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MEETING REPORT

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*N. Petrovsky*

Department of Endocrinology, Finders Medical Centre, Bedford Park, Australia

**CONTENTS**

MVA vaccines from EBS ..... 801
An update on projects from NasVax ..... 801
A novel human papillomavirus (HPV) vaccine ..... 802
An update on NASVAC ..... 802
The first hepatitis E vaccine – HEV-239 ..... 802
Vaxine's Advax™ adjuvant ..... 803
Vaccines in India ..... 803
A malaria vaccine from BIKEN ..... 803
*Helicobacter pylori* as a vaccine delivery vector ..... 804
REGiMMUNE's ReVax technology ..... 804

## MVA VACCINES FROM EBS

Stephen Lockhart from Emergent Biosolutions (EBS) introduced the company and discussed modified vaccinia Ankara (MVA) vector-based vaccines in development. EBS was founded in 1998 to acquire a derelict anthrax vaccine plant and its current major product is an old-fashioned anthrax vaccine, which is sold to the U.S. Government, with 2010 revenues of US $286 million and a market capitalization of approximately $600 million. EBS has subsidiaries in Singapore, the U.K. and Germany; it acquired the German company ViVacs in 2006, which provided access to the MVA pipeline. The company is also developing monoclonal therapeutics for cancer and autoimmunity. The MVA virus was isolated in Turkey in the 1950s and has been passaged 570 times in primary chicken embryo fibroblasts, resulting in a 15% loss of its genome and causing it to be incapable of replicating in mammalian cells. The MVA strain licensed by EBS was used in Germany as a licensed prevaccine for smallpox and was administered to approximately 120,000 people as a predose prior to their immunization with vaccinia. The first recombinant MVA virus was described in 1992 and EBS is now attempting to develop this platform as a way of delivering a variety of different vaccine antigens. The MVA virus expresses early and late proteins, including any recombinant proteins added to its genome, but then fails to repackage in mammalian cells. Homologous boosting with MVA favors an antibody response, whereas a heterologous boost favors a T-cell response. MVA has been studied as a delivery vehicle for antigens from tuberculosis (TB), influenza, malaria, HIV, hepatitis C virus (HCV), respiratory syncytial virus (RSV), measles, anthrax, plague, Epstein-Barr virus (EBV) and oncology antigens. The manufacturing of MVA requires an avian cell substrate and is currently performed using primary chicken embryo fibroblasts, which is extremely expensive; thus, EBS is currently exploring continuous avian cell lines as an alternative for MVA production. The viral vector MVAtor is an isolate of MVA, the genome of which has been fully sequenced.

EBS has entered into a collaboration with Oxford University, known as the Oxford Emergent Tuberculosis Consortium, formed in 2008, to further develop a TB vaccine, the MVA-85A vaccine (Oxford Emergent Tuberculosis Consortium/TB-VAC), which was prepared at Oxford University and is designed to boost T-cell responses in individuals primed with Bacillus Calmette-Guérin (BCG) or by prior infection with TB. A phase IIb study in infants was undertaken in 2009 and a phase IIb study of MVA-85A was due to commence in HIV-infected adults in July 2011, with funding from a range of foundations, including the Wellcome Trust, Aeras Global TB Vaccine Foundation and the Bill & Melinda Gates Foundation. In an individual primed with BCG, the MVA-85A vaccine induced up to 2,000 interferon gamma (IFN-γ) ELISPOTs per million cells as a peak response, with approximately 500 ELISPOTs long term; however, it is still not known whether these correlate with protection. The safety of the MVA vaccine has been tested in people with latent TB, and there is no evidence that it reactivates latent TB or increases the progression of HIV in HIV-positive individuals.

EBS's MVA vector has also been used to deliver conserved influenza antigens as part of a new joint venture, EPIC Bio, formed between EBS and Temasek Life Sciences Ventures in Singapore. This joint venture will exploit intellectual property developed by Temasek that has identified protective epitopes from the influenza virus. Due to constraint variability, a trivalent vaccine has demonstrated the potential to cover 95% of strains of pandemic influenza viruses. Other projects that the joint venture is undertaking include vaccines based on the MVA platform against enterovirus 71, dengue virus and EBV infections.

