specificity for the heavy chain of secreted, but not surface expressed antibody. Using these new reagents, we characterized the kinetics and magnitude of the antibody secreting cell response elicited by infection of ferrets with pandemic H1N1 using FCM and ELISPOT respectively. Assembly of multi-parametric FCM panels incorporating these newly identified markers will enable more detailed characterization of the innate and adaptive immune response elicited by infection or vaccination in the ferret model.
[O9.4]

**Development of a safe, tolerable and efficacious gene-based immunoprophylaxis delivery strategy to protect against RSV**


Respiratory syncytial virus (RSV) is a massive medical burden in infants, children and the elderly worldwide, and an effective and safe RSV vaccine remains an unmet need. Here we report a novel immunization strategy to deliver a pDNA vaccine candidate (pGX2303), encoding RSV-F. pGX2303 was delivered to the skin using a surface electroporation device (SEP) to specifically target epidermal cells in clinically relevant experimental models. We demonstrate the utility of this strategy to target epidermal Langerhans cells, and elicit robust cellular and humoral immune responses in the host. In the cotton rat RSV challenge model we demonstrated complete resistance to pulmonary infection after delivering a single low dose of pGX2303. In contrast to the formalin-inactivated RSV (FI-RSV) vaccine, no enhanced lung inflammation upon virus challenge was observed in the pGX2303 vaccinated group. In summary, the data presented outlines the pre-clinical development of a highly efficacious, tolerable and safe non-replicating gene-based vaccine strategy against RSV.

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[O9.5]

**Formulation of the RSV fusion protein with a novel combination adjuvant mediates the induction of long-term immune memory and disease protection**

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Respiratory syncytial virus (RSV) is one of the most serious respiratory pathogens in young children and elderly. Therefore, an effective RSV vaccine needs to be developed for these populations. Previously, we reported the development of a novel RSV vaccine formulation consisting of a truncated version of the RSV fusion protein formulated with a TLR agonist and an immunostimulatory peptide in a polyphosphazene carrier system (ΔF/TriAdj). The ΔF/TriAdj induced robust, balanced immune responses in several animal models including mice, cotton rats and lambs. These responses were still fully protective from RSV infection one year after vaccination in mice. The safety of the ΔF/TriAdj was established in cotton rats. In addition, ΔF/TriAdj induced both cell-mediated and humoral immune responses in neonatal mice and lambs, even in the presence of RSV-specific maternal antibodies. Recently, we have started to elucidate the mechanism of action of the ΔF/TriAdj. When used as a mucosal adjuvant with the fusion protein of RSV, TriAdj promoted the production of chemokines and inflammatory cytokines, followed by recruitment of several immune cell populations including dendritic cells, macrophages and neutrophils, to the upper and lower respiratory tract. This was correlated to the induction of local humoral and cell-mediated immune responses, including production of large numbers of IgA-secreting B cells as well as IFN-γ secreting CD8 T cells, in mice. In addition, the TriAdj promoted increased germinal centre reactions, which in turn lead to effective B cell activation in the lungs following intranasal vaccination. Furthermore, ΔF/TriAdj induced higher gene expression of activation-induced cytidine deaminase, which is a key players in IgA class switch recombination. In summary, the ΔF/TriAdj has potential as a safe and effective vaccine against RSV.

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Broad Cross-Protective Anti-Hemagglutination Responses Elicited by Influenza Micro-Consensus DNA Vaccine

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Despite the routine development and distribution of seasonal influenza vaccines, influenza remains an important pathogen contributing to significant human morbidity as well as mortality each year. The seasonal variability of influenza creates a significant issue for vaccine development of matched seasonal strains that afford protection from infection and disease based on serotype matching. It is appreciated that the globular head of the HA antigen contained in the vaccines generates antibodies that result in HAI activity that are considered major correlates of the protection against a particular strain. Due to genetic changes in the HA protein, however, new vaccine strains are needed to be developed continually to match the new HA antigen of that seasons virus. A distinct advantage in seasonal vaccine development would be if a small group of antigens could be developed that could span many seasons without needed to be replaced due to genetic drift of the HA antigen. Here, we utilized a synthetic microconsensus approach to design four distinct plasmid-encoding influenza H1 HA micro-consensus antigens; each containing a consensus sequence that was generated based on analysis of a subset of the broader collection of primary sequences of H1 HA antigens deposited in the NCBI database. We first confirmed the immunogenicity of each individual micro-consensus plasmid delivered by EP in mice. Then we combined these plasmids as a single vaccine formulation and evaluated its immunogenicity in guinea pigs and rhesus macaques. We observed that this influenza DNA vaccine containing four plasmid-encoding micro-consensus H1 HA immuogens was capable of inducing protective HAI titers ($\geq 1:40$) against a wide range of unmatched H1N1 viruses that span the past 25 years of seasonal vaccine strains as well as unmatched pandemic H1N1 isolates, including the highly pathogenic Spanish Flu virus, A/South Carolina/1/18 in guinea pigs and non-human primates as well. The protective HAI titers induced by microconsensus immunogens are fully functional in vivo and the immunized ferrets were completely protected from A/Mexico/InDRE4487/2009 virus infection and any morbidity associated with lethal challenge. These results are encouraging that a limited easy-to-formulate collection of antigens can be developed that can span seasonal vaccine changes allowing for continued immune protection.

Design and Characterization of a COBRA H1N1 HA Vaccine in a Pre-Immune Ferret Model

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One of the challenges for developing influenza A vaccines is the diversity of antigenically distinct isolates. Previously, our group described a novel hemagglutinin (HA) for H1N1 influenza derived from a methodology termed computationally optimized broadly reactive antigen (COBRA). Nine prototype H1N1 COBRA HA proteins were developed and tested in naïve mice and ferrets using a virus-like particle (VLP) format for the elicitation of broadly-reactive, universal antibody responses and protection against viral challenge. These candidates were designed to recognize H1N1 viruses isolated within the last 10, 20, and 30 years. In addition, several COBRA candidates were designed based on sequences of H1N1 viruses spanning the past 100 years, including modern pandemic H1N1 isolates. Four of the 9 H1N1 COBRA HA proteins (X1, X3, X6, and P1) had the broadest HAI activity against a panel of 17 H1N1 viruses. In order to mimic vaccination in adult humans with prior influenza exposure, these vaccines were then tested in a pre-immune ferret model. Ferrets were infected with different seasonal H1N1 viruses and allowed to recover for 12 weeks prior to vaccination with wild type or COBRA VLP vaccines. The COBRA vaccinated pre-immune ferrets had an increase in HAI activity against the more viruses in the target panel compared to non pre-immune, naïve ferrets and pre-immune ferrets vaccinated with wild-type vaccines. These ferrets also show a greater level of protection against infection with a pandemic H1N1 isolate and little or no detectable viral replication in their lungs compared to wild type or naïve ferret controls. Pre-immune ferrets vaccinated with COBRA VLP had a greater potential to induce a robust, broadly reactive antibody responses than naïve ferrets or pre-immune ferrets vaccinated against a wild type influenza strain.
EFFICACY OF ADENOVIRUS VECTOR-BASED MULTI-EPIPOTE VACCINE AGAINST A/H5, A/H7 AND A/H9 AVIAN INFLUENZA VIRUSES
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Since 1996, there have been numerous reports of human infections with avian influenza A viruses of subtypes H5N1, H7N7, H7N9 and H9N2. Although human-to-human transmission has been infrequent and limited, genetic reassortment between avian and human/porcine influenza viruses or mutations in some of the genes leading to virus replication in the upper respiratory tract in humans could result in the generation of a novel pandemic influenza virus strain that not only would infect but also effectively transmit among the human population which would have little or no immunity to the new virus. Current vaccines against these viruses do not provide effective cross protection against antigenically distinct strains of H5, H7 and H9 influenza viruses. Hence, newer vaccine approaches with the potential to induce both humoral and cellular immune responses that confer protection against a broad range of influenza viruses emerging from avian reservoirs are needed. To enhance the breadth of protective efficacy against avian influenza viruses, we developed an egg-independent adenovirus vector-based multi-epitope (ME) vaccine approach by including the relatively conserved immunogenic domains of H5, H7, and H9 influenza proteins [M2 ectodomain (M2e), HA fusion domain (HFD), T cell epitope of nucleoprotein (TNP) and HA α-helix domain (HαD)]. Immunization of mice with ME vaccine formulations led to development of humoral immune responses against the M2e, HFD or HFD domains and cell-mediated immune response against the TNP domain. There was significant reduction in the lung viral titers when immunized mouse groups were challenged with antigenically distinct H5, H7 or H9 avian influenza viruses suggesting that the ME vaccine approach provided a broad protective against avian influenza viruses. Since the nature of the next pandemic influenza virus is unknown, this vaccine approach could significantly lower morbidity, hinder transmission and prevent mortality in a pandemic situation before a strain-matched vaccine can be produced.

Upper Respiratory Tract (URT) Administration of a Replication-Competent Ad4-H5-Vtn Vaccine Induces Durable Neutralizing Antibody Responses in Humans
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Replication-competent recombinant vectors provide more prolonged exposure to transgene-encoded antigens than non-replicating vectors, which may more efficiently drive serum neutralization potency, antibody somatic hypermutation (SHM), affinity maturation, and long-lived plasma cells. We report results of a Phase 1 trial with a replication competent adenovirus type 4 encoding influenza H5 Hemagglutinin (HA). Live Ad4-H5-Vtn was administered in an oral capsule (10¹⁰ virus particles (VP)), tonsillar swab or nasal spray (10³-10⁸ VP). Nasal, rectal, and tonsillar swabs were collected up to 28 days after vaccination and VP shedding was quantified by qPCR. Plasma and PBMC were collected on day 0 and weeks (w) 4, 8, 26, and 52. B cells were sorted using fluorescently-labeled HA probes. Immunoglobulin genes were cloned, sequenced and re-expressed to measure SHM, neutralization potency and breadth. H5 mAb affinity was measured by surface plasmon resonance. A subset of volunteers was boosted with a recombinant H5 protein vaccine (90 μg) at 52+ weeks. Plasma from boosted patients was collected at day 0, w 4 and w 8 post-boost.

In most URT immunized volunteers, viral shedding was detected in nasal, tonsillar, and rectal swabs for 2-4 weeks. In orally immunized volunteers, only rectal shedding was detected. URT immunizations effectively induced H5 Ab responses at 8 w (median ID₅₀ = 835 (tonsillar) and 423 (nasal)) compared to oral immunization (180) which remained stable for 26 w. B cells that bound both H1- and H5-probes were expanded by 8 w. Remarkably H5-monospecific B cells continued to expand at 26 w. Compared to the germline, SHM of H5 mAbs increased from 1.0 % at 4 w to 5.9% at 34-52 w, while some mAbs were mutated up to 40%. The binding affinity and neutralizing potency of H5 mAbs increased concurrently. Some mAbs neutralized both group 1 and 2 influenza viruses. The URT administration of live Ad4 recombinants is a promising platform for the induction of antibodies against influenza.
An Inactivated Rabies Virus–Based Ebola Vaccine, FILORAB1, Adjuvanted With Glucopyranosyl Lipid A in Stable Emulsion Confers Complete Protection in Nonhuman Primate Challenge Models
Matthias Schnell, Drishya Kurup

The 2013–2016 West African Ebola virus (EBOV) disease outbreak was the largest filovirus outbreak to date. Over 28,000 suspected, probable, or confirmed cases have been reported, with a 53% case-fatality rate. The magnitude and international impact of this EBOV outbreak has highlighted the urgent need for a safe and efficient EBOV vaccine. To this end, we demonstrate the immunogenicity and protective efficacy of FILORAB1, a recombinant, bivalent, inactivated rabies virus–based EBOV vaccine, in rhesus and cynomolgus monkeys. Our results demonstrate that the use of the synthetic Toll-like receptor 4 agonist glucopyranosyl lipid A in stable emulsion (GLA-SE) as an adjuvant increased the efficacy of FILORAB1 to 100% protection against lethal EBOV challenge, with no to mild clinical signs of disease. Furthermore, all vaccinated subjects developed protective anti-rabies virus antibody titers. Taken together, these results support further development of FILORAB1/GLA-SE as an effective pre-exposure EBOV vaccine.

Clinical Assessment of a Bivalent DNA Vaccine for Hemorrhagic Fever with Renal Syndrome Caused by Hantavirus Infections
Connie Schmaljohn, Drew Hannaman, Kristopher Paolino, Jay W. Hooper

Objective: Demonstrate safety and efficacy of DNA vaccines against hemorrhagic fever with renal syndrome (HFRS).
Methods: We developed DNA vaccines for HFRS expressing the Gn and Gc genes of HTNV or PUUV. To assess the clinical safety and immunogenicity of the DNA vaccines, we conducted Phase I studies using gene gun or intramuscular electroporation (IM-EP) delivery devices and have now advanced into Phase 2 clinical testing.
Results: The gene gun study demonstrated that the HTNV and PUUV DNA vaccines delivered as separate administrations were safe and immunogenic; however, the low overall seroconversion rate (~50%) indicated the need for improved vaccine delivery. Consequently, we conducted a Phase I study using Ichor Medical Systems IM-EP TriGrid Delivery System. All vaccinations were administered at a 2 mg total dose and comprised HTNV DNA alone, PUUV DNA alone, or a combined vaccine (1 mg each HTNV and PUUV DNAs). Neutralizing antibody responses were detected in 5/9 or 7/9 of individuals receiving three doses of the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 and 3/9 subjects developed neutralizing antibodies to PUUV or HTNV, respectively, signifying potential vaccine interference. To address this, we modified the HTNV vaccine to reflect the codon bias of humans and to remove elements known to negatively impact expression or mRNA stability. We initiated a Phase 2a dose ranging study with the modified mixed DNA vaccines in July, 2014, which will conclude in December, 2016. 120 subjects were randomized into four vaccination groups, and all vaccinations have been completed. The subjects received 1 mg or 2 mg of the mixed DNAs either on Days 0, 28 and 56, or on Days 0 and 56, with an optional 6-month booster. Although group assignments will remain blinded until the study conclusion, preliminary analyses of serum samples from completed subjects indicate that the modified HTNV/PUUV vaccines can consistently induce responses against both viruses.
Conclusion: The HTNV and PUUV DNA vaccines delivered by IM-EP are safe and immunogenic in humans. Preliminary evidence indicates that observed interference issues can be mitigated through vaccine refinement, permitting further development of this HFRS vaccination strategy.
Discovery of Toxoplasma gondii vaccine candidate antigens
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Toxoplasma gondii is an obligate intracellular protozoan parasite that causes congenital toxoplasmosis, as well as other serious clinical presentations, in immune compromised humans. The parasite has also been recently linked to behavioral diseases in humans and other mammalian hosts. A vaccine is required to protect the human host, while a transmission blocking vaccine for the definitive host (Felidae cats) would limit the spread of infection to humans and other mammalian species that are susceptible to toxoplasmosis in nature.

The selection of the antigen(s) to be used in vaccine formulations is challenging owing to the large number of potential vaccine antigens encoded in the parasite genome. Efforts to date using conventional approaches have led to disappointing results at relatively late stages of the development pipeline. To address this issue, novel vaccine antigens are being discovered in attempts to enhance vaccine development studies.

We have focused on the discovery of vaccine candidate antigens for vaccine formulations against toxoplasmosis using a high throughput protein microarray screening approach. Here, protein microarrays are probed with well characterized serum samples from humans and animal models. To date, microarrays containing > 2,870 candidate exon products of T. gondii have been probed with sera collected from patients from Turkey with acute and chronic toxoplasmosis. In the present study, we wished to evaluate the mouse as a surrogate for the human immune response, and as a potential model for human vaccine development. The aim, therefore, was to quantify the overlap in the mouse profile and human antibody profiles published previously. For this, a reactive antigen list of 240 antigens recognized by murine IgG and IgM was identified using sera from mice which were administered orally with viable tissue cyst and oocysts. Analyses of screening data prioritized 56 proteins. Among them GRA5, GRA6, GRA7, GRA8, ROP1, and MAG1 have shown strong immunogenicity in both mouse and human antibody profiles. We propose these six antigens represent priority candidates for preclinical development of a vaccine against toxoplasmosis in mice.
**P1** Development of a Process for Production and Purification of Virus-Like Particle (VLP) Vaccines

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Virus-like particle (VLP) vaccine candidates have received increased attention following their successful use for vaccines against human papilloma virus and hepatitis B virus. VLPs are a versatile technology for creation of new vaccines, but their large scale production requires high titer production, recovery, and purity; this drives the need for continual process improvement to meet market demand. In this study, a platform for production and purification of a hepatitis C VLP-based vaccine candidate was evaluated in collaboration with Instituto de Biologia Experimental e Tecnológica (IBET), Portugal. The VLP vaccine candidate was produced in an insect cell expression system using disposable bioreactor technology, and cell culture attributes were compared with those from a glass stirred tank bioreactor culture. Cell culture harvests from both systems were subsequently purified to assess the impact of upstream production on final product purity and quality. The downstream train was improved through the selection of an appropriate anion exchange resin to reach 70% recovery and effective baculovirus reduction. In addition, appropriate depth filtration and ultrafiltration technologies were assessed and selected. Altogether, this case study lays the foundation for a fully GMP-compliant, scalable process that can be implemented for clinical and commercial production of VLP vaccine candidates.

**P2** Formulation of bivalent conjugate vaccine against Salmonella typhi and Salmonella paratyphi A with or without adjuvant

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A Bivalent subunit vaccine protecting against Paratyphoid as well as Typhoid fever will provide a solution to the increasing number of Typhoid fever cases in developing countries. A novel method for handling the Vi precipitate using 0.2µm sterilizing grade filters to trap and wash the Vi and then, re-solubilisation, allow the Vi to pass through the filter was developed. The final yield of purified Vi was approximately 250µg/mL of culture broth and the bulk concentrates paired with the specifications defined in the WHO recommendations for Vi polysaccharide vaccine. Vi is then conjugated to Diphtheria Toxoid (DT) and is highly immunogenic.

The O-specific polysaccharide (OSP) derived from the lipopolysaccharide (LPS) is a protective antigen of Salmonella enterica serovar Paratyphi A. Highly yielding and cost-effective solubilization and diafiltration methods were used in the purification of OSP without use of any hazardous chemicals. The yield of purified OSP was greater than 800 mg/L of culture broth and meets the WHO acceptance criteria for polysaccharide vaccines. OSP is conjugated to different carrier protein like Diphtheria Toxoid (DT), Pneumococcal Surface Protein Antigen (PspA) and Tetanus Toxoid (TT). OSP-TT conjugate is giving best immune response in comparison to other conjugates.

Monovalent conjugates of Vi-DT and OSP-TT are highly immunogenic but formulation of these conjugates in a Bivalent conjugate with both components was showing inhibition in immune responses against both antigens. To overcome the inhibition two different adjuvants like Aluminium hydroxide (ALUM) and Double Mutant Heat-Labile Toxin (dmLT) were used in different concentrations. Result suggests dmLT have better adjuvant effect and is able to strongly boost the immune response of both the Antigens. The adjuvant effect of dmLT was further established by cytokine studies. Vi-DT and OSP-TT are the two major antigens in the bivalent vaccine formulation and a reduction in the production cost by providing a high yielding method could potentially make is an affordable vaccine candidate for the developing countries.

