THE IMPACT OF VACCINES WORLDWIDE AND THE CHALLENGES TO ACHIEVE UNIVERSAL IMMUNIZATION

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Key Words: immunization programme, vaccine impact, data quality, ownership, life-course

This presentation will provide an overview of the current status of the global immunization programme using available published and non-published data from WHO Member States and review the pathways to alleviate the main barriers towards achieving universal immunization during this era of the UN Sustainable Development Goals.

In 1974, the establishment of the WHO Expanded Programme on Immunization marked a turning point in the large-scale use of vaccines. Today, more children than ever are being reached with immunization; polio is on the verge of being eradicated, the WHO model list of vaccines now includes twenty-two vaccines for all ages that countries can choose from. The health impact is evident with the continued decline of under-five mortality due to vaccine-preventable diseases from roughly 4million deaths in 2000 to less than 2million deaths in 2015. Overall, WHO estimates that vaccines prevent 2-3 million deaths each year. The broader benefits of vaccines are also well documented.

In 2011, the Global Vaccine Action Plan for this Decade of Vaccines was produced with the ambitions to close the equity gap in vaccine coverage and to unleash the vaccines vast potentials. An independent assessment of the GVAP implementation was carried out by the WHO Strategic Advisory Group of Experts (SAGE) on Immunization which expressed strong concerns that most countries were off track to achieving their immunization goals. The SAGE requested strong actions to tackle challenges such as poor data quality and use that hinder understanding and corrective actions; affordability and supply of vaccines that remain problematic; and the basic failures of health systems that repeatedly miss opportunities to offer vaccinations including during the disruptive situations created by civil conflicts and major disease outbreaks.

Looking forward, while taking pride in the progress made, there is need to secure a much stronger leadership and ownership by the countries as well as continued greater international solidarity to harness the full potential of vaccines throughout the life course by 2030.

STRUCTURE-BASED VACCINES FOR RESPIRATORY VIRUSES

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Respiratory syncytial virus (RSV) is an orthopneumovirus in the family Pneumoviridae. The fusion glycoprotein (F) is responsible for mediating viral entry and is the major antigenic target for RSV vaccine development. There are two major RSV subtypes, A and B, defined largely by genetic variation in the G glycoprotein.

Recent advances in defining the structure of F-specific NT-sensitive epitopes and the structure of the prefusion (pre-F) and postfusion (post-F) conformations of F have led to a better understanding of neutralizing mechanisms, the serological responses to natural RSV infection and vaccination, pathogenesis of disease, mechanisms of viral inactivation, and the importance of targeting pre-F surfaces with the vaccine antigen. The RSV program has also informed the development of improved vaccine antigens for other viruses that use class I fusion proteins like the coronavirus spike, influenza hemagglutinin, Ebola glycoprotein, HIV-1 gp160, and the F of other paramyxoviruses. The talk will review the structure and function of F, and describe the design, antigenicity, immunogenicity, and initial clinical data for the DS-Cav1 candidate RSV subunit vaccine based on a stabilized version of prefusion RSV F. In addition, antigen design strategies for coronaviruses and influenza will be reviewed.

mRNA VACCINES: ON THE PROGRESS FROM PROMISE TO REALITY

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Messenger RNA offers unparalleled breadth and depth for the discovery and development of novel drugs and vaccines. The potential to direct specific tissue translation of both wild type and engineered intracellular, membrane-bound, and secreted proteins (and combinations thereof), coupled with rapid transition from preclinical to clinical development, has enabled Moderna and its partners to progress several development candidates into the clinic. This potential is being realized by the parallel development of different modalities (e.g. prophylactic and therapeutic vaccines, paracrine and systemic drugs). This talk will review the update on the vaccines pipeline and scientific and engineering accomplishments enabling pharmaceutical development.

RNActive®-AN mRNA-BASED VACCINE TECHNOLOGY FOR NEXT GENERATION PROPHYLACTIC VACCINES

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In recent years, messenger RNA (mRNA) based technologies have increasingly been applied in vaccine development. RNActive®, an mRNA based vaccination technology developed by CureVac, which uses sequence-optimized, unmodified mRNA, promises a powerful new platform for the development and production of prophylactic vaccines against infectious diseases. RNActive® vaccines have achieved promising results against a variety of viral pathogens such as influenza, rabies, Ebola and respiratory syncytial virus in several animal models. In previous studies we showed that intradermal application of RNActive® vaccines was able to confer protection against lethal influenza and rabies virus challenge infection in mice and induced protective levels of functional antibodies against both viruses in domestic pigs. In a first-in-human trial, the RNActive® vaccine against rabies, which was formulated with the cationic protein protamine, appeared safe with a reasonable tolerability and was able to induce boostable virus neutralizing antibodies (VNTs) after intradermal needle free injection whereas needle based injection was ineffective. This study provided important guidance for the development of improved vaccine candidates. Here, we describe a lipid nanoparticle (LNP) formulation of RNActive® vaccines that is able to induce potent immune responses when applied intramuscularly using low doses (µg) of mRNA. Vaccination of mice with this RNActive® vaccine encoding for influenza hemagglutinin or rabies glycoprotein led to the induction of both, humoral and cellular immune responses. Further experiments showed that the vaccine was able to induce potent and long lasting immune responses against influenza HA as well as high titers of rabies virus neutralizing antibodies in non-human primates following intramuscular administration by needle.

