VACCINES: REACHING FOR HIGHER BRANCHES AFTER THE LOW HANGING FRUIT HAS BEEN PICKED

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Vaccines represent one of the most significant advances by medicine in addressing infectious diseases being responsible for the elimination of a few and the virtual eradication of several other scourges. In addition, medicine is also beginning to appreciate the potential applicability of vaccines beyond infectious diseases to areas such as oncology. During any emerging infectious disease public health event, availability of a vaccine figures prominently in the assessment of the capability to address the threat most effectively. A more rapid deployment of new vaccines would enable more effective management of emerging threats. Efforts to streamline manufacture and production of vaccines have yielded some success. Vaccination enhancements such as temperature stability, adjuvants, and alternative delivery devices are needed for improving overall effectiveness. At the same time, animal model testing of candidate vaccines have displayed variable success in predicting immunogenicity and efficacy in humans. Examples of efforts in these latter arenas will be discussed to highlight the potential of newer technologies and approaches that may further improve the capability to bring vaccines forward towards clinical evaluation in a timelier manner to impact responses to emerging infectious diseases.

A UNIVERSAL INFLUENZA VIRUS VACCINE CANDIDATE CONFERS PROTECTION AGAINST PANDEMIC H1N1 INFECTION IN FERRETS

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Key Words: Influenza virus, universal vaccine, ferrets, challenge model

Influenza viruses can cause severe disease and mortality in humans. Due to constant change in their immuno-dominant antigenic sites they can evade adaptive immune responses. Current seasonal influenza virus vaccines therefore require annual re-formulation and re-administration to confer protection from circulating viruses. Additionally, these vaccines cannot protect against novel pandemic influenza virus strains. Novel vaccination approaches attempt to refocus antibody responses towards more conserved domains like the hemagglutinin stalk. Antibodies against the stalk domain are broadly-reactive and can neutralize multiple influenza virus subtypes. However, the stalk domain is immuno-subdominant and not preferentially targeted by the immune system. In this study, we tested if a vaccination strategy based on influenza viruses expressing chimeric hemagglutinins (cH) that contain exotic, divergent head domains, but a conserved H1 stalk domain could induce cross-protective antibody responses in ferrets. We compared a heterologous live-attenuated virus (cH8/1N1) prime followed by an inactivated split virus (cH5/1N1) boost combination approach to two doses of split-virus vaccines (cH8/1N1/cH5/1N1) and the impact of adjuvant on the immune response. Additionally, a ‘standard of care’ control group received 2 rounds of a human trivalent influenza virus vaccine. We found that all universal influenza virus vaccination approaches were successful at inducing stalk-reactive antibody responses in serum. Virus replication was limited to the nasopharynx in the live attenuated/split vaccine groups and nasal wash titers were lower than in the 'standard of care' control group. No virus replication was detected in the lungs of attenuated/split vaccinated ferrets, while the 'standard of care' group had similarly high titers as an unvaccinated control group. Our findings demonstrate that - using a chimeric hemagglutinin based heterologous attenuated/split combination strategy - our candidate universal influenza virus vaccine can successfully protect ferrets from pandemic H1N1 infection. The data support further development of this vaccination approach and advancement into clinical trials.

SINGLE-CELL ANALYSIS OF INFLUENZA A VIRUS-INFECTED CELLS FOR THE OPTIMIZATION OF CELL CULTURE-BASED VACCINE PRODUCTION

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Key Words: influenza, single-cell analysis, vaccine manufacturing, mathematical modeling

Individual cells generally show a large cell-to-cell variability in their properties, such as the cell cycle stage or protein content. Moreover, cellular biochemical reactions are subject to stochastic effects which can increase cell-to-cell heterogeneity. Yet, how this variability affects virus replication, which comprises noisy reactions itself, remains largely elusive. We conducted single-cell analysis of influenza A virus (IAV)-infected cells to investigate cell-to-cell heterogeneity in virus replication in detail. Single Madin-Darby canine kidney cells, infected with influenza virus A/Puerto Rico/8/34 (H1N1), were isolated in 384-well plates by using a limiting dilution approach. After incubation, we quantified virus titers in the supernatant by the plaque assay and intracellular genomic viral RNAs (vRNAs) by real-time RT-qPCR. Our experiments reveal a surprisingly high variability in IAV replication. Progeny virus yields ranged from 1 to 970 plaque-forming units per cell and intracellular vRNA levels spanned three orders of magnitude. With the assistance of stochastic mathematical modeling, we show that two types of molecular noise affect virus titers: (i) extrinsic noise, which can arise by cell-to-cell variability and (ii) intrinsic noise, originating from stochastic effects during viral RNA synthesis. Furthermore, we demonstrate that the heterogeneity in IAV infection is apparently not generated by the genetic diversity of the infecting virus population; and defective interfering particles affected only the infectivity of progeny virions. In addition, our simulations suggest that random degradation of viral genomes can result in a large fraction of non-productive cells at a low multiplicity of infection. The observed large cell-to-cell variability in IAV replication supports the notion that population-derived measurement data do not accurately represent viral infections. Moreover, characterizing high-yielding cells at the single-cell level may enable us to derive strategies for the optimization of cell culture-based vaccine manufacturing processes.

Reference: Heldt and Kupke et al. (2015) Nature Commun 6, 8938

DEVELOPMENT OF TYPHAX, A *SALMONELLA* TYPHI VI POLYSACCHARIDE PROTEIN CAPSULAR MATRIX VACCINE

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Matrivax Research & Development Corporation is researching and developing a novel technology termed Protein Capsular Matrix Vaccine (PCMV) as an alternative to polysaccharide-protein conjugate vaccines. In a PCMV, polysaccharide antigens are entrapped in a cross-linked protein 'carrier' matrix. This process is simpler than conjugate vaccines and should yield polysaccharide vaccines that elicit T_H-cell 'memory', are highly efficacious, and less expensive to manufacture.

Typhoid fever, caused by *Salmonella enterica* serovar Typhi, is a disease that afflicts ~16 million people worldwide, resulting in 600,000 deaths, annually. Although typhoid fever vaccines are commercially available, there are significant limitations. Ty21a is an oral vaccine that requires a multi-dose regimen; whereas Typherix® and TyphimVi® are parenteral and associated with local reactogenicity. The existing typhoid vaccines confer variable, ~70%, protective efficacy, do not protect young children (<2 years old), and are not used for routine immunization.

As proof of concept for PCMV technology, the Vi polysaccharide of *Salmonella* Typhi was entrapped in a CRM197 cross-linked matrix to make a Vi PCMV (Typhax). Typhax adjuvanted with Adju-Phos® was evaluated for safety and immunogenicity in three distinct animal models. In murine immunogenicity studies, a three dose regimen of 0.05 µg of Typhax administered intramuscularly (IM) elicited an anti-Vi IgG antibody response in mice that was ~64-fold above that induced by Vi alone. Rabbits immunized IM with a three dose regimen of 0.1 µg, 2.5 µg and 10 µg of Typhax developed a dose dependent response. Similarly, a dose dependent response was observed in non-human primates (NHP) immunized IM with a two dose regimen of either 2.5 µg or 10 µg of Typhax eliciting an anti-Vi IgG antibody GMT ~20-fold and ~50-fold higher, respectively, above baseline.

Animal Model	Typhax Dose Level	Fold increase in anti-Vi IgG antibody response
Mouse	0.05 µg	64
Rabbit	0.1 µg	2.4
	2.5 µg	28
	10 µg	34
NHP	2.5 µg	~20
	10 µg	~50

Recently, Matrivax executed a murine preclinical immunogenicity study with a proprietary adjuvant. In this study, a three dose regimen of 0.05 µg adjuvanted Typhax elicited a 3.3-fold higher anti-Vi IgG antibody response than the same Vi PCMV adjuvanted with Adju-Phos® and 207-fold higher than that elicited by Typhim Vi®.

A Phase 1 clinical trial for Typhax will begin in 1Q2016. In this study, 0.5 µg, 2 µg and 10 µg doses of Typhax adjuvanted with Adju-Phos® will be evaluated for safety and immunogenicity. In 2Q16, a second Phase 1 clinical trial is scheduled to evaluate Typhax in combination with a novel proprietary adjuvant. Preclinical immunogenicity data in mice, rabbits, and non-human primates as well as key GMP manufacture and release data of Typhax will be reviewed. Moreover, the concept of the Vi PCMV as a cornerstone of a multivalent Salmonella vaccine will be discussed.

HIGH PREVALENCE OF PRE-EXISTING AND BROADLY CROSS-REACTIVE ANTIBODIES IN THE SEROLOGICAL REPERTOIRE FOLLOWING INFLUENZA VACCINATION

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Key Words: serological repertoire, proteomics, pre-existing, cross-reactive

Vaccines convey protection by stimulating B cells to produce a diverse repertoire of antigen-specific antibody proteins for an extended period of time. While antibodies in circulation following vaccination are critical for protection to influenza infection, the identities and biochemical properties of the individual antibodies that constitute the polyclonal serum response remain unknown. Here, we used recently developed techniques to directly analyze the composition of four human donors' serum antibody repertoires to each of the three monovalent strains in the 2011-2012 seasonal trivalent inactivated vaccine. A time-course analysis reveals that >60% of the post-vaccination serum response comprised of antibodies which pre-existed in sera prior to the vaccination that became boosted. Our data also show unexpected prevalence (>30%) of antibodies cross-reactive between H1 and H3 in sera. Characterization of recombinant antibodies led to a discovery of a novel class of broadly cross-reactive, but non-neutralizing antibodies. These antibodies displayed exceptional binding breadth to group 1 and group 2 hemagglutinins by recognizing a newly-identified conserved epitope in the head domain that is exposed only in monomeric form of the hemagglutinins. These antibodies conferred protection in lethal challenge mice models. Additionally, we identified a group of stem-binding antibodies with broad binding and neutralization in pseudo-virus neutralization assays. Some of these antibodies were abundant in serum. This is the first-ever report providing an extensive molecular-level description of the human serum antibody response to seasonal flu vaccination, and collectively, this data provide unprecedented insights on the serological response to influenza vaccination that have direct implication for the design of immunogens for a future universal flu vaccine.

