

# Recombinant vaccines

## Strategies for candidate discovery and vaccine delivery

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: March 12<sup>th</sup> 2010

As both genomic and post-genomic analyses of pathogens and disease has advanced the production of successful recombinant vaccines we now require the strategies to both \* select appropriate molecules and \*deliver these vaccines effectively to stimulate protective immune responses.

Both candidate discovery and the tools for delivering vaccines effectively will be explored and discussed at this one day event

This meeting has CPD accreditation

- 9:00 – 9:45      **Registration**
- 9:45 – 10:00    **Introduction by the Chair:** *Sarah C. Gilbert*, Reader in Vaccinology, Jenner Institute, Oxford
- 10:00- 10:30    **Overview of candidate selection, vaccine manufacturing and delivery**  
*Dr David Simpson*, Process Development Manager, Eden Biodesign, Liverpool, UK  
This presentation will provide an overview of vaccine candidate selection, characterisation and in particular the difficulties encountered during early stage development in defining meaningful indicators of function. Indeed, vaccines by nature are often complex which has required the development of both novel analytical techniques and orthogonal approaches for their characterisation. However, our increased knowledge of closely related vaccine products has facilitated the development of combined platform production processes and appropriate analytical methodologies which can be deployed to expedite the manufacture of this often critical therapeutic product class.
- 10:30 – 11:00    **A flexible Stabilisation Technology for use with Vaccines and Proteins**  
*Dr Stephen Ward*, Development Director at Stabilitech, UK  
Improved stability against temperature excursions as applied to vaccines and biopharmaceuticals would have a significant and obvious impact upon temperature-controlled distribution. Stabilitech Ltd has overcome thermostability issues for a wide range of viruses, vaccines and biopharmaceuticals. A proprietary excipient system shall be discussed which, when mixed with suspensions of target vaccine or protein enhances the stability of the products markedly, and prevents thermal damage both when stored at elevated temperatures or during multiple freeze thaw cycles. The technology can be readily incorporated into cGMP manufacturing processes and uses standard equipment.
- 11:00 – 11:30    **How responses to subunit vaccines against *Salmonella* help dissect T and B cell contributions to protective immunity**  
*Dr. Adriana Flores-Langarica*, University of Birmingham, UK  
Here we show when antibody and T cell responses to subunit vaccines are effective against Salmonella. We show antibody restricts early colonisation and bacteraemia, but these effects are highly antigen-dependent. Inappropriate antibody either has neutral or negative effects on protection. T cell contributions are markedly different. Early after infection T cells do not promote intracellular clearance, but contribute by promoting IgG switching. Later, vaccination enhances Th1 responses and bacterial clearance, despite inducing Th2 priming. Together, this suggests that subunit vaccines should be selected primarily on levels of protective antibody induced and not on their capacity to promote T cell responses.
- 11:30 – 11:35    **Speakers photo**
- 11:40 – 12:00    **Mid-morning break**
- 12:00 – 12:30    **FMD vaccine development**  
*Dr David Paton*, Institute for Animal Health, UK  
Current FMD vaccines are killed whole virus preparations and several billion doses are used annually, mainly in South America and Asia. These vaccines have been very successful in helping to control and eradicate FMD in many parts of the world including Europe. For future emergency use in case of FMD outbreaks in disease-free regions, priority improvements would be in onset rate of protection, DIVA compatibility and safety of production. In the case of FMD control and eradication from endemically affected countries, better vaccine stability and longer duration of

protection are desired, along with low cost of production. Progress in meeting these objectives will be reviewed along with ongoing work to better understand immune responses to FMD infection and vaccination.

12:30– 13:00

### **Methods for delivery of GM vaccines to stimulate appropriate, protective responses**

*Dr David JM Lewis, St George's Hospital, UK*

Genetic Modification offers the potential to rationally design live vaccines that are safe and effective, by attenuating harmful traits in otherwise pathogenic viruses and bacteria, making them safe for delivery to humans and animals. While live vaccines derived from pathogens offer the attractions of inducing robust cellular and humoral immunity that may be long-lasting; and immune responses in mucosal sites of colonisation or invasion; a constant issue is the balance between efficacy (immunogenicity/colonisation) and safety (reactogenicity/invasion). In addition, the use of vector organisms to carry foreign genes offers the potential for multi-valent combination vaccines in one-shot, but here the issues of level of foreign gene expression and levels of immunity induced are encountered. Finally, live vaccines must circumvent or overcome adaptive (specific) and innate (non-specific) immune and anatomical barriers before they can bring about a response. The personal clinical experience with bacterial pathogen GM vaccines gained over 6 clinical trials with *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae* and hybrid strains, will be reviewed to demonstrate these issues, together with practical issues of Phase 1 trial design that can influence safety and efficacy readouts.