**P3** NLRC4-driven immunogenicity of a novel rMVA vaccine encoding flagell and the induction of mucosal immune responses.

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Modified vaccinia virus Ankara (MVA) is a highly attenuated poxvirus, used as a safe viral vector for the generation of vaccines against infectious diseases and cancer. We wanted to determine if we could improve MVA immunogenicity in the context of mucosal vaccination. We therefore developed a genetically-encoded recombinant (r) MVA using the mucosal adjuvant flagellin (Flg) and the model antigen ovalbumin (OVA). We administered the vaccine vector intranasally (i.n.) and determined the immunogenicity of rMVA-Flg at systemic and mucosal sites, with particular interest in the respiratory and the gastro-intestinal (GI) tract and the role of the NLRC4 inflammasome.

Following i.n. immunization with rMVA-Flg, enhanced CTL responses were observed in both spleen and lungs compared to rMVA alone, with enhanced frequency of cytokine-producing CTL (IFN-g, TNF-a) in spleen and lungs of i.n. rMVA-Flg-immunized mice. Humoral responses from i.n. rMVA-Flg- immunized mice were also increased with higher ag-specific IgG2c in the serum. The hallmark of mucosal immunity is production of local IgA at mucosal sites. In the BAL of i.n. rMVA-Flg-immunized mice, higher IgA titers were observed, together with increased IgG and IgG2c titers. Moreover, IL-1b could be detected in the BAL of wt mice whilst a complete loss was observed in Nlrc4-/- mice. In addition BAL IgA responses were also abrogated in i.n. rMVA-Flg-immunized Nlrc4-/- mice. These findings support a role of NLRC4 in rMVA-Flg-induced immune responses.

Furthermore, we report migration of OVA-specific CD8+ T cells to the mLN of i.n. rMVA-immunized mice, with an increased migration pattern and gut-homing receptor expression (α4β7, CCR9) when using rMVA-Flg, suggesting the induction of a GI immune response. This was consistent with the presence of IgA in the intestinal lavages of those mice. Similarly, GI immune responses were abrogated in Nlrc4-/- mice, and a loss of intestinal ag-specific IgA was demonstrated. Altogether these data indicate an essential role of the NLRC4-inflammasome in rMVA-Flg immunogenicity.
**P4 Broadened Immunity and Protective Responses with an Emulsion-Adjuvanted H5 COBRA-VLP Vaccine**

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A number of challenges for developing a protective pre-pandemic influenza A vaccine still exist including predicting the target influenza strain and designing the vaccine for an immunologically naïve population. Manufacturing and supply of the vaccine would also require implementing ways to increase coverage for the largest number of people through dose-sparing methods, while not compromising the potency of the vaccine. Previously, our group described a novel hemagglutinin (HA) for HSN1 influenza derived from a methodology termed computationally optimized broadly reactive antigen (COBRA). This is the first report describing a strategy combining a COBRA-based HA vaccine with an oil-in-water emulsion, resulting in a dose-sparing, immunologically broadened, and protective response against multiple HSN1 isolates. Here we show that different emulsion-based adjuvants enhance the magnitude and breadth of antibody responses with both a wild-type H5 HA and an H5 COBRA VLP vaccines. The COBRA HA VLP, combined with an emulsion adjuvant, elicited HAI specific antibodies against a larger panel of HSN1 viruses. Furthermore, 10-fold less antigen was required to induce significant HAI titers with adjuvanted vaccine compared to non-adjuvanted vaccine alone.

**P5 Two potential recombinant rabies vaccines expressing canine parvovirus virion protein 2 induce immunogenicity to canine parvovirus and rabies virus**

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Both rabies virus (RABV) and canine parvovirus (CPV) cause lethal diseases in dogs. In this study, both high egg passage Flury (H5P-Flury) strains of RABV and recombinant RABV carrying RABV glycoprotein (G) gene were used to express the CPV virion protein 2 (VP2) gene, and were designated rHEP-VP2 and rHEP-dG-VP2 respectively. The two recombinant RABVs maintained optimal virus titration according to their viral growth kinetics assay compared with the parental strain HEP-Flury. Western blotting indicated that G protein and VP2 were expressed in vitro. The expression of VP2 in Crandell feline kidney cells post-infection by HEP-VP2 and HEP-dG-VP2 was confirmed by indirect immunofluorescence assay with antibody against VP2. Immunogenicity of recombinant rabies virus was tested in Kunming mice. Both HEP-VP2 and HEP-dG-VP2 induced high level of rabies antibody compared with HEP-Flury. Mice immunized with HEP-VP2 and HEP-dG-VP2 both had a high level of antibodies against VP2, which can protect against CPV infection. A challenge experiment indicated that more than 80% mice immunized with recombinant RABVs survived after infection of challenge virus standard 24. Together, this study showed that recombinant RABVs expressing VP2 induced protective immune responses to RABY and CPV. Therefore, rHEP-VP2 and rHEP-dG-VP2 might be a potential combined vaccine for RABY and CPV.

**P6 Chimeric Virus-Like Particle Vaccines Displaying Conserved Enterovirus 71 Epitopes Elicit Protective Neutralizing Antibodies in Mice through Divergent Mechanisms**

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Enterovirus 71 (EV71) is a major causative agent of hand, foot and mouth disease, which frequently occurs in young children. Since there are 11 subgenotypes (A, B1 to B5, and C1 to C5) within EV71, an EV71 vaccine capable of protecting against all of these subgenotypes is desirable. We here report the vaccine potential and protective mechanism of two chimeric virus-like particles (VLPs) presenting conserved neutralizing epitopes of EV71. We show that fusions of hepatitis B core antigen (HBc) with the SP55 or SP70 epitope of EV71, designated HBcSP55 and HBcSP70, respectively, can be rapidly generated and self-assembled into VLPs with the epitopes displayed on the surface. Immunization with the chimeric VLPs induced carrier- and epitope-specific antibody responses in mice. Anti-HBcSP55 and anti-HBcSP70 sera, respectively, were able to neutralize in vitro multiple genotypes and strains of EV71. Importantly, passive immunization with anti-HBcSP55 or anti-HBcSP70 sera protected neonatal mice against lethal EV71 infections. Interestingly, anti-HBcSP70 sera could inhibit EV71 attachment to susceptible cells, whereas anti-HBcSP55 sera could not. However, both antisera were able to neutralize EV71 infection in vitro at the post attachment stage. The divergent mechanism of neutralization and protection conferred by anti-SP70 and anti-SP55 sera is in part attributed to their respective ability to bind authentic viral particles. Collectively, our study not only demonstrates that chimeric VLPs displaying the SP55 and SP70 epitopes are promising candidates for a broad-spectrum EV71 vaccine but also reveals distinct mechanisms of neutralization by the SP55- and SP70-targeted antibodies.

**P7 Structural basis for recognition of human enterovirus 71 by a bivalent broadly neutralizing monoclonal antibody**

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Enterovirus 71 (EV71) is the main pathogen responsible for hand, foot and mouth disease with severe neurological complications and even death in young children. We have recently identified a highly potent anti-EV71 neutralizing monoclonal antibody, termed D5. Here we investigated the structural basis for recognition of EV71 by the antibody D5. Four three-dimensional structures of EV71 particles in complex with IgG or Fab of D5 were reconstructed by cryo-electron microscopy (cryo-EM) single particle analysis all at subnanometer resolutions. The most critical EV71 mature virus-Fab structure was resolved to a resolution of 4.8 Å, which is rare in cryo-EM studies of virus-antibody complex so far. The structures reveal a bivalent binding pattern of D5 antibody across the icosahedral 2-fold axis on mature virion, suggesting that D5 binding may rigidify virions to prevent their conformational changes required for subsequent RNA release. Moreover, we also identified that the complementary determining region 3 (CDR3) of D5 heavy chain directly interacts with the extremely conserved VP1 GH-loop of EV71, which was validated by biochemical and virological assays. We further showed that D5 is indeed able to neutralize a variety of EV71 genotypes and strains. Moreover, D5 could potentially confer protection in a mouse model of EV71 infection. Since the conserved VP1 GH-loop is involved in EV71 binding with its uncoating receptor, the scavenger receptor class B, member 2 (SCARB2), the broadly neutralizing ability of D5 might attribute to its inhibition of EV71 from binding SCARB2. Altogether, our results elucidate the structural basis for the binding and neutralization of EV71 by the broadly neutralizing antibody D5, thereby enhancing our understanding of antibody based protection against EV71 infection.
P8 Genetic polymorphism of varicella-zoster virus in in vitro cell passaging and attenuation*
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Varicella-Zoster Virus (VZV) is a member of herpesviruses, with a genome size about 125 kbp. Primary infection of VZV results in varicella and reactivation from latency often leads to zoster. Both varicella and zoster can be prevented by live attenuated vaccines, but the molecular mechanism of attenuation is not clearly understood. In this study, genetic polymorphism of VZV vaccine and clinical strains were investigated in order to get an insight into the molecular mechanism of attenuation. High throughput sequencing data of vaccine strains and clinical strains with high and low in vitro cell passaging histories were analyzed. Genetically polymorphic site (GPS) was defined as the genome site where the proportion of minor base exceeds 2.5%. The number of GPS was higher in vaccine strains than clinical strains, and increased in high passaged strains. The GPS was not distributed evenly among the genome, and appeared to be clustered in ORF11, ORF14, ORF22, and ORF62/71. The most prevalent pairs of major and minor base were A/G, C/T, G/A, and T/C, where major bases shown as capital and minor bases were shown as small letters. Finally, a read-through mutation was detected in 15~20% vaccine strains due to A -> C or T -> G substitution resulting in a change of a stop codon to Gly. Further studies of genetic polymorphism in vaccine and high-passage clinical strains will help to elucidate the molecular mechanisms of VZV attenuation.

P9 Optimization of Production for Vesicular Stomatitis Virus (VSV) in suspension serum-free culture medium at high cell density
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During the last decade, oncolytic viruses such as vesicular stomatitis virus (VSV) has gained tremendous popularity as an efficient cancer vaccines. One major obstacle for using this type of virus for cancer therapy is the relatively high cost of manufacturing and the difficulty to obtain good viral titer using culture devoid of animal derived components. Although large-scale production of VSV is mostly performed using adherent cells, in some instances production has also been reported in suspension of Vero cells in the presence of fetal calf serum. Recently we have developed two stable cell lines, 293SF-36F (derived from HEK293 cells) and SF-BMAdR cells (a variant of A549 that expresses the E1 region of human adenovirus). These two cell lines were adapted to grow efficiently in suspension culture and serum-free medium. In this report we evaluated the production of a recombinant VSV expressing the green fluorescent protein (VSV-GFP) in these two stable cell lines. Virus titers, expressed as infectious particle (IP) per ml, were measured by TCID₅₀ or by plaque assay on 293A cells. At a relatively low cell density of 500,000 cells per ml, 293SF-36F produced 4.7X more infectious particles than SF-BMAdR cells. More than 50% of produced viruses were extra cellular and could be recovered from the supernatant of infected cells. By concentrating the cells before infection through low speed centrifugation, a titer of 3-4x10¹² IP/ml using both cell lines was obtained. There was a linear correlation between virus titer and cell density up to 2X10⁶ cells per ml. However at concentration of 3X10⁶ cells per ml virus titer was reduced 2.7 times compared with 2X10⁶ cells per ml. We also developed a fed-batch process using an in-house medium and Feed in which 293SF-36F cells grew up to 10⁷ cells per ml. Shifting the temperature from 37°C to 34°C at infection time improved VSV titer up to 5 times. Under optimized conditions (cell density, temperature and multiplicity of infection), 293SF-36F cells produced VSV-GFP at titer of 5-10X10¹² extracellular IP/ml. In conclusion, our data demonstrated that 293SF-36F cells, for which a cGMP master cell bank is available, is an performant cell line for the scale up of VSV production for clinical application.

P10 Construction and Functional evaluation of a shuttle Plasmid Vector for Lactococcus lactis delivery genes into mammalian cells: in vitro and in vivo assays.

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Lactococcus lactis (L. lactis) is broadly developing as a safe alternative for delivery, directly inside of eukaryotic cells, a eukaryotic DNA expression vector for DNA vaccine or therapeutical vehicle for different molecules. This work describes a new plasmid called pEXU (Extra Chromosomal Unit) for DNA delivery to be express in Eukaryotic cells, and its functionality was evaluated either in vitro and in vivo assays, pEXU vector has remarkable features: the prokaryotic region offers the capacity to replicate either in Escherichia coli (E. coli) and in Acid Lactic Bacteria (LAB) by theta origin replication, providing a good structurally and segregationally stability. The eukaryotic region, allows the cloning of different antigens of interest under the control of the pCMV eukaryotic promoter to be expressed by a host cell.

The eGFP ORF, of reporter gene, was cloned into pEXU (pEXU:eGFP) and its functionality was evaluated by transfection into mammalian cells (Chinese hamster ovary Cells) by fluorescence microscopy and flow cytometry. The pEXU:eGFP plasmid was stabilized by electrotromination in L. lactis MG1363 strains. This strain (L. lactis MG1363 (pEXU:eGFP)) had been administrated by gavage into gastrointestinal tract of BalbC mice and the expression of eGFP was analyzed by confocal microscopy. The pEXU vector had been shown an excellent stability either in E. coli, as well as in L. lactis, the eGFP protein had been expressed successfully either, in vitro and in vivo analyze, being a good candidate to delivering efficiently, several kind of genes into eukaryotic cells. In vitro and in vivo data presented in this report support the hypothesis that pEXU vector carrying by native L. lactis is an efficient system of delivering DNA into mammalian cells.

P11 Applied Modeling of Immune Senescence in Rodents and Non-Human Primates

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Age confers various changes to humoral and cell mediated immune responses to both infections as well as active immunization. Decline in normal immune function, collectively known as immunosenescence, is also accompanied with the loss of efficacy for both preclinical as well as marketed vaccines. System biological analyses are beginning to be applied to vaccines administered to both young and aged populations. Recent advances in whole transcriptome sequencing has enabled an interdisciplinary approach that can be utilized to examine complex molecular interactions and to probe immune biomarkers specific for driving immunity, reactogenicity and mechanisms by which the innate and adaptive immune system responds to vaccines. By assessing age differences in animal species widely used in vaccine discovery and distinguishing their unique immunosignatures, novel ways to understand the responsiveness to specific vaccines as well as strategies to compensate for loss of effectiveness can be employed. Additionally, a systems biology approach can be applied in early clinical development to derisk undesirable events.

Here we describe our initial steps to explore developing translatable animal models that mimic human signatures known to correlate with ineffective responses to vaccine administration and then propose mitigative approaches.

(1) We first demonstrate bioage differences in rodents and monkeys. The results will allow us to design studies to experimentally recapitulate what was described clinically, in order to establish robust translational animal models that can be utilized to address strategies to overcome lack of vaccine efficacy due to increased age

(2) Additionally, we describe early attempts showing the profound impact that age has on our preclinical models and the path we will take in order to build gene modules in order to identify promising biomarker sets that can that can ultimately be applied in the clinical setting.
**P12** Vaccination with a human parainfluenza virus type 3 chimeric FHN glycoprotein formulated with a novel adjuvant induces protective immunity.

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Human parainfluenza virus type 3 (hPIV-3) is a major cause of lower respiratory disease i.e. bronchiolitis, bronchiolitis or pneumonia, in infants and young children. Among all hPIVs, hPIV-3 is most virulent and associated with significant morbidity and mortality. Presently there is no safe, effective and licensed vaccine against hPIV-3. This virus encodes two major glycoproteins, F (fusion) and HN (haemagglutinin-neuraminidase), which represent major vaccine candidates. To produce an effective subunit vaccine, a chimeric F-HN glycoprotein consisting of the N-terminal ectodomain of the F protein linked to the HN protein without transmembrane domain, as well as secreted forms of the individual F and HN glycoproteins, were expressed in mammalian cells and purified. Cotton rats were immunized intramuscularly with F-HN or mixtures of F and HN proteins (F+HN) formulated with poly I:C and an innate defense regulator peptide in polyphosphazene (TriAdj). Significantly higher levels of systemic virus-neutralizing antibodies were observed in cotton rats immunized with F-HN/TriAdj compared to animals immunized with the combination of F and HN proteins (F+HN/TriAdj). After hPIV-3 challenge, essentially no virus was detected in cotton rats immunized with F+HN/TriAdj. In conclusion, formulation of chimeric F-HN protein with a novel adjuvant has potential for development of a safe and effective vaccine against hPIV-3.

**P13** Peptide Nanoparticle-based Vaccine Induces Immune Responses Against the Infection of High Pathogenicity Avian Influenza Virus in Chickens

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Subunit vaccines are generally less immunogenic than whole organism vaccines. Approach to address this issue is to display repetitive antigen in a potent adjuvant formulation. Self-Assembling Protein Nanoparticle (SAPN) technology is an excellent platform to achieve this goal for developing a universal Avian Influenza Virus (AIV) vaccine. Two conserved antigens (M2e and Helix C) are repetitively displaying on our SAPN surface. To generate self-adjuvanted SAPN, flagellin peptide was built into each SAPN. In this study, self-adjuvanted M2e/HeIcF-SAPNs can stimulate TLR5 in vitro in a dose dependent manner. Specific Pathogen-Free Chickens vaccinated with M2e/HeIcF-SAPN induce significantly higher titers of antibodies than unvaccinated one. Antibodies from chickens vaccinated with the self-adjuvanted vaccine are significantly more neutralizing toward H5N2 in vitro than those from animals vaccinated by the unvaccinated one. Importantly, anti-sera induced by the self-adjuvanted vaccine also neutralize heterozygous subtypes of AIVs in vitro. SPF chickens vaccinated with M2e/HeIcF-SAPNs were protected from challenge with H5N2 high pathogenicity avian influenza virus. We have shown 63% and 35% survival rates in chickens vaccinated intramuscularly and orally, respectively when compared to the unvaccinated control group at 2 weeks post challenge. Our data together indicates our M2e/HeIcF-SAPNs is a potent vaccine for AIV.

**P14** Access and Barriers to Adult Pneumococcal Vaccine in Argentina, Brazil, and Mexico

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**Background:** Pneumococcal vaccination of older adults, 60 years of age and older, is recommended in Argentina and Mexico, but not recommended in Brazil. Additionally only Argentina finances the vaccine for older adults. The vaccination rates among adults remain low despite the increasing burden of pneumococcal disease in the adult population. This trend indicates the need for renewed vaccination education and programming and highlights the importance of identifying barriers and successes surrounding current adult vaccination implementation for pneumococcal disease.

**Methods:** We aimed to identify influencing factors to uptake and access to adult pneumococcal vaccination in 3 Latin American Countries. A review of literature from 2005 to 2015 was conducted on barriers and facilitators of adult vaccination against pneumococcal disease. We searched Scopus, PubMed, and EconLit using terms focused on pneumococcal disease, barriers to vaccination, and vaccine access and distribution. Countries were selected from the Latin America region (LATAM) based on previous public health successes, including Argentina, Brazil, and Mexico. Grey literature, including WHO position papers on pneumococcal disease, further supplemented the available data.

