

The 5J8 DMAb was expressed at  $\mu\text{g/mL}$  levels in serum of both nude and immune-competent mice. Serum DMAb produced from muscle in vivo were functional in vitro - with the ability to bind influenza HA, block hemagglutination of red blood cells, and neutralize influenza virus. Serum DMAb expression levels approximate those required for protection. Influenza challenge studies of mice treated with 5J8 DMAb are underway.

DMAb provide an important new approach to immune therapy. DNA has an excellent safety profile and averts challenges of pre-existing serology associated with many viral vectors. Here, we demonstrate that DNA can be used to deliver consistently high levels of potent monoclonal antibodies for protection against a viral pathogen.

#### 434. Adoptive Treg Cell Therapy Using Factor VIII-Specific CAR Regulatory T Cells Regulates Anti-Factor VIII Immune Responses in Hemophilia A Mice

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The immune response to factor VIII (FVIII; F8 in constructs) protein limits the effectiveness of treatments for hemophilia A (HemA) patients. Our previous studies demonstrated that regulatory T cells (Tregs) play a pivotal role in modulation of anti-FVIII immune responses. In particular, adoptive transfer of Tregs isolated from FVIII-primed HemA/Foxp3 mice attenuated anti-FVIII immune responses induced by gene transfer of FVIII plasmid in HemA mice. For developing adoptive Treg therapy, we successfully expanded FVIII-sensitized polyclonal Tregs using an FVIII-specific expansion protocol in vitro and showed that these cells had increased FVIII-specific suppressive activity compared with nonspecific Tregs. However, FVIII-specific Tregs in the polyclonal population are still in very small numbers. In this study, we explored the strategy to generate FVIII-specific Tregs using the chimeric antigen receptor (CAR) approach. Lentiviral vector (LV) incorporating a high-binding anti-FVIII antibody-derived variable region (scFv) linked to signaling and costimulatory moieties of immune receptors (third generation CAR) and fused with a murine Foxp3 cDNA (F8CAR-Foxp3-LV) was prepared and used to transduce murine CD4<sup>+</sup>T cells. Flow cytometry analysis confirmed extracellular scFv and intracellular Foxp3 expression in transduced cells (F8CAR-Tregs). In vitro suppressive assay showed that transduced CD4<sup>+</sup>T cells had significantly higher FVIII-specific suppressive activity than untransduced cells towards FVIII-specific CD4<sup>+</sup> effector T cells (Teffs). In addition,  $1 \times 10^6$  transduced cells and untransduced cells were adoptively transferred into HemA mice. One day after cell transfer, the treated mice were challenged with FVIII plasmid injected hydrodynamically. The anti-FVIII antibody titers are evaluated overtime. It is expected that F8CAR-Foxp3-LV transduced cells will prevent or decrease the production of anti-FVIII antibodies. We have also prepared a LV incorporating only the F8CAR region (F8CAR-LV). CD4<sup>+</sup>CD25<sup>-</sup> and CD4<sup>+</sup>CD25<sup>+</sup> cells isolated from HemA mice were transduced with F8CAR-LV to generate FVIII-specific Teffs and Tregs, respectively. In vitro FVIII-specific suppressive assays using CD4<sup>+</sup> Teffs from FVIII-primed HemA mice or F8CAR-LV transduced Teffs as responder cells are performed to compare the suppressive function of F8CAR-LV transduced CD4<sup>+</sup>CD25<sup>+</sup> cells and F8CAR-Foxp3-LV transduced CD4<sup>+</sup> cells. CD4<sup>+</sup>CD25<sup>+</sup> cells isolated from FVIII-primed HemA mice and untransduced CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup> cells from HemA mice are used as control cells. These experiments compare the extent of suppression towards specific F8CAR Teffs and polyclonal FVIII-specific Teffs as well as the potency of suppressive function between two differently engineered F8CAR Tregs. Finally, adoptive transfer experiments into

HemA mice using the transduced and control cell populations are performed to evaluate their in vivo function to protect the HemA mice from anti-FVIII antibody production. We anticipate that compared with nonspecific and polyclonally expanded Tregs, FVIII-specific CAR Tregs will exert superior suppressive activity towards anti-FVIII immune responses without triggering systemic immune suppression.

#### 435. Skin Delivery of a RSV Vaccine with Surface Electroporation Provides Full Protection from Lower Respiratory Disease in the Cotton Rat

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

#### 436. Developing a Synthetic DNA Vaccine for an Emerging Pathogen - Middle East Respiratory Syndrome

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**Background:** Middle East Respiratory Syndrome (MERS) was first reported in 2012 in Saudi Arabia when a patient died from severe respiratory disease caused by a novel betacoronavirus, MERS-CoV. Through November 2015, there have been 1618 confirmed global cases of MERS-CoV infection and 579 deaths reported to the World Health Organization (WHO). Currently, no vaccine or specific treatment is available and patients are treated with supportive care based on their clinical condition. While most MERS cases occur in and around Saudi Arabia, the recent outbreak in Korea highlights the potential for this disease to spread beyond the immediate region. A vaccine is needed to prevent future disease caused by MERS-CoV.

**Methods:** A synthetic DNA MERS vaccine was generated using a consensus sequence of the MERS spike protein. Mice, dromedary camels, and non-human primates (NHP) were immunized with MERS-vaccine by intramuscular injection followed by electroporation. Cellular immune responses were measured by flow

cytometry and IFN $\gamma$  ELISpot. Humoral immune responses were measured by ELISA and neutralizing antibody (nAb) assay. Following immunization, NHPs were challenged with infectious MERS-CoV (EMC/2012) and monitored for signs of infection by clinical scoring and examinations. Viral load was measured by qRT-PCR and tissue sections were stained with H&E.