13:00 – 14:00

### **Lunch**

14:00 – 14:30

### **Can Mucosal Vaccines make Needles a Thing of the Past?**

*Dr. Valerie Ferro, University of Strathclyde, Scotland*

We have developed a delivery system composed of non-ionic surfactant vesicles and bile salts (bilosomes), which protect entrapped antigen against degradation in the gastrointestinal tract. This delivery system removes the need for live or attenuated vaccines and is ideal for oral delivery of synthetic peptides and recombinant proteins. An IgA response is induced locally, while systemically the immune response can be directed towards a Th1 or Th2 bias by altering the size of the vesicles, irrespective to the pathogenic origin of the antigen.

14:30 – 15:00

### **Recombinant avipoxvirus vaccines**

*Dr Michael A. Skinner, Imperial College London,*

Viruses of the avian poxvirus (avipoxvirus) genus represent a diverse and divergent group of poorly characterised viruses. Borrowing approaches developed for Vaccinia virus, Fowlpox virus (the type species) was developed as a live recombinant vaccine vector for use in poultry. Like the other well-known member of the genus, Canarypox virus, Fowlpox virus only causes disease in birds but it is nevertheless able to infect mammalian cells, express proteins and induce immune responses, both humoral and cellular, which may in some circumstances be protective. Blocked during morphogenesis (or before) in mammalian cells, they have extremely high safety profile as live recombinant vaccine vectors for use in humans and other mammals, as demonstrated in numerous clinical trials (recombinant Canarypox virus, used in prime-boost vaccination with recombinant protein, has recently returned the first indications of partial success in an HIV vaccine trial). Relatively low potency has been addressed by using prime-boost regimes and by co-expression of host cytokines or co-stimulators. New generation vectors may involve deletion of avipoxvirus immunomodulators to improve efficacy safely. The focus of our current work is to identify appropriate immunomodulators for deletion.

15:00 – 15:30

### **Afternoon Tea/Coffee**

15:30 – 15:40

### **Construction and Evaluation of Safe Live Attenuated Cholera Vaccine, VCUSM21P**

*Murugaiyah Chandrika, School of Health Sciences, Universiti Sains Malaysia, Malaysia*

Cholera is a major health issue, affecting millions of lives annually. In light of the recurrent outbreaks of cholera, there is a pressing need for the development of vaccines that allow rapid mass vaccination. In this study, we have designed a genetically modified vaccine candidate, VCUSM21P. VCUSM21P was found not to disassemble the actin of HEp2 cells. Mouse colonization assay was used to determine VCUSM21P colonization ability *in vivo*. Rabbit ileal loop assay was performed to evaluate the reactogenicity caused by it. The immune responses provoked by it and its protective efficacy were evaluated in a rabbit model. VCUSM21P colonized the mice intestine approximately 1 log lower than that of the Wild Type (WT) strain. In the ileal loop assay using non-immunized rabbits, fluid accumulation was found in loops injected with  $1 \times 10^6$  and  $1 \times 10^8$  colony forming unit (CFU) of WT. Unlike the WT challenge,  $1 \times 10^6$  and  $1 \times 10^8$  colony forming unit (CFU) of VCUSM21P did not cause any reactogenicity in non-immunized rabbits. Oral immunization using  $1 \times 10^{10}$  CFU of VCUSM21P induced both IgA and IgG against Cholera Toxin (CT) and O139 lipopolysaccharides (LPS). The serum vibriocidal antibody titer had a peak rise of 2560 fold on week 4. The reactogenicity caused by the WT in rabbits immunized with  $1 \times 10^{10}$  CFU of VCUSM21P was found to be reduced as evidenced by absence of fluid in loops administered with  $1 \times 10^2$ - $1 \times 10^7$  CFU of WT. In the 'Removable Intestinal Tie Adult Rabbit Diarrhoea (RITARD)' experiment, the non-immunized rabbits were found unprotected against a lethal

challenge with  $1 \times 10^9$  CFU WT. However, 100% of immunized rabbits survived the WT challenge during the RITARD experiment exceeding 5 days, without any symptomatic diarrhoea. Immunohistochemical, histopathological and ultrastructural examination of non-immunized rabbits' ileum challenged with WT revealed severe damages involving the microvilli, villi and lamina propria. But less severe damages were noted in the ileum of rabbits immunized with VCUSM1P following the WT challenge. Attenuated VCUSM21P vaccine could be used for vaccination program against potentially fatal endemic or emerging cholera by *V. cholerae* O139.