## AN UPDATE ON PROJECTS FROM NASVAX

Data on NasVax's oral anti-CD3 antibody vaccine for autoimmune and inflammatory disease were presented by Rom Eliaz. An oral anti-CD3 antibody was initially licensed from Centocor Ortho Biotech (part of Johnson & Johnson) that had been investigated in

**Correspondence:** Nikolai Petrovsky, Department of Endocrinology, Finders Medical Centre, Bedford Park 5042, Australia. E-mail: nikolai.petrovsky@flinders.edu; nikolai.petrovsky@flinders.edu.au.

phase I and II clinical trials. The phase I study was undertaken in 18 healthy subjects who were given 5 daily oral doses of anti-CD3, which was shown to be safe. Evidence suggested that regulatory T-cell markers, including transforming growth factor-β (TGF-β), were increased, and this led to a phase IIa study performed at the Hadassah Medical Centre in Israel in patients (N = 36) with non-alcoholic steatohepatitis (NASH), who received the OKT3 murine anti-CD3 antibody (0.2, 1 or 5 mg) for 30 days. Evidence of reduction in liver function tests, glucose tolerance and triglyceride levels was observed in nine individuals; however, no weight data were presented, suggesting that the active anti-CD3 therapy may have caused nausea and weight loss, and thus, an apparent improvement in metabolic syndrome parameters. However, Johnson & Johnson stopped production of the OKT3 antibody, and NasVax is making other arrangements to develop a fully humanized antibody against anti-CD3 which can then be taken back into clinical studies in patients with NASH to confirm the original findings.

NasVax has also been developing a universal protein vaccine against pneumococci. A proteomics approach was used to narrow down an initial 140 candidates to 7 protein targets, which have been incorporated into a chimeric fusion protein. In 2008, NasVax acquired Protea to gain access to their pneumococcal vaccine intellectual property, and in 2009, GlaxoSmithKline (GSK) was granted an option to obtain an exclusive license for this project. NasVax has received 2.4 million euros (approx. US $3.5 million) from GSK for an option fee over the pneumococcal project, with milestone payments of 17 million euros (approx. US $24.7 million) and further royalties on product sales. Other NasVax projects include the MAb BBS-1 for the treatment of Alzheimer's disease, which is in preclinical development, and a liposomal vaccine adjuvant, VaxiSome, which is in a nonexclusive license agreement with Novartis.

## A NOVEL HUMAN PAPILLOMAVIRUS (HPV) VACCINE

Pele Chong (National Health Research Institutes) presented data on current vaccine programs. The Taiwanese Government has asked the Institutes to develop an HPV vaccine, ideally a therapeutic vaccine that would work in patients with established infection. The Institutes' strategy involves a lipoprotein vaccine based on the late-expressed HPV antigens E6 and E7, which are tumor-associated antigens, and includes the production of lipoproteins that are able to bind to Toll-like receptors (TLRs), thereby enhancing vaccine immunogenicity. The lipoproteins are expressed in *Escherichia coli*, but these generally provide low yields and incomplete lipid modification. A unique *E. coli* strain, C43 (DE3), which provides higher yields of lipoprotein, and a leader sequence from antigen 473 (AG473) from *Neisseria meningitidis* that contains a motif for lipidation have been identified. A minimal sequence of 20 amino acids of AG473 is required for lipidation, and thus the Institutes has taken out a patent on this sequence. Further patents on the purification of lipoproteins have been filed. The lead product, rlipoE7m, has been shown to include both diacyl and triacyl lipoproteins, and when incubated with dendritic cells, induced upregulation of costimulatory molecules, including CD40, CD86 and IL-12. In human leukocyte antigen (HLA) transgenic mice, the rlipoE7m lipoprotein induced IFN-γ ELISPOTs against E7 protein and these responses were not seen in *TLR2* knockout mice, which is consistent with the lipoproteins signaling via Toll-like receptor 2. The rlipoE7m protein inhibited the growth of cervical cancers in a mouse model, whereas the unlipidated E7 protein was not effective. The rlipoE7m vaccine was also able to protect prophylactically against tumor development.