VIRUS-LIKE PARTICLE VACCINES AGAINST BK AND JC POLYOMAVIRUSES

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Key Words: VLPs, polyomavirus, neutralizing, PML, nephropathy

Nearly all healthy adults are asymptomatically infected with human polyomaviruses. In immunosuppressed individuals, the infection can reactivate and cause disease. BK polyomavirus (BKV) frequently damages transplanted kidneys and causes severe bladder disease in bone marrow transplant patients. JC polyomavirus (JCV) causes a lethal brain disease, PML, in individuals on various immunosuppressive therapies. PML also affects immunodeficient individuals, including AIDS patients.

The outer capsid proteins of polyomaviruses are structurally similar to the capsids of human papillomaviruses (HPVs). Building on the success of the NCI's HPV virus-like particle (VLP) vaccine technologies, we have developed VLP vaccines targeting BKV and JCV. Preclinical testing in a monkey model indicates that the BKV and JCV VLP vaccines share the HPV vaccines' exceptionally potent immunogenicity. Given our knowledge of the role that antibodies play in ameliorating polyomavirus pathologies, the new VLP vaccines are likely to protect at-risk patients against the development of BKV-induced urinary tract disease and JCV-induced brain disease. Each year, roughly 30,000 Americans join wait-lists for kidney transplantation. Additionally, roughly 300,000 Americans per year are diagnosed with diseases that might be treated with bone marrow transplantation. Emerging evidence indicates that antibody-producing plasma cells elicited by the BKV vaccine will persist after bone marrow transplantation and the vaccine should thus provide protection against post-transplant hemorrhagic cystitis.

The highly effective multiple sclerosis therapy Tysabri (natalizumab) is associated with up to 2% risk of PML side effects. Rituxan (rituximab), which is used for treatment of rheumatoid arthritis and certain types of lymphoma, carries a black box warning for PML and a dozen additional immunosuppressive therapies are also known or suspected to have PML side effects. The JCV vaccine should be a useful preventive adjunct for these popular immunotherapies.

Since there are currently no effective treatments for BKV or JCV diseases, the candidate vaccines seem likely to qualify for FDA's Accelerated Approval Program. The NCI is currently seeking industry partners.

MOLECULAR QUALITY ENGINEERING FOR LOW COST VACCINE PRODUCTION

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Key Words: Molecular engineering, product quality, functional genomics, transcriptomics, *Pichia pastoris*,

Vaccines based on recombinant proteins provide a compelling case for low cost products with broad global accessibility. Protein immunogens are typically derived directly from native sequences found in bacterial or viral pathogens, and may not be well-suited for efficient expression in recombinant hosts. Native immunogens may also suffer from numerous challenges during expression that impact their quality or efficient production, including truncation, aggregation and poor stability. These challenges can lead to inefficiencies in manufacturing of subunit protein vaccines.

Typically, recombinant vaccine manufacturing processes are complex, serial batch operations requiring extensive quality testing throughout to ensure product integrity. In response to the Gates Foundation's Grand Challenge for Innovations in Vaccine Manufacturing for Global Markets, we are co-developing the ULTRA program for flexible, low cost vaccine products. This program aims to develop platform processes for production of recombinant vaccines. We believe that molecular design of the antigens provides a critical handle in improving antigen quality, manufacturability, and product stability, all of which could enable potent, low-cost vaccines. Addressing potential manufacturing challenges early on in product development should enable simple integrated processes for antigen production while minimizing costs associated with quality testing.

To this end, we are demonstrating our platform approach with a recombinant trivalent subunit vaccine for rotavirus currently in clinical development. We chose to express the three VP8 subunits in *Pichia pastoris* to take advantage of the high titers of secreted proteins and minimal process-related contaminants typically experienced with this organism—critical features when developing simple intensified processes to meet our cost targets of $0.15/dose. Initial expression results showed the rotavirus antigens were poorly expressed and suffered from *N*-terminal truncation and aggregation—all of which were also observed in a previously developed *E. coli*-based process. We have deployed a two-pronged approach toward improving the manufacturability of these antigens. First, we used a functional genomics approach to identify bottlenecks experienced during cellular expression of the antigens. RNA-sequencing is a mature, inexpensive and acccessible technique for yeast that can indicate host- or sequence-derived bottlenecks in antigen transcription, translation and expression. Second, we made direct sequence changes to the antigens to mitigate specific quality challenges, such as aggregation. Iterations of this approach have enabled robust titers of rotavirus antigens with improved quality. This framework for incorporation of molecular engineering early in development provides a useful model for improving target product profiles that include manufacturability for low-costs, while maintaining immunogenicity.

SINGLE-CELL ANALYSIS UNCOVERS A NOVEL INFLUENZA A VIRUS-DERIVED DEFECTIVE INTERFERING PARTICLE FOR ANTIVIRAL THERAPY

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Key Words: Single-cell analysis, antiviral agent, OP7, defective interfering particle, bioprocess engineering

Single-cell analysis of virus-infected cells (Heldt and Kupke et al., 2015) enables the characterization of individual highly productive cells, which may support strategies to improve cell culture-based vaccine production. However, the definition of poor producer single cells can also yield valuable information. Here we show that low-productive single Madin-Darby canine kidney (MDCK) cells, infected with influenza A virus (IAV) of strain A/PR/8/34 (PR8), were affected by a yet unrecognized form of defective-interfering particle (DIP). Conventional DIPs (cDIPs) typically contain a deleted form of the viral genome and are therefore unable to reproduce in an infection. However, upon complementation by the co-infection with fully infectious standard virus (STV), interference with the normal viral life cycle can be observed. Interestingly, considering their ability to suppress STV replication, cDIPs are of growing interest for clinical application, i.e. for their use as antivirals (Dimmock and Easton, 2014).