15:40– 16:10 **Clinical trials of a novel influenza vaccine**

*Sarah C. Gilbert*, Reader in Vaccinology, Jenner Institute, Oxford

Currently licensed influenza vaccines are designed to induce antibodies against the polymorphic external proteins of the virus, chiefly haemagglutinin. They can achieve 80% effectiveness when the vaccine strain and circulating virus are well matched, but substantially less when the circulating virus changes, and are never as effective in those aged 65. Completely new versions are required to protect against possible pandemic viruses. The Jenner Institute is testing a new type of influenza vaccine using the conserved internal proteins of the virus to boost cellular immunity to all subtypes of influenza A with a single vaccine. Cellular immunity is an important component of natural immunity to influenza virus and the clinical trials are designed to examine the safety, immunogenicity and efficacy of this new type of influenza vaccine.

16:10 - 16:40 **Optimizing vaginal responses to HIV vaccines**

*Professor Robin Shattock*, St Georges University of London, UK

16:40 - 17:00 **Chairman's summing up**

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**Facebook** - <http://www.facebook.com/group.php?gid=70847076549>

**Twitter** - <http://twitter.com/Euroscicon/>

About the Chair

After a first degree in Biological Sciences at the University of East Anglia and a PhD in the Biochemistry Department at the University of Hull, **Dr Sarah Gilbert** worked on pentose metabolism in brewing yeasts at the Brewing Research Foundation, then the Leicester Biocentre, followed by four years at Delta Biotechnology in Nottingham, working on production of recombinant human blood proteins in yeast before moving to Adrian Hill's group at the University of Oxford in 1994. Dr Gilbert's chief research interest is the development of vaccines that work by inducing strong and protective T cell responses. Following the discovery that heterologous prime-boost immunisation, in which the same antigen is delivered in first one vector and then another, is highly immunogenic in pre-clinical studies, she designed antigen inserts for, and produced DNA, MVA and fowlpox vaccines which were then manufactured for clinical trials in both the malaria and tuberculosis vaccine clinical programmes, and is now leading the flu vaccine programme.

About the speakers

**Dr. Valerie Ferro**, studied Biochemistry at the University of Aberdeen, then completed PhD at University of St Andrews (1989). Took up position as a Postdoc at the University of Strathclyde working on peptide vaccines. Is currently a lecturer at the Strathclyde Institute of Pharmacy and Biomedical Science. Since 1991 has commercialised a number of vaccines and delivery systems for benefit of both human and animal health, with applications in infectious disease as well as non-infectious conditions.

**Dr David JM Lewis** qualified in Medicine University of Wales 1983, postgraduate medical education in General (Internal) Medicine in S Wales, followed by training in microbiology at London School of Hygiene & Tropical Medicine, and Infectious Diseases at St George's, Tooting. Took up position at St George's 1988, to carry out research on mucosal immunology, vaccines and HIV. Appointed Consultant Physician 1994, Professor of Clinical Vaccinology & Medicine 2006. Specialises in translational immunology and vaccine studies, especially mucosal vaccines; genetically modified live organisms; and vaccines against TB, HIV and enteric infections.

**Dr. Adriana Flores-Langarica** received her PhD. in Immunology by the The National School of Biological Sciences in Mexico in 2005, from where she moved to The Rockefeller University to work in antigen targeting to dendritic cells and adjuvant strategies. She carried on working on DC biology at the University of Oxford for two years. In 2008 she moved to the University of Birmingham to work with Dr. Cunningham studying how Th1 and Th2 responses are induced to subunit vaccines and pathogens.

**Dr David Simpson** is responsible for Eden's process development capabilities, its progression, client programs and technical transfer activities and is supported by a highly experienced team in areas of molecular biology, cell line/strain development, fermentation and purification development. His combination of scientific, product and commercial knowledge has allowed David to drive the development of platform processes that can be rapidly deployed to support pre-clinical and clinical development programs across a range of product classes. Prior to joining Eden, David worked in academia and with numerous biotechnology/pharmaceutical companies to develop client specific expression systems incorporating proprietary technologies for use in clinical development programs.

**Dr Stephen Ward** joined Stabilitech in 2009, bringing over 14 years of complex biological development and vaccine manufacturing experience into the company. He enjoys converting early research ideas into regulatory compliant products for patients. He has developed and validated scaleable, commercial manufacturing processes for recombinant biologicals and cell-based medicines, including international cold chain and clinical supply. Commercial/academic multi-disciplinary programmes to predict the effects of manufacturing stress upon product efficacy have been of recent interest, including companion biomarker identification. Prior to joining Stabilitech, he played a key role in the whole cell immunotherapy vaccine programme at Onyvox, and also worked on the hepatitis immunotherapy delivery platform at Medeva. His PhD and early academic research at St Bart's Hospital Medical School and Imperial College London focused on recombinant vaccine design and mucosal protection using attenuated bacterial delivery systems.