UPSTREAM AND DOWNSTREAM PROCESS DEVELOPMENT OF A VERO CELL-BASED YELLOW FEVER VACCINE

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Key Words: Yellow-fever vaccine, Vero cells, serum-free bioreactor cultivation, chromatographic purification, membrane adsorbers and multimodal resins

Yellow fever (YF) is a lethal viral disease that is endemic in some tropical regions of South America, Central America and Africa. An attenuated vaccine produced in embryonated eggs is available since the 1930's and is known to be highly effective and safe. However, after large vaccination campaigns in the 2000's, reports of rare, but serious adverse events have stimulated Biomanguinhos/FIOCRUZ, who produces the current attenuated 17DD vaccine, to develop a new, inactivated vaccine.

Over the last years, through a partnership of the Federal University of Rio de Janeiro and Biomanguinhos/FIOCRUZ, both upstream and downstream processes were developed. The upstream process was established based on Vero cell cultivation on microcarriers in serum-free medium, using stirred-tank bioreactors. The first studies were carried out in spinner flasks to select the microcarrier type and the serum-free medium. Also, statistical DOE tools were used to study the infection step, varying the moiety of infection and the time of infection. This process was then scale-up to stirred-tank bioreactors and further optimized regarding microcarrier concentration, stepwise medium addition, dissolved oxygen level/sparging intensity, impeller configuration and time of harvest. The final upstream process that was established results in virus titers of 10^8 pfu/mL within a time frame 144h post inoculation of the cells in the bioreactor.

The downstream process was designed priorizing chromatographic techniques, aiming at achieving high purity levels and extensive removal of process-related critical contaminants, such as DNA and host-cell proteins (HCP), as preconized by the regulatory authorities. For the capture step, both cation- and anion-exchange chromatographies were evaluated. A Q membrane adsorber process was selected and the best operational conditions in terms of pH, temperature, buffers and washing strategies were determined. For the second purification step, three techniques were evaluated: multimodal chromatography, ultrafiltration/diafiltration, and hydrophobic interaction chromatography using a HIC membrane adsorber. The multimodal resin showed the best results, and operational conditions of this step were further optimized. The final 2-step yellow-fever virus purification process resulted in an overall yield of 52% and residual HCP of 350 ppm (0.05%). Residual DNA was 1.2 ng per dose, considering the dose established based on animal studies, and is in agreement with the limit recommended by the World Health Organization (<10 ng/dose). Electrophoretic analysis (SDS-PAGE) of the purified samples showed a band corresponding to 96% of identified proteins with molecular mass of 56 kDa, which is the expected mass for the virus envelope protein (E). Anti-E Western blot (WB) showed a single band, confirming the identity of the samples. No band was revealed in the anti-HCP blot, confirming the low HCP levels quantified.

The developed process allows the production of a new, high-purity yellow-fever vaccine through a scalable technology, which is better suited than egg-based technology to meet emergency demands in case of epidemics and is useful in the current scenario of increasing worldwide demand YF vaccine.

FAST-TRACK LENTIVIRAL VECTOR UPSTREAM PROCESS DEVELOPMENT: LEVERAGING HIGH-THROUGHPUT PROCESS MONITORING, SINGLE-USE BIOREACTOR SCALABILITY

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The design and applications of recombinant viral vector have significantly increased over the past years for various therapeutic areas such as gene therapy, cell therapy and vaccines. To respond to this growing demand, viral vector production processes should demonstrate robustness and scalability. Thus a high-throughput development method has been implemented to allow fast-track process optimization and scale-up of non-replicative lentiviral vector production by transient transfection.

A state-of-the-art process development strategy was put in place to develop robust and highly-productive lentiviral vector production platforms by transient transfection of cell suspension in serum-free conditions. Labscale representative models are developed allowing parallel large number of experiments. Designs of experiments are applied to identify interaction between identified or selected optimized parameters. Disposable solutions are implemented all along the process to decrease development timelines and provide flexibility for panel vectors manufacturing. High-throughput process monitoring tests were also developed to support associated analytical needs.

Comparative analysis of results observed within different scales models are presented and discussed, demonstrating good scalability from 15-mL micro-bioreactor up to 200L production scale.

HOLLOW FIBER-BASED HIGH-CELL-DENSITY AND TWO-STAGE BIOREACTOR CONTINUOUS CULTIVATION: OPTIONS AND LIMITS TOWARDS PROCESS INTENSIFICATION FOR VIRUS PRODUCTION

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Key Words: Perfusion, scale-down, cell-specific virus yield, modified vaccinia virus Ankara (MVA), influenza virus.

Availability of suspension cell lines and culture media for expansion of up to 20×10^6 cells/mL provide perfect starting points to develop process intensification strategies for vaccine production. Modern hollow fiber-based perfusion systems accomplish up to 500 $\times10^6$ cells/mL in CHO cell cultivations. Reaching 10 to 20 fold higher cell concentrations, while keeping cell specific virus yields constant, could make processes with very low cell specific virus yields (10-100 viruses/cell) already to feasible processes. Therefore, all possible process strategies using new media, cell lines and reactor equipment need revisiting.
Data obtained from the production of the modified vaccinia Ankara virus strain MVA-CR19 as well as influenza A/PR/8 virus in either hollow fiber-based high-cell-density (HCD) cultivations (using an alternating tangential flow (ATF) perfusion system) or in two-stage bioreactor continuous cultivations of the suspension cell line AGE1.CR.pIX are presented and critically discussed. Options and limits are highlighted to allow an evaluation of both approaches with respect to scale-up and application to other virus-host cell systems.
Both process strategies were successfully scaled-down into shaker flasks allowing parallel experiments. Accordingly, perfusion and semi-perfusion at a feeding rate of 0.05 nL/cell×d led to concentrations of AGE1.CR.pIX cells above 60×10^6 cells/mL with neither limitation nor overload of nutrients. For infections in 50 mL, a combined strategy comprising an initial fed-batch phase followed by a periodic virus harvest phase resulted in the highest product concentration. Compared to a conventional batch process at 4 to 8×10^6 cell/mL, maximum titer increased more than 10-fold. Additionally, a 3-fold increase in both cell-specific yield (virus/cell) and volumetric productivity (virus/L×d) could be obtained. The subsequent scale-up into a 1 L bioreactor with ATF perfusion was equally successful and besides allowed re-evaluation of hollow-fiber cut-off.
Alternatively, a small scale semi-continuous two-stage cultivation system (100 mL scale, two shaker flasks) was established as an approximation for a genuine continuous bioreactor set-up (1 L scale, two-stage stirred tank bioreactor). MVA virus production at both scales resulted in stable titers of MVA-CR19 virus (approx. 1×10^8 IU/mL) for over 18 days suggesting an absence of the “von Magnus effect” compared to influenza virus. PCR analysis confirmed stable maintenance of the recombinant transgene in a MVA-CR19.GFP virus. Such a system may be of interest for continuous production of recombinant MVA-based vaccines and gene therapy vectors in the future.

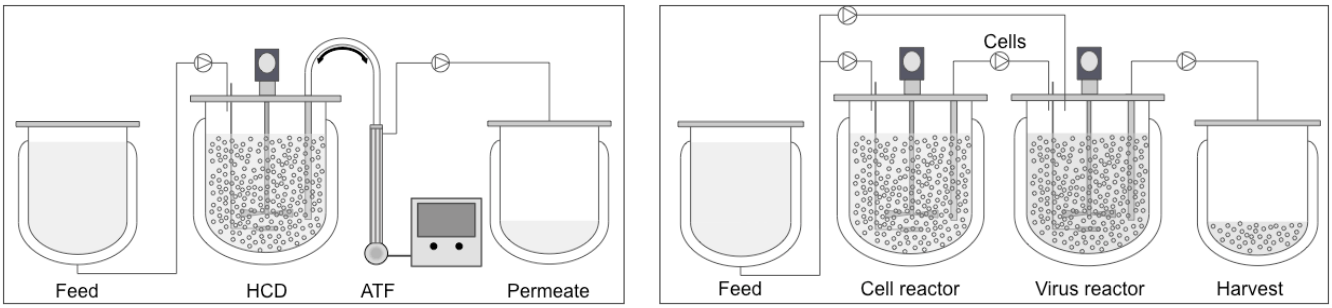

Figure 1: Scheme of a hollow fiber-based HCD and a two-stage bioreactor continuous virus production.

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A LIVE ATTENUATED RSV VACCINE, PROCESS DEVELOPMENT STUDIES

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Key Words: Design of experiments, Vero cell, single use bioreactor, density gradient ultracentrifugation, lyophilization.

Respiratory Syncytial Virus (RSV) is the leading cause of lower respiratory tract disease in infants and young children. A vaccine to prevent the high burden of disease caused by RSV is urgently needed, but not available.