**Results:** Policies on reimbursement for pneumococcal vaccinations in adults are varied for adults in LATAM and current WHO recommendations lack evidence to support prioritizing vaccination among adults over the age of 50. Affordability, education among society and healthcare workers (HCW), and lack of knowledge of disease burden contributed to lower vaccine uptake among adults across the region. However, patient education and distribution programs have contributed to success. A vaccination program targeted at adults in Brazil, including media and healthcare involvement, led to a decrease in pneumonia and influenza-related mortality by 26.3% in São Paulo over 5 years. Similarly, public awareness campaigns and financial incentives in Mexico and Argentina have increased vaccination coverage among adults.

**P14** (Continued)

**Conclusion:** The barriers of cost, vaccine knowledge among society and HCW, and lack of knowledge of disease burden highlight the shortcomings identified in current research. Increased awareness and financial support for adult vaccines may further diminish adult mortality across the three countries. With the proportion of adults over the age of 60 growing worldwide, public health professionals need to take a proactive approach to decrease vaccine preventable disease burden in this population. Current research suggests that focused funding and education efforts can reduce disease burden. In order to reduce the burden of pneumococcal disease in the region, further investigation and programs are needed.
P15 Needle free intradermal delivery of a polyvalent influenza DNA vaccine induces cross-reactive humoral and cellular immune responses in pigs

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disc: Needle-free vaccination of growing pigs with the optimized DNA vaccine resulted in specific, dose-dependent immune response. Both the antibody-mediated and the recall lymphocyte immune responses demonstrated high reactivity against vaccine-specific strains and cross-reactivity to vaccine-heterologous strains.

Conclusion: The results suggest that polyvalent DNA influenza vaccination can provide a strong tool for broad protection against swine influenza strains threatening animal as well as public health. In addition, the needle-free administration technique used for this DNA vaccine will provide an easy and practical approach for the large-scale vaccination of swine.

P16 Production of a Nanoplasmid™ with a Large Gene insert using the HyperGRO™ Fermentation Process

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Plasmid based DNA vaccines are emerging as a promising alternative to traditional vaccines due to several advantages, including faster production of DNA plasmids using E. coli. However, further increases in transgene expression are needed to meet efficacy requirements for various non-viral gene therapy and DNA vaccination applications. While existing minicircle DNA technology has been shown to offer improved levels and durations of transgene expression by removal of the bacterial region from the plasmid, low manufacturing yields may be a barrier to widespread use of minicircle DNA for vaccination.

Nature Technology Corporation’s (NTC’s) minimalized Nanoplasmid™ vectors utilize RNA-OUT (Rou1) antibiotic-free selection and replace the large 1000 bp pUC replication origin with a novel, 300 bp, R6K-derived mini-origin. Reduction of the spacer region linking the 5’ and 3’ ends of the transgene expression cassette to <500 bp remarkably increases plasmid-mediated transgene expression. Host strains expressing heat-inducible, high copy R6K replication (Rep) proteins have been developed for selection and propagation of Nanoplasmid. This is an additional Nanoplasmid safety factor since mini-origin vectors can only replicate within the engineered Rep protein-expressing E. coli host strain.

With years of expertise in plasmid production, NTC and VGXI have successfully implemented NTC’s HyperGRO™ fed-batch fermentation process for traditional plasmid and Nanoplasmid production at yields >1 g/L. However, production of plasmids containing large antigen-coding inserts may have various challenges. Bacterial cell machinery may not be able to produce high cell growth during fermentation due to a large gene insert in the plasmid. A modified HyperGRO process was developed by NTC to overcome this cell growth inhibition. Plasmid rNano1, a 6689 bp Nanoplasmid with a gene insert of 5018 bp, was successfully produced by VGXI using NTC’s modified HyperGRO process, with high end cell density of OD600 90.1 and volumetric yield of 0.698 g/L.

Further host strain engineering to repress plasmid copy number during biomass growth resulted in rNano1 volumetric yield of 2.4 g/L after 42°C induction of high copy Nanoplasmid amplification, which is near the highest published fermentation yield for any plasmid.

P17 Feasibility of varicella-zoster virus MAV06 strain as an antigen for fluorescent antibody to membrane antigen test

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Backgrounds: Fluorescent antibody to membrane antigen (FAMA) test is gold standard method to represent protective immunity of varicella vaccine. However, FAMA titer could be influenced by several conditions such as antigen type (VZV strains), viral titer, and reading conditions (reference antibody, magnification of microscope, intensity of fluorescent light), etc. In this study, to assess the feasibility of using MAV06 vaccine strain as a FAMA antigen, we examined whether FAMA antibody titers are affected by different types of antigen strains

Methods: Three VZV strains, namely MAV06 (vaccine strain for SuduVax, Green Cross Corp., South Korea), vOka (vaccine strain for Varilrix, GSK.), and Ellen (wild type) were used for FAMA test antigens. WHO international standard VZV IgG immunoglobulin (NIBSC, W1044) was used as a reference antibody. Forty-five children (mean age: 12 month) were enrolled in this study and vaccinated with SuduVax (Green Cross Company, South Korea). Antibody titers of 45 pre- and 5 weeks post-vaccination paired sera were measured by FAMA tests with three different strains.

Results: Geometric mean antibody titers (GMTs) of 45 pre-vaccination sera were 21.56, 21.78, and 21.09 in the FAMA tests with MAV06, vOka, and Ellen strains, respectively. Therefore, GMTs of pre-vaccination sera were measured by three different viral strains were all FAMA negative level. Geometric mean antibody titers (GMTs) of 45 post-vaccination sera were 21.38, 21.15, and 21.24 in the FAMA tests with MAV06, vOka, and Ellen strains, respectively. GMTs of post-vaccination sera measured by the three different viral strains were not significantly different (p>0.05).

Conclusion: To evaluate vaccine immunogenicity, vaccine companies generally use their own vaccine antigens in their assay systems. However, protective immunity against wild type virus infection should be considered for vaccine efficacy. FAMA titers measured using the MAV06 strain were similar to those measured using the vOka vaccine strain or a wild type strain. Therefore, both MAV06 and vOka strains are acceptable as FAMA antigens for the evaluation of varicella immunity.
**P18** Lymphocytic choriomeningitis virus vectors expressing gB and pp65 - a new bivalent vaccine that protects against congenital cytomegalovirus infection

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A vaccine against congenital cytomegalovirus (CMV) infection is an unmet medical need. Subunit glycoprotein B (gB) and pp65 vaccines have been evaluated in the clinic, but it is unclear whether additive protection can be conferred by simultaneous vaccination with both antigens. Hookipa Biotech AG is developing a new bivalent vaccine based on non-replicating lymphocytic choriomeningitis virus (rLCMV) vectors expressing gB(dCt), a cytoplasmic tail-deleted gB, and pp65 from human CMV. Sera from mice immunized with rLCMV-gB(dCt) were significantly more potently neutralizing than sera from mice vaccinated with gB protein with an emulsion adjuvant. Immunization of mice with rLCMV-pp65 elicited significant T cell responses. Combination of the gB(dCt) and pp65 vectors in a bivalent formulation showed immunogenicity to each component equivalent to the monovalent formulations in mice. Due to the species specificity of CMV, the congenital guinea pig cytomegalovirus (GPCMV) model was used for proof-of-principle. rLCMV vectors encoding guinea pig homologues of pp65 and gB(dCt) elicited significant immune responses and no loss of immunogenicity was noted with a bivalent formulation. Vaccination with the bivalent rLCMV-vectored GPCMV gB(dCt) and pp65 conferred 91% protective efficacy against pup mortality, and provided better protection against pup mortality, maternal viremia and congenital transmission than vaccination with the individual vectors or recombinant gB with Freund’s adjuvant. In sum, rLCMV vectors elicited strong immune responses against gB and pp65, and vectors expressing the GPCMV homologues of these antigens conferred protection in a congenital infection model. Clinical trial material was produced according to cGMP using a scalable process and preclinical safety studies confirmed the inherent safety of these non-replicating vectors. A double-blind dose-escalating first-in-human trial will start in June 2016. An update on the clinical trial will be provided at the conference.

**P19** Peptide Nanoparticle-based Vaccine for Infectious Bronchitis Virus

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Infectious bronchitis virus (IBV) causes respiratory disease in poultry as well as affecting avian renal and reproductive systems. Controlling of IBV is mainly based on vaccination program. Current available live attenuated or killed vaccines have been challenged by their effectiveness due to large numbers of IBV variants and lack of cross-protection. In our study, to address this issue, we designed novel IBV vaccines by using a highly innova-tive platform called self-assembled peptide nanoparticle (SAPN). One of the major genome encodes of IBV is a spike (S) protein. The S protein is post-translationally cleaved into the outer S1 and the membrane bound S2 proteins. To generate a potent vaccine the best conserved and protective B and T cell epitopes should be combined into a highly immunogenic epitope delivery and presenting system. S1 protein comprises major antigenic determinants that induce neutralizing antibodies which make it a major target of vaccine design. The S protein of IBV contains two coiled-coil sequences. We engineered this coiled-coil sequence onto the trimeric coiled-coil of several versions of our current SAPNs. We repetitively present epitopes of spike protein of IBV in a conformational specific manner. Importantly, we also co-present flagellin on our SAPN to make it self-adjuvant. Immunogenicity study is assessed with assays including ELISA, virus neutralization, and lymphocyte proliferation. Vaccine efficacy will be evaluated by challenging with different virulent strains of IBV in future.

**P20** Adipose tissue as a novel target for electroporation-mediated delivery of DNA vaccines

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To date, most in vivo electroporation (EP)-enabled DNA vaccinations have been designed to target skeletal muscle or skin, while the subcutaneous adipose layer has generally been viewed as an electrical barrier dividing the two. Here, we present evidence that adipose tissue can be successfully targeted for EP DNA vaccination, and may offer unique advantages from a DNA delivery perspective. First, finite element modeling software was used to predict electric field distribution within subcutaneous adipose tissue clamped between parallel plate electrodes. Next, in vivo treatments were performed into subcutaneous fat pads of Hardy guinea pigs, and the treatment protocol was optimized based on expres-sion of the reporter construct, green fluorescent protein. Dye injection studies demonstrated that the injectate preferentially travels down collagenous septa surrounding adipose lobes, and these observations were consistent with GFP transfection patterns. To demonstrate secretion of encoded proteins, adipose-targeted EP treatment was performed using DNA encoding monoclonal antibodies (dMAbs), which led to detectable systemic levels of protein. Finally, adipose-targeted EP DNA vaccination of plasmid encoding H1N1 nucleoprotein was shown to be immunogenic. Compared to traditional intramuscular routes, adipose-targeted EP DNA vaccinations may offer tolerability advantages due to the lower voltages, shallower injections, and noninvasive electrodes used.

**P21** Heterologous Boost with Synthetic Nanofiber Vaccines Augments BCG-primed Cellular Immunity against Mycobacterium tuberculosis

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Pulmonary tuberculosis (TB) caused by Mycobacterium tuberculosis (MtB) is a global health concern with nearly 8 million infections and 1.5 million deaths each year. Cell mediated immunity is necessary for host defense against TB and multifunctional CD8+T cells (IFN-g+TNF-a+IL-2)+ have been associated with lower reactivation risk and enhanced control of active infection. Bacillus Calmette- Guerin (BCG) is the only vaccine against TB and has limited protection efficacy, which wanes post ado-lescence. Since boosting with BCG is contraindicated, heterologous booster vaccines that augment T cell immunity in the lungs of BCG-vaccinated individuals are an attractive alternative.

We developed a vaccination strategy based on self-assembling peptide nanofibers presenting MtB-specific CD8+ or CD4+ T cell epitopes that, when injected into mice, induce high frequency and antigen-specific effector memory T cells producing IFN-g and IL-2. Co-assembled nanofibers bearing CD8+ T cell epitopes and a toll-like receptor (TLR) 2 agonist elicited an 8-fold expansion in multifunctional CD8+ T cell populations producing IFN-g+TNF-a+IL-2. Intranasal immunization with peptide nano-fibers was safe and well tolerated in mice leading to increased antigen-specific CD8+ T cell population in the lungs. Aerosol challenge with MtB in BCG-primed and nanofiber-boosted mice provided an additional 0.5-log CFU reduction in lung bacterial load and also prevented bacterial dissemination to the liver suggesting enhanced protection compared to BCG alone. Together, these data suggest that heterologous prime-boost with BCG and peptide nanofiber-based MtB vaccines induces cell mediated immunity in the lung, reduces bacterial burden and dissemination, and potentially provide a safer alternative for boosting BCG-primed immunity.
Porcine circovirus type 2 (PCV2) is the causative agent of postweaning multisystemic wasting syndrome characterized by progressive weight loss, respiratory symptoms, jaundice, and has a significant economic impact on pig industry. Vaccines against PCV2 have been studied intensely and found to be effective in decreasing mortality and improving growth in swine populations. This study aims to seek for a novel effective way to elevate the immune response of PCV2 vaccination. The eukaryotic expression vector expressing pig interleukin-23 (IL-23) was constructed and entrapped with chitosan nanoparticles and then was intramuscularly co-immunized to 3-week-old piglets with PCV2 vaccine. Before and after vaccination on 0, 1, 2, 4, 8 and 12 weeks, their weights were measured and blood were collected respectively to test the changes of immune cells, specific antibody and the expression levels of immune genes by ELISA and quantitative RT-PCR. The results indicated that weight and leukocytes, red cells, platelet, hemoglobin in blood mounted significantly. The co-vaccinated group recorded higher specific antibody titer and more CD4+ and CD8+ T cells up to 12 weeks post vaccination compared to the control (P<0.05). The expression of IL-23 gene in vivo can enhance the immune response to PCV2 vaccine and has the potential to be developed into a safe and effective adjuvant to promote the immunity of pig against PCV2 infection.

**P22** The enhanced immune responses of pigs to PCV-2 vaccine by inoculation with chitosan nanoparticles of recombinant pig interleukin-23 gene

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Porcine circovirus type 2 (PCV2) is the causative agent of postweaning multisystemic wasting syndrome characterized by progressive weight loss, respiratory symptoms, jaundice, and has a significant economic impact on pig industry. Vaccines against PCV2 have been studied intensely and found to be effective in decreasing mortality and improving growth in swine populations. This study aims to seek for a novel effective way to elevate the immune response of PCV2 vaccination. The eukaryotic expression vector expressing pig interleukin-23 (IL-23) was constructed and entrapped with chitosan nanoparticles and then was intramuscularly co-immunized to 3-week-old piglets with PCV2 vaccine. Before and after vaccination on 0, 1, 2, 4, 8 and 12 weeks, their weights were measured and blood were collected respectively to test the changes of immune cells, specific antibody and the expression levels of immune genes by ELISA and quantitative RT-PCR. The results indicated that weight and leukocytes, red cells, platelet, hemoglobin in blood mounted significantly. The co-vaccinated group recorded higher specific antibody titer and more CD4+ and CD8+ T cells up to 12 weeks post vaccination compared to the control (P<0.05). The expression of IL-23 gene in vivo can enhance the immune response to PCV2 vaccine and has the potential to be developed into a safe and effective adjuvant to promote the immunity of pig against PCV2 infection.
Oil-in-water (OW) emulsions are potent adjuvants for induction of broad neutralizing antibody responses against the influenza hemagglutinin (HA) protein in mice.

Targeting the type 6 secretion system of *Yersinia pestis* for the development of novel live-attenuated vaccine candidates

*Y. pestis* is classified as a Tier-1 select agent by the CDC and a re-emerging human pathogen by the WHO as it remains a prevalent public health threat in many regions of the world. Unfortunately, there is no FDA-approved vaccine against plague that is available and treatment of *Y. pestis*-inflicted patients is limited to antibiotic therapy. However, isolation of multi-antibiotic resistant strains from patients with plague and engineering of antibiotic-resistant strains of *Y. pestis* by several countries to be used as a biological warfare agent, represent a trigger to evaluate novel prophylactic treatment options such as new generation vaccines. Since both humoral and cell-mediated immune responses seem critical in providing protection against *Y. pestis* infections, specifically pneumonic plague, live-attenuated vaccines, which generate both immune responses and are highly immunogenic, offer a substantial advantage over subunit vaccines, which primarily generate antibody-mediated responses and provide highly variable antibody titers in humans. In order to offer a substantial advantage over subunit vaccines, which primarily generate antibody-vaccine candidate strains, over 5,000 transposon signature-tagged mutants of *Y. pestis* CO92 were screened (T6SS), with no assigned function in plague pathogenesis. Generation of a set of a (component of the T6SS apparatus identified in signature-tagged mutagenesis screen) CO92 were screened for their ability to induce broadly neutralizing antibodies against antigenically distinct historical seasonal H1N1 strains, multiple different adjuvating formulations were tested: OW emulsions, alum-based adjuvants (with or without TLR agonists), saponins and poly(D,L-lactico-glycolic acid (PLGA) nanoparticles. A recombinant HA from the H1 influenza strain A/New Caledonia/20/1999 was co-administered with the various adjuvants twice, 4 weeks apart. Formulations were administered subcutaneously, intramuscularly, or intradermally, according to the recommended route for the adjuvant being tested. HA-specific binding antibodies were measured using a multiplex binding assay, and functional antibodies were measured through Hemagglutination Inhibition (HAI) assay. The strongest binding, highest autologous HA-specific HAI titers (1:1280, 1:640) and the maximal breadth of response (6 strains with HAI titer ≥ 40 from years 1986 to 2007) were detected in mice receiving HA vaccine formulated with squalene-based OIW emulsions (AF03 or Addavax). Quil-A, a semi-purified Quillaja extract containing saponins, also induced high HAI titers comparable to OIW emulsions, but resulted in reduced breadth as compared to OIW emulsions (5 strains with HAI titer ≥ 40 from years 1991 to 2007). The purified saponin, QS-21, was less potent than Quil-A. Alum-based adjuvants (ALG or AlPO4) were also less potent, reaching a HAI titer of 1:320. The addition of a TLR 7/8 agonist to AlPO4 or addition of a TLR9 agonist to AlOH did not impact the peak response, and did demonstrate strong HAI titers after the 1st immunization suggesting a more rapid induction of immunity. Vaccination with PLGA nanoparticles containing both TLR4 and TLR7/8 ligands produced HAI titers of 1:320 against the antigen, but resulted in an even narrower response, eliciting an HAI titer ≥ 40 against only 4 strains (1995-2007). Immunization with a single TLR agonist using a clinically tested methodology (intradermal immunization of HA with topical Imiquimod as adjuvant) produced lower binding and HAI titers than any other adjuvant tested. In conclusion, recombinant HA co-formulated with squalene-based OW emulsions have consistently been the most potent adjuvant for induction of HA-specific antibodies in our murine model. The Sanofi Pasteur team will continue to evaluate the potential of these and other adjuvants for use in a broadly protective influenza vaccine.