Single-cell infection experiments revealed a surprisingly high variability in IAV replication with progeny virus yields that ranged from 0 to roughly 1000 plaque-forming units (PFU) per cell. Intriguingly, low-productive cells (0-10 PFU) displayed an abnormal phenotype, which was caused by the co-infection of a subpopulation of virus, in the following termed OP7 virus. Sequences of the genomic viral RNA (vRNA) of OP7 virions showed a significant amount of nucleotide substitutions in one of the eight vRNA segments, affecting its promotor, encoded proteins and virus packaging signals. We showed that these alterations were all directed towards the predominant genomic replication and packaging of the mutated vRNA over other genome segments. Concurrently, OP7 virions lacked a large fraction of other vRNAs, which constitute its defect in virus replication. Finally, co-infection experiments showed strong interference of OP7 virus with IAV replication, as indicated by a dramatic reduction in the infectivity of released virions. This interference was directed against relevant homologous and heterologous IAV strains, including strains of the current influenza season. Furthermore, we demonstrated interference in human cell lines.

Therefore, OP7 virions are a novel form of IAV-derived DIPs with a non-deleted but mutated genomic RNA segment. First, it seems reasonable to investigate the presence of OP7 virions in seed virus preparations, as they can reduce virus titers in a production process, similar to cDIPs (Frensing et al., 2014). Second, OP7 virus may be used for antiviral therapy. As they are not able to reproduce on their own, they may be administered to organisms with no harm. The presence of OP7 virions can then inhibit the propagation of IAV of a natural infection. In addition, the induction of the innate immune response, observed upon infection with OP7 virus, can even further promote the antiviral effect. In the future, the design of efficient production systems for OP7 virions and the execution of animal trials may facilitate its utilization as a novel antiviral agent.

References

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Patent

Patent pending for usage of OP7 virions as an antiviral agent

PERSISTENT ANTIBODY CLONOTYPES DOMINATE THE SERUM RESPONSE TO INFLUENZA FOLLOWING REPEATED VACCINATION OVER MULTIPLE YEARS

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Key Words: Antibody repertoire, longitudinal vaccine response, proteomics, serological imprinting

We used Ig-Seq, a liquid chromatography tandem mass spectrometry (LC-MS/MS)–based serum antibody proteomics methodology, to determine the clonal composition and dynamics of the H1N1 California/7/2009 (CA09) hemagglutinin (HA)-reactive antibody repertoire over 5 years in a well-characterized donor from whom a large number of homosubtypic and heterosubtypic neutralizing monoclonal antibodies had been previously isolated by B cell analysis. The donor was infected with the CA09 strain in 2009 and immunized annually for the next five years with seasonal influenza vaccine which contained the CA09 strain. We find that the serological repertoire in this donor was highly static, with a modest number (24) of persistent antibody clonotypes, detected in serum for at least 4 out of 5 years, accounting on average for 72.6 ± 10.0% of the repertoire to the CA09 HA. These persistent antibodies: (i) displayed a higher degree of somatic hypermutation relative to antibodies that could be detected in the serum transiently (i.e. lasted less than 1 year in serum); (ii) comprised a significant fraction that also bound to HA from a phylogenetically distant H5N1 A/Vietnam/1203/2004 (VT04) strain, a hallmark of stem-binding antibodies due to the lack of homology between CA09 and VT04 in the head region of HA and (iii) perhaps most strikingly, but consistent with the wealth of heterosubtypic neutralizing antibodies that had previously been identified from this donor, some of the most abundant persistent antibody clonotypes, including the dominant clone that accounted on average for 18.6 ± 12.3% of the serum titer across 5 years, neutralized both the CA09 and VT04 influenza strains. Our analysis highlights the magnitude of ‘serological imprinting’ in the donor’s serum response to CA09, indicates that seasonal vaccination can further reinforce a stable serological memory and finally suggests that once elicited, antibodies cross-reactive between CA09 and VT04 with heterosubtypic neutralization activity, thus likely to bind to HA-stem, can persist for many years, which is a fundamental goal of universal influenza vaccines.

Pan-HA ANTIBODIES CONFER PROTECTION IN MICE AGAINST INFLUENZA

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Key Words: Influenza, Hemagglutinin, Universal antibodies, Adeno-associated virus, CHO

The elderly population is one of the most vulnerable groups to influenza infection and influenza-related complications. Unfortunately, vaccination exhibits reduced efficacy in this population. Immunization and treatment with passive antibody transfer could therefore be a valuable alternative.

In this project, we have generated antibodies against a constant region of hemagglutinin (HA), the main protein found at the surface of the virus. In vitro, two lead candidates (mAb 10A9 and mAb 11H12) were able to detect strains belonging to 13 subtypes of Influenza A as well as B strains (1). In order to facilitate large scale production, stable CHO pools were generated for pan-HA antibody production. Biophysical characteristics of antibodies produced in CHO cells were similar to the ones of antibodies generated using mouse hybridomas. The antibodies were further tested in a mouse model of influenza to evaluate their protective potential.