A live attenuated respiratory syncytial virus (RSV) vaccine for intranasal delivery is currently under development at Intravacc. The vaccine concept comprises a live Glycoprotein-complemented RSVΔG virus. This G-RSVΔG virus is generated by proliferation of an RSVΔG on G-expressing Vero cells. The vaccine thus contains virus particles that have the G-protein on their surface but not in their RNA genomes. This recombinant virus is highly attenuated compared to wild type RSV and therefore presents a live attenuated vaccine candidate for RSV infection.

A vaccine production process has been setup for the production of Phase I clinical lots. In short, the production process steps are: cell and virus culture, clarification, continuous flow density gradient ultracentrifugation, ultra/diafiltration, filling and lyophilization.

An example of process development is the design of the cell and virus culture method. Using the statistical design of experiment approach the virus culture has been optimized to both virus yield and harvest quality. As RSV is a filamentous virus, the optimization of harvest quality with respect to purification opportunities is pivotal. This DoE was done at lab-scale bioreactors (2-L) and the chosen conditions were successfully scaled-up to 50-L single use bioreactors. Preparation of preclinical and clinical lots is done at this scale.

The pre-clinical studies were successful. In the cotton rat model, the G-RSVΔG vaccine is safe, immunogenic and protects against challenge with wild type RSV. The following step, a clinical Phase I study, is planned.

GENETIC ENGINEERING OF VACCINE MANUFACTURING CELL LINES ENHANCES POLIOVIRUS AND ENTEROVIRUS 71 PRODUCTION

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Key Words: Poliovirus Vaccine, RNAi, CRISPR, Cell Engineering.

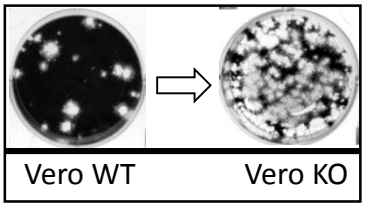

Figure 1 – Plaque Assays. Knockout of individual host genes dramatically enhances poliovirus production

Vaccine manufacturing costs and production limitations represent two fundamental challenges facing researchers, public health officials and vaccine manufacturers committed to global health solutions. To address these issues, we have investigated whether the cell lines employed by vaccine manufacturers can be engineered to enhance vaccine virus production. As a first step in a proof-of-principle study, a genome-wide RNA Interference (RNAi) screen was conducted to identify host gene modulation events that increased Sabin 2 poliovirus (PV) replication. Primary screen hits were validated in a Vero vaccine manufacturing cell line using both attenuated and wild type poliovirus strains. This approach identified multiple single and dual gene knockdown events that increased PV titers >20-fold and >50-fold, respectively. Top candidate genes did not affect virus antigenicity, cell viability, or cell doubling times. Moreover, CRISPR/Cas9-mediated knockout (KO) of the top three targets created stable cell substrates with improved viral vaccine strain production (Figure 1). Interestingly, silencing of several genes that enhanced PV replication also boosted replication of enterovirus 71, a clinically relevant virus for which vaccines are being targeted. The discovery that host gene modulation can markedly increase virus vaccine production identifies a strategy to address current public health and industry challenges.

ADJUVANTS IN PRECLINICAL AND CLINICAL DEVELOPMENT: THE DO AND DON'T

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It is clearly accepted that all vaccines are adjuvanted (endogenously: part of the pathogen or exogenously: added to the antigen formulation) except for a few vaccines such as liquid Hib or non aluminic Meningitis vaccines .It is also recognized that the vast majority of recombinant vaccines if not all, will require an adjuvant to induce an immune response which will be adequate in quality and quantity. Over the past 3 decades, adjuvants have become increasingly important and central to the development of new and improved vaccines. Although the introduction of exogenously adjuvanted vaccine has been slow and steady, 7 licensed vaccines based in the adjuvanted approach are now licensed, and the number eaching late development phase continue to increase.

Their introduction into vaccines has added complexity to the development of vaccines in terms of formulation, preclinical and safety evaluation both preclinically and clinically.

Challenges however remain and should be understood and overcome when embarking in the development of a new adjuvant antigen combination. It is only the combination of the right antigen and the right adjuvant that will lead to an efficacious vaccine, in that sense, one cannot be assessed without the other, in particular in the context of the formulation. Special consideration need to be taken when defining and evaluating the formulation aspect of the vaccine.The preclinical evaluation needs to take into account the nature of the adjuvant used and its potential differences between species. Understanding adjuvant mode of action has therefore become crucial, with the realization that differences existed between animals and humans making extrapolation between preclinical and clinical not always possible. The use of adjuvants in new vaccines has highlighted the need to define preclinical and clinical safety evaluation methods as compared to therapeutic small molecules

This presentation will review the challenges faced with the development of adjuvanted vaccines, from their inception to current days, and path that could be followed.

COMBINING DOE WITH AN EMPIRICAL APPROACH TO IMPROVE VACCINE FORMULATION DEVELOPMENT

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Key Words: vaccine, formulation, stability

Vaccine development and formulation is based upon understanding of the candidate's target product profile, and leverages knowledge and experience in pharmaceutical products. Developing an optimal formulation takes into account factors such as ensuring route of administration, product stability, and identifying Quality Target Product Profiles that are suitable for the product's intended use. Based upon such knowledge and experience, multiple excipient formulations may be first identified for initial screening. Achieving the QTPP is a measure of results, and can be achieved with screening formulations by aggressive accelerated stability study conditions to enable the final formulation components to be down-selected. This presentation highlights what has proven to be a successful approach to formulation development of a vaccine product.

Product design and formulation selection criteria focused on attributes associated with lyophilized preparations of a live attenuated vaccine candidate, and included accelerated stability studies. Beginning with an understanding of optimal buffer and formulation parameters in solution, an initial screening of 8 lyophilized formulations were evaluated. A single formulation was selected for further development where the relative concentration of each of the components was tested using a definitive DoE screen. Lyophilized samples were prepared, behavior during processing evaluated, finished product attributes assessed and dried state accelerated stability was monitored by measuring potency. Statistical methods were used to predict the interaction and synergistic effects of each of the formulation components. Results of the evaluations and assessments of the varying concentrations verified the predictive analysis, leading to selection of the most promising formulation that met or exceeded the initial defined QTPP. Use of statistical methods were then used to refine the DoE central composite design, to build on the understanding of the design space for further evaluation of the final formulation.

DEVELOPMENT OF A THERMOSTABLE ID93 + GLA-SE VACCINE USING A DESIGN OF EXPERIMENTS (DOE) APPROACH

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Key Words: Adjuvant, Lyophilization, Tuberculosis, Formulation Development

Next-generation rationally-designed vaccine adjuvants represent a significant breakthrough to enable development of vaccines against challenging diseases including tuberculosis, HIV, and malaria. New vaccine candidates often require maintenance of a cold-chain process to ensure long-term stability and separate vialing to enable bedside mixing of antigen and adjuvant. This presents a significant financial and technological barrier to worldwide implementation of such vaccines. Herein we describe the development of a single-vial lyophilized thermostable tuberculosis vaccine comprised of an antigen (ID93) and an oil-in water emulsion adjuvant (GLA-SE), using a design of experiment (DOE) approach. Stabilizing excipients were identified, and the effect of various factors were evaluated to determine optimized formulations that minimized GLA and ID93 degradation, particle size growth, and pH change, while optimizing cake quality. Formulations were identified that are stable at elevated temperatures. Further this vaccine retains the ability to elicit both antibody and TH1 responses against the vaccine antigen and protect against experimental challenge with Mycobacterium tuberculosis. These results represent a significant breakthrough in the development of vaccine candidates that can be implemented throughout the world without being hampered by the necessity of a continuous cold chain or separate adjuvant and antigen vials.

CONTROLLED, PULSATILE RELEASE OF THERMOSTABILIZED INACTIVATED POLIO VACCINE FROM PLGA-BASED MICROSPHERES

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Key Words: Drug delivery; single-administration vaccines; controlled release kinetics; polio vaccine stability

Many vaccines, such as the inactivated polio vaccine (IPV), must be administered in several doses for full efficacy. Because patient access is a major challenge for vaccination efforts in developing countries, administering multiple doses per patient is impractical in those areas. Single-administration vaccines would greatly improve efforts to vaccinate populations in Third World countries, and the World Health Organization (WHO) Expanded Program for Immunization describes an ideal vaccine as one that is heat-stable, requires only one shot, and is easy to administer. Although already existing technologies, such as microspheres composed of poly(lactic-*co*-glycolic acid) (PLGA), are able to encapsulate vaccines and release them over an extended period of time up to several weeks, they are not able to maintain antigen stability over the longer time intervals *in vivo*. Vaccines such as IPV, however, are known to be unstable at elevated temperature, such as the 37°C environment of the body, as well as in the acidic environment of the degrading PLGA microspheres.

We identified excipients that stabilize IPV at elevated temperature over time as well as against other stresses that the vaccine would face during encapsulation in PLGA microspheres. We then showed that PLGA-based microsphere formulations can co-encapsulate IPV along with stabilizing excipients and release D-antigen active IPV over the course of weeks in vitro. The pH-sensitive material Eudragit E PO was doped into the microsphere formulation and controlled the PLGA degradation rate in a concentration-dependent, easily tailored manner while also protecting IPV from acid damage. The best formulations released IPV in two separate bursts, with the total release equivalent to two standard clinical doses (Figure 1), mimicking the delivery of two boluses approximately one month apart.