Development of a Functional Assay to Assess the Ability of Vaccines to Induce Cellular Cytotoxic Immune Responses

After vaccination, antigen-specific immunologic responses of cytotoxic T lymphocytes can be measured by the IFN-γ ELISpot assay or phenotyping of activated CD8+ T cells by flow cytometry. Although these assays can detect the presence of CD8+ T cells resembling CTLs, they are correlative. A functional assay directly confirms induction of cytolytic activity. To address this, a flow cytometry based functional killing assay has been developed to measure downstream caspase activity in target cells following delivery of a lethal hit by vaccine specific cytotoxic lymphocytes. The assay output, or the number of target cells with activated caspase activity, is determined by the quantity of antigen specific CD8+ T cells added to target cells [effector:target (E:T) ratios of 50:1, 10:1, 0:1]. We tested the killing assay with PBMCs defined to be responsive to influenza, CMV and EBV (CEF) as well as PBMCs isolated from patients in a clinical study immunized with our therapeutic HPV16/18 candidate vaccine, VGX-3100, delivered by in vivo electroporation. Effectors exhibited CTL activity as evidenced by the increased presence of caspase 3/7 in target cells loaded with CEF or HPV peptides. Target cells loaded with non-related OVA peptides did not induce CTL activity above background in effectors, demonstrating the antigen-specificity of the assay. The functional killing assay provides a way to evaluate cell-mediated cytolysis with high antigen specificity as an alternative to the radioactive chromium release assay. This assay is a good addition to the current immunologic monitoring assays and a multifactor analysis of effector cell response after vaccination may help evaluate the immunological potency of a vaccine.

Generation of T6SS cluster deletion mutants as well as other single effector deletion mutants have resulted in varying levels of attenuation in the pneumonic murine model of plague. Further studies will focus on the development of combinatorial deletion mutants and their evaluation in providing protection against subsequent pneumonic plague infection and for their ability to elicit humoral and cell-mediated immune responses. Accomplishment of these studies will allow us to have new vaccine candidates in the developmental pipeline for diverse human population groups.
P28 Mucosal immunization of a flagellin-adjuncted Hgp44 vaccine potentiates immune responses to prevent alveolar bone loss in a Porphyromonas gingivalis infection model

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Chronic periodontitis is an inflammatory disease caused by the interplay between polymicrobial biofilm of subgingival region and host factors. Porphyromonas gingivalis is one of major pathogens associated with periodontitis. The immunization approach to prevent periodontal disease using P. gingivalis-specific antigens is recently attracting substantial attention. Since the oral mucosa is the major site of bacterial colonization, a strong mucosal vaccine using a P. gingivalis-specific antigen should induce better protection against periodontal disease. We have previously reported that bacterial flagellin, which is an agonist of Toll-like receptor-5 (TLR5), serves an excellent mucosal adjuvant for various vaccines. In the present study, we employed 44-kDa gingipain adhesion/hemagglutinin (Hgp44) domain of Arg-gingipain A (RgpA) as a mucosal antigen for periodontitis vaccine and tested mucosal adjuvant activity of a flagellin by measuring protective immune responses in a P. gingivalis infection model. First we confirmed that P. gingivalis Hgp44 domain was well recognized by the host immune system and inactivated P. gingivalis-immunized mice raise robust Hgp44-specific antibody responses.

P28 (Continued)

We performed intranasal (IN) and sublingual (SL) immunizations to evaluate the potential of Hgp44 polypeptide antigen as a mucosal periodontitis vaccine. IN immunization induced significantly higher Hgp44-specific IgG titer in the serum than did SL administration, and co-administration of a flagellin (Vibrio vulnificus FlaB) potentiated the serum antibody responses in both IN and SL vaccinations. Interestingly, anti-Hgp44-specific IgA titer in saliva was comparable in IN and SL vaccinations. Co-administered flagellin significantly potentiated the secretory IgA responses in saliva. Furthermore, mice administered a mixture of Hgp44 and FlaB via IN and SL routes manifested significant reductions in alveolar bone loss induced by live P. gingivalis infections. Overall, mucosally co-administered flagellin potentiates the Hgp44-specific immune response in both systemic and mucosal compartments and prevents alveolar bone loss in a P. gingivalis infection model.

P29 Field efficacy study of a novel ready-to-use subunit vaccine against porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus

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Concurrent infection with porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) is known as one of the major causes for porcine respiratory disease complex (PRDC). Dual infection with PCV2 and PRRSV is consistently to have more severe clinical presentations and pulmonary lesions than infection with PCV2 alone or PRRSV alone. Epidemiological analyses have revealed that coinfection with PCV2 and PRRSV is most commonly observed in field cases. The objective of this study was to evaluate the safety and protective efficacy of a new combination subunit vaccine containing PCV2 and PRRSV antigens (PCCOM™ PCV2 and PRRS subunit vaccine) in laboratory studies and under field conditions. In safety studies, no vaccine related significant adverse reactions were recorded throughout the study. A transient increase of body temperature was recorded in studies 4 hours after vaccination, but the increase in body temperature lasted no longer than 24 hours. Gross necropsy and histopathological examination did not reveal any abnormalities at the injection site in vaccinated pigs. IFN-gamma release assay was used for evaluation of the cell-mediated immune response. The results show that the vaccinated pigs significantly increased the IFN-gamma levels. In challenge studies with the individual pathogens, the vaccinated pigs significantly not only reduced the PCV2 and PRRS viremia but also reduced the PCV2 load in lymphoid tissue and PRRSV-induced lung lesions. In a placebo-controlled field trial on a farm where both PCV2 and PRRS were present, vaccination of piglets at 3 and 4 weeks of age resulted in a reduction of PCV2 and PRRS viremia. In addition, a positive effect on the average daily weight gain in the finishing phase was observed. It can therefore be concluded that this new ready-to-use combination subunit vaccine is safe and efficacious against PCV2 and PRRSV single and combined infections.

Keywords: Porcine circovirus type 2; Porcine reproductive and respiratory syndrome virus; Combination subunit vaccine

P30 Sensitivity and specificity two commercial varicella-zoster virus glycoprotein ELISA kits for the evaluation of immunity induced by varicella vaccine

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Backgrounds: Fluorescent antibody to membrane antigen (FAMA) test has been considered as a reference method to measure protective antibody against varicella-zoster virus (VZV). Despite the high sensitivity and specificity of FAMA test, laborious and fastidious nature of the test limited availability of FAMA test only in a few laboratories. In this study, sensitivity and specificity of two commercial glycoprotein ELISA (gpELISA) kits were evaluated as alternative tests for handling large amount of vaccination samples.

Methods: Antibody titers of 45 children’s pre- and post-vaccination (SuduVax) paired sera were measured by FAMA test (with MAV06 strain as antigen) and gpELISA test. VaccZyme™ VZV glycoprotein IgG ELISA kit (Binding Site, UK) and Serion classic VZV IgG kit (SerionVirion, Germany) were used for gpELISA test.

Results: Sensitivity and specificity of VaccZyme™ kit were 34.1% and 100% (kappa: 0.361) when the kit guideline (susceptible to infection level <100 mIU/ml) was applied. However, they were 73.2% and 95.9 % (kappa: 0.703) when cut-off value was adjusted as 50 mIU/ml according to 2006 European Sero-Epidemiology Network 2 (ESEN2) guideline for the diagnosis of varicella using conventional ELISA. Sensitivity and specificity of Serion kit were 45% and 100 % (kappa: 0.485) when we adopted Serion kit guideline (negative <50 mIU/ml). Pearson correlation coefficient (r) of VaccZyme™ kit and FAMA tests was 0.807. The r of Serion classic VZV IgG kit and FAMA test was 0.777.

Conclusion: Both of commercial gpELISA kits showed high positive correlation with FAMA test. However, sensitivity were very low when the kit’s own cut-off values were applied. When 2006 ESEN2 guideline was applied to VaccZyme™ kit, sensitivity and strength of agreement were increased. If commercial VZV gpELISA kits would be applied to evaluate varicella vaccine immunity, long term follow-up clinical data of vaccinated children seemed to be necessary for the adjustment of gpELISA kit cut-off value.
**P31 Development of a flagellin-adjuvanted anti-tauopathy vaccine targeting paired helical filament conformation**

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Alzheimer disease (AD) and related disorders called tauopathies, are characterized by the pathological aggregation of tau proteins in the human brain leading to neurofibrillary degeneration and dementia. Tau is a highly soluble, natively unfolded microtubule (MT)-associated protein. The “repeat-domain” of Tau (TauRD) is responsible for the Tau–MT interaction, and also play pivotal role in the pathological aggregation of hyperphosphorylated tau proteins. Assembly of TauRD is much faster than that of full length tau into “paired helical filaments” (PHFs) in vitro conditions. Given that tau oligomers are known to be acutely neurotoxic, TauRD would serve as a plausible vaccine candidate for preventive and immunotherapeutic against tauopathies. In the present study, we expressed TauRD in E. coli, and formulated with a potent mucosal adjuvant FlaB, a flagellin from Vibrio vulnificus, for intranasal immunization. Recombinant TauRD polyepitope formed β-sheet aggregates as revealed by native-PAGE and thioflavin S staining. TauRD adjuvanted with FlaB induced Th2-dominant humoral immune responses with a significant induction of TauRD-specific neutralizing antibodies in serum and also induced strong IgA responses in serum and feces. FlaB-mediated antibody responses were toll like receptor 5 (TLR5) dependent, which was abrogated in TLR5-/- mice. Interestingly, we found that the antisera induced by 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 FlaB-mediated antibody responses would serve as an effective anti-norovirus mucosal vaccine, providing protective immune responses in mucosal and systemic compartments neutralizing the native virus.

**P32 Recombinant norovirus specific P domain protein fused with the mucosal adjuvant FlaB induces protective conformational 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) polyepitope formed small particles and induced a robust humoral immune response when administered through intranasal route. Moreover, the mixture of the Pd protein with the mucosal adjuvant FlaB (Pd+FlaB) significantly enhanced the antibody response that was further enhanced when Pd was fused with FlaB (Pd-FlaB) as a fusion protein vaccine. Pd-FlaB, as well as Pd+FlaB 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. FlaB-mediated antibody responses were toll like receptor 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.

**P33 Rational design of novel potential vaccine adjuvants – New class of TLR4 agonists**

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For the identification of novel yet unknown classes of TLR4 agonist we had performed virtual high throughput screening of 120 000 compounds from, Zinc database (a free database of more than 4 000 0000 commercially available drug-like compounds for virtual screening). For narrowing the broad spectrum of available compounds we have utilized an open source software Screening Assistant 2. We have screened circa 120 000 compounds from Zinc utilizing the resources of Czech national supercomputing center and finally obtained 10000 compounds which were subsequently docked into the active spot of TLR4 and bonding energy between actives spot of TLR4 and ligand for each compound was calculated. From this vHTPS screening we have obtained 60 compounds with high bonding energy to the receptor TLR4. Calculated bonding energy of these compounds is higher than (-12) Kcal/mol.

We have chosen lead compound from virtual docking and evaluated its capability to affect human TLR4 on cell line, (HEK-Blue™-hTLR4 (Invivogen, France)) that express Human TLR4 receptor. We have found that our lead possesses about 30% activity of MPLA and human TLR4 on cell line, (HEK-Blue™-hTLR4 (Invivogen, France)) that express Human TLR4 receptor. We have found that our lead possesses about 30% activity of MPLA and human TLR4 on cell line, (HEK-Blue™-hTLR4 (Invivogen, France)) that express Human TLR4 receptor. We have found that our lead possesses about 30% activity of MPLA and human TLR4 on cell line, (HEK-Blue™-hTLR4 (Invivogen, France)) that express Human TLR4 receptor. We have found that our lead possesses about 30% activity of MPLA.

**P34 Effective stabilization of a live viral vaccine by lyophilized and liquid SPS® formulations - example of a PRRSV vaccine in animal health**

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One main problem with vaccines is the need for a cold chain to maintain efficacy and safety as well as a sufficient shelf life. Live viral vaccines and live viral vectors are even more prone to stability issues especially in liquid formulations in comparison to lyophilized presentations. However, liquid formulations do offer relevant advantages in terms of processing and production, are more convenient to use and are less error-prone as reconstitution is not needed. Recently, a novel proprietary formulation technology platform SPS® for the stabilization and protection of proteins and other biologics including vaccines has been proven to stabilize biological macromolecules in dry and liquid formulations as well as during storage at ambient temperatures and different kinds of physical and chemical stresses.

In this study, we evaluated the effects of SPS® (liquid and dry) on the TCID50 of a live virus (PRRSV, arteriviridae; enveloped RNA virus; 45-70 nm) vaccine which is used against porcine reproductive and respiratory syndrome (PRRS). Although a liquid-formulated live virus vaccine is urgently needed, currently, PRRSV is only available in a lyophilized form because of severe stability issues in liquid formulations.

Our data show that SPS®-based, lyophilized formulations of PRRSV vaccine is stable at 5 °C comparable to the control and with 18 months even longer than most marketed live PRRSV vaccines. Moreover, the SPS®-based liquid viral PRRSV vaccine demonstrated 1 year stability at 5 °C that was higher than control and comparable to lyophilized presentations.

Data indicates that SPS® technologies enable live PRRSV vaccine production as liquid formulation. Such a product would represent the first liquid live PRRSV vaccine in the animal health market which might provide significant benefits in terms of convenience and especially in terms of manufacturing costs since costly lyophilisation processes could be avoided. Stability tests beyond 365 days are currently ongoing.
P35 A Multi-Agent Lassa and Ebola Virus DNA Vaccination Strategy Fully Protects Guinea Pigs against Simultaneous Infection

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The unprecedented large-scale outbreak of Ebola hemorrhagic fever in the heart of what was previously known as the Lassa fever hyperendemic geographical region of Africa has raised many concerns about the current lack of available medical countermeasure for both of these deadly pathogens. The Ebola outbreak was occurring at the same time as a Lassa fever outbreak and numerous cases of co-infection in humans were identified. Though the outcomes and pathogenesis of Lassa fever and Ebola hemorrhagic fever differ, often the early presentation of both diseases in humans is indistinguishable, which is further complicated if an individual is brought to clinic infected with both viruses. The development of countermeasures for prevention or treatment that could be effective against both infections is advantageous. We have developed optimized DNA vaccines against both Lassa fever and Ebola hemorrhagic fever viruses that have been shown to be protective individually in guinea pig and nonhuman primate models. In this study, we combined these two DNA vaccines into a single, multi-agent platform. Strain 13 guinea pigs received two vaccinations, spaced four weeks apart and consisting of 50 µg of each DNA vaccine or a mock vaccine at discrete sites. Five weeks following the second vaccinations, guinea pigs were exposed to lethal doses of Lassa virus, ebola virus, or a combination of both viruses simultaneously. None of the vaccinated guinea pigs, regardless of challenge virus and including the co-infected group, displayed weight loss, fever or other disease signs, and all survived to the study endpoint. All the mock-vaccinated guinea pigs that were infected with Lassa virus, and all but one of the EBOV-infected mock-vaccinates succumbed.

P36 NGS Analysis of Vaccine-induced Antibody Responses in a Mouse Model

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Background: The diversity of a vaccine-induced antibody response depends on several factors, chiefly the selection and clonal expansion of naïve B cells carrying recombinant immunoglobulin genes that can recognize antigen presented to them in lymphoid organs. We analyzed the antigen-specific B cell population’s clonal composition in terms of their CDR3 and V-gene usage. We seek to understand whether this clonal composition is variable or sharing common patterns among individual mice immunized with the same vaccine regimen.

Methods: Mice were immunized with 2 doses of plasmid DNA encoding HIV gp120 or vector control and subsequently boosted with recombinant gp120 protein. PBMCs on day 7 after the 1st DNA vaccination, and splenocytes on day 4 after protein boost were isolated. Splenocyte samples were sorted into CD138+ (plasmablasts) and CD138- (resting B cells) by FACS. B-cell RNA was extracted and cDNA amplicons covering the entire variable region of the gamma and kappa chains were generated by 5 RACE followed by PCR, and prepared for Ion Torrent NGS. Reads over 400 bases long and with high quality score were selected for analysis and submitted to the IMGT High V-quest server.

Results: The output data were clustered into clonotypes based on unique CDR3 and V-gene usage. BLASTp was utilized to generate pairwise alignments of the unique CDR3s from different mice. The B-cell population expanded by the vaccine displayed a relatively strong clonality: a large proportion of the plasmablast reads was assigned to a small number of unique CDR3s. In addition, several highly ranked CDR3s were shared among different mice. Frequency distributions of V-gene usage were generated and V-genes that were significantly overrepresented in plasmablast samples as compared to resting B-cell samples were noted. Interestingly, this expanded V-gene pattern was relatively different in different individual mice in spite of having received the same vaccine regimen (private specificities). Nevertheless, there were several instances in which the same V-genes were expanded in different mice (public specificities). An unexpected pattern was noteworthy: the kappa chain V-gene usage in CD138- splenocytes was remarkably conserved among individual mice, whereas CD138 + splenocytes did not show such a high degree of conservation although a few public specificities arose.

Conclusions: The clonal composition of antigen-specific cell populations upon vaccination is highly polarized towards a small number of clones, and it is not identical between mice that received the same immunization regimen. A few CDR3s and V-genes are shared among the responding populations in different mice. This leads us to believe that a large number of different naïve B-cell clones have the potential to recognize antigen and expand, but upon vaccination only a subset of those B cells do encounter antigen and acquire an advantage over the rest, leading to expansion.

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In order to determine if the multi-agent vaccination strategy could protect against both viruses if exposures were temporally separated, we held the surviving vaccinates in BSL-4 for approximately 120 days to perform a cross-challenge experiment in which guinea pigs originally infected with Lassa virus received a lethal dose of Ebola virus and those originally infected with Ebola virus were infected with a lethal dose of Lassa virus. All guinea pigs remained healthy and survived to the study endpoint. This study clearly demonstrates that a multi-agent vaccination strategy can be highly effective in protecting against individual virus exposures, but could also fully protect in a mixed-infection environment.
**P37 Mosaic Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Vaccines Induce Broad Cellular Immune Responses in Swine to Diverse Strains**

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Porcine reproductive and respiratory syndrome (PRRS) causes significant economic losses due to reproductive failure in pregnant pigs and respiratory disease mainly in young pigs. Because of the remarkably high genetic and antigenic diversity of the causative agent PRRSV, an RNA virus, it is very challenging to control this disease with current vaccines. In order to achieve broad protection towards such a divergent virus, two mosaic sequences were designed based on 748 ORF5 (glycoprotein S) sequences using the mosaic T Cell Vaccine Tool Suite from the Los Alamos National Laboratory. The mosaic sequences were then used to construct two DNA vaccines. The mosaic sequences were overall closer in amino acid identity to a collection of strains that are different from VR2332 by at least 10%. The T cell epitope coverage was broadened when two mosaics were made. Expression of the mosaic ORF5 sequences was verified in E. coli BL21 (DE3) cells by western blot. Three vaccination/challenge trials were performed in pigs to evaluate the immunogenicity and efficacy of the vaccines. The vaccines were delivered by gene gun (Trial 1), electroporation (Trial 2) or complexed to liposome (Trial 3). Lymphocyte proliferative responses were detected in virus-stimulated peripheral blood mononuclear cells (PBMC) of mosaic-vaccinated pigs.

**P38 Improving the potency of DNA-based cancer vaccines using Syn-Con® Technology**

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Inovio synthetic consensus (SynCon®) technology is a multi-phase DNA vaccine design strategy. Previously, we have demonstrated that DNA vaccines designed by SynCon® increased breadth of vaccine-induced cellular and humoral immune responses against infectious diseases. Conversely, breaking tolerance appears to be a big challenge for cancer vaccine development. Instead of using germline sequences, we incorporated Syn-Con® design strategy into our cancer vaccine design. We hypothesized that by introducing small amino acid differences into cancer antigen germline sequences, Syn-Con® antigens should exhibit stronger ability to break tolerance.