## AN UPDATE ON NASVAC

Data on the therapeutic hepatitis B virus (HBV) vaccine candidate NASVAC (NASTERAP), under development by the Center for Genetic Engineering and Biotechnology (CIGB), were presented by Gerardo Guillen. Historically, GSK developed a therapeutic vaccine based on the HBV surface antigen and ASO2 adjuvant; however, despite this vaccine being able to induce antibodies against the surface antigen, it was unable to control the virus. In work performed at CIGB in collaboration with researchers at Vaxine, a vaccine based on a combination of HBV surface and core antigens given both intramuscularly and intranasally was tested in mice and was shown to be highly immunogenic and capable of inducing a high number of IFN-γ ELISPOTs. This research then led to the development of NASVAC, which was shown to induce a strong response to HBV core antigen, with no evidence of any induction of liver damage, even after 10 immunizations, when tested in HBV surface antigen transgenic mice. These data alleviated fears that the vaccine may trigger liver damage in infected subjects, and thus, a phase I clinical study was performed in Cuba in healthy volunteers who received the nasal component of the vaccine containing both HBV surface and core antigens. The surface antigen was produced in yeast and the core antigen produced in *E. coli*. In this study, 75% seroconversion to surface antigen and 100% seroconversion to core antigen was observed with the nasal vaccine alone. Subsequently, a phase II clinical trial was undertaken in Bangladesh, involving 20 patients with chronic HBV infections aged 18-52 years, with 7 HBeAg-positive and 13 HBeAg-negative individuals. The patients initially received five doses of intranasal vaccine with 100 μg of surface antigen and 100 μg of core antigen given every 2 weeks, and subsequently received the same regimen of nasal antigen in addition to five immunizations with an intramuscular formulation of core and surface antigens plus an alum adjuvant. After initial intranasal priming, significant elevations in IL-8, IL-1, TNF and IL-6 were seen in HBeAg-negative, but not HBeAg-positive subjects. After 1 year, 9 of 18 of the subjects completing the study demonstrated complete viral clearance of HBV DNA, with 8 of the 11 subjects in the HBeAg-negative group having complete clearance compared with 2 of 7 subjects in the HBeAg-positive group. This was also associated with normalization of transaminases. A phase III study was recently begun in Bangladesh, in which PEGylated interferon will be compared with the NASVAC vaccine regimen. The current aim is to register the vaccine in 2013.

## THE FIRST HEPATITIS E VIRUS (HEV) VACCINE – HEV-239

Ng Mun Hon (Xiamen Innovax Biotech) discussed the experience of developing the world's first HEV vaccine, HEV-239 (Hecolin). A phase III study of HEV-239 was completed in 2009 in 100,000 subjects with a 3-dose regimen of 30 μg administered intramuscularly at 0, 1 and 6 months. A per-protocol efficacy of 100% was obtained, with an efficacy of 95.5% after 1 year in subjects receiving one or more doses. The vaccine was well tolerated. This vaccine is based on proprietary technology in which the HEV surface antigen has been modified to

assist expression and enable the vaccine to assemble into a viral-like particle. Currently one-third of the world's population become infected with HEV at some point in their life. There are four major genotypes. Genotypes 1 and 2 are found in hyperendemic areas, are water-borne and cause high mortality in pregnant women, with an estimated mortality rate of 25%. Genotypes 3 and 4 are found in endemic areas and infection occurs via infected meat from pigs, as these are the main reservoir, and mainly affects elderly men. Current target populations for the HEV-239 vaccine include high-risk individuals, including pregnant women, young children, people with chronic liver disease, people with organ transplants, HIV-infected individuals and otherwise immunocompromised patients.