**Results:** Immunization of mice with MERS-vaccine induced strong humoral and cellular responses. Mice produced strong binding antibody (bAb) titers and nAb titers. A strong, polyfunctional, CD4 and CD8 T cell response was detected against multiple epitopes across the MERS spike protein. Immunization of dromedary camels induced the production of MERS spike specific antibodies and nAbs. Immunization of NHPs induced strong bAb titers and nAb titers and a strong CD4 and CD8 T cell response. NHPs immunized with multiple vaccination regimens were also protected from signs of disease upon challenge with infectious MERS-CoV and showed a greater than 3 log reduction in viral load after challenge compared to unvaccinated animals.

**Conclusions:** A consensus DNA MERS-vaccine was able to generate both a strong T cell and neutralizing antibody response in multiple animal models, including camels, a natural host for MERS-CoV and a probable source of human infection. MERS-vaccine was also able to protect NHPs from an infectious MERS-CoV challenge. These results demonstrate the promise of this consensus DNA MERS-vaccine as a candidate for vaccine development.

#### 437. A Light-Producing Model of Infection-Related Preterm Birth

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##### Background:

Preterm birth is increasing in incidence and current therapies are relatively ineffective. It is responsible for >1million neonatal deaths per annum worldwide and long term complications in survivors. Approximately 50% of PTBs are preceded by microbial invasion of the intrauterine space; current clinical management centres on diagnosis of intrauterine bacterial presence by identifying the resultant inflammatory response. To investigate the relationship between intrauterine bacterial presence and inflammation we developed two separate gene technology approaches:

1. Intravaginal bioluminescent bacteria to measure bacterial ascent into the uterus which mimics the ascending vaginal infection seen in preterm birth.
2. Lentiviral gene transfer of an NFkB activated luciferase reporter construct to allow bioluminescent imaging of the subsequent systemic NFkB response.

##### Methods:

An NFkB response element was cloned into a lentivirus vector upstream of the genes encoding a codon-optimised firefly luciferase. High titred virus was injected intravenously at birth to neonatal female C57BL/6 J-Tyrc-2J mice to achieve luciferase expression predominantly in the liver (to monitor systemic inflammatory response). These mice received *Escherichia coli* (non-pathogenic K-12, MG1655 with integrated luxABCDE operon) intra-vaginally once reaching adulthood and intraperitoneal lipopolysaccharide (LPS) three weeks later. Luciferase expression was monitored by whole body bioluminescence imaging. Local inflammation was determined using H&E, ICAM-1 (Intracellular adhesion molecule 1) and Ly6g immunohistochemistry and enzyme-linked immunosorbent assays for serum and uterine TNF- $\alpha$  and IL1- $\beta$  cytokines.

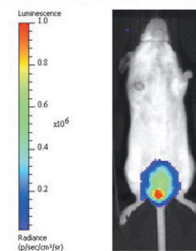
##### Results:

Bioluminescent imaging revealed that C57BL/6 J-Tyrc-2J mice were the most susceptible mice breeds for modelling of ascending vaginal infection with *E.coli* luxABCDE operon. Intraperitoneal LPS induced an NF-KB response in the liver by biosensing ( $p<0.01$ ), however intravaginal *E.coli* administration induced no response. There was evidence of uterine inflammation with an upregulation of ICAM-1 and neutrophils.

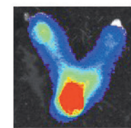
##### Conclusion:

Although it is possible to detect LPS-induced NFkB inflammation in the liver by biosensing, ascending vaginal infection induced no response. This highlights the clinical challenge of identifying bacterial presence, confined to the uterus, using systemic markers. This model can be used to test new treatments for the prevention of PTB.

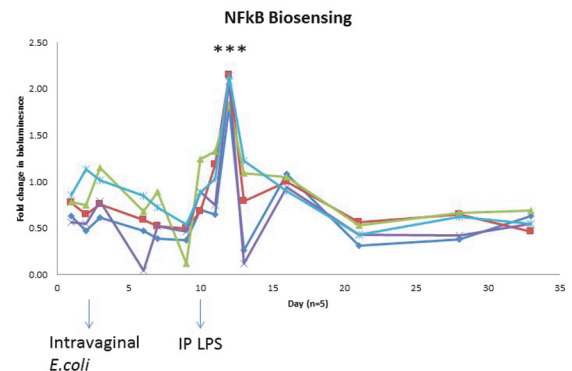
**Figure 1. *E.coli* luxABCDE operon bioluminescence at 24h**



**Figure 2. Tracking bacterial ascent**



Ascending infection disease modelling using the : NF-Kb activated luciferase reporter construct - inflammation induces a bioluminescence response.



#### 438. Human, Pig and Mouse IFITMs Partially Restrict Pseudotyped Lentiviral Vectors

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Lentiviral vectors are increasingly used in clinical trials to treat genetic diseases. Our research has focused on strategies to improve lentiviral gene transfer efficiency in the airways. Previously we demonstrated that a feline immunodeficiency virus (FIV)-based lentiviral vector pseudotyped with the baculovirus envelope glycoprotein GP64 (GP64-FIV) efficiently transduced mouse nasal epithelia *in vivo* but transduced mouse intrapulmonary airways with ten-fold less efficiency. Here, we demonstrate that a family of proteins with antiviral activity, interferon induced transmembrane proteins (IFITMs), are more highly expressed in the mouse intrapulmonary airways as compared to the nasal airways. Using GP64 and VSV-G pseudotyped FIV, we show that expression of mouse IFITM1, IFITM2, and IFITM3 restricts gene transfer. Further we show that both