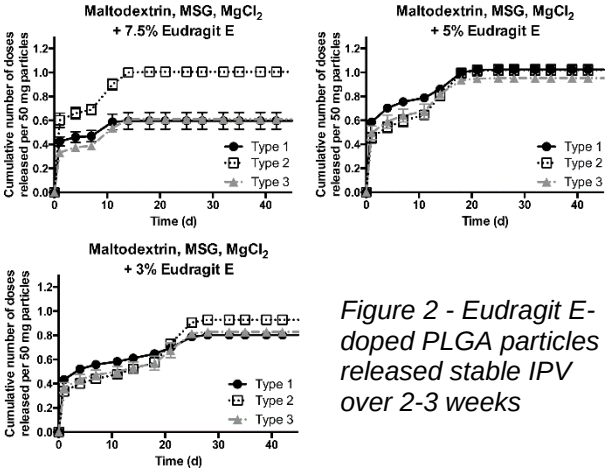

Figure 2 - Eudragit E-doped PLGA particles released stable IPV over 2-3 weeks

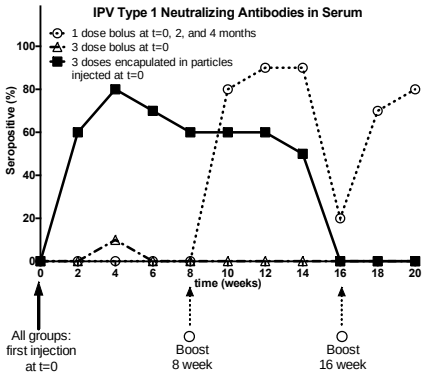

Figure 3 - Encapsulated IPV elicits a more robust neutralizing response than a bolus of IPV.

Leading formulations from the *in vitro* release studies were administered intramuscularly (IM) to rats, and antibody titers were measured over time. A single bolus was not sufficient to generate any measurable neutralizing antibody response against poliovirus serotype 1, the least stable of the three serotypes, and only with multiple bolus doses spread over 2-4 months did we detect protective levels of antibodies in the animals, with a peak of 90% protection (Figure 2). In contrast, only one injection of IPV encapsulated in microspheres was enough for 80% seroprotection within 4 weeks. In addition, while protective levels after bolus IPV injections fall to 20% within 8 weeks of the last administration, encapsulated IPV required only a single administration and conferred protection of >50% for at least 14 weeks. By reducing the number of necessary administrations to elicit a more long-lasting and robust protective response, this technology could be a tool to aid in the eradication of polio and could serve as a platform technology applicable to other infectious diseases for the improvement of global health.

CURRENT TECHNOLOGIES FOR ADVANCING HIV VACCINES

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Key Words: HIV vaccine, recombinant, viral vectors.

Development of an effective AIDS vaccine is a global priority. However, the extreme diversity of HIV along with host factors that prevent the elicitation of protective immune responses, continue to hinder vaccine development. Breakthroughs in understanding of the biology of the transmitted virus, the structure and nature of its envelope trimer, vaccine-induced CD8 T cell control in primates, and host control of broadly neutralizing antibody elicitation have given rise to new vaccine strategies. This presentation will cover promises and challenges associated with development of both prophylactic and therapeutic approaches to combat HIV with recent practical examples of technological approaches behind encouraging developments.

APPLICATIONS OF DNA VACCINE TECHNOLOGY TOWARDS DIFFICULT IMMUNE TARGETS

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Major improvement in DNA vaccine technology over the past decade has reinvigorated this platform which has conceptual advantages over traditional vaccine platforms. In humans prior generations of DNA vaccines were poorly immunogenic. Through multiple improvements including synthetic optimization, genetic adjuvant technology with enhanced EP delivery this technology exhibits improved performance. These synthetic DNA vaccines drive immune responses similar or superior to live viral vectors. We present data in animal models and in human studies that illuminate their immune potency and clinical efficacy targeting both established infection and in prevention approaches to EID.

ADVANCING THE MRNA THERAPEUTICS PLATFORM FOR VACCINES

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Messenger RNA (mRNA) can be encoded for a target protein and designed to be taken up by the cells in specific tissues and organs. Once delivered, like native mRNA in healthy individuals, mRNA acts as cellular software directing ribosomes to express proteins or antibodies within targeted tissues. mRNA holds the potential to address medical needs that are not treatable through current approaches. In this talk the ability of mRNA vaccines to express a variety of antigens in vivo and to induce a robust immune response will be discussed.

VACCINE BASED IMMUNOTHERAPY REGIMEN FOR THE TREATMENT OF PROSTATE CANCER

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A successful cancer vaccine will activate the host immune system to induce a balanced T- and B-cell response to tumor-specific antigens that can lead to tumor regression. Effective cancer vaccines will need to overcome several challenges to accomplish this goal, such as to break tolerance to the tumor associated antigens which are mostly self-antigens, induce and maintain high tumor-specific T-cell titers to keep the immune pressure of cytolytic killing of tumor cells as well as to overcome the immune suppressive tumor micro-environment to keep the T cells active at the tumor site. We have applied the lessons learned in the field to the development of a multi-component cancer immunotherapy regimen that has entered clinical testing at the beginning of 2016 for the treatment of patients with prostate cancer. The Vaccine Based Immunotherapy Regimen (VBIR) consists of a chimpanzee adenovirus prime vaccination administered intra-muscularly followed by DNA plasmid boost vaccinations delivered with an electroporation device. With each vaccination low dose of an anti-CTLA4 antibody (tremelimumab) is administered subcutaneously, in close proximity of the vaccine draining lymph nodes. The AdC68 and DNA express the three prostate cancer antigens PSA, PSMA and PSCA. Lastly, to counter the immune suppressive tumor micro-environment, Sutent is added to the regimen to lower myeloid derived suppressor cells at doses that are lower than the approved clinical dose or anti-PD-1 to interfere with the PD-L1/PD-1 signaling pathway.

DEVELOPMENT OF IMMUNOTHERAPEUTIC IMMUNIZATION FOR VIRUS INFECTIONS AND CANCER

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Key Words: Immunotherapy, Cell mediated immunity, Viruses, Cancer

Vaccines to prevent virus infection have generally been successful in preventing epidemic childhood infections. However, immunization to resolve persisting viral infection or to eradicate cancer cells expressing non-self antigen has proven challenging when naturally induced immune responses have proven insufficient. In many clinical trials, only minimal efficacy has been demonstrated. We have used animal models of persisting infection to study better immunotherapy strategies. In a model of Herpes Simplex infection, infection can be controlled by inducing immune responses in skin rather than muscle, and by biasing those responses to favor T cell responses over antibody, enabling clearance of otherwise lethal infection. In a model of persisting infection with papillomavirus, we have shown that hyper proliferative epithelium expressing papillomavirus proteins secretes cytokines attracting immunoregulatory lymphocytes, and these suppress cytotoxic T cell responses. Local immunosuppression can be overcome by removing inhibitory cytokines, by administering checkpoint blockade inhibitors, or by preventing epithelial proliferation and consequent attraction of regulatory cells. Clinical trials based on these findings, planned or underway, will be discussed.

RSV VACCINES FOR THE YOUNG AND THE OLD

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Key words: Respiratory Syncytial Virus, F protein

Respiratory Syncytial Virus (RSV) is a single-stranded RNA virus of the family *Paramyxoviridae*. RSV causes a significant burden of respiratory disease in the most vulnerable members of the population: young children and older adults, particularly those with pulmonary and cardiovascular – related co-morbidities. RSV presents many scientific and clinical challenges, and despite half a century of research no broad-based prophylactic vaccine for RSV has been approved. Since the 1998 approval of Synagis® (palivizumab), a monoclonal antibody for the prophylaxis of premature and other high-risk infants, we have developed new technologies and approaches to expand RSV prophylaxis to all vulnerable members of the population. MEDI8897 is a second-generation monoclonal antibody for prophylaxis of newborns. The mAb targets the recently identified 'Site 0' epitope on the pre-fusion form of the RSV fusion (F) antigen and binds to the virus with 100-fold higher affinity than palivizumab. Combined with modifications for half-life extension and formulation for intramuscular injection, MEDI8897 is intended for all infants entering their first RSV season, as well as children with chronic heart & lung conditions entering their second RSV season. The burden of RSV illness in children remains significant beyond the first season, and for this we are pursuing various approaches for active vaccination. Options for protecting this population will be discussed in the context of the scientific and clinical challenges inherent in active vaccination for RSV in children. MEDI7510 is a protein subunit vaccine candidate designed for older adults and is formulated with a TLR4-based adjuvant. In a Phase I clinical trial of older adults, MEDI7510 was safe and well tolerated, and neutralizing antibody titers were boosted above pre-vaccination levels. T-cell responses against the RSV-F antigen were also boosted above pre-vaccination levels. A Phase II trial of Medi7510 is in progress.

DEVELOPMENT, MANUFACTURING, AND SUPPLY OF MSD'S EBOLA VACCINE

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Ebola was first discovered in 1976 and is a member of the filoviridae family of viruses. There are multiple strains of Ebola and infection can lead to hemorrhagic fever and death. In March 2014, a historic Ebola outbreak occurred in three Western African countries, Guinea, Liberia, and Sierra Leone. Over 28,000 cases were reported and led to more than 11,000 deaths, more than ten times the amount of cases compared to all past outbreaks combined. On August 8th 2014, the World Health Organization declared a Public Health Emergency of International Concern (PHEIC). Merck Sharp & Dohme (MSD) partnering with NewLink Genetics entered into an exclusive worldwide licensing agreement to research, develop, manufacture, and distribute an investigational Ebola vaccine candidate based on recombinant Vesicular Stomatitis Virus (rVSV) technology. Working with multiple partners, Merck Sharp & Dohme have brought forward an efficacious vaccine candidate from Phase I trials in October 2014, to Phase III consistency studies by August 2015. This presentation will provide background into Merck Sharp & Dohme's strategy to bring the vaccine to licensure, product development activities to scale-up the process from clinical to commercial, and the challenges faced during product development.