To demonstrate the principles, a WT1 vaccine was designed with incorporation of Syn-Con® technology (SynCon®WT1), sharing approximately 92% sequence identity with mouse germline WT1. A plasmid encoding mouse germline WT1 (mWT1) was created as a control. The mice received a total of 4 immunizations that were administered 2 weeks apart. IFN-γ ELISpot assay was performed to evaluate the vaccine-induced cellular immune responses. The results revealed that vaccination with SynCon®WT1 was able to break tolerance and induced robust cellular immune responses against SynCon but more importantly mouse native WT1 peptides (~200 SFU/10⁶ splenocytes). In contrast, there were little to no cellular responses in mice immunized with native mWT1 illustrating that vaccination with SynCon®WT1 was capable of breaking tolerance. In an acute myeloid leukemia (mWT1-C1498) tumor challenge model, vaccination with SynCon®WT1 was able to slow tumor growth compared to mice vaccinated with mWT1. We further tested the ability of Syn-Con® based approach to break tolerance in non-human primates. A SynCon®STEAP1 (human Six-Transmembrane Epithelial Antigen of Prostate) that is approximately 95% identical to the rhesus macaque STEAP was designed. Rhesus macaques were either immunized with a plasmid encoding SynCon®STEAP1 or a plasmid encoding a human STEAP1 that shares 99% sequence identity with rhesus macaque STEAP (huSTEAP1). Monkeys were immunized three times and cellular immune responses were evaluated by ELISpot assay. Similar to what we observed in mice, compared to huSTEAP1, immunization with SynCon®STEAP1 elicited much stronger immune responses (420 SFU/10⁶ vs 20 SFU/10⁶). Taken together, we demonstrated that Syn-Con® technology can be tailored to design potent DNA vaccines against cancer with the potential to significantly improve the ability of DNA vaccines to break self-tolerance.

**P39 A novel DNA vaccine targeting Fibroblast Activation Protein (FAP) breaks tolerance and synergizes with anti-cancer immune therapy in mice**

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Fibroblast Activation Protein (FAP) is a membrane-bound enzyme with gelatinase and peptidase activity that is up-regulated in cancer-associated fibroblasts in over 90% of human carcinomas. Previous studies have shown that ablation of FAP-expressing cells from transgenic mice attenuates tumor growth and synergizes with other immune therapies such as immune checkpoint blockade. Groups have additionally shown that T cells expressing chimeric antigen receptors targeting FAP slow tumor progression; however, in some mouse strains these CARs cause lethal toxicity. There is therefore a need to develop safer therapies targeting FAP. In this study we designed a novel consensus-based FAP DNA vaccine to help break tolerance. We hypothesized that this FAP vaccine could break tolerance and synergize with other immunotherapies to induce anti-tumor immunity without causing overt toxicity. Upon delivery of our FAP DNA vaccine intramuscularly followed by electroporation (EP), we detected IFN-γ and TNF-α CD8 T cell responses (p<0.05), and TNF-α and CD4 responses (p<0.05) against native mouse FAP peptides, indicating that our vaccine is capable of breaking tolerance. Furthermore, we detected cytotoxic CD107a/IFN-γ/T-bet triple-positive CD8 T cells that are reactive to native FAP peptides (p<0.05). In a prostate cancer model, vaccination with FAP alone or the prostate cancer antigen PSMA alone did not significantly alter tumor growth. However, vaccination with a combination of FAP and PSMA significantly attenuated tumor growth (p<0.0005), with final tumor volumes that were approximately 9 times smaller than tumors in naïve mice. Thus, our results support the use of FAP vaccines to target the tumor microenvironment in combination with immune therapies targeting the tumor cells themselves. We are investigating additional combination therapies with our FAP vaccine to modulate tumor infiltrating lymphocytes and to broaden responses to additional tumor antigens.
P40 Rationally Designed Immunogens Targeting HIV-1 gp120 V1V2 Induce Distinct Conformation—specific Antibody Responses in Rabbits

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The V1V2 region of HIV-1gp120 harbors a major vulnerable site targeted by a group of broadly neutralizing mAbs such as PG9 through strand—strands recognition. However, this epitope region is structurally polymorphic, as it can also form a helical conformation recognized by RV144 vaccine—induced mAb CH58. This structural polymorphism is a potential mechanism for masking the V1V2 vulnerable site. Designing immunogens that can induce conformation—specific Ab responses may lead to vaccines targeting this vulnerable site. We designed a panel of immunogens and engrafted the V1V2 domain into trimeric and pentameric scaffolds in structurally constrained conformations as well as fused to an Fc fragment to mimic the unconstrained V1V2 conformation. We tested these V1V2—scaffold proteins for immunogenicity in rabbits using a DNA prime protein boost regimen and assessed the responses by ELISA and competition assays. Our V1V2 immunogens induced distinct conformation—specific Ab responses. The Ab responses induced by structurally unconstrained immunogens only competed with mAb PG9, suggesting helix—recognizing Abs. The V1V2—focused Ab responses induced by the structurally constrained immunogens competed with mAb PG9, suggesting strand—recognizing Abs. The Ab responses induced by the structurally constrained immunogens were more broadly reactive and had higher titers than those induced by the structurally unconstrained immunogens. Our results demonstrate that immunogens presenting the different structural conformations of the gp120 V1V2 vulnerable site can be designed, and that these immunogens induce distinct Ab responses with epitope conformation specificity. Therefore, these structurally constrained V1V2 immunogens are vaccine prototypes that target the V1V2 domain of the HIV—1 envelope.

P41 A mucosal vaccine for bovi mastitis using cholera toxin A2/B adjuvant

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Shumans and animal species. Mastitis is a prevalent inflammatory disease of mammary tissues in cows, and chronic, contagious mastitis is mainly caused by Staphylococcus aureus. This infection is difficult or impossible to treat with antibiotics, and has a significant economic impact on agriculture worldwide. An effective multivalent vaccine to prevent S. aureus infection would improve animal health and also reduce dependence on antibiotics. The surface of S. aureus contains microbial surface proteins critical for establishing infection and adhesion in the host. The iron-regulated surface determinant A (IsdA) and clumping factor A (CifA), are conserved adhesins that are promising vaccine candidates. We have previously reported the construction of an IsdA-cholera toxin A2/B fusion (IsdA-CTA2/B) and the ability of this vaccine to stimulate antigen-specific responses after mucosal delivery in mice. Here we report, the results of two immunogenicity clinical trials on cows using IsdA-CTA2/B and CifA-CTA2/B fusions. The cows were intranasally inoculated with IsdA-CTA2/B + CifA-CTA2/B at the dry off period. Cellular immune responses were detected from bovine serum using flow cytometry analysis. Humoral immune responses were also examined by ELISA on serum, milk, and nasal washes. Results from clinical trials indicate a significant increase in antigen-specific CD4+ T cells in vaccinated cows versus unvaccinated, and a significant increase in antigen-specific humoral responses in milk in vaccinated cows. We also report the identification of immunogenic proteins from S. aureus using 2-DE (two-dimensional electrophoresis) & MALDI-TOF/TOF (mass spectrometry) techniques, followed by serological proteome analysis (SERRA). These studies were undertaken to isolate novel antigens for potential incorporation into a multivalent vaccine.Staphylococcus aureus is a pathogenic gram-positive bacterium, affecting both . Overall current results indicate positive immunogenic potential of the IsdA- and CifA-CTA2/B fusions to induce antigen-specific responses after intranasal delivery in cows. Continued exploration of immunogenic antigens, using affinity chromatography and immune co-precipitation, will promote the identification of additional vaccine antigens to generate an effective vaccine for bovine mastitis.

P42 Cross-talk between innate and acquired immunities: contribution of two signaling pathways to the induction of influenza antigen-specific antibody responses by DNA immunization

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Background: It is well established in both animal and human studies that DNA immunization is able to induce protective antibody responses against various subtypes of influenza viruses. However, the mechanism for DNA vaccines remains to be fully understood. It has been proposed that the DNA vaccination confers an intrinsic adjuvant effect which may have the involvement of the innate immune system. Previous studies suggested DNA TLR9 sensing pathway mainly activated by the CpG motifs of DNA vaccines. However, the mechanisms of cytosolic DNA sensing pathways remain unclear. Recently, we studied the roles of the inflammasome (AIM2 - absent in melanoma 2 protein) and Type I Interferon (STING - stimulator of IFN genes) pathways in the induction of hemagglutinin (HA) antigen-specific antibody response by an influenza HA DNA vaccine.

Methods: In this study, Aim2, STING, IRF3, IRF7 and other gene knockout mice as well as wild type controls were immunized with a DNA vaccine expressing the hemagglutinin antigen (HA) of 2009 pandemic H1 subtype influenza virus. The HA-specific antibody responses were evaluated by ELISA; the HA-specific B cell and T cell responses were measured by IFN-γ ELISPOT and B cell ELISPOT, respectively. A panel of pro-inflammatory inflammatory cytokines in immune mouse serum collected at different time points were measured by cytometric bead arrays.

Results: The anti-H1HA antibody responses were impaired in Aim2 knockout mice. While deletion of IL-1 and IL-18 signaling did not significantly affect the anti-HA antibody responses, Aim2-dependent pyroptotic cell death greatly affects the immune response generated by DNA vaccination. We further investigated the roles of the putative DNA sensor cyclic GMP–AMP synthase (cGas), as well as the downstream IFN regulatory factors (IRF) 3 and 7 in IFN induction and Ag-specific immune responses elicited by DNA vaccination. DNA vaccine–induced, IRF7-dependent signaling, as part of the Sting pathway, was critical in generating both innate cytokine signaling and Ag-specific B and T cell responses. In contrast, Irf3 was not as critical as expected in this pathway and, more surprisingly, immune responses elicited by DNA vaccines were not c-Gas-dependent in vivo.

Conclusions: Both Aim2 pathway and cGAS-Independent STING/IRF7 Pathway play an important role in shaping the antigen specific antibody response elicited by DNA vaccines. These previously unrecognized pathways are important innate immune mechanisms responsible for the generation of Ag-specific immune responses by DNA vaccination.

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**P43 Optimized Bivalent and Trivalent GP DNA vaccine formulations afford protection against lethal Ebola virus challenge in non-human primates and induce long-term immune responses post-immunization.**

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The recent Ebola virus disease (EVD) outbreak (2013-2016) was the most severe and wide-spread occurrence that has been reported in the past 40 years. Over 28 000 cases were confirmed, although these numbers are likely an under-estimate of real figures. There are no licensed vaccines or therapeutics for prevention of EVD however various experimental candidates are currently in ongoing clinical trials. Although promising, several Ebola vaccine human trials have reported adverse events including fever, arthritis & joint pain, and blistering, which could impair delivery to certain populations or limit certain applications. To address some of these concerns, we designed multivalent DNA vaccines as an alternative vaccine approach that is serology independent and has a clean safety profile. We designed 3 novel synthetic Zaire Ebola virus (EBOV) glycoproteins (GP) and combined them into a single Bivalent or Trivalent vaccine formulation along with plasmid-encoded IL-12 (pIL-12) as an adjuvant.

By inducing mucosal and systemic immune responses through the respiratory tract, we will convert nicotine into an immunogenic compound that can be administrated intranasally in a mouse model.

**P44 NICOTINE VACCINE INNOVATION: improvement of vaccine efficacy by inducing mucosal and systemic immune responses**

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**Objective:**

Tobacco use is a worldwide epidemic which contributes to an overall decreased quality of life in addition to emotional and economic burdens. Currently over 1 billion people smoke with an estimated 6 million deaths annually. Despite knowing for decades that smoking is prominently linked to cancer, cardiovascular diseases and overall decreased health, the addiction to nicotine makes it difficult to quit smoking. Because of the low success rates of smoking cessation products that are currently on the market, our team is developing the next stepping stone of nicotine vaccines to address unmet needs in prevention and treatment of tobacco smoking. In theory, antibodies produced by a vaccine can then rapidly bind to nicotine inhaled from cigarettes in the lung before it reaches the bloodstream and prevent it from reaching the brain; where it exerts its effect.

**Methods:**

Using a novel peptide/bacterial derived adjuvant particle platform which stimulates IL-12 production, a potent mechanism for mucosal immunity, the team will convert nicotine into an immunoogenic compound that can be administrated intranasally in a mouse model. Female BALB/c mice 6-8 weeks of age were vaccinated once every 3 weeks vaccine and sera was collected 2 weeks post-vaccination. Post-mortem, sera, lung washes and tissues were collected to assess immunological and toxicological responses.

**Results:**

The data demonstrates that our vaccine induces robust levels of anti-nicotine IgA and IgG in the mucosae and anti-nicotine IgG systemically in a mouse model. These antibodies are long lasting (sustainable for at least 8 months) and in high enough quantities [IgG titers of 10^6 (410-430 μg/mL)] to theoretically be able to neutralize the amount of nicotine present after more than one cigarette. In addition, our conjugate nicotine vaccine is able to induce immunological memory against nicotine and it does not induce toxicity in the lung or chronic inflammation.

**Conclusion:**

By inducing mucosal and systemic immune responses through the respiratory tract, we have demonstrated our vaccine design is the next generation of vaccines as a nicotine addiction therapeutic. Based on our proof of concept, the needle-free platform could also be employed to develop other vaccines for drug addiction treatments related to cocaine, methamphetamine and heroin.

**P43 (Continued)**

Antibody and T cell responses were assayed following different injection, dosing, and interval spacing regimens in cynomolgus macaques (n=4-5/group). High levels of sero-conversion were observed after a single immunization (83%) and 100% after 2 injections with either vaccine formulation. Animals were challenged with a lethal dose of the outbreak strain EBOV Guinea-Makona (1000TCID50, 7-U virus). 100% of animals receiving the Trivalent GP DNA vaccine formulation with a 3-injection regimen at 4 week intervals were fully protected against lethal challenge. These animals were fully protected against clinical signs of disease and did not have elevated blood cell counts or blood chemistry. Interestingly, 2-injection regimens with the Bivalent GP and Trivalent GP DNA vaccine formulations afforded 75% and 50% protection, respectively. In a separate group of animals, strong antibody and T cell responses are detectable several months following the last vaccine dose. Monitoring of long-term immunne responses is ongoing in these animals. Overall, the data strongly supports the protective efficacy of Bivalent and Trivalent GP DNA vaccine formulations in NHPs.
**P45 Evaluation of Next-Generation Nanoplasmid Vectors for Alphavirus and Filovirus DNA Vaccines**

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There remains a need for FDA-licensed vaccines to protect against intentional and natural human infections caused by the alphaviruses Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV) and the filoviruses Ebola virus (EBOV) and Marburg virus (MARV). To this end, we have developed highly promising DNA vaccines expressing codon-optimized envelope glycoprotein (GP) genes of these viruses in the traditional eukaryotic expression vector pWRG7077. When delivered by electroporation, these DNA vaccines elicited robust virus-specific antibody and cellular immune responses and provided high levels of protection against homologous virus challenge in multiple animal species. However, our previous studies have not focused on the potential for modifying the vector backbone to enhance the immunogenicity of these DNA vaccines. Nature Technology Corporation has developed miniaturized Nanoplasmid™ expression vectors that are smaller than traditional plasmids used for DNA vaccination. Consequently, these vectors have improved uptake and persist longer in transfected cells leading to increased transgene expression, which can result in enhanced immune responses and improvements in immunological memory. To evaluate the potential benefit for alphavirus and filovirus DNA vaccines, we engineered the nanoplasmid vector to express the codon-optimized GP genes of VEEV, EEEV, WEEV, EBOV, and MARV and also generated similar DNA vaccines for all of these viruses using nanoplasmid vectors that also encode a retinoic acid-inducible gene 1 (RIG-I) RNA agonist or the RIG-I agonist plus CpG RNA, which provide vector-mediated activation of innate immune pathways that can improve DNA vaccine-induced adaptive immunity. The results of in vitro analyses demonstrated that the expression levels of the VEEV, EEEV, WEEV, EBOV, and MARV GP antigens from the nanoplasmid DNA vaccines were increased as compared to the pWRG7077-based DNA vaccines. Furthermore, intramuscular vaccination of mice with the VEEV and EBOV nanoplasmid DNA vaccines resulted in increased virus-specific antibody and cellular immune responses as compared to the pWRG7077-based DNA vaccines. Taken together, our preliminary results indicate that the nanoplasmid vector may have inherent advantages over the traditional pWRG7077 vector for alphavirus and filovirus vaccines. We are currently performing more in-depth immunogenicity studies prior to evaluation of the relative protective efficacies of these DNA vaccines in established animal models of alphavirus and filovirus infection.

**P46 Polyvalent DNA Prime/Protein Boost Improved the Breadth of Both HIV-1 Env-specific Binding and Neutralizing Antibody Responses in Rabbits**

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**Background:** The diversity of primary HIV-1 isolates requires potential HIV vaccines to achieve broad coverage against circulating strains in a targeted population or geographic region. Although great efforts have been devoted, it still remains challenge in eliciting broadly neutralizing antibodies (bNAb) by vaccination mimicking those bNAb isolated from HIV-1 infected people.

**Method:** In the current study, we explored the value of polyvalent principle and DNA prime-protein boost (D-P) regimen, using gp120 immunogens from three main circulating subtypes (ThB, BC, and AE) in China. Rabbits were immunized with monovalent DNA/monovalent gp120 protein (D-P groups), monovalent DNA/3-valent protein (D-3P groups), 3-valent DNA/3-valent protein (either 3D-3P Mix or 3D-3P Sequential), or empty vector/3-valent protein (V-3P Protein). The magnitude, specificity and breadth for both binding and neutralizing activities in immune rabbit sera were characterized by ELISA, peptide microarray and neutralization assays.

**Results:** We detected a vaccine strain-matched dominance pattern in monovalent D-P groups and improved cross-clade responses in the D-3P groups for binding to gp120 immunogens, gp70-V1V2 scaffolds, and linear epitopes. The highest binding breadth was seen in the 3D-3P groups. Neutralizing activity showed similar patterns. In particular, the 3D-3P Mix group (3-valent DNA primes given simultaneously) showed higher neutralization breadth than the 3D-3P Sequential group (3-valent DNA primes given sequentially), and significantly higher than the V-3P Protein group (p=0.017) and the monovalent D-P groups (p=0.05).

**Conclusion:** Our data demonstrate a clear advantage of the polyvalent DNA prime/polyvalent protein boost regimen for enhancing the gp120-specific binding breadth and neutralizing antibody responses against HIV-1.

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**P47 The iVAX Toolkit: An in silico platform for epitope discovery, triage and analysis with applications to human and animal vaccine development**

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In silico prediction of immune response to emerging infectious diseases and cancers can accelerate the design of novel and next generation vaccines. We have leveraged computing power, genomic data, and advanced immunoinformatics tools to identify T cell epitopes that harness productive T cell immune mechanisms for generation of safe and effective vaccines. 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 VaccineCAD algorithm, simultaneously minimizing deleterious junctional epitopes that may be created in the linking process.