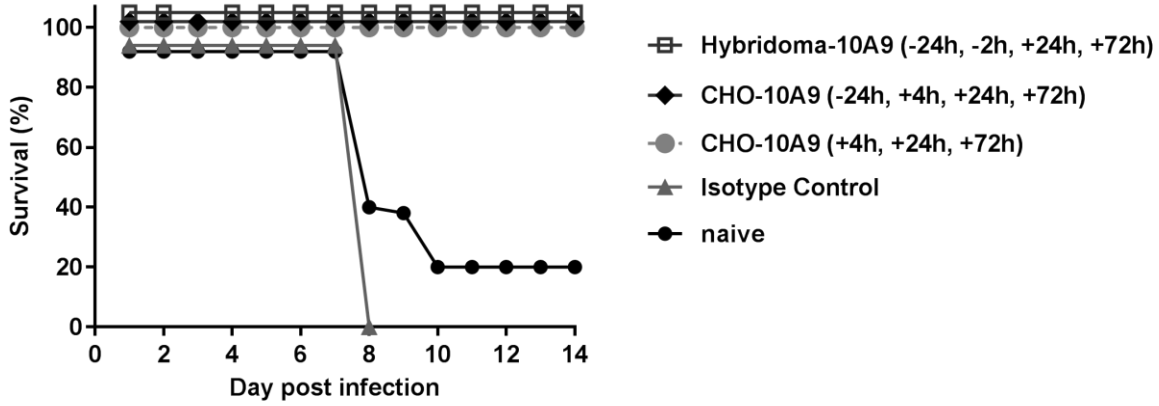

Figure 1 – Protective effect of 15 µg/g body weight of mAb 10A9 (produced from mouse hybridoma or CHO cells) against H3N2 A/Hong Kong/8/68 in BALB/c mice (n= 5 per group).Mice were infected with 10^4PFU virus by intranasal challenge. The different injection times relative to infection are shown in the legend in brackets.

The antibodies offered protection against influenza regardless of their production platform (Figure 1). The CHO-produced antibody was tested in prophylactic mode (first injection 24hrs before the challenge) and as a therapeutic (first treatment 4hrs after viral infection). In both cases, all mice survived. Other viral strains and treatment regimens have to be tested but these preliminary results are encouraging.

Finally, multiple repeated administrations of the mAb in at-risk population would be impractical and too expensive. Therefore, recombinant Adeno-Associated-Virus (rAAV) is being considered as a delivery system. Using rAAV would allow delivering the genes encoding for the mAb and ensuring their long-term expression. The heavy chain and light chain of the pan-HA antibodies are expressed on the same cassette and transfected into suspension HEK293SF cells, along with two other plasmids to form the AAV particles. After purification, the AAV will be injected in mice for long term production of the antibodies (experiments ongoing). This would represent a cost effective delivery route in immunocompromised or elderly individuals.

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THE STORY OF A SUCCESSFUL BIOTECH (AD)VENTURE: THE DEVELOPMENT OF FLUBLOK

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Biography:
Dr. Manon M.J. Cox, MBA founded NextWaveBio early 2018 following her departure from Protein Sciences Corporation where she led the development of Flublok®, the only FDA approved recombinant influenza vaccine. In August 2017 Sanofi completed the acquisition of Protein Sciences Corporation where she served as President and Chief Executive Officer since April 2010 and Director since 2008. She joined Protein Sciences in 1998 as Director of Business Development and became Chief Operating Officer in 2003. She serves on the Scientific Advisory Boards of Epivax Oncology and Pall BioPharmaceuticals, the Board of Directors of the Netherlands-America Foundation, where she is a member of the Executive Committee and Chairman of its Education Committee, and the Board of Trustees of St. Joseph's University. Before Protein Sciences, she was with Gist-brocades; a large Dutch company specialized in fermentation, where she played a key role in the development of Lipomax® and held various management positions most recently in New Business Development, and before that in Production and Research and Development. Prior to joining Gist-brocades she worked as a Molecular Biologist on the development of a PCR screening test for cervical cancer at the University of Amsterdam. Dr. Cox has received many honours and awards recognizing her stature as a leader in innovation and influenza including receiving a Doctorate in Humane Letters honoris causa from St. Joseph University and the Woman of Innovation award from the Connecticut Technology Council. In 2015 she was elected fellow of the International Society of Vaccines. Dr. Cox holds a Doctorate from the University of Wageningen, received her MBA with distinction from the University of Nijenrode and the University of Rochester, NY and holds a Doctorandus degree in Molecular Biology, Genetics and Biochemistry from the University of Nijmegen, The Netherlands.

Abstract:
I joined Protein Sciences in the late nineties for the people, the products, and the technology. Gale Smith was one of the inventors of the baculovirus technology and Dan Adams one of the founders of the biotech industry. The company was actively involved in vaccine development and the baculovirus expression system was their technology platform. What would be a better place for further learning and driving change in the vaccine industry?

The baculovirus expression system was already an established tool for the production of complex proteins. But we realized that we needed to bring our technology to maturation by taking a product forward through approval by the Food and Drug Administration (FDA) as none of the partners for whom we were developing vaccines using our technology was going to do this for us. An influenza vaccine seemed to be a perfect target as the company had already generated preliminary human clinical data in the mid nineties supporting the hypothesis that a recombinant hemagglutinin (rHA) protein could prevent influenza.