THIRD GENERATION VACCINE FOR WORLD ERADICATION OF POLIOMYELITIS

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Key Words: Poliovirus, Hyperattenuated strains, IPV production process, animal component-free medium, platform process

Great efforts have been undertaken by the World Health Organization to achieve eradication of poliomyelitis, a paralytic disease. At present, two different vaccines are available: inactivated polio vaccine (IPV) developed by Salk based on chemical inactivation of the virus and oral polio vaccine (OPV) developed by Sabin based on live attenuated virus strains. The risks associated with IPV concern the safety of the production process as it is based on highly virulent wild type strains, and in contrast, the OPV risks are associated with the reversibility of the attenuated viruses to a transmissible paralytic form. There is therefore a need for a new generation polio vaccines capable to overcome outbreaks and manufacturing risks.

With the evolution of molecular virology of Sabin vaccine strains, it is now possible to design extremely genetically stable and hyperattenuated strains without the associated reversion risks. Sabin poliovirus strains were therefore genetically modified giving rise to the third generation of polio vaccine strains [1, 2].

In the present work we have explored the possibility of using the already well-established IPV production process, developed at our site [3] and integrated worldwide [4] for the production and manufacturing of third generation of IPV strains. Specifically, we have produced third generation vaccines in animal component free medium and at 50-L pilot scale. The product obtained did show acceptable yields and was immunogenic in rats. Together, our results indicate that the third generation vaccine strains produced under the flexible platform process are potential candidates which provide increased biosafety during manufacturing which is necessary after polio eradication. In addition, the flexibility and scalability of the process constitute a platform for the production of a large range of vaccines worldwide.

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IMPROVING GLOBAL HUMAN HEALTH THROUGH NOROVIRUS VIRUS-LIKE PARTICLE MANUFACTURING

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Norovirus infection is the most common cause of acute gastroenteritis in the U.S., estimated to afflict 21 million people per year. For scale-up manufacturing of a norovirus vaccine candidate, the baculovirus expression system has been used in 1000 L working volume stirred-tank bioreactors for production of two distinct norovirus virus-like particles (VLP). Downstream processing using methods for enveloped virus inactivation and a series of orthogonal chromatography steps results in norovirus VLP that contain low residual levels of host-cell protein, host cell and baculovirus DNA, and are free of replicating baculovirus. Emphasis has been placed on employing single-use technologies including disposable bags for media storage and sample collection, disposable bioreactors for VLP production, capsule filters for VLP harvest, and membrane chromatography for VLP capture. Takeda Vaccine's VLP manufacturing process results in quantities of highly purified VLP required to support upcoming Phase III trials and early commercial launch

HOW IS VACCINE R&D PIPELINE STRATEGY GOING TO EVOLVE FOR PHARM INDUSTRY…?

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The pharmaceutical industry has gone through huge changes since the early 2000s. Faced with patent expirations, increased cost of development, reduced R&D productivity and increased price pressure, the pharmaceutical industry had continuously to rethink its model to keep its success, and continues to do so. In the presentation, we'll review how the model has evolved over time, and continues to evolve from Pharma 1.0 (traditional model), through Pharma 2.0 (2005-2010) to pharma 3.0 (> 2020).

National decision makers and Health care payers in the industrialized world are heavily focusing on the importance of cost effectiveness of public health interventions. We'll discuss how, in this context, the importance of prevention and vaccines will grow as pressure on health care budgets increases, and the global population grows and ages. Breakthrough in better understanding of the immune pathways, availability of new vaccine technology platforms to successfully trigger these pathways, will be key contributors to vaccination become one of the key pillars of future healthcare, both in prevention, and, later on, in disease interception.

CHALLENGES IN THE DEVELOPMENT AND SCALE-UP OF A PURIFICATION PROCESS FOR AN ATTENUATED LIVE VIRUS VACCINE CANDIDATE

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Key Words: Live Attenuated Virus, Single-Use Bioreactor, Purification, Closed Sterile Processing

Prophylactic live attenuated vaccines (LAV) have been successfully developed for multiple viral disease targets, offering an advantage over subunit vaccine approaches by simultaneously stimulating innate, humoral and cellular immune responses. However, the development of manufacturing processes for robust production of LAVs at commercially viable scales can be challenging, particularly because of the need to use novel and/or adherent cell lines, the inefficient performance of conventional chromatography for processing large viral particles, and the complexity of product characterization. Further adding to these challenges, closed-system aseptic processes are required for those viruses too large for terminal sterile filtration, thereby limiting processing options and complicating process logistics at commercial scale. Highlighting these challenges, we present here on the development of a scalable, fully sterile, purification process for a large, enveloped, live attenuated virus having multiple glycoprotein complexes. During the development of this vaccine the process was changed from a static cell culture process to one that is amenable to scaling in a stirred tank single-use bioreactor. This change presented challenges for the purification process, requiring modifications of the process separation techniques including evaluation of new unit operations of various separation modes such as membrane and monolith absorbers, resin chromatography, selective precipitation, large pore tangential flow filtration, and centrifugation. Critical to this evaluation was the understanding of the adaptability of these unit operations to closed sterile processing, with a premium placed on industry ready, single-use technologies. Through this process development effort, a scalable, sterile, purification process was defined that met targets for purity and yield.

INSECT CELLS PLATFORMS FOR FAST PRODUCTION OF PSEUDO-TYPED VLPS FOR DRUG AND VACCINE DEVELOPMENT

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Keywords: Insect cell expression; Flipase-mediated cassette exchange; Baculovirus infection; HIV Gag-VLPs; GPCRs; Vaccines.

Expression systems capable of delivering high concentrations of membrane proteins in their native structure are essential in the vaccine field as well as in drug discovery. In this work, we took advantage of insect cell expression and site-specific gene integration based on flipase-mediated cassette exchange (FMCE) technology to generate cell platforms for efficient production of membrane proteins on the surface of a protein scaffold, namely enveloped virus-like particles (VLPs). The expression of membrane proteins concomitantly with capsid proteins of enveloped viruses (e.g. HIV Gag or influenza M1) will enable their capturing in lipid rafts of the cellular plasma membrane and their display on the surface of budding VLPs, thus providing a native conformation for downstream assays.

Parental insect *Sf*-9 and High Five cells were randomly tagged with GFP-fused Gag or M1 proteins and FACS enriched with cells tagged in genomic “hot-spots” supporting high expression. A linker including a Flp recognition target (FRT) site was used to allow posterior removal of the marker gene from the particle through cassette exchange. By confocal microscopy we could observe that Gag localizes preferentially at the plasma membrane whereas M1 disperses within the cell. Upon promoting Flp-mediated recombination in the tagging populations, cassette exchange was well succeeded, allowing to recover cells tagged in loci supporting FMCE. We are currently evaluating the capability of both core proteins as scaffolds to display GPCRs (e.g. beta-2 adrenergic receptor) and Influenza HA proteins. For the latter, we will present recent results on the feasibility of combining stable and baculovirus-mediated expression of HA in insect High Five cells for production of multi-HA influenza enveloped VLPs towards the development of an “universal” vaccine. This strategy surpasses standard methods for production of multivalent Influenza VLPs such as coinfections or the use of larger, unstable vectors. Overall, modular insect cells platforms are being generated to be readily adaptable for production of a broad range of VLP-based vaccines as well as receptor display particles for drug screening or antibody discovery.

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DETERMINING WHETHER ADSORPTION STATE IS A CRITICAL ATTRIBUTE IN ALUMINUM ADJUVANTED VACCINES

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Aluminum based adjuvants have a long history of safe and effective use in vaccine products. When developing vaccine formulations incorporating aluminum adjuvants it is important to understand whether adsorption state is a critical attribute of the vaccine. Investigations initiate with obtaining an understanding of how the antigen of interest interacts with aluminum adjuvant surfaces. The balance of attractive versus repulsive interaction forces (including electrostatic, ligand exchange, and hydrophobic interactions) determines the resulting adsorption state of the antigen. Once the interaction of aluminum adjuvant and antigen is understood, formulations can be prepared along the spectrum of aluminum adjuvant surfaces to obtain various levels of adsorption. These formulations are evaluated in a relevant animal model to determine the impact of adsorption state on potency of the vaccine. The impact of adsorption state on potency determines whether antigen adsorption state is a critical attribute of the vaccine formulation. Further development can then optimize the formulation to maintain safety, stability, and efficacy over the desired shelf life of the vaccine product.

Assessment of the criticality of adsorption state was evaluated for the development of a vaccine targeting *Streptococcus pyogenes* (group A strep) to determine that adsorption to an aluminum containing adjuvant was critical to optimize potency of the vaccine. Process and analytical techniques used to determine antigen/adjuvant interactions, produce variable aluminum surfaces, as well as vaccine formulation for *in vivo* potency determination will be discussed. Understanding how to determine the criticality of antigen adsorption state early in the development process allows for rapid development of a robust vaccine formulation.