## VAXINE'S ADVAX™ ADJUVANT

An update on Vaxine's proprietary polysaccharide Advax™ adjuvant, which is currently in phase II/III clinical development across a range of indications, including seasonal and pandemic influenza, HBV and allergy, was provided by Nikolai Petrovsky. Vaxine, first established in 2002, is based in Adelaide, Australia, and focuses on the development of vaccines against a wide range of infectious and chronic disease indications. Vaxine has subsidiaries in Japan and the U.S., in addition to over 50 research partnerships and collaborations around the globe. Vaxine's lead products include vaccines against seasonal and pandemic influenza, Japanese encephalitis, West Nile virus, hepatitis, malaria, rabies, severe acute respiratory syndrome (SARS) and allergy. The company also has a veterinary division that is commercializing vaccines and vaccine adjuvants tailored to specific animal health problems. In July 2009, Vaxine undertook a world-first clinical trial in Adelaide of the first recombinant pandemic H1N1 2009 (swine flu) influenza vaccine using recombinant hemagglutinin produced by its U.S. partner Protein Sciences Corporation (codevelopers of the vaccine). This trial confirmed the ability of the Advax™ adjuvant technology to enhance the effectiveness of recombinant pandemic influenza vaccines, but also showed the advantages of a recombinant vaccine approach over traditional egg-based pandemic influenza vaccines in terms of the speed with which the new vaccine could be produced and introduced to the clinic. Advax™, which is based on delta inulin, has now been administered to over 1,000 humans without any safety or tolerability issues, and is thus a promising replacement for traditional alum adjuvants in most protein and polysaccharide conjugate vaccines. The only antigens for which Advax™ has not been shown to enhance immunogenicity are unconjugated polysaccharide antigens, as these are T-cell-independent antigens, suggesting that a major part of the action of these adjuvants is via enhanced T-cell help. At this meeting, Vaxine won the 2011 Vaccine Industry Excellence (VIE) Award for the most innovative Asian biotech, reflecting its success in introducing multiple new vaccine technologies into the clinic in recent years.

## VACCINES IN INDIA

Rayasam Prasad from Biological E discussed the vaccine projects undertaken at the company, which started producing vaccines in 1968 and has been the major supplier of Expanded Programme on Immunization vaccines to the Indian Government. There is major growth potential in the vaccines markets in emerging countries with the increasing use of vaccines; these countries also have a high level of population growth and the greatest market need for interventions to prevent infectious diseases. The current Indian birth rate is 25 million babies per year and China's birth rate is 16 million babies per year. Biological E has licensed an inactivated Japanese encephalitis (JE) vaccine, IC-51 (JE-PIV, Ixiaro®), from Intercell, and has recently completed phase II pediatric clinical trials in India, and is currently undertaking a phase III clinical trial, with a planned launch of the JE vaccine in India by the end of 2011. It was noted that all global pharmaceutical companies need to acquire a partner in China and India if they wish to access these markets and thereby extend the life of their current vaccine products.

## A MALARIA VACCINE FROM BIKEN

The malaria vaccine BK-SE36 (BIKEN), which has recently completed a phase IIb clinical trial in Uganda, was introduced by Toshihiro Horii (Osaka University). This vaccine targets serine repeat antigen (SERA), a malaria erythrocytic-stage antigen that is approximately 1,000 amino acids in length and is processed into 4 fragments by the protease domain that lies outside the cell wall and is therefore accessible to antibodies. Antibodies against SERA result in agglutination of merozoites, leading to their removal from the circulation. The major impediment in the development of SERA antigen as a malaria vaccine has been the difficulty in expressing the recombinant protein, principally because the native nucleotide sequence encoding the protein does not express well in eukaryotic hosts. An artificial synthetic gene that is codon-optimized for expression in *E. coli* has been generated. This gene has also been modified by removing the seven repeats in the protein sequence that cause high hydrophobicity. Antibodies to the larger SE47 construct of SERA inhibit the growth of malaria parasites in culture, and this is increased by the addition of complement. Epidemiological studies support a role for immunity to SERA in the control of malaria; children presenting with malaria-associated fever have no measurable antibodies against SERA, whereas children without fever generally have high antibodies to SERA. In further support, there is a strong inverse correlation between serum levels of antibody to SE47 and the level of parasitemia. The number of individuals with SERA antibodies in Africa increases with age, which is consistent with adults being more resistant to malaria than children.

An SE36 antigen was produced by BIKEN in *E. coli* under GMP and was formulated with aluminum hydroxide adjuvant to form BK-SE36. This was administered to chimpanzees in a dose range of 10-450 μg as two intramuscular injections, which were sufficient to generate high antibody levels. After 12 months, a booster dose of 10 μg was administered. Immunizations were next performed in squirrel monkeys, which are susceptible to *Plasmodium falciparum* infection. Squirrel monkeys were given two doses of BK-SE36 (50 μg) in alum. The BK-SE36-immunized animals had a maximum level of 5% parasitemia after challenge compared with levels of 10-20% parasitemia seen in the control animals. Furthermore, the monkeys that had been immunized prior to challenge had an antibody boost against BK-SE36 following infection not seen in the control monkeys and which may have played a role in controlling the parasitemia.