**P47 (Continued)**

JanusMatrix, a specially tailored homology analysis tool that examines pathogen/host sequence similarity at the MHC-TCR interface for any given peptide, predicts potentially cross-reactive epitopes, allowing candidate sequences with potential host cross reactivity to be preferentially excluded from vaccine constructs. 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. This tool also successfully identified the cross-reactive epitope between the MAGE A3 immunotherapeutic and human titin implicated in two fatalities among melanoma and myeloma clinical trial participants, highlighting the importance of personalized prediction with patient-specific HLA in mind.

Recent emergence of H7N9 influenza illustrates the challenges associated with ‘standard’ approaches to vaccine development, while modern cancer vaccine research has underscored the danger of auto-reactive vaccines and immunotherapeutics. These studies provide an opportunity to apply 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. Academic and commercial collaborations are welcomed and encouraged.
**P48 Innovative Cancer Neo-Epitope Classification Strategy for T Cell Epitope Selection for Mutanome-Directed Cancer Vaccination**

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T cell epitopes bearing tumor-specific mutations discovered using whole-exomic and transcriptomic sequencing of tumor-normal pairs stimulate T cell-mediated processes that lead to tumor regression. Although neo-epitope prediction using computational methods rapidly identifies epitope candidates in the mutanome, a large proportion of neo-epitopes proves to be non-immunogenic. Innovative computational tools validated for infectious disease targets can be applied to enhance design of personalized cancer immunotherapies by classification of predicted epitopes by potential for mounting a tumor-specific response. Tumor-specific epitopes with potential to stimulate naïve T cells and epitopes that may cross-react with antigens experienced by regulatory T cells raised in infection are valuable targets. Epitopes with potential to activate regulatory T cells trained on self and commensal antigens are counter-indicated.

We developed the JanusMatrix algorithm that parses query sequences into MHC-facing and T cell receptor (TCR)-facing sequences and screens sequence databases to identify MHC ligands that share TCR faces with host-related proteins. A database of human protein sequences is available to identify tumor-specific epitopes that may reduce anti-tumor activity by sequences that activate regulatory T cells (Tregs) trained in the thymus on self-antigens. Similarly, tumor-specific epitope candidates are screened using databases composed of antigens derived from human commensals or pathogens to identify epitopes that, respectively, may detrimentally or beneficially cross-react with T cells raised over the course of an individual’s immune history. Neo-epitope candidates are ranked according to MHC binding potential, TCR face homology.

**Results:** Several human-like T cell epitopes from HIV Env protein were identified by JanusMatrix analysis. One epitope shares a TCR-face with a large number of human leukocyte antigen class I molecule sequences and is found in both the HIV-1 E and B Env antigens used in the ‘moderately effective’ HIV RV144 trial. Compared with non-human-like HIV epitopes, human-like HIV peptides, and their human analogs increased the frequency of CD4+CD25+CD39+FoxP3+ Tregs. Human-like HIV-1 Env T cell epitopes suppressed tetanus toxoid-induced total CD4+ T cell proliferation.

**Conclusions:** HIV-1 may be camouflaged from human immune response by T cell epitope sequences that avert or regulate effector T cell responses through host tolerance mechanisms. The impact of human-like epitopes on vaccine immunogenicity and efficacy deserves consideration in future HIV vaccine development programs.

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**P49 HIV-1 Env T Cell Epitopes That Mimic Human Sequences May Induce Treg-mediated Tolerance**

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**Introduction:** Major obstacles to HIV-1 vaccine development include the delayed development and short life of broad neutralizing antibodies against the critical antigen envelope (Env). Defining immune mechanisms responsible for these impediments and designing immunogens that overcome them are a key focus of the HIV-1 vaccine field. We recently discovered that viruses that cause chronic infection might escape human immune response by mutating their epitopes to resemble “human-like” amino acid sequences, which may be recognized by autologous regulatory T cells (Tregs), thus may actively suppress the immune response to themselves. We hypothesize that HIV-specific humoral responses may be modulated by induction of Treg by human-like HIV-1 T cell epitopes.

**Methods:** We used an immunoinformatics tool, JanusMatrix, to scan ~3000 HIV-1 clade B Env sequences from the LANL database to locate highly conserved human-like T cell epitopes. PBMC from de-identified HIV-healthy subjects were cultured with human-like HIV-1 peptides, their corresponding human analogs, and non-human-like HIV peptide in vitro. The frequency and phenotype of the responding T cells were analyzed using flow cytometry. The suppressive function of human-like HIV-1 peptides was evaluated in an in vitro bystander T cell assay.

**Results:** Several human-like T cell epitopes from HIV Env protein were identified by JanusMatrix analysis. One epitope shares a TCR-face with a large number of human leukocyte antigen class I molecule sequences and is found in both the HIV-1 E and B Env antigens used in the ‘moderately effective’ HIV RV144 trial. Compared with non-human-like HIV epitopes, human-like HIV peptides, and their human analogs increased the frequency of CD4+CD25+CD39+FoxP3+ Tregs. Human-like HIV-1 Env T cell epitopes suppressed tetanus toxoid-induced total CD4+ T cell proliferation.

**Conclusions:** HIV-1 may be camouflaged from human immune response by T cell epitope sequences that avert or regulate effector T cell responses through host tolerance mechanisms. The impact of human-like epitopes on vaccine immunogenicity and efficacy deserves consideration in future HIV vaccine development programs.
P50 Adjuvant-specific Enhancement of the Germinal Center and Mucosal Response to Electroporated HIV-1 DNA Immunogens


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Most licensed vaccines rely on the production of antibodies that mediate protection from disease. The germinal center is a dynamic cellular substructure found in lymphoid organs where follicular helper T cells (Tfh) provide the necessary proliferation stimuli and cytokine signaling that promote antibody class-switching, somatic hypermutation, and affinity maturation of B cell receptors. Here, mature B cells differentiate into long-lived antibody-secreting cells and memory B cells, instituting the characteristic humoral memory response to vaccination. DNA vaccines have demonstrated great potential as vaccine immunogens and in vivo electroporation (EP) has enhanced this immunogenicity, however the germinal center response to EP DNA remain uncharacterized. Due to its critical role in the development of long-lived humoral responses and memory, understanding the germinal center response (GCR) to DNA vaccine antigens is important, and an innovative means to identify effective molecular adjuvants that drive long-lived functional anti-HIV humoral immunity. Using multicolor flow cytometry, antigen-specific ELISA and ELISpot, and Immunofluorescence staining of GC architecture, we have demonstrated that the immunogenicity of HIV-1 consensus envelope (env) immunogens can be enhanced by the inclusion of molecular chemokine adjuvants that promote the formation of germinal centers and drive mucosal homing.

P50 (Continued)

We have developed HIV envelope (env) based consensus immunogens delivered intramuscularly with in vivo electroporation in the presence of plasmid encoded IL-12, which has been demonstrated to enhance Tfh function, or mucosa-associated epithelial chemokine (MEC) which recruits IgA positive B cells to the mucosa. Mice were vaccinated twice, separated by two weeks with either HIV-1 env DNA alone, HIV-1 env DNA with MEC, or HIV-1 env DNA with IL-12. The GCR to HIV-1 env DNA antigens alone is suboptimal inducing fewer env-specific GC B cells and fewer TFH cells compared to mice vaccinated with HIV-1 env DNA + IL-12 (0.5% envA+envC+ for HIV-1 env DNA alone versus 2.6% for HIV-1 env DNA + IL-12); however the addition of MEC surprisingly did not enhance the peripheral GCR to HIV-1 env DNA, as no significant differences in GC B cells or TFH numbers were observed between mice vaccinated with DNA alone or DNA + MEC, it does increase the levels of HIV-1 specific IgA in serum, vaginal washes and fecal pellets (1600ng/ml compared to 1200ng/ml with env alone in serum, 30ng/ml compared to 19ng/ml with env alone in vaginal wash and 100ng/ml versus 30ng/ml in fecal pellets), suggesting analysis of the mucosal GC response is warranted. While IL-12 administration enhanced the peripheral GCR to HIV-1 env DNA as expected, it did not enhance IgA levels in the serum of vaccinated animals suggesting this adjuvant does not enhance mucosal humoral immunity. These data represent to our knowledge, the first careful examination of the GCR to EP-delivered DNA-based vaccine antigens in combination with molecular adjuvants. This work is funded by the WW Smith Charitable Foundation, Pennsylvania Department of Health Care and NIH P01AI071379.

P51 Engineering Chimeric Virus-Like Particles for use as Vaccine Antigens

Danielle Basore, Thomas Jordan, Carolyn Barcellona, Emilie Mausser, Kanthi Bommarredyy, Donna Crane, Rajesh Naz, Sharon Isern, Scott Michael, Chris Bystroff

Viruses-like particles (VLPs) are stable scaffolds that can be used to carry antigenic peptides. The major capsid protein L1 of the Human Papilloma Virus spontaneously forms a VLP 55nm in size. Antigenic peptides of varying sizes may be inserted into the β5 loop while conserving the assembly of the particle. HPV-VLPs generate high levels of long-lived serum plasma B-cells and memory B cells.

We are focused on producing a universal Dengu vaccine and an anti-sperm contraceptive vaccine. In designing the Dengu vaccine, the antigen must be conserved across serotypes and generate neutralizing antibodies. The Fusion loop of the Envelope protein forms a VLP 55nm in size. Antigenic peptides of varying sizes may be inserted into the β5 loop while conserving the assembly of the particle. HPV-VLPs generate high levels of long-lived serum plasma B-cells and memory B cells.

We have developed HIV envelope (env) based consensus immunogens delivered intramuscularly with in vivo electroporation in the presence of plasmid encoded IL-12, which has been demonstrated to enhance Tfh function, or mucosa-associated epithelial chemokine (MEC) which recruits IgA positive B cells to the mucosa. Mice were vaccinated twice, separated by two weeks with either HIV-1 env DNA alone, HIV-1 env DNA with MEC, or HIV-1 env DNA with IL-12. The GCR to HIV-1 env DNA antigens alone is suboptimal inducing fewer env-specific GC B cells and fewer TFH cells compared to mice vaccinated with HIV-1 env DNA + IL-12 (0.5% envA+envC+ for HIV-1 env DNA alone versus 2.6% for HIV-1 env DNA + IL-12); however the addition of MEC surprisingly did not enhance the peripheral GCR to HIV-1 env DNA, as no significant differences in GC B cells or TFH numbers were observed between mice vaccinated with DNA alone or DNA + MEC, it does increase the levels of HIV-1 specific IgA in serum, vaginal washes and fecal pellets (1600ng/ml compared to 1200ng/ml with env alone in serum, 30ng/ml compared to 19ng/ml with env alone in vaginal wash and 100ng/ml versus 30ng/ml in fecal pellets), suggesting analysis of the mucosal GC response is warranted. While IL-12 administration enhanced the peripheral GCR to HIV-1 env DNA as expected, it did not enhance IgA levels in the serum of vaccinated animals suggesting this adjuvant does not enhance mucosal humoral immunity. These data represent to our knowledge, the first careful examination of the GCR to EP-delivered DNA-based vaccine antigens in combination with molecular adjuvants. This work is funded by the WW Smith Charitable Foundation, Pennsylvania Department of Health Care and NIH P01AI071379.

P52 A novel synthetic CD40L plasmid adjuvant generates unique anti-HPV DNA vaccine induced responses that impact tumor growth

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The use of plasmid adjuvants encoding cytokine, chemokine or immune modulators to tailor vaccine-induced response is a strength of the DNA vaccine platform. Due to its role in both innate and adaptive immunity, we hypothesized that the co-delivery of plasmid encoded CD40 ligand (CD40L) could increase DNA vaccine responses. In its natural form, CD40L can occur as either a surface bound form or a cleaved/solubilized form. Thus, we sought to determine if different forms of pCD40L can influence cellular and humoral responses when co-delivered with a HPV16 DNA vaccine expressing the oncogenic proteins E6 and E7. Mice were immunized with HPV DNA with or without synthetic optimized plasmids expressing various forms of CD40L followed by electroporation (EP). Mice that received the soluble form of CD40L (sCD40L) exhibited significantly higher antigen specific CD8+ T cell responses including IFN-γ, IL-2 and TNF-α expression as well as slight increases in CD4+ T cells and antibody responses. These responses were also maintained into memory. Conversely, the surface bound as well as the wild-type form of CD40L blunt vaccine-induced responses compared to vaccine alone. Time course analysis revealed that 11 days after primary immunization, CD8+ tetramer specific (H-2D+ HPV16 E7 (RAHYNVTFL)) T cells in mice immunized with sCD40L averaged around 18% compared to vaccine alone at 4%. These responses were partially dependent on CD4+ T cell help and were functionally similar to responses observed post final immunization. We have also observed similar vaccine-induced immune responses when sCD40L was combined with other cancer vaccines including mouse telomerase reverse transcriptase (mTERT). Upon therapeutic tumor challenge, mice immunized with HPV + sCD40L displayed significant tumor regression compared to vaccine alone or naïve animals. Additionally, when sCD40L was included the ratio of tetramer specific CD8+ T cells to CD4+ Tregs infiltrating the tumor was significantly higher compared to vaccine alone or naive group. These results demonstrate the power of using a plasmid adjuvant encoding a synthetic optimized sCD40L in a DNA vaccine. Additional studies in other animal cancer models are important for determining the possible broad importance of this approach for cancer immune therapy.
P53 Prophylactic Intradermal Delivery of INO-4201 Drives Robust Ebola Glycoprotein Specific Humoral and Cellular Immune Responses in Healthy Volunteers in an Open Label Phase I Trial


As of April 2016, the CDC reports that the West Africa Ebola virus (EBOV) outbreak focused in Sierra Leone, Liberia and Guinea resulted in over 28,000 suspected cases of infection and over 11,000 total deaths. This outbreak underscores the need for a protective vaccine against Ebola that is capable of generating sterilizing immune responses. To date, no specific vaccine immune correlate has been associated with protection against Ebola.

Thus, the development of a vaccine that generates robust immune responses from both the humoral and cellular arms of the adaptive immune system stands as the best approach to achieve full protection. We have developed INO-4201; a plasmid-based prophylactic vaccine encoding the Zaire Ebola glycoprotein (GP), for the prevention of Ebola infection.

INO-4201 is a consensus antigen, whose design encompasses genetic variability from previous outbreak strains (1976-2014) in order to establish a wide breadth of immune coverage for possibly divergent Ebola virus variants. Administration of a 3-dose regimen of INO-4201 was well tolerated in healthy volunteers with no Grade 3 or Grade 4 SAEs noted. Assessment of immune responses generated by INO-4201 after a 2mg intradermal administration using the Cellectra in vivo electroporation device in these volunteers revealed the induction of robust Ebola GP-specific antibody and CD4+ as well as CD8+ T cell responses. Specifically, 100% seroconversion, as gauged by binding ELISA, was detected after only two doses of INO-4201. The reciprocal geometric mean endpoint titer at that time was 39,664.20 and was boosted by administration of the third dose to 46,968.00. Examination of the EBOV GP specific T cell response as assessed by interferon gamma (IFNγ) ELISPOT revealed a mean peak response magnitude of 295.3 SFU per 10^6 PBMCs, suggesting immunization also engaged the cellular arm of the immune system.

INO-4201 was well tolerated in healthy volunteers with no Grade 3 or Grade 4 SAEs noted. Further analysis of cellular immune activity as measured by Flow Cytometric intracellular cytokine staining indicated that immunization with INO-4201 drove statistically significant increases in in the production of IFNγ or Tumor Necrosis Factor alpha (TNFα) in both the CD8+ T cell compartment (p=0.001, two tailed Wilcoxon signed-rank test) and CD4+ T cell compartment (p=0.004, two tailed Wilcoxon signed-rank test). Taken together these data confirm that intradermal administration of INO-4201 using the Cellectra device is both well tolerated and immunogenic as assessed by both humoral and cellular EBOV GP-specific immunocytoassays. These results indicate that INO-4201 is a strong candidate for further clinical development of a prophylactic Ebola vaccine.

P54 A Combined DNA encoded anti-toxin monoclonal antibody and antigenic vaccine approach for prevention of morbidity and mortality associated with acute severe and recurrent Clostridium difficile infection

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Clostridium difficile is a pathogen that causes intestinal disease, with the symptoms of infection ranging from diarrhea to pseudomembranous colitis and toxic megacolon, which are often fatal. Clostridium difficile infection (CDI) is the largest Hospital Acquired Infection in the US and most of the developed world. A CDC funded study estimated that there were approximately 453,000 cases of, and 29,000 deaths due to CDAD in 2011.

CDI is primarily mediated by two large enterotoxins, A and B, which glycosylate Rho proteins, and cause actin disruptions and intestinal cell death. Our laboratory has developed a DNA vaccine to elicit a humoral response to the C-terminal receptor binding domains (RBD) of these two enterotoxins, subsequently providing active protection in a murine model, as well as demonstration of passive immunity using hyperimmune sera from vaccinated non-human primates into mice with efficacy being tested via a lethal spore challenge. In addition to active immunization strategies to protect against severe acute and recurrent CDI, passive immunization has been shown to impart protection from acute infection. The administration of monoclonal antibodies against the two toxins A and B significantly reduced the rate of recurrence of CDI, in a phase II clinical trial.

We plan to use a clinically relevant aging murine model to test our hypothesis, using a previously validated lethal spore challenge to analyze vaccine efficacy, and also a second model of recurrent CDI. We have established an aging mouse model that shows mice >18 months of age have a greater susceptibility to sublethal doses of C. difficile UK1 hypervirulent spores, and this is associated with lower anti-toxin antibody post infection and increased intestinal inflammation.

We plan to test co-delivery of passive immunization (DMab-tox A and DMab-toxB) and active (toxA-RBD and toxB-RBD3) immunization will simultaneously ameliorate symptoms of acute disease and generate long lasting immunity, thus protecting against recurrent CDI in high risk individuals, including the elderly.

We plan to test co-delivery of passive immunization (DMab-tox A and DMab-toxB) and active (toxA-RBD and toxB-RBD3) immunization on subsequent outcomes in recurrent CDI models in aged vs young adult mouse models.

P54 (Continued)

Individuals are colonized within 14 days of a hospital stay, often showing symptoms of CDI in 21 days (abstract Gupta et al., at 3rd International Clostridium difficile Symposium, 2010). Therefore, an immune response to an active vaccine antigen would need to be induced rapidly in order to protect high risk individuals in a hospital setting, effectively creating a temporal niche for passive immunization. In addition, active immune strategies are warranted as the goal is to induce an anti-toxin memory immune response to protect individuals against recurrence of infection.

Here, we present data showing the expression of our DMAb plasmids in vitro, as well as functionality of the antibody in an ex vivo toxin neutralization assay. We hypothesize that a combination approach of passive immunization (DMab-tox A and DMab-toxB) and active (toxA-RBD and toxB-RBD3) immunization will simultaneously ameliorate symptoms of acute disease and generate long lasting immunity, thus protecting against recurrent CDI in high risk individuals, including the elderly.

We plan to use a clinically relevant aging murine model to test our hypothesis, using a previously validated lethal spore challenge to analyze vaccine efficacy, and also a second model of recurrent CDI. We have established an aging mouse model that shows mice >18 months of age have a greater susceptibility to sublethal doses of C. difficile UK1 hypervirulent spores, and this is associated with lower anti-toxin antibody post infection and increased intestinal inflammation.