ANALYTICAL CHARACTERIZATION OF HUMAN CYTOMEGALOVIRUS VACCINE AND VACCINE INDUCED HUMORAL RESPONSES

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Key Words: HCMV, vaccine, characterization, pentameric complex, epitope

Congenital infection of human cytomegalovirus (HCMV) is one of the leading causes of non-genetic birth defects, and development of a prophylactic vaccine against HCMV infection is of high priority for public health. Merck has a Phase I clinical trial of a replication-defective live HCMV virus vaccine with restored expression of the pentameric complex gH/gL/pUL128-131. In this presentation, we will describe the in-depth characterization of the soluble pentameric complex. We will discuss how the characterization of the pentameric complex increases our understanding of the immune dominant component of the vaccine and how it enables us to evaluate the quantity and quality of humoral responses elicited by the vaccine.

MULTI-TASKING AN INACTIVATED INFLUENZA VACCINE TO PROVIDE RAPID INNATE IMMUNE SYSTEM-MEDIATED AND SUBSEQUENT LONG-TERM ADAPTIVE IMMUNITY AGAINST INFLUENZA AND SECONDARY PNEUMOCOCCAL INFECTIONS

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Key Words: influenza, inactivated vaccine, adaptive immunity, innate immunity, Streptococcus pneumonia

The threat to global health posed by influenza warrants continued efforts to improve the protective capability of influenza vaccines particularly against outbreaks of novel strains. Both innate and adaptive immune systems differ in mechanism, specificity and times at which they take effect. The innate immune system responds within hours of exposure to infectious agents while adaptive immunity takes several days to become effective. Here we show, by using a simple lipopeptide-based TLR2 agonist, a low dose of an inactivated detergent-split influenza vaccine can be made to simultaneously stimulate and amplify both systems in animals to provide immediate antigen-independent anti-viral protection mediated by innate immune responses while giving the adaptive immune system time to effect long-term antigen-dependent immunity (Chua et al. 2015). This immediate effect protects against both homologous and serologically distinct heterologous viral strains within a day of administration for up to a week. The enhancement of the adaptive immune response is characterized by the induction of high levels of hemagglutinin and neuraminidase-inhibiting antibodies against homologous virus as well as viral nucleoprotein-specific primary $CD8^+$ T cell responses, which act to reduce disease severity associated with heterologous viral infection and significantly mitigate the severity of infection caused by contact-dependent transmission. Results from the use of antibody deficient and $CD8^+$ T cell depleted animals also indicate that the heterologous immunity bestowed by this vaccine co-formulation is attributed to robust recall T cell-mediated responses. Additionally, we also demonstrate that vaccination can significantly lessen the impact of secondary infections with *Streptococcus pneumonia* by reducing (i) viral-associated pulmonary bacterial burdens, (ii) levels of pro-inflammatory cytokines that normally accompany co-infection and (iii) the vascular permeability of the pulmonary tract thereby preventing systemic bacterial infection (Mifsud et al. 2015). These protective effects are achieved using a considerably smaller dose of vaccine than is usually required to induce biologically active antibody responses in animals. The value of this cost-effective method coupled with its ease of implementation and conferring of dual functionality on influenza vaccines could be especially beneficial for improving community protection particularly during periods between an outbreak and when a vaccine becomes available or in scenarios when there is imperative for mass vaccination against a strain to which the population is immunologically naïve.

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CORRELATIONS OF ANTIBODY RESPONSE PHENOTYPE TO GENOTYPE REVEALED BY MOLECULAR AMPLIFICATION FINGERPRINTING

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Key Words: vaccine profiling, antibody repertoire, next-generation sequencing, systems immunology

It has long been possible to measure the phenotype of antibody responses (antigen-specific titers) through conventional serological assays (e.g., ELISA). In contrast, the ability to measure the genotype of antibody responses has only recently become possible through the advent of high-throughput antibody repertoire sequencing (Ig-seq), which provides quantitative molecular information on clonal expansion, diversity and somatic hypermutation. However, Ig-seq is compromised by the presence of bias and errors introduced during library preparation and sequencing and thus prevent reliable immunological conclusions from being made. By using synthetic antibody spike-in genes, we determined that Ig-seq data overestimated antibody diversity measurements by up to 5000-fold and was less than 60% accurate in clonal frequency measurements.

In order to overcome the widespread inaccuracies in Ig-seq, we developed a method known as molecular amplification fingerprinting (MAF). This consists of stepwise incorporation of unique molecular identifiers (UID), which starts with tagging first-strand cDNA during reverse transcription with a reverse-UID (RID), thus providing a unique tag to each transcript. Importantly MAF continues by tagging each DNA-RID molecule during multiplex-PCR amplification with a forward-UID (FID). This fingerprint of amplification for each molecule allowed us to implement an algorithm to normalize multiplex-amplification bias effects. We used several bioinformatic steps for error correction, this resulted in nearly absolute (98%) or absolute (100%) correction of intraclonal and clonal variants, respectively (Fig. 1a, b). Furthermore, by tagging molecules during amplification we were able to develop a novel algorithm for bias correction, this resulted in 98% accuracy of antibody clonal frequencies (Fig. 6c, d).

In order to probe the relationship between antibody genotype and phenotype, we have performed extensive *in vivo* experiments from immunized mice. Specifically, we modulated titer (phenotype) by varying the number of immunizations (boosts) and in parallel performed MAF Ig-seq to assess genotype through a series of quantitative metrics. These metrics describe clonal selection, expansion, and somatic hypermutation. Importantly, we also used different protein antigens, this allowed us to determine how epitope complexity impacted the genotype of antibody repertoires. Finally, by applying multivariate modeling using MAF-corrected data, we were able to predict the immune status of individual antibody clones. This extensive systems-based analysis demonstrates how accurate Ig-seq provides insight on the genotype and phenotype of humoral immunity.

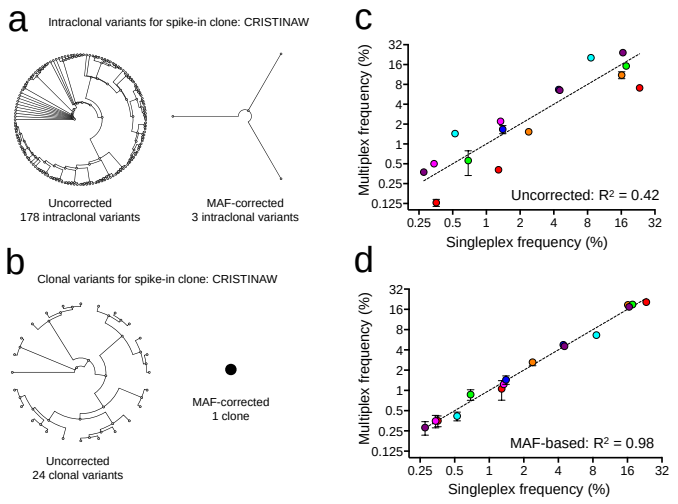

Figure 1. MAF error and bias correction validation. (a) Phylogenetic trees before and after MAF-error correction of intraclonal variants for a single spike-in example clone. (b) Phylogenetic trees before and after MAF-error correction of clonal variants (CDR3 a.a.) for a single spike-in example clone. (c) Correlation of uncorrected spike-in clonal frequencies from multiplex-PCR versus singleplex-PCR results in an $R^2 = 0.42$. (d) Correlation of MAF bias corrected spike-in clonal frequencies from multiplex-PCR versus singleplex-PCR results in a significantly improved $R^2 = 0.98$.

IMMUNE ENGINEERING ENHANCES H7N9 VACCINE IMMUNOGENICITY BY REGULATORY T CELL EPITOPE DELETION IN HEMAGGLUTININ

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Key Words: influenza, T cell epitope, regulatory T cell, antibody, humanized mice

Avian-origin H7N9 influenza is a novel influenza group A virus that emerged in humans in China in 2013. H7N9 influenza hemagglutinin (HA) elicits weak neutralizing antibody responses in natural infection and vaccination. Limited helper T cell response could explain the poor immunogenicity observed [1]. We hypothesize a T cell epitope in H7-HA stimulates regulatory T cells (Tregs) capable of suppressing crucial signals needed for protective antibody production. Furthermore, deletion of the epitope without perturbing neutralizing B cell epitope structures may increase H7-HA immunogenicity to produce an optimized vaccine.

Immunoinformatics tools were used to identify H7N9 class II HLA epitopes with high potential to cross-react with Tregs educated on human antigens. In peripheral blood leukocyte cultures, H7N9 epitopes with significant human homology expanded CD4+CD25+FoxP3+CD39+ Tregs and reduced IFNγ secretion when co-incubated with other H7N9 epitopes with low potential cross-reactivity [2]. We applied this finding to design an antigenically improved H7-HA based on Anhui/01 by introducing three modifications to recombinant HA (rHA) that delete a highly conserved Treg activating epitope. Engineered rHA (Opt1 rH7-HA) demonstrated both preserved antigenicity and improved immunogenicity in humanized mice. Monoclonal antibodies raised against wild type H7-HA recognized Opt1 rH7-HA with affinity equivalent to the wild type protein, suggesting that modifications did not induce significant structural perturbations. Similarly, human polyclonal sera demonstrated identical binding profiles against Opt1 and wild type rH7-HA. Vaccination of immunodeficient mice reconstituted with human PBMCs (N=8) using non-adjuvanted 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. In a related study, HLA-DR3 transgenic mice were immunized with Alum-formulated H7N9 virus-like particles containing either Opt1 or wild-type H7-HA and hemagglutinin inhibition (HAI) titers were measured. Opt1 rH7-HA stimulated protective levels of 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 vaccine.