The baculovirus technology was perfectly suited to support the annual updates required for the influenza vaccine as only the baculovirus would need to be modified. Finally, the 1998 H5N1 bird flu outbreak in Hong Kong had clearly revealed the limitations of the egg-based manufacturing process used for the production of influenza vaccines. The National Institutes of Health (NIH) told us that we were the only company in the world that could develop a vaccine "in time" and we met their expectations delivering doses in just eight weeks. However, what seemed to be pretty simple and straightforward project, became a 14-year trajectory that ultimately led to FDA approval of the first recombinant influenza vaccine, named Flublok.

The development of Flublok taught us three important lessons: 1) Use of proven platform technology may lead to new, potentially better products but with the inherent uncertainty it will delay product availability; 2) Allow the regulatory process to focus on safety and evidence of protection in animals, with confirmatory efficacy evidence to be gathered post introduction; and 3) Ensure that adequate funds are available for development.

The speaker will share her experience and the "ups and downs" in the development of Flublok.

ACCELERATING BIOPROCESS DEVELOPMENT BY ANALYSIS OF ALL AVAILABLE DATA: A USP CASE STUDY

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Key Words: IPV, MVDA, Vero cells, Polio.

Bioprocess development generates extensive data from different unit operations and it generally includes large datasets (e.g. time series, quality measurements). By analyzing all available data, bioprocess development can be accelerated. This can only be achieved by having a clearly defined data logging and analysis strategy. Here, we present a case study using available data from the development and optimization of the upstream process (USP) of Sabin inactivated polio vaccine (IPV) using animal component free medium.

IPV production using attenuated Sabin strains instead of wild type polio viruses is an initiative supported by the World Health Organization. This change is favorable to reduce the risk of outbreaks during IPV production. Optimizing this process using only animal free components reduces operational costs and lowers the risk of adverse effects related to animal derived compounds.

During the process development, 40 bioreactors at scales ranging from 2.3 to 16 L were run. For optimization and robustness studies, design of experiments (DoE) was performed and several USP operational parameters were varied. These included operational mode (batch vs semi-batch), multiplicity of infection (MOI) and time of infection (TOI). This data was routinely analyzed using factors based on DoE methodology.

With the new strategy, it became possible to scrutinize all data from the 40 USP development runs in a single data study. The total data package that was analyzed; this included the DoE response parameters, all offline data (e.g. cell, substrate and product concentrations), all data generated by the bioreactor control systems (T, pH, DO, DOCO), and derived calculations (specific rates like μ and q_{glu}). This analysis showed which parameters were most important regarding the bioreactor performance. This USP case study showed that with the new strategy a more detailed, reliable and exact view on the most important parameters regarding bioreactor performance could be obtained.

In order to do this, a feature based approach supported by the inCyght® software was utilized. It consisted of logging all data into a database, which was used to determine data integrity for all variables and batches. Exact phase information (cell growth, virus production phase) and other meta information are transferred into the database for each batch. This allowed outliers to be visually determined and certain variables to be excluded from the analysis (i.e. those that did not fluctuate). Univariate outlier detection technique was used to further determine outliers. Principal component analysis (PCA) was used to gain a multivariate process understanding and partial least squares (PLS) regression was performed to identify correlations. This result determined the best subset of variables to be fitted by using multiple linear regression (MLR). Future experiments will focus on the relevant parameters highlighted by this approach.

This strategy was applied for the analysis of previously produced data. Further development will use this data analysis methodology for continuous accelerated process development, intensified DoE and integrated process modelling.

PURIFYING VIRUSES WITH A SHEET OF PAPER: SINGLE-USE STERIC EXCLUSION CHROMATOGRAPHY AS A CAPTURE PLATFORM FOR VACCINE CANDIDATES

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Key Words: Membrane chromatography; single-use; virus purification; downstream processing; platform technology

Steric exclusion chromatography (SXC) is a method in which a crude sample is mixed with polyethylene glycol (PEG) and fed to a hydrophilic stationary phase. Selectivity in SXC is strongly influenced by the target species' size, so it is particularly well suited for purification of large biomolecules such as viruses and virus-like-particles. The product is captured without a direct chemical interaction thanks to the mutual steric exclusion of PEG between the product and the stationary phase (cellulose membranes with micron-sized pores). Product elution is achieved by removing the PEG from solution, and can theoretically be made in any buffer system. The low cost of the cellulose membranes allows this operation to be single-use.

Using SXC, we have achieved virtually full recovery of several viruses produced in serum-free mammalian cell culture: influenza virus, yellow fever virus, and Modified Vaccinia Ankara (MVA) virus. For influenza virus, four different strains were produced separately in MDCK cell suspension cultures using either chemically defined medium or serum-free medium. Full recovery of all strains was observed using identical SXC conditions (loading with 8% PEG-6000) for both infectious and chemically inactivated virus particles. Coupling a nuclease treatment for DNA digestion prior to SXC, dsDNA was depleted >99.98%. The column capacity in terms of the viral hemagglutinin antigen was at least 50 mg m^{-2}. In the case of yellow fever virus, two attenuated strains used for commercial manufacture were produced separately in adherent Vero cells grown in serum-free medium. Full recovery of infective virus titer for both strains was attained using 10% PEG-6000 for sample load. The elution fraction was concentrated >100-fold compared to the feed with the very high titer of 6×10^9 plaque forming units, equivalent to ≈100 000 doses. Total recovery was also observed for MVA virus loaded at 4% PEG-6000; produced in an avian cell line in chemically defined medium, the SXC elution pools contained $\approx3.7\times10^9$ virions as estimated by $TCID_{50}$ assay.