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P54 (Continued)
**P55 Exploratory Characterization of the Immune Response Induced by ACC-001, a Vaccine Directed at β-amyloid, in Alzheimer Disease Patients**

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Alzheimer’s disease (AD), the most common form of dementia, is the 6th leading cause of death in the US. Antibody-mediated clearance of Aβ, one of the pathological hallmarks of AD, is being evaluated through either passive or active immunotherapy. ACC-001, an N-terminal Aβ1-7 peptide conjugate vaccine, was evaluated in Phase 2 trials in both mild-to-moderate and early AD patients. When administered with adjuvant (QS-21) ACC-001 elicited consistently higher peak and sustained anti-Aβ IgG titers compared to ACC-001 alone. We have further characterized the immune response of 41 patients treated with ACC-001 over time from 3 Phase 2 trials (ClinicalTrials.gov Identifiers: NCT01227564, NCT00498602 (US); EudraCT #: 2006-002061-39). Epitope mapping was performed by an ELISA assay designed to measure the binding of antibodies to overlapping peptides that spanned the first 8 residues of the Aβ sequence. Each peptide was offset by one amino acid. Interestingly epitope mapping of the sera showed that ACC-001 immunization could elicit an oligoclonal antibody response in contrast to the previously observed N-specific response induced by full length Aβ peptide immunization (AN1792, aggregated Aβ1-42). Affinity of the sera to the Aβ peptide, measured by Solution Equilibrium Affinity, did not change over time. Information gained in these studies may contribute to the optimization of future AD vaccine approaches.

**P56 M2SR: A Single Replication Next Generation Live Influenza Vaccine**

Pamuk Bilsel

Influenza vaccines have remained virtually unchanged for decades. The most commonly used vaccine is the inactivated influenza vaccine (IIV). Despite annual updating to match the circulating influenza strains, the effectiveness of IIV is only 60% across the population as a whole and much less for the elderly. A vaccine with greater efficacy that also provides broad-spectrum immunity across all segments of the population, i.e., a universal vaccine, would present a transformative achievement for public health and pandemic preparedness.

FluGen’s M2SR (Single Replication) vaccine candidate has achieved these characteristics in animal models. We have shown that the M2SR vaccine provides broad-spectrum, long-lasting cross-protection against multiple influenza subtypes (H3N2, H1N1 and H5N1) in mice and ferrets. The M2SR vaccine virus exploits the best features of both inactivated and live attenuated influenza vaccines (LAIV). Like the inactivated vaccine, it elicits a strong humoral response against the major target of neutralizing antibody, the hemagglutinin (HA). Similar to the LAIV, it is administered intranasally to mimic a natural infection and induce broad-spectrum immunity including mucosal and cell-mediated responses. The novelty of M2SR is that the vaccine virus presents multiple antigen targets to the immune system like a wild-type virus and activates the immune system without shedding of vaccine virus.

**P57 High-Throughput Sequencing Identifies Novel and Conserved microRNAs in Response to EV71 and CA16 Infection in Peripheral Blood Mononuclear Cells**

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Enterovirus 71 (EV71) and Coxsackieviruses A16 (CA16), as the predominant etiological agents of hand, foot, and mouth disease (HFMD), both belong to the human enterovirus A species of the Picornaviridae family and share similar genetic homology, but the clinical manifestations of HFMD caused by the two viruses still have some discrepancies. Furthermore, the underlying mechanisms leading to these differences remain unclear. MicroRNAs (miRNAs) participate in numerous biological or pathological processes, including host responses to viral infections, by targeting messenger RNAs (mRNAs) for translational repression or degradation. Here, we focused on the different changes of miRNAs expression in peripheral blood mononuclear cells (PBMCs) infected by EV71 and CA16 at different points by high-throughput sequencing analysis. The results showed that 106 known and 13 novel miRNAs have significantly differences and 35 miRNAs among them presented opposite tendency in EV71- and CA16-infected samples. GO analysis of the predicted targets exhibited that 14 in biological process, 10 in molecular function and 8 as cellular components. Pathway enrichment analyses revealed that the predicted target genes were involved in 104 pathways.

**P57 (Continued)**

Subsequently, the regulatory networks of the 35 significantly differentially expressed miRNAs associated with the transcription factor, predicted targets, biological process and pathways were built and suggested that the complex regulatory mechanism of miRNAs during the infection phase. Ultimately, hierarchical GO categories of the predicted targets involved in immune system process were analyzed, mainly including macrophage activation, immune response, antigen processing and presentation, response to interferon-gamma, B cell mediated immunity and complement activation. The results indicated that the innate and adaptive immunity induced by EV71 and CA16 infection may be markedly distinct. Therefore, this is the first report of miRNAs expression profiles of EV71 and CA16 infection in PBMCs by high-throughput sequencing, and our findings could provide useful clues to help elucidate the differences of host-pathogen interactions in EV71 and CA16 infection and even might offer novel therapy targets for EV71 and CA16 infection.
Pathologic and immunologic characteristics of coxsackievirus A16 infection in rhesus macaques
Xialong Zhang

Coxsackievirus A16 (CA16) is a major pathogen of human hand, foot and mouth disease (HFMD) with unclear pathogenesis. Observation of CA16 infection in rhesus macaques 6-8 months of age revealed the presence of characteristic vesicles in the oral mucosa and limbs in combination with viremia and positive viral loads in the tissues, suggesting that these animals are capable of reflecting the pathologic process of the viral infection. Immunologic analysis indicated a defective immune response, which included undetectable neutralizing antibodies and IFN-γ specific memory T cells in macaques infected by CA16, and the existing neutralizing antibodies in macaques immunized with the inactivated vaccine were surprisingly unable to protect against virus challenge despite the presence of a positive T-cell memory response against viral antigens. These immunologic characteristics were presumed to be related to the viral infection identified in the pre-conventional dendritic cell (pre-cDC) population and might give rise to an unprotected state of infected or immunized macaques against repeated virus challenge. For the development of a vaccine against CA16, which is responsible for approximately 40% of human HFMD, an effective animal model is capable of providing various indicators of pathologic processes during viral infection and developing an immune response induced by the inactivated vaccine. The pathologic and immunologic characteristics of CA16 infection described here in rhesus macaques suggest a defective immune response induced by the viral infection, which implies that the conventional inactivated CA16 vaccine might be ineffective at preventing this disease.

Antigen Presenting Cells Drive Specialized Immune Responses of CD4 Effectors
Devarajan P, Vong AM, Bautista BL, Castonguay CH, Swain SL

We have defined the “Memory Checkpoint” as the period when CD4 effector T cells (TEFF) require cognate antigen (Ag) recognition for further differentiation into CD4 memory T cells. Using an influenza A virus (IAV) infection model, we find that generation of CD4 cytotoxic T cells (ThCTL) from TEFF requires MHC Class-II restricted Ag at this checkpoint, 5-7 days after initial infection. Ag recognition at this checkpoint also promotes CD4 follicular helper T cell (TFH) generation. We use transgenic mouse models in which Ag presentation is restricted to specific antigen presenting cells (APC), bone marrow chimeras and addition of various APC subsets at the memory checkpoint, to analyze which APC drive TEFF to TFH vs. ThCTL. We find that distinct APC, during this checkpoint, promote different effector fates resulting in generation of distinct effector subsets. While, as expected, B cell APC are necessary for TFH responses, the APC subset(s) that promote ThCTL generation are not yet clear. Our data indicate that a specialized hematopoietic subset of CD11c+ APC, that are preferentially localized in the infected tissue, is required at this checkpoint, to support ThCTL generation. Once we identify the APC subset(s) needed, we plan to target Ag to particular APC at the checkpoint, to favor distinct functional subsets and also to promote functional specialization of T cell memory. We plan to evaluate the function of distinct effector and memory subsets we obtain in clearing IAV and promoting longterm immunity. We suggest this may allow us to tailor the quality of the effector response and thus tailor vaccines to better protect against distinct pathogens.

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Autophagy facilitates EV71 and CA16 replication via suppressing IFN-I production and promoting viral receptors expression
Jie Song, Yajie Hu, Jiaqi Li, Huwen Zheng, Ruotong Ning, Jingjing Wang, Lei Guo, Haijing Shi, Lichun Wang, Qihan Li*, Longding Liu*

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Hand, foot, and mouth disease (HFMD) is a serious global epidemic that is mainly caused by enterovirus 71 (EV71) and coxsackievirus A16 (CA16), which trigger significantly different clinical symptoms. However, the underlying mechanisms leading to these differences are still unknown. This study aims to investigate the possible mechanisms that result in these differences by comparing the genes related to the production of type I interferons in normal human airway epithelial cells (16HBE). The results show that the gene expression levels of TRK3, MAVS, MDAS, MyD88, IRF7, IFNo and IFNα were significantly increased after EV71 infection. However, TRK3 and IRF3 were markedly decreased after CA16 infection, and TRK7, MAVS, RIG-I, MyD88, IRF7, IFNo and IFNα are no significant differences. Additionally, viral copy number and viral titer suggest that CA16 replicates more efficiently than EV71 in 16HBE. Subsequently, we focused on the reason about the differences of IFN-I production induced by EV71 and CA16 infection. Autophagy acts as a direct effector for protection against pathogens, as well as a modulator of pathogen recognition and downstream signaling in innate immune responses.
P61 The analysis of mode of action of Hydroxypropyl-β-cyclodextrin (HP-β-CD) as a mucosal adjuvant

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Understanding of the mode of action of adjuvant is very important to design safe and effective adjuvants. So far, we have investigated the adjuvant activity of Hydroxypropyl-β-cyclodextrin (HP-β-CD) as a vaccine adjuvant, and we reported the efficacy of HP-β-CD as a subunit flu vaccine adjuvant. Furthermore, we recently reported that HP-β-CD function as a mucosal adjuvant by intranasal (IN) administration. However, the detailed mechanism of the mode of action of HP-β-CD in immune response is yet unknown. In this study, we investigated the mechanism of adjuvant activity of HP-β-CD by IN administration, using several gene-deficient mice.

Mice were immunized by IN administration twice with ovalbumin (OVA) mixed with or without HP-β-CD one week period. Sera and bronchoalveolar lavage fluids (BALF) were taken a week after last immunization. The adjuvant activity was estimated as antibody responses by ELISA. We found that the 7μg/μl and My088α-μg/mice immunized by HP-β-CD + OVA showed decreased levels of OVA-specific IgG responses compared to their littermates. TBK1 is known to be a downstream signaling molecules for DNA sensing, and we observed the release of dsDNA, which is dead cell-derived factors (DAMPs), in BALF, indicating that DAMPs contributed the adjuvanticity of HP-β-CD by IN administration. In addition, the level of OVA-specific IgG responses was significantly decreased in Cd4-cre βCat−/− mice as a model of Folllicular helper CD4 T cell (Thf)-deficient mouse.

Taken together, these results suggest that DAMPs released by HP-β-CD is administration are involved in the development of Thf cells. Thf plays an important role in mucosal immune responses elicited by IN HP-β-CD administration.

P62 Regulatory system: safety and efficacy of vaccines

*Menta KUCUKU MSc., PhD
*National Agency for Medicines & medical Devices

Today the number and types of vaccines in immunization schedule and in the market are increased. The vaccination applied at first day of life and continues in different ages during life in healthy people. It is the reason that the safety and efficacy of vaccines are very important and much discussed point today. Some countries time after time face unfavorable situation for vaccination. Today, people can get information for immunization and vaccines in different ways; journal, internet, TV, social media, newspaper, discussion with each other etc. Not always the information is right, but unfortunately this can cause panic and damage the system of immunization in the country and sometimes in the world. It is very important to prepare the situation in advance which mean not to allow space for misunderstanding and misinterpretation of vaccines safety and efficacy. The regulatory systems has a responsibility to ensure the safety and efficacy of vaccines because the injection of ineffective and poor quality of products sometimes can cause very serious problems even and death and undermine confidence in the health system, health professionals, pharmaceutical manufacturers and distributors. Another problem of poor quality and non effective vaccines has a financial wasted cost. National Regulatory Authority (NRA) or National Control Laboratories (NLC) are different and their consideration for benefit/risk in their country is different based on vaccine supply, disease prevalence and severity, their specific epidemiological situation, which must be taken in consideration when making a decision. WHO coordinates efforts directed towards achieving international consensus, but NRAs ultimately make decisions based on benefit/risk assessment for their population [1].

The conclusion: Strengthening of regulatory function in every country avoids misinterpretation and misunderstanding of safety and efficacy of vaccines. Second exchange experiences and information between regulatory agencies for safety and efficacy of vaccines can prepare the situation in advance and protect immunization system and public confidence.

P63 Evaluation of conditions for local irritation after intranasal administration of poly I:C in Crt:CD rats

Tetsuo Nakano
Kyowa Hakko Bio Co., Ltd.

Polyinosinic:polycytidylic acid (poly I:C) activates the mammalian innate immune system by interacting with both Toll-like receptor 3 (TLR-3) and RIG-I-like receptors (RLRs), inducing the synthesis of type-I interferon (IFN) and proinflammatory cytokines. The strength and period of biological activity of poly I:C leading to toxicity depend on the nucleotide length. How the nucleotide length of poly I:C can be controlled remains a limitation to its application as a vaccine adjuvant. To address this issue, we developed an uneven-structured poly I:C, “uPIC100,” which was designed to fix and stabilize the nucleotide lengths based on poly I:C structure. Intranasal administration of uPIC100 combined with either a split or subunit antigen of H1N1pdm09 influenza virus induced protective immunity against H1N1pdm virus challenge and elicited specific IgG (in serum) and sIgA (in nasal wash) inductions in BALB/c mouse models (presented by NIIID at the 19th annual meeting of JSV).

Intranasal poly I:C administration can cause olfactory inflammation. It was reported that intranasal administration of poly I:C induced transient inflammatory and degenerative changes in the olfactory area of mice [Kanasra et al, Cell Tissue Res (2014)]. To evaluate the strength of local irritation after intranasal administration of poly I:C, we examined the conditions in Crt:CD rats, using a long-nucleotide length poly I:C (long-PIC).

Single high-dose administration of 250-1000 μg/rat of long-PIC in the left nasal cavity of rats did not induce obvious systemic adverse events in the hematology test. However, submucosal inflammation with inflammatory cell infiltration of the left nasal cavity was observed in a dose-dependent manner, based on histopathological findings on day 1 after administration. This inflammation was quickly reduced at 3 days and nearly disappeared by 7 days after administration. The intensity of inflammation was in accordance with that of hyperplasia of NALT, indicating that inflammation was caused by innate immunity induction from long-term PIC administration. We are currently testing repeated-dose intranasal administration of uPIC100 under the same conditions, which will be reported at this conference.

P64 Mucosal adjuvant SF-10 mimicking human pulmonary surfactant induces CD8+ T cell response with formation of potent effector and central memory

Hyejin Kim

We previously reported that intranasal administration of a synthetic human pulmonary surfactant (SSF) with a carboxy vinyl polymer (CVP) as a viscosity improver, renamed SF-10, shows a potent mucosal adjuvanticity. SF-10 induces both systemic and mucosal adjuvanticity with balanced IgG1 and IgG2a responses in sera and Th1 and Th2 type cytokines in nasal lymphocytes in mice suggesting the involvement of cell-mediated immune response by SF-10. In this study, we assessed whether influenza virus antigen combined with SF-10 induces CD8+ T cell-mediated protection against influenza virus. We initially evaluated the effects of antigen combined with SSF on cross-presentation in bone marrow derived-dendritic cells (BMDCs). Administration of OVA-SSF up-regulated TAP expression in BMDCs, and the stimulated BMDCs by OVA-SSF increased an activation of OT-1 CD8+ T cells in a dose-dependent manner. Intranasal administration of OVA-SF-10 in C57BL/6 mice also induced CD8+ T cell proliferation and their activation significantly in cervical lymph nodes. Indeed, many antigen-specific CTLs (Tetramer+CD8+T cells) were detected in C57BL/6 mice after intranasal vaccination of OVA-SF-10. Similar findings were detected in mice administrated with hemaggulitin (HA)-SF-10. Furthermore, we found up-regulated granzyme B expression in CD8+ T cells in the splenocytes of Balb/c mice vaccinated intranasally with HA-SF-10 and their high cytotoxicity against target cells pulsed with HA peptide dose-dependently. Frequencies of HA-specific effector (CD44highCD62Llow) and central (CD44highCD62Lhigh) memory CD8+ T cells were increased significantly by vaccination with HA-SF-10 compared with the treatment with HA alone at 45 days after the final vaccination and these memory sustained in high level even after 6 months.

These results suggested that SF-10 adjuvant promotes activation of CD8+ T cell via cross-presentation, and induces cell-mediated immune responses for protection against influenza virus infection with relatively high formation of the effector and central memory CD8+T cells.
P65 MDCK Proliferation and Flu Production in Suspension Culture on Various Microcarriers

Name: Jerome Jacques
Media Research & Technology, Lonza Walkersville, Inc, 8830 Biggs Ford Road, Walkersville, MD, 21793, USA

Cell-based influenza vaccine production is quickly growing in the vaccine industry to meet the threat of pandemic outbreaks and to eliminate health concerns associated with egg protein allergies. Madin Darby Canine Kidney (MDCK) is a commonly used cell line for production of influenza virus and the cell-based manufacture of inactivated flu vaccines. Expansion of MDCK cells allows for rapid response and quick scale up compared to the traditional egg based vaccine manufacturing process. This study examines the feasibility of using microcarrier-based cell culture for the expansion of MDCK cells in 3D. Several media were compared for their ability to support MDCK cell growth in planar and suspension culture as well as their ability to support flu virus production from MDCK cells. ProMDCK (2D) and ProMDCK (3D) (Lonza) supported excellent cell proliferation and virus production in both planar culture and on multiple types of microcarriers. The preparation and usage of the various microcarriers were based on the manufactures’ recommendations and were evaluated for ease of use, ability to support cell proliferation and virus production, and ability to support MDCK expansion without cell dissociation in disposable culture systems.

P66 Duration of immunity against yellow fever in Brazilian children with a single dose of vaccine in their first two years of life

Tatiana Guimarães de Noronha1, Luiz Antonio Bastos Camacho1, Olinido Assis Martins-Filho1, Ana Carolina Campi-Azevedo1, Sheila Maria Barbosa de Lima1, Marcos da Silva Freire1, Reinaldo de Menezes Martins1, José Geraldo Leite Ribeiro2, Jandira Aparecida Campos Lemos2, Maria de Lourdes de Sousa Maia1
Oswaldo Cruz Foundation
State Department of Health of Minas Gerais

Yellow fever (YF) is an acute febrile infectious disease caused by a flavivirus, transmitted by mosquitoes. Because of its clinical severity and potential for spread, it is subject to the International Health Regulations. Vaccination is the most important measure for prevention and control of disease in humans. It is recommended by the World Health Organization (WHO) for residents of endemic areas, ideally in routine vaccination of children between 9 and 12 months of age, or in immunization mass campaigns and vaccination of travelers to risk areas. In 2013, the WHO discontinued the recommendation of booster doses every 10 years, indicating a single dose as sufficient for lifelong protection. Considering the reports of sizable proportions of seronegative adults 10 years after one dose, and lack of seroconversion in some children 30-45 days after vaccination, a study was set out to assess the duration of immunity to yellow fever in children vaccinated in the first two years of life. The serologic status (negative, indeterminate and positive), and geometric mean titers (GMT, inverse dilution) of neutralizing antibodies against yellow fever obtained by PRNT method (Plaque Reduction Neutralization Test), was assessed across categories of time after yellow fever vaccination. The strength of association of seropositivity with time was assessed by the odds ratio (OR) taking recent vaccination (1-6 months) as reference. A total of 824 children from a metropolitan area in Southeast Brazil, with date of yellow fever vaccine documented in vaccination cards were tested. A decreasing trend in the proportion of seropositivity (SP), and geometric mean antibody titers was inversely associated to time since vaccination. The level of immunity dropped markedly across time periods: from 88.7% seropositive (GMT: 47.9; 38.3-59.9) in newly vaccinated to 42.2% seropositive (GMT: 8.6; 7.1-12.1) in the subgroup vaccinated 73-100 months before. These data reinforce the need for revaccination of children living in areas with yellow fever virus circulation reported in humans or in other primates. The data also supported the change of a booster dose to 4 years of age for those primarily vaccinated for yellow fever in the first two years of life.