Epitope-driven approaches to vaccine design that carefully consider T cell subsets primed in immunization promise to enhance vaccine efficacy.

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VACCINATION AS A TOOL TO REDUCE ANTIMICROBIAL RESISTANCE WORLDWIDE

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Antimicrobial resistance is a global human and animal health concern that is influenced by the non-appropriate use of antimicrobial agents in both human and veterinary medicine, as well as in the plant sector. To combat antimicrobial resistance, the World Organisation for Animal health (OIE) has developed science-based intergovernmental standards and guidelines covering terrestrial animals and aquaculture.

The OIE also contributed to the development of the WHO Global Action Plan on Antimicrobial Resistance, adopted in 2015 by the World Health Assembly. The 180 Member Countries of the OIE expressed their support to this plan of action through a Resolution, unanimously adopted in May 2015.

As a contribution to the global actions to address antimicrobial resistance, and in consideration of the use of vaccines to prevent diseases as one of the possible options to reduce the use of antimicrobial agents at the global level, the OIE convened an *ad hoc* Group on Prioritisation of Diseases for which Vaccines could Reduce Antimicrobial Use in Animals in April 2015.

Animal diseases for which availability and use of vaccines could reduce the use of antimicrobial agents in animals were identified and recommendations were made to better target research programmes for new or improved vaccines. The Group focused on pigs, poultry and fish as a first step and reviewed the main reasons for antibiotic use. Key diseases, including some viral diseases, driving antibiotic use in animals were considered, and areas for research, where investment could lead to new or improved vaccines with the potential to reduce antibiotic use were identified.

The outcome of this work, which will be presented with more details, was the development of tables of ranked priority diseases per species considered with the aim of providing direction to policy makers and research communities and industry on where to invest to reduce the need for antimicrobial use in animals with a focus on vaccines.

References:
Report of the *ad hoc* Group on Prioritisation of Disease for which Vaccines could reduce Antimicrobial Use in Animals (Annex 5
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STRUCTURAL-BASED DESIGNED MODULAR CAPSOMERE COMPRISING HA1 AS LOW-COST POULTRY INFLUENZA VACCINE

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Key Words: influenza, vaccine, poultry, capsomere, polyomavirus

Influenza is a severe respiratory tract infection caused by influenza viruses. The increasing number of highly pathogenic avian influenza (HPAI) virus outbreaks, generally H5 and H7 subtypes, underlines the threat of a possible pandemic. The recent HPAI H5 outbreak in domestic poultry and wild birds in US from December 2014 to June 2015, affecting almost 50 million birds and resulting in $3.3 billion economic losses due to the death and culling of poultry, has demonstrated the lack of capabilities to control the rapid spread of avian influenza. Poultry vaccination has been shown to not only reduce the virus spread in animals but also reduce the virus transmission to humans, preventing potential pandemic development. However, existing vaccine technologies could not respond to a new virus outbreak rapidly and at a cost and scale that is commercially viable for mass poultry vaccination. Here, we developed modular capsomeres, building blocks of virus-like particle, as a low-cost poultry influenza vaccine. Modified murine polyomavirus (MuPyV) VP1 capsomere was used to present structural-based designed influenza Hemagglutinin (HA1) antigen. Six constructs of modular capsomeres presenting three truncated versions of HA1 and two constructs of modular capsomeres presenting non-modified HA1 have been generated. Modular capsomeres presenting HA1 were successfully produced in stable forms using *Escherichia coli*, without the need for protein refolding process. This adjuvanted modular capsomere (CapHA1) induced strong antibody responses (almost 10^5 endpoint titre) when administered into chickens, similar to titres obtained in the group administered with insect cell-based HA1 proteins. Based on our process simulation, 320 million doses of modular capsomere vaccines can be produced in 2.3 days, at a cost of less than 1 cent per dose. The result presented here indicated that this platform for bacterially-produced modular capsomere could potentially translate into a rapid-response and low-cost vaccine manufacturing technology suitable for poultry vaccination.

DEVELOPMENT OF A VACCINE BASED ON RECOMBINANT SUBUNIT PROTEINS TO PROTECT HUMANS AND ANIMALS AGAINST FILOVIRUS DISEASE

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Key Words: Ebola, filovirus, recombinant, subunit, preclinical

Ebola Virus Disease (EVD) is the most prominent example of filovirus disease and as a zoonotic virus fits the characteristics of a neglected tropical disease. Despite being characterized as a Category A Priority Pathogen by NIH/NIAID over a decade ago, EVD lacked public and private research resources due to the absence of a commercial market. Previously, outbreaks of limited scale linked to transmission from livestock or wild animals into the human population occurred in the endemic areas located in the forested regions of Central Africa and the Philippines (for *Reston ebolavirus*), therefore other public health threats garnered more attention. This changed recently in 2013-2015 when an Ebola virus (EBOV) outbreak of increasing size in several West African countries started to reveal the true epidemic potential that filovirus infections can have when entering an urban setting in a highly mobile society. As typical in an epidemic with a significant number of infectious patients traveling within and from the endemic area, the disease was also exported outside the outbreak region as has been shown with introductions into Nigeria, Mali, and the United States (amongst other countries averting in-country transmission from imported cases). This demonstrated the threat posed to the global public health systems if spread of Ebola or a related filovirus cannot be contained at its source.

We have produced three soluble recombinant filovirus glycoproteins (GP) and the matrix proteins of EBOV (VP24 and VP40) using the Drosophila S2 cell expression system. For each antigen, a specific immunoaffinity chromatography method was developed to allow purification to purity levels >90%. The immunogenicity of recombinant subunits and admixtures formulated with or without clinically relevant adjuvants was subsequently evaluated in mice, guinea pigs and macaques.

Strong antigen-specific IgG titers as well as virus neutralizing titers were observed after administering two or three doses of adjuvanted formulations. In mice and non-human primates subunit proteins were also shown to elicit cell mediated immune responses. Analysis of secreted cytokines in batch-cultured, antigen-stimulated splenocytes or PBMC's demonstrated antigen-induced Th1 and Th2 type responses.
Recombinant vaccine candidates were tested in mice for protection against challenge with mouse-adapted EBOV. All vaccine formulations containing EBOV GP generated protective responses and serum transfer from such animals into naïve mice demonstrated that humoral immunity alone can be fully protective. Furthermore, the transfer of immune splenocytes into naïve mice showed that recombinant GP and VP24 subunits elicit functional T cell responses that lead to protection against live virus challenge.

Immunogenicity and efficacy studies in guinea pigs were focused on optimized antigen dosing, antigenic balance and adjuvantation. Multiple formulations consistently produced strong antibody responses and demonstrated 100% protective efficacy in the EBOV guinea pig model.

Results from studies in two species of non-human primates suggest that vaccination with GP+VP40+VP24 and an emulsion-based adjuvant consistently produces high anti-EBOV IgG and virus neutralizing titers. This prevents viremia subsequent to live virus challenge and protects animals from terminal EBOV disease. These studies suggest that we have defined a viable Ebola virus vaccine candidate based on non-replicating viral subunits.

Current efforts in our laboratory are focused on defining correlates of protection to allow clinical development of a monovalent vaccine candidate for protection against EVD and further formulation optimization towards a trivalent, recombinant subunit vaccine with protective efficacy against EBOV, *Sudan ebolavirus* (SUDV) and *Marburgvirus* (MARV) infection.

HOW TO DELIVER NEW VACCINES UNDER VERY SHORT TIMELINES : THE ZAPI PROJECT

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The ZAPI project is the first One Health project in the European Innovative Medicine Initiative (IMI) program. Its objective is to define and validate unique methodologies that can be applied when new zoonotic infectious diseases will occur in Europe or other regions of the world. The project is supporting an entirely new approach for “anticipating the unexpected”, and is defining new ways to alleviate the technical constraints which are typically encountered with the classical vaccine development processes.

The ZAPI project involves a total of 20 partners, from academic, biotech, and industry origin. One important feature of ZAPI is that it is “framed by an industrial mindset” from the start, in order to ensure the delivery of vaccine products that can effectively be manufactured at large scale. This quite ambitious objective will eventually be achieved through a succession of breakthroughs. The ordered sequence of these technical steps will define a “universal methodology” which can be used on the new emerging viruses. As the key deliverable of the ZAPI project, this methodology will reduce very significantly the timelines for the manufacturing cycles of vaccine batches.

The different constraints and steps of this “vaccine product by design” approach will be described.

FROM VIRUS DISCOVERY TO INTERVENTION

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The vast majority of emerging and re-emerging pathogens in humans are of animal origin. Most if not all of this growing number of threats have their origin in wildlife, while humans are exposed either directly or through indirect domestic animal contacts. After having crossed the species barrier, the pathogen may directly start spreading efficiently among humans, like Ebola virus, or may need to adapt to further allow efficient intra-species transmission, like H5N1 avian influenza virus. Effective and economical ways of protecting mankind from emerging diseases are best based on combatting zoonotic pathogens at the animal source. The “One Health” concept creates awareness of the major opportunities that exist to protect public health through policies aimed at controlling these pathogens at the level of their animal hosts, or more specifically, at the interface between humans, animals and their environments. Implementation of these policies places those who have regular contacts with domestic animals, like owners, handlers and veterinarians in the front line together with people who regularly come into contact with wildlife and their environment. Investment in the establishment of strategies that allow rapid development and implementation of specific diagnostic, vaccine and anti-microbial strategies is crucial in this respect. Therefore the “One Health” concept highlights the importance of integration between medical and veterinary disciplines to combat the threat of emerging pathogens from the animal world.