**Dr Michael A. Skinner** leads the Vaccine Vector Group in the Department of Virology (Faculty of Medicine) at Imperial College London (St Mary's Campus). The group is currently working on two BBSRC-funded, inter-related topics: (i) avian innate immunity, in particular the antiviral type I interferon system, and viral mechanisms to modulate the host responses (BBSRC-funded, in collaboration with Prof. Steve Goodbourn, St George's University of London), and (ii) modified, improved recombinant Fowlpox virus FP9 vaccine vectors, to be tested in poultry (against avian influenza H5N1) and hopefully in mammals. Scientific background initially in the molecular biology of human viral pathogens, specifically: HIV (tat/TAR interactions, at the MRC Laboratory of Molecular Biology, Cambridge), poliovirus (analysis of the role of secondary structure of 5' non-coding RNA in neurovirulence and vaccine attenuation, with Prof Jeff Almond, in Leicester then Reading), coronaviruses, in the pre-SARS era (with Prof Stuart Siddell, then in Würzburg, Germany). Extended interest to veterinary viruses at the Institute for Animal Health (IAH), studying avian poxviruses (their molecular biology & as candidate recombinant vaccine vectors) also economically important emerging pathogens of poultry, specifically: a novel retrovirus (Avian leukosis virus subgroup J) a birnavirus ('very virulent' Infectious bursal disease virus), with funding from the BBSRC, the EC, the British Egg Marketing Board Research and Education Trust and Oxxon Pharmaccines. At IAH also collaborated with Prof Adrian Hill & Dr Sarah Gilbert (University of Oxford) on the use of FP9 as a recombinant vector for malaria vaccination in humans.

**David Paton** is a veterinarian who graduated from Cambridge University in 1984 and after three years working in veterinary practice in UK and Australia has spent the last 23 years as a veterinary virologist specialising in the epidemiology, diagnosis and control of various viral livestock diseases. In 2001, he joined the Institute for Animal Health (IAH) as Head of the Department for vesicular disease control that includes the foot-and-mouth disease (FMD) world reference laboratory. In 2007 he was appointed Head of the FMD Programme at IAH.

*This meeting was **organised by Euroscicon** ([www.euroscicon.com](http://www.euroscicon.com)), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by '**BioPark**' ([www.biopark.co.uk](http://www.biopark.co.uk)), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

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**Construction and Evaluation of Safe Live Attenuated Cholera Vaccine, VCUSM21P**

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Cholera is a major health issue, affecting millions of lives annually. In light of the recurrent outbreaks of cholera, there is a pressing need for the development of vaccines that allow rapid mass vaccination. In this study, we have designed a genetically modified vaccine candidate, VCUSM21P. VCUSM21P was found not to disassemble the actin of HEp2 cells. Mouse colonization assay was used to determine VCUSM21P colonization ability *in vivo*. Rabbit ileal loop assay was performed to evaluate the reactogenicity caused by it. The immune responses provoked by it and its protective efficacy were evaluated in a rabbit model. VCUSM21P colonized the mice intestine approximately 1 log lower than that of the Wild Type (WT) strain. In the ileal loop assay using non-immunized rabbits, fluid accumulation was found in loops injected with  $1 \times 10^6$  and  $1 \times 10^8$  colony forming unit (CFU) of WT. Unlike the WT challenge,  $1 \times 10^6$  and  $1 \times 10^8$  colony forming unit (CFU) of VCUSM21P did not cause any reactogenicity in non-immunized rabbits. Oral immunization using  $1 \times 10^{10}$  CFU of VCUSM21P induced both IgA and IgG against Cholera Toxin (CT) and O139 lipopolysaccharides (LPS). The serum vibriocidal antibody titer had a peak rise of 2560 fold on week 4. The reactogenicity caused by the WT in rabbits immunized with  $1 \times 10^{10}$  CFU of VCUSM21P was found to be reduced as evidenced by absence of fluid in loops administered with  $1 \times 10^2$ - $1 \times 10^7$  CFU of WT. In the 'Removable Intestinal Tie Adult Rabbit Diarrhoea (RITARD)' experiment, the non-immunized rabbits were found unprotected against a lethal challenge with  $1 \times 10^9$  CFU WT. However, 100% of immunized rabbits survived the WT challenge during the RITARD experiment exceeding 5 days, without any symptomatic diarrhoea. Immunohistochemical, histopathological and ultrastructural examination of non-immunized rabbits' ileum challenged with WT revealed severe damages involving the microvilli, villi and lamina propria. But less severe damages were noted in the ileum of rabbits immunized with VCUSM1P following the WT challenge. Attenuated VCUSM21P vaccine could be used for vaccination program against potentially fatal endemic or emerging cholera by *V. cholerae* O139.