P67 Clinical protection and sporozoite-inhibitory antibodies against heterologous malaria parasite strains following P. falciparum sporozoite immunization under chemophrophylaxis (CPs) in humans


Department of Medical Microbiology, Radboud university medical center, Nijmegen, The Netherlands * Current institution: Innatoss Laboratories, Oss, The Netherlands
† These authors contributed equally to this work

Long-lasting and sterile homologous protection against the malaria parasite Plasmodium falciparum can be achieved by immunization of malaria-naive human volunteers under chemophrophylaxis with P1N54 sporozoites delivered by mosquito bites (ChemProphylaxis with Sporozoites; CPS-immunization). In this study, we explored whether CPS-immunization induces protection from challenge with heterologous P. falciparum clones; 24 volunteers were CPS-immunized and subjected to challenge infection with either a homologous (P1N54) or a genetically distinct heterologous parasite strain (P1N153 or P1N166). While volunteers were completely protected against challenge with P1N54, protection against P1N153 or P1N166 infection was only observed in 2/10 and 1/9 CPS-immunized volunteers, respectively, with another 6/10 volunteers challenged with P1N153 experiencing delayed parasitemia. CPS P1N54-induced antibodies possessed cross-strain functionality, but were less capable to inhibit heterologous P1N153 or P1N166 sporozoites to infect fresh primary hepatocytes in vitro than homologous P1N54 sporozoites. The combined data demonstrate that CPS-immunization induces better homologous than heterologous protection as reflected by functional antibody activity in vitro.

P68 A Tetravalent Sub-unit Dengue Virus Vaccine Formulated with Ionizable Cationic Lipid Nanoparticle induces Significant Immune Responses in Rodents and Non-Human Primates

Gokul Swaminathan1, Elizabeth A. Thorlый1, Kara S. Cox1, Jeffrey S. Smith2, Jayanthi J. Wolf3, Marian E. Gindy2, Danilo R. Casimiro1 and Andrew J. Bett*1
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Dengue virus has emerged as an important arboviral infection worldwide. As a complex pathogen, with four distinct serotypes, the development of a successful Dengue virus vaccine has proven to be challenging. Here, we describe a novel Dengue vaccine candidate that contains truncated, recombinant, Dengue virus envelope protein from all four Dengue virus serotypes (DEN-80E) formulated with ionizable cationic lipid nanoparticles (LNPs). Immunization studies in mice, Guinea pigs, and in Rhesus macaques, revealed that LNPs induced high titters of Dengue virus neutralizing antibodies, with or without co-administration or encapsulation of a Toll-like Receptor 9 agonist. Importantly, LNPs were also able to boost DEN-80E specific CD4+ and CD8+ T cell responses. Cytokine and chemokine profiling revealed that LNPs induced strong chemokine responses without significant induction of inflammatory cytokines. In addition to being highly efficacious, the vaccine formulation proved to be well-tolerated, demonstrating no elevation in any of the safety parameters evaluated. Notably, reduction in cationic lipid content of the nanoparticle dramatically reduced the LNP’s ability to boost DEN-80E specific immune responses, highlighting the crucial role for the charge of the LNP. Overall, our novel studies, across multiple species, reveal a promising tetravalent Dengue virus sub-unit vaccine candidate.
P69 Infection-permissive immunity against influenza virus provided by vaccination prevents loss of alveolar macrophages and affects virus-induced cross-reactive cellular immune responses during subsequent influenza infections.

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Background:

Conventional influenza vaccines aim at the induction of virus-neutralizing antibodies. This requires vaccine efficiencies that are not always reported for vaccines. Moreover, virus neutralization by antibodies is limited in time due to antigenic drift or shift or because antibody levels diminish. We wondered to what extent infection-permissive immunity provided by a classical influenza virus vaccine could modulate disease and virus-induced immune responses in the absence of neutralizing antibodies. We first focused on alveolar macrophages (AM), innate immune cells that are transiently lost during influenza infection in the mouse-influenza challenge model. Later we focused on how vaccine-induced infection-permissive immunity affects induction of cross-reactive CD8+ T cells by virus infection, as well as their T cell receptor (TCR) repertoire.

Methods:

C57 bl6 or Balb/c mice were vaccinated intramuscularly with trivalent inactivated virus vaccine (TIV, equivalent of 3ug HA). Three weeks after immunization, mice were challenged sublethally with homologous H1N1 virus. Lung virus titers were quantified at 3dpi and 7dpi. At 7dpi, we also quantified alveolar macrophages in vaccinated and control-vaccinated animals. For rechallenge experiments, mice were infected with H3N2 virus four weeks after primary challenge. Cross-reactive CD8+ T cell responses directed against the influenza nucleoprotein were measured at different days post primary and secondary infection. The effect of vaccination on the TCR Vbeta-region bias of D6NP366-specific CD8+ T cells in lung tissue and blood was investigated at 8 days post secondary infection.

Results:

TIV vaccination did not result in detectable HI titers and did not prevent morbidity, however, it correlated with lower viral lung titers and faster recovery after homologous challenge. Alveolar macrophages were completely abolished at 7dpi in negative control mice, but not in TIV-vaccinated mice. TIV vaccination still allowed the induction but also affected levels of NP-specific CD8+ T cell responses, as well as the TCR Vbeta-region bias after secondary challenge with heterosubtypic virus.

Conclusion:

Suboptimal TIV vaccination cannot prevent morbidity but results in modulation of disease and host responses after homologous infection. Infection-permissive immunity provided by suboptimal TIV vaccination still allows the induction of cross-reactive T cell responses upon virus infection, which correlates with protection against heterosubtypic virus. These results suggest that suboptimal vaccination with conventional influenza vaccines may still positively modulate disease outcome, thereby still allowing induction of heterosubtypic immunity by virus infection.

P70 Longitudinal Antibody Repertoire Analysis of a Patient Immunized with an MF59™-adjuvanted Pandemic 2009 H1N1 Vaccine


Since its emergence as a pandemic strain in 2009, A/California/07/09 (and antigenically similar strains) has accounted for the H1N1 component of seasonal influenza vaccines. At its height, estimates suggested that one in five people worldwide were infected with this flu strain, due in large part to its dissimilar antigenicity to the concurrent and pre-pandemic circulating H1N1. Thus, much of the immunity that developed as the result of either infection or vaccination would theoretically be de novo. In 2010, a clinical study was conducted where seven adult patients received a single dose of inactivated, monovalent subunit vaccine adjuvanted with MF59™. The antigen used was generated from the candidate vaccine virus X-181, the egg-derived H1N1 high-growth reassortant derived from A/California/07/09. Sera and cells were collected prior to immunization (Day 0) and at Days 8, 22, and 202 post-vaccination. Memory B cells (MBCs) from the samples taken on Days 0, 22, and 202 were sorted for binding to hemagglutinin (HA), a glycoprotein found on the surface of influenza virus particles which mediates cell entry. Samples taken at Day 8 were sorted for plasmablasts, all of which were harvested. This study structure provided a unique opportunity to trace the patients’ antibody and serological responses to a mostly novel antigen in real time, to assess for the presence of broadly protective antibodies, and to determine which responses were converted into long-term memory. Here, we provide an in-depth analysis of the longitudinal antibody repertoire of a single patient from that study.

In total, 29 MBC samples were isolated from Day 0; 217 plasmablasts from Day 8; 63 MBCs from Day 22; and 128 MBCs from Day 202.
**P71 Delaying apoptosis enhances immunogenicity of MVA-based vaccinations**

Lynette S. Chea1, Linda S. Wyatt2, Bernard Moss2, Rama R. Amara1

1Emory Vaccine Center, Department of Microbiology and Immunology, and Yerkes National Primate Research Center, Emory University, Atlanta, Georgia, USA; 2Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Background: The development of an HIV vaccine to protect from infection is critical to prevent the spread of HIV/AIDS. Modified vaccinia Ankara (MVA) is an immunogenic, attenuated poxvirus being developed as a viral vector for multiple vaccines. However, MVA-infected cells undergo rapid apoptosis leading to faster clearance of antigens. Here, we introduced the anti-apoptotic gene, B13R, into MVA and tested its effects on antigen persistence and immunogenicity of MVA.

Materials & Methods: Human, NHP, and mouse cells were infected with recombinant MVA or MVA-B13R expressing SIV Gag Pol and HIV clade C Env (SHIV), and monitored for caspase 3 activation and cell membrane permeability as markers of apoptosis progression. To determine immunogenicity, mice were immunized intramuscularly with MVA/SHIV and MVA-B13R/SHIV and SHIV-specific cellular and humoral responses were characterized.

Results: MVA-B13R infected Hela cells were protected from chemically induced apoptosis better than WT MVA confirming functionality of B13R (p<0.05). To understand how MVA-B13R behaves in other species, human and NHP peripheral blood mononuclear cells and splenocytes from mice were infected. MVA-B13R/SHIV infected monocytes from human, NHP, and mice had less cells in the late apoptotic stage compared to MVA/SHIV infected cells; NHP B cells and CD8+ T cells also exhibited a similar trend. Following immunization of BALB/c mice, we observed 2- to 4-fold higher Env gp140-specific serum antibodies in mice immunized with MVA-B13R/SHIV compared to MVA/SHIV at the peak of response (p<0.05). The higher antibody titers were associated with higher Env-specific CD4+ T cell responses.

Conclusions: These results demonstrate that MVA-B13R infected cells show delayed apoptosis compared to MVA and this effect can lead to augmented HIV Env antibody responses in mice. The preliminary immunogenicity data suggests that delayed apoptosis contributes to the enhanced immunogenicity of MVA-B13R. Preclinical NHP studies will elucidate the potential of MVA-B13R vector as an HIV vaccine candidate.

**P71 (Continued)**

**P72 The causative agent of a new emerging infection of small ruminants - Hobi like virus can be spread with the live vaccine against Peste des Petits Ruminants (PPRV).**

Anoyatbekova A.M., Alexeyenkova S. V., Dias Himenes K. A., Yurov K. P.

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Introduction: Currently, spreading of an emerging infection becomes permanent. To a great extent it is promoted by the expanding international economic relations. This fact encourages veterinary service of Tajikistan to tighten the control program aimed at preventing a number of infectious diseases, including PPRV of small ruminants. PPRV was first described in 1942 in the Ivory Coast, West Africa.

In countries which are not free from PPRV vaccine prevention is an essential component of control program. However, in some cases, the vaccination does not provide sufficient immune protection of sheep and goats against infection. In this regard there was a necessity to study the characteristic of the vaccines used in Tajikistan for vaccination of small ruminants against PPRV, using genetic methods.

Materials and methods

Vaccines. Vaccines were obtained from commercial firms.

RT-PCR and nucleotide sequences. RT-PCR for the identification of genes encoding polypeptides N and F of PPRV of small ruminants; RT-PCR for identification of non-structural polypeptide NS3 genes of BVDV performed in accordance with the recommendations of the OIE Terrestrial Manual, 2013. Sequencing PCR products were carried out on the Genetic Analyzer (Applied Biosystems) in “Syntol” company.

Phylogenetic analysis. Partial F gene sequences of PPRV and NS3 genes of BVDV were aligned by using the “FASTA3” (European Bioinformatics Institute) with the referent strains from the database INSAD. Clustering pattern was determined by neighbor-joining method using the MEGA 6.0 program.

Results and discussion

When using primers flanking the polypeptide fragment F gene found out PPR virus DNA specific fragment of 371 bp. Furthermore, electrophoresis showed a positive result of amplification with primers flanking the gene fragment of the non-structural polypeptide NS3 of BVDV.

In order to clarify the results, we have performed the determination of the nucleotide sequences. Comparative analysis with the computer program «FASTA3» nucleotide sequences of the BVDV revealed the common origin of the strain with the BVDV genotype 3 (Hobi-like virus), registered in the INSD.

Thus, in commercial vaccine against PPRV revealed BVDV genotype 3- Hobi like virus. Hobi-like pestivirus is a new pestivirus, which was detected in a batch of commercial fetal bovine serum of Brazilian origin.

Conclusion

It has been found out a new pestivirus BVDV genotype 3 – Hobi like virus as a contaminant of live vaccines which might give unsatisfactory results of immunization of sheep and goats against PPRV.
Zika virus (ZIKV) is an emerging *Aedes* mosquito-borne Flavivirus that was first identified in Uganda in 1947. The symptoms of ZIKV disease include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild and short in duration. However, in recent outbreaks, ZIKV has been shown to be associated with clusters of microcephaly and other neurological disorders, including Guillain-Barré syndrome. Zika virus can spread from a pregnant woman to her fetus and has been isolated from the brain of a fetus with central nervous system abnormalities. In a study of pregnant women in Brazil, fetal abnormalities were detected by Doppler ultrasonography in 29% of the ZIKV-positive women and in none of the ZIKV-negative women. No vaccine currently exists that can control the spread of ZIKV disease. Therefore, development of an effective vaccine is urgently needed. Antibodies against the envelope (E) protein of other Flaviviruses such as West Nile (WNV), Japanese Encephalitis (JEV) and Dengue have shown to be effective in neutralizing those viruses and confer protection against challenge infection. The E protein is therefore a prime vaccine candidate for ZIKV. We have previously developed vaccine candidates for WNV and JEV based on recombinant baculoviruses expressing a truncated form of the WNV or JEV E proteins. To rapidly develop an effective ZIKV vaccine, we have designed E protein with deletions of stem and transmembrane domains termed as E\text{DST}. developed a scalable process, produced a GMP batch, and characterized the E\text{DST} protein using a battery of biophysical, biochemical and immunological assays. In addition, we have developed alum formulations for E\text{DST} and initiated an immunogenicity study in out-bred Swiss Albino mice with and without alum to evaluate the ability of E\text{DST} protein to induce neutralizing antibody responses. We will present the purification, characterization and immunogenicity results obtained for the vaccine candidate (E\text{DST} protein) against the ZIKA virus.
The International Society for Vaccines is an organization of professionals in the diverse disciplines of vaccinology. It engages, supports and sustains its membership through education, communication, and public information with the goal to advance human and animal health through immunization science and vaccination.

Founded in 1994
Re-organized in 2008

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# 2016 International Society for Vaccines Annual Congress

## Sunday, 2 October 2016

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<td>9:30-10:00</td>
<td>Coffee Social  <em>(Foyer of Grand Ballroom)</em></td>
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| 9:55-10:00 | Opening Remarks  *(Grand Ballroom)*  
ISV Congress Co-chairs: Margaret Liu, Frédéric Tangy, David Weiner, Anne De Groot |
| 10:00-12:15 | Plenary Session One: Vaccine Challenges  
Panel: Challenges of Making Emerging Diseases Vaccines  
Session Chairs: Shan Lu and Margaret Liu |
| 12:15-13:30 | Lunch  *(Harbor View Ballroom and Commonwealth Ballroom)*          |
| 13:30-15:30 | Concurrent Session 1: Mechanisms of Immune Efficacy  
Session Chairs: Nik Petrovsky and Peter Nara  
Concurrent Session 2: DNA Vaccines  
Session Chair: David Weiner  
Concurrent Session 3: Zoonoses and Animal Models for Evaluating Vaccine Protection  
Session Chairs: Cyril Gay and Bin Wang |
| 15:30-16:00 | Coffee Break  *(Foyer of Grand Ballroom)*                      |
| 16:00-18:00 | Plenary Session 2: Insights into the Immunology of Vaccines and Immunotherapy  
Session Chairs: Adolfo Garcia-Sastre and Anna-Lise Williamson |
| 18:15-20:00 | Poster Session and Reception  *(Palm Garden)*                      |

## Monday, 3 October 2016

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| 8:00-10:00 | Plenary Session 3: Global Health Issues and Vaccine Approaches  
Session Chairs: William Jacobs and Yvonne Maldonado  
Keynote Speaker: Victor Dzau, Biosecurity and Winning the War against Future Epidemics |
| 10:00-10:30 | Coffee Break  *(Foyer of Grand Ballroom)*                      |
| 10:30-12:45 | Concurrent Session 4: Institut Pasteur Special Session  
Session Chairs: Christiane Gerke and Shan Lu  
Concurrent Session 5: Vaccines and Biologicals: Production and Efficacy  
Session Chairs: Phil Krause and Britta Wahren  
Concurrent Session 6: JSV/KVS: Adjuvant and Immunomodularity Principles for Mucosal vs. Systemic Immunity  
Session Chairs: Hiroshi Kiyono and Baik Lin Seong |
| 12:45-13:45 | Lunch  *(Harbor View Ballroom and Commonwealth Ballroom)*          |
| 13:00-14:00 | Poster Session 2  *(Palm Garden)*                                |
| 14:00-14:45 | ISV Annual Meeting  *(Grand Ballroom)*                           |
| 14:45-15:15 | Coffee Break  *(Foyer of Grand Ballroom)*                        |
| 15:15-17:30 | Plenary Session 4: Immunization of Individuals in Special Physiological Circumstances  
Session Chairs: Sam Katz and Jeffrey Ulmer |
| 19:00-22:00 | ISV Congress Dinner (tickets required)  *(New England Aquarium)*   |

## Tuesday, 4 October 2016

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| 8:00-10:00 | Plenary Session 5: Insights into Vaccine Targets and Effective Immune Responses  
Session Chairs: Flor Munoz and Marie-Paule Kieny |
| 10:00-10:30 | Coffee Break  *(Foyer of Grand Ballroom)*                        |
| 10:30-12:45 | Concurrent Session 7: New and Transformed Vaccine Technologies (KVS/JSV session)  
Session Chairs: Joon Rhee and Julia Lynch  
Concurrent Session 8: Emerging and Re-emerging Diseases; VaxRen Session  
Session Chairs: Anne De Groot and Connie Schmaljohn  
Concurrent Session 9: Vaccines Against Respiratory Pathogens  
Session Chairs: Ted Ross and Linda Klavinsks |
| 12:45-13:45 | Lunch  *(Harbor View Ballroom and Commonwealth Ballroom)*          |
| 13:45-14:00 | ISV Award Ceremony  *(Grand Ballroom)*                           |
| 14:00-16:00 | Plenary Session 6: One Health and Emerging Diseases  
Session Chairs: Frédéric Tangy and Margaret Liu |
| 16:00-16:15 | Closing Remarks and Introduction of 2017 ISV Annual Congress  *(Grand Ballroom)*   |