SYSTEMS BIOLOGY APPROACHES TO STUDY HUMAN VACCINATION RESPONSES

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Key Words: Systems Immunology, Computational Biology, Correlates of Vaccination Responses, Human Immunology, Single Cell Genomics

The move toward precision medicine has highlighted the importance of understanding biological variability within and across individuals in the human population. In particular, given the prevalent involvement of the immune system in diverse pathologies, an important question is how much and what information about the state of the immune system is required to enable accurate prediction of future health and response to medical interventions. Towards addressing this question, our and others' recent studies using vaccination as a model perturbation and systems-biology approaches are beginning to provide a glimpse of how natural population variation together with multiplexed, high-throughput measurement and computational analysis can be used to uncover predictors of vaccination response quality in humans. Here I discuss our recent work in this emerging field, with emphasis on baseline correlates of vaccination responses, as well as highlighting relevant features of study design, data generation, and computational analysis.

STRUCTURE-BASED VACCINE DESIGN: LESSONS FROM RSV F

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The use of atomic-level information in the design of immunogens has created new candidate vaccines against important pathogens. From our work on the fusion glycoprotein from respiratory syncytial virus (RSV), we have learned several important lessons that may be generally applicable to other structure-based vaccine-design efforts. This talk will summarize these lessons and the latest advances in the field, as well as highlight opportunities for future development.

INDUCTION OF ANTIGEN-SPECIFIC IMMUNOLOGICAL TOLERANCE WITH SYNTHETIC NANOPARTICLE VACCINES

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Keywords**:** Immunotolerance, tolerogenic vaccine, nanoparticle, Treg, uricase

The context in which dendritic cells encounter antigen can determine the outcome of the immune response. Conventional vaccines provide antigen in the context of an adjuvant to stimulate antigen-specific immune responses. We have recently engineered nanoparticles to present antigen in the context of a tolerogenic signal provided by rapamycin to induce antigen-specific immune tolerance. These self-assembling, biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles containing rapamycin together with either co-encapsulated antigen or admixed with free antigen are capable of inducing durable antigen-specific tolerance that control adaptive immune responses and withstand multiple immunogenic challenges with antigen. We demonstrate that administration of tolerogenic nanoparticles through multiple routes (e.g. subcutaneous and intravenous) inhibits the activation of antigen-specific T cells and B cells while inducing antigen-specific regulatory cells. Tolerance induction is dependent on the encapsulation of rapamycin, as free rapamycin is ineffective. In a model of experimental autoimmune encephalomyelitis, tolerogenic nanoparticles dosed therapeutically at the peak of disease completely inhibited disease relapse. Immune tolerance could be adoptively transferred to naïve mice in this model. The use of tolerogenic nanoparticles can also be applied for the prevention of anti-drug antibodies (ADAs) to biologic therapies. The development of ADAs is a common cause for treatment failure and adverse events, such as hypersensitivity reactions, associated with biologic therapies. We have demonstrated immune tolerance induction to a variety of antigens, including coagulation factor VIII in a model of hemophilia A, anti-TNF monoclonal antibody in a model of spontaneous arthritis, pegylated uricase in uricase deficient mice and in nonhuman primates, and adeno-associated vectors used in gene therapy. Tolerogenic nanoparticle therapy for the prevention of ADAs against pegylated uricase in the treatment of gout is currently being evaluated in Phase 1 clinical trials.

UNIVERSAL AND IN-PROCESS ANALYTICAL TOOL FOR INFLUENZA QUANTIFICATION USING A LABEL-FREE TECHNOLOGY

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Key Words: Multivalent VLPs, in-process HA quantification, sialic acid receptors, Octet RED, BLI technology.

Virus-like particles (VLPs) have become a promising solution for influenza pandemics, leading to an increasing interest on the development of VLP purification processes. However, the analytical methods used to detect and quantify VLPs are not yet able to keep up with the downstream progress. Currently, quantification relies on traditional methods such as hemagglutination (HA) assay, Single Radial Immunodiffusion (SRID) assay or Neuraminidase (NA) enzymatic activity assays. However, these analytical technologies are time-consuming, cumbersome and are only reliable for final product quantification and characterization, posing challenges for efficient downstream process development and monitoring. [1]

Here we report a label-free tool that uses Biolayer interferometry (BLI) technology applied on an octet platform to detect and quantify Influenza VLPs at all stages of downstream processing (DSP). Human (α2,6-linked sialic acid) and avian (α2,3-linked sialic acid) biotinylated receptors associated with streptavidin biosensors were used, in order to quantify HA content [2] in several mono- and multivalent Influenza VLP strains. The applied method was able to detect and quantify HA from crude sample up to final VLP product. The resulting concentration values are similar to HA quantification method.

BLI technology showed promising results as a high throughput analytical method with high accuracy and improved detection limits, when compared to traditional approaches. Moreover, it eliminates the need of fresh erythrocytes and reduces user variations on the quantitation. This simple and fast tool allowed for robust real-time results, which is crucial for in-line monitoring of DSP. Since the main goal of the work performed is to improve process control as well as monitoring, it may be used as a PAT (process analytical technology) tool.

[1] THOMPSON et al., Virology Journal, 10:141, 2013.
[2] CRUSAT et al., Virology, 447, 326-337, 2013.

APPLICATIONS OF HIGH-THROUGHPUT SINGLE B-CELL SEQUENCING TO ACCELERATE RATIONAL VACCINE DESIGN

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Key Words: Vaccine response analysis, HIV vaccines, antibody repertoire, high-throughput sequencing

Understanding the antibody repertoire response to vaccination is critical for the rational design and evaluation of experimental vaccines. Immune receptors comprise two chains encoded by separate mRNA strands and thus conventional NextGen sequencing fails to identify the native pairings encoded by individual lymphocytes. To overcome this limitation, we are applying recent technical advances in high-throughput sequencing of complete antibodies (i.e., paired heavy and light chain sequencing) to generate a quantitative understanding of experimental vaccine performance and to accelerate vaccine design. We apply repertoire-based metrics of vaccine-elicited antibodies to evaluate and select promising candidate immunogens for inducing HIV-1 Envelope-specific VRC01-class antibodies. The VRC01 class of broadly neutralizing antibodies have been observed in multiple individuals and targets the HIV CD4 binding site via a common recognition motif that requires specific features in both heavy and light chains (e.g., VH1-2 heavy chain V-gene and a short, $\leq$5 amino acid light chain CDR3). We are using paired heavy and light chain sequencing to quantify the performance of various candidate HIV immunogens for inducing VRC01-class broadly neutralizing HIV antibodies in transgenic mouse models. We are also elucidating the ontogeny of antibodies in vaccinated and naturally infected human subjects and animal models via interrogation of paired heavy and light chain antibody sequences and antibody synthesis/testing of promising clones, including experimental influenza vaccine trials and a Phase I Ebola vaccine trial. These next-generation immunoanalytic approaches are providing detailed molecular feedback regarding experimental vaccine performance to accelerate vaccine design efforts against pathogens of major public health importance.

PLANT-BASED TECHNOLOGIES TO ENABLE RAPID RESPONSE TO EBOLA OUTBREAK

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Key Words: Ebola virus, recombinant vaccine, therapeutic monoclonal antibody.

Ebola is a rare and deadly disease caused by infection with a strain of Ebola virus (Zaire, Sudan or Marburg). Ebola is spread through a direct contact with body fluids of infected patients. The recent Ebola outbreak affected multiple countries in West Africa and clearly demonstrated a need for development of technologies enabling production of adequate supply of vaccines and therapeutics to treat infected patients. We have undertaken efforts to develop methods and produce plant-based recombinant vaccine as well as a series of monoclonal antibodies to fight Ebola infection.

Several approaches were carried out to develop vaccines based on recombinant soluble E-protein and on enveloped or non-enveloped (CP-based) virus-like particles. Preliminary results demonstrating challenges and opportunities for further development of plant-expressed Ebola vaccines will be discussed.

In parallel efforts, we have developed a robust plant-based expression platform for expression of monoclonal antibodies against Zaire and Sudan strain of Ebola. We established and optimized expression system and process for rapid production of these antibodies in quantities sufficient to enable pre-clinical evaluation and stability studies. Initial characterization of selected Ebola antibodies will be presented.

CAUSES, CHALLENGES AND SOLUTIONS FOR DEVELOPING VACCINES FOR LOW-INCOME SETTINGS

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Vaccines are one of the most successful health interventions in history. They have been the primary means of eradication of smallpox and the near eradication of polio, and have led to the decrease in mortality and morbidity for millions. Yet even today, hundreds of thousands of children are dying every year around the globe from vaccine preventable diseases, such as pneumonia and rotavirus. More are dying as well from diseases where safe and effective vaccines have not yet been developed and commercialized, such as HIV, TB, and malaria. These diseases are disproportionately affecting people in countries that can least afford effective interventions. This talk will explore some of the causes and potential solutions to the challenges of developing, manufacturing, and commercializing vaccines for the poor, including technological innovations in vaccine development and manufacturing, and how partnerships with innovation and technology-focused organizations in both the public and private sectors are advancing these goals. Specific examples of how the Bill & Melinda Gates Foundation is engaging to bring about positive progress in vaccine development, manufacturing, and supply will be discussed.