In conclusion, SXC can drastically reduce process development in terms of time and equipment requirements. The convenience of purifying different virus strains using similar chromatography conditions is almost impossible to match by other methods, as are the high product recoveries typically achieved with SXC. The latter gives space to include additional polishing operations without risking low overall process yields. We deem membrane-based SXC as a promising platform technology for capturing viruses and virus-like particles in vaccine manufacturing.

VERO SF TECHNOLOGY PLATFORM: STRATEGY FOR RAPID AND EFFECTIVE VACCINE DEVELOPMENT ; FLAVIVIRUS VACCINES CASE STUDY

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Key Words: Vero cell, Flavivirus, platforms, high-throughput.

As a world leader in vaccine development, *Sanofi Pasteur* has acquired a strong expertise in the development and manufacturing of Vero SF-based vaccines, including against diseases of the Flavivirus family.
To develop this innovative platform and provide a fast response to new viral epidemics, the vaccine manufacturing process development strategies have considerably evolved over the past decade. Toolboxes dedicated to high-throughput development have been designed and optimized to provide rapid response and effective vaccine availability.

For the sake of speed and efficiency, development strategies have been reorganized by platforms. Theses platforms, such as screening, modelling, process monitoring, bioreactor, have themselves been completely redesigned to allow fast implementation of all development phases up to industrial scale.
Major investments have been made in automated bioreactors and on-line analytics that enable high-throughput studies to support process definition, product characterization, ultimately moving to scale up and clinical manufacturing. Additional focus has been placed on chemically defined media definition, suitable for both cell culture and viral amplification, thus allowing higher generic development and simpler process optimization.

Part of the platform considers automated bioreactors Ambr15 and 250 implemented and combined to scale up models up to 200 Liters. These models have been characterized to reduce time dedicated to scale-up studies and validation. Overall development timelines have been greatly reduced and the optimization phases reduced by a few months.

‘Funding : Sanofi Pasteur’

BIOPROCESS INTENSIFICATION FOR PRODUCTION OF A PESTE DES PETITES RUMINANTS VIRUS (PPRV) VACCINE

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Key Words: PPRV vaccine, *In situ* cell detachment, Perfusion, Scale-up.

Peste des Petites Ruminants Virus (PPRV) is a highly contagious disease affecting small ruminants in Africa and Asian countries, with negative/significant economic impact. Aiming to eradicate the disease, targeted by the Food and Agriculture Organization for 2030, a novel and scalable PPRV vaccine production process is clearly needed. Built upon work previously done at iBET, a new production process is herein proposed using Vero cells growing on microcarriers, serum-free medium (SFM) and stirred-tank bioreactors (STB). This includes a new method for cells detachment from microcarriers, and perfusion culture for reducing turnaround time.

The PPRV vaccine production process was developed in the 2L BIOSTAT® DCU-3 and the 20L BIOSTAT® Cplus STB (both from Sartorius) using Nigeria 75/1 strain. Engineering correlations (energy dissipation rate, shear stress and Kolmogorov Eddy size) were used to optimize culture conditions in the 2 L STB and to scale-up the process to the 20 L STB. Vero cells were adapted to grow in ProVero™-1 SFM (Sartorius). A new enzymatic and mechanical method for *in situ* cell detachment from microcarriers was designed. Perfusion was evaluated in the 2 L STB (equipped with internal spin-filter) in order to reduce seed-train preparation time. PPRV were clarified using depth filtration (Sartopure PP3, Sartorius). Process scalability was validated in the 20 L STB.

Vero cells were adapted to ProVero™-1 SFM, reaching growth rates similar to serum-containing cultures (0.03 h^{-1}). The new *in situ* cell detachment method was successfully implemented, with yields above 80%. A two-fold increase in maximum cell concentration was obtained using perfusion when compared to batch culture. Combining perfusion with the new *in situ* cell detachment method enabled the scale-up to 20 L STB directly from a 2 L STB, surpassing the need for a mid-scale platform and thus reducing seed-train preparation time. Infectious PPRV titers increase over culture time in both 2 L and 20 L STBs, reaching maximum values of 4.5-4.9x10^6 $TCID_{50}$/mL at day 4-5 post-infection. The potential of depth filtration for PPRV clarification was confirmed; comparable PPRV recovery yields after clarification (85-90%) were obtained in both STBs.

Overall, the novel and scalable vaccine production process herein proposed has the potential to assist the upcoming PPR Global Eradication Program (PPR GEP), to which iBET already contributes as partner in the PPR Global Research and Experts Network (PPR GREN), and thus support the One Health concept.

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DEVELOPING VACCINES FOR LOW RESOURCE SETTINGS THROUGH PRODUCT DEVELOPMENT PARTNERSHIPS (PDPs)

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Development of new vaccines for low resources settings is driven not only by the unmet medical need but also by addressing issues related to vaccine access, availability, affordability, acceptability and sustainability by the multitude of stakeholders involved in the research, development, introduction and scale-up of vaccines for low- and middle income countries (LMICs). The first gap that needs to be traversed is the valley of death between discovery and deployment (http://dx.doi.org/10.1038/453840a). Product Development Partnerships are one mechanism that has been successfully used to accelerate development of vaccines for LMICs. Two case studies will be reviewed: the meningitis A vaccine, MenAfriVac® and the malaria vaccine, Mosquirix®. A second valley of death has been identified in the development of the malaria vaccine (http://dx.doi.org/10.1016/S0140-6736(16)30394-4). To traverse this second valley death, a different way of thinking about vaccine development may be required.