A phase I/II study was undertaken in Uganda, in which one group of adults received three 50-μg doses of BK-SE36 vaccine at 3-week intervals and a second group received three 100-μg doses of BK-SE36. The only significant adverse event was swelling at the site of

injection in 13 of 15 subjects, which disappeared within several months. All subjects achieved seroconversion; however, antibody titers were higher in patients from group 2, who achieved higher antibody levels after two doses of vaccine than those patients in group 1 after three doses of 50 μg. In an extension study in Uganda, individuals aged 6-20 years were immunized with the same BK-SE36 vaccine, with the surprising finding that only children between 6 and 10 years of age showed an antibody response to the vaccine: 70% of the subjects in this age range had a > 2.5-fold increase in antibody titers at the end of the study; however, no responses were seen in patients over 10 years of age. The lack of response in the older subjects was thought to be due to tolerance induced by exposure to malaria parasites. Consideration is currently being given to reformulating the BK-SE36 vaccine with an alternative adjuvant to determine whether this can overcome the lack of immunogenicity in subjects over 10 years of age, or, alternatively, to target the vaccine to children under the age of 6.

## *HELICOBACTER PYLORI* AS A VACCINE DELIVERY VECTOR

Barry Marshall (Ondek), a previous recipient of the Nobel Prize for Medicine for his role in the discovery of *H. pylori* and the identification of its role in gastric ulcers, presented data on adapting *H. pylori* as a vaccine delivery vector for oral vaccines. Ondek has recently completed a human phase I study in which healthy volunteers were infected with a variety of different strains of *Helicobacter* to identify those strains that were well tolerated but generated persistent infections. The most common adverse events reported were burping, nausea and vomiting, in addition to adverse reactions to antibiotics used at the end of the study to eradicate the colonized *Helicobacter*. In animal models, transgenic *Helicobacter* has been shown to induce IgG against pertussis antigen encoded by *Helicobacter*, and the next step will be to undertake studies to confirm the ability of a *Helicobacter* vector to successfully deliver a vaccine antigen and induce immunity in a human host. Ondek won the 2011 VIE Award for most successful therapeutic vaccine development in Asia.

## REGIMMUNE'S REVAX TECHNOLOGY

A collaboration between RIKEN and REGiMMUNE (a spin-out from RIKEN) was discussed by Yasuyuki Ishii (RIKEN). The Japan-based company REGiMMUNE is developing agents based on the α-galactosylceramide product KRN-7000, a natural killer (NK) T-cell ligand. This technology is known as ReVax and comprises KRN-7000, which, when added to an allergen and administered intravenously, induces tolerance to the coadministered allergen, and could thus be a potential treatment for allergies and autoimmune disease. More recently, a second technology, termed LaserVax, has been developed that is based on the same KRN-7000 platform. This product is being promoted as a vaccine adjuvant that can be injected parenterally or given intranasally. When combined with ovalbumin, LaserVax was less effective than ASO4 adjuvant; however, the combination of LaserVax with aluminum hydroxide resulted in a higher antibody response than ovalbumin with ASO4. When given intranasally, LaserVax induces an IgA response against coadministered antigen. Other products in development include RGI-1001, an application of ReVax against Cedar polinosis; RGI-2001 (Multivax, ToleroVax), which is due to begin a phase I/II clinical trial in the U.S. to induce tolerance against donor cell transplants and has been shown to improve survival in graft versus host disease in a mouse model through the induction of $CD4^+$ forkhead box protein $P3^+$ T cells in the spleen; RGI-3010, which involves the administration of insulin with ReVax to induce tolerance to insulin and thereby prevent type 1 diabetes; and RGI-4100, which involves the administration of myelin basic protein with ReVax to treat multiple sclerosis.

## DISCLOSURES

The author is the Research Director of Vaxine Pty. Ltd., which has commercial interests in Advax™ vaccine adjuvant technology.

The website for this meeting can be found at: http://www.terrapinn.com/2011/wvcasia/index.stm.