CONTINUOUS PURIFICATION OF CELL CULTURE-DERIVED INFLUENZA A VIRUS PARTICLES THROUGH PSEUDO-AFFINITY MEMBRANE CHROMATOGRAPHY

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Key Words: continuous chromatography, influenza vaccines, membrane adsorbers, sulfated cellulose

Continuous manufacturing is a relevant trend in biopharmaceutical production to reduce the process footprint and to improve the process economy. Vaccines against world-spread diseases, such as influenza, should benefit in particular from such an approach, given the increasing demand for seasonal vaccines and the need for a fast response in case of a pandemic outbreak. Upstream processing of viral vaccines has seen important progress in continuous production of viral vaccines [1], which further supports the development of hybrid or fully continuous flow-schemes for downstream processing.

In this work, we implemented a multi-column strategy for the chromatographic purification of a continuous feed stream of cell culture-derived influenza A virus particles using sulfated cellulose membrane adsorbers (SCMA). The use of SCMA for batch purification of influenza A virus particles is well described [2] as well as the process conditions required for a successful separation [3]. This facilitated the transfer of this chromatography technique from batch to continuous-mode. Using a 3-device set-up, we reproducibly purified cell-culture derived influenza A/Puerto Rico/8/1934 virus particles during 10 cycles. Each cycle comprised load, wash, elution and re-equilibration of all three devices. The virus hemagglutinin activity (HA) average yield obtained was 67% ± 11%, with contaminant removals above 70% for total protein and 99.8% for DNA, respectively. Moreover, the contaminant content relative to the eluted HA were 1.0 ± 0.1 $\mu g_{total\ protein}$/kHAU and 3.5 ± 0.7 ng_{DNA}/kHAU. These are similar to those achieved for comparable batch runs. In addition, based on the breakthrough curves [Fig. 1], the SCMA were challenged to about 69% of their estimated static binding capacity. Compared to traditional batch operation (capacity challenge at 70% of the dynamic binding capacity, $DBC_{10\%}$), continuous operation of the SCMA saves at least 10% of the processing time.
Overall, the implementation of this continuous chromatography approach for the purification of viral particles would result in a considerable reduction of plant footprint, buffer consumption, and operating costs. Yields and contamination levels achieved support the future use of membrane chromatography as a platform solution, especially suited for low-cost vaccine manufacturing.

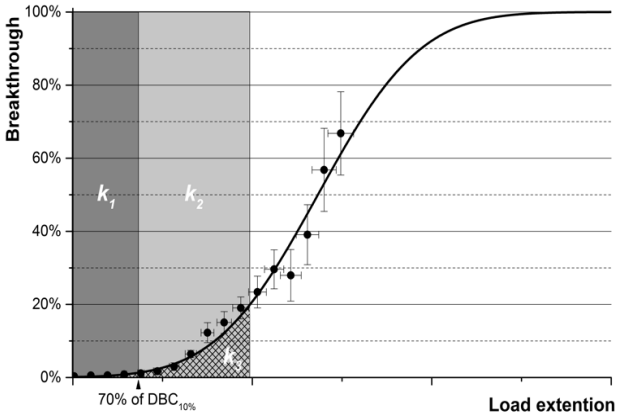

Figure 2 – A continuous multi-column strategy for the purification of influenza A virus using SCMA increases to overall capacity used per device (k_2), in comparison to the traditional capacity challenge used in batch mode (k_1). The virus particles loss on the flow through of the main load device are caught by the following device (k_3).

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INTEGRATED SCALABLE CYTO-TECHNOLOGY FOR RECOMBINANT PROTEIN BIOPROCESSING

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Key Words: Process intensification, Flexible facility, continuous bioprocessing, yeast

Biological knowledge of infectious diseases and other diseases for which vaccines may provide therapeutic benefits, such as cancer, is growing at an accelerated pace. The implications of this knowledge are improved stratification of diseases, possibilities for personalized treatments, and explicit understanding of protective immune responses to be elicited by vaccines. With this knowledge, it is becoming increasingly feasible to engineer vaccines for specific responses rather than relying on empirical development. Despite this potential, the challenge of routine, low-cost manufacturing of vaccines creates a barrier to transforming health care in both high- and low-resource countries. Vaccines today do not benefit from well-defined, platform-like processes for manufacturing, and concepts such as continuous bioprocessing remain largely within the realm of biopharmaceutical products.

The InSCyT platform is an advanced prototype manufacturing system that provides integrated and automated production and purification of multiple protein therapeutics. The system allows end-to-end manufacturing of 100's to 10,000's of doses of recombinant protein drugs in days. It uses a state-of-the-art approach to process design and implementation that takes advantage of a fast-growing, tractable microbial host (*Pichia pastoris)* and continuous processing for automated, hands-free purification through simple 2- or 3-stage chromatographic processes. The platform design is highly modular, allowing facile process development and process deployment for multiple products. This feature emerges from the predictable behavior of the fermentation and cell culture fluids, and rapid cloning of new molecules, that together facilitate fast development of entirely new processes in weeks. To date, this system has been used to reproducibly manufacture high-quality human growth hormone (hGH), granulocyte-colony stimulating factor (G-CSF), and interferon-α2b (IFN-α2b) in an integrated, automated manner. The speed of production using the InSCyT prototype allows volumetric productivities that compare favorably to those for mammalian-based production.

This talk will outline the design and capabilities of the InSCyT system, demonstrate the quality of biologic drugs made to date on the system, and outline opportunities for advancing the platform to provide new capabilities in manufacturing recombinant proteins for use in vaccines. As part of a Gates Foundation-funded Grand Challenge called ULTRA, we have begun to assess the feasibility of manufacturing millions of doses of a trivalent recombinant rotavirus vaccine annually on a small-scale production system like InSCyT. Integrated bioprocessing enabled by systems such as these could offer potential advantages for routine production in local regions with minimal infrastructure, and for democratization of manufacturing capacity for new vaccine concepts and personalized treatments in cancer.

LOW-COST CELL-BASED PRODUCTION PLATFORM FOR SEASONAL AND PANDEMIC INFLUENZA VACCINES

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Key Words: suspension MDCK, avian influenza, seasonal vaccines.

Influenza-related illnesses have caused an estimated over million cases of severe illness, and it has about hundred thousands of deaths worldwide annually. Traditionally these vaccines are produced in embryonated chicken eggs. However, in the case of a pandemic outbreak, this egg-based production system may not be quickly enough to meet the surging demand. The efficacy associated with egg-based vaccines are low in recently years. The raising concerns with egg-derived vaccines is resulting in the spurred exploration of alternatives. MDCK cells are becoming as an alternative host to embryonated eggs for influenza virus propagation. Although MDCK cells were considered to be a suitable host for the virus production, their inability to grow in suspension still limits the process of scale-up and their production capability.

Table 1. Virus titers of pandemic and seasonal influenza vaccine strains grown in sMDCK cells.

Virus	Source	HA unit/50 µL	
		received	adapted
A/Vietnam/1194/2004 (H5N1)	NIBSC	512	989
A/Anhui/1/2013 (H7N9)	Egg-derived	256	996
A/Singapore/GP2050/2015 (H3N2)	VIDRL	n.a	n.a.
B/Brisbane/63/2014 (Victoria)	Cell-derived	1337.2	1708.8
B/Brisbane/9/2014 (Yamagata)		838.2	1552.9

In this study, NHRI, Taiwan and Irvine Scientific, USA has developed a suspension MDCK cell line and proprietary medium (BalanCD simple MDCK). The cell concentration of the suspension MDCK (sMDCK) cells up to 2×10^6 cells/ml after 96 hrs was obtained, and the doubling time of 30-35 hrs was found very similar to the adhesion MDCK (aMDCK)cells cultivated on microcarriers (5g/L). In addition, no fresh medium replacement was necessary in the sMDCK cell culture during the cell growth stage. The H7N9 candidate vaccine virus (NIBRG-268 which was derived from A/Anhui/1/2013) was used and infected both sMDCK and aMDCK cells with a low multiplicity of infection. The harvest of viruses was collected on day three post infection. The HA titers in sMDCK and aMDCK cells were reached to 996 and 574 HA units/50ul, respectively. The results show that the HA titers from both pandemic and seasonal vaccine strains cultured in sMDCK cells are high (see table 1). A similar finding was found in the small-scale bioreactor. The animal study also showed higher immunogenic response over the antigen produced from the aMDCK cell culture. Based on the overall recovery yield (30%) and the consumable cost from medium usage, the production cost is similar to the egg process.

In summary, a new adapted sMDCK cell line was developed. This sMDCK cultured using chemical-define (CD) medium can remain the similar growth rates as aMDCK cells but showed higher viral titers in both pandemic and seasonal influenza strains. Thus, this new combined technology of sMDCK with specifically-optimized CD medium could provide a low-cost vaccine production to the bottlenecks for establishing a large-scale cell culture using adherent MDCK cells.

POLYMER GRAFTED CHROMATOGRAPHY MEDIA FOR DIRECT CAPTURE AND HIGH-RESOLUTION PURIFICATION OF ENVELOPED VIRUS-LIKE PARTICLES

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Key Words: Virus-Like Particles; Anion Exchange Chromatography; Polymer Grafted Chromatography Media; Chromatography Beads.

Usually, the downstream processing of viruses and virus-like particles (VLP) does not include conventional chromatography media (beads) in the capture and/or purification steps. For large biomolecules, the binding capacity of conventional resins is limited to the outer surface of the beads. We developed a purification process based on polymer-grafted media, which allows a swift purification of HIV-1 VLP from CHO cell culture supernatant. The dynamic binding capacity is one order of magnitude lower than convective media but still in the range of 5-7x10^{11} particles/ml packed bed, which is unexpectedly high. For that reason, the binding mechanism was studied in detail. As expected, transmission electron micrographs showed that the VLPs only adsorb at the outer surface of the beads. This was corroborated by confocal microscopy using florescence labelled VLPs by incorporating cell membrane label. In batch update experiments, we observed a biphasic behavior with a fast uptake within minutes followed by a slow adsorption within hours. Desorption was also occurring very fast within minutes. Modeling linear gradient elutions with different gradient slopes showed that the number of effective charges involved in the adsorption is in the range of 30 and the adsorption is not really affected by salt. This explains why VLPs can be directly loaded from culture supernatant without further preconditioning. Scalability is not an issue, because these polymer grafted media can be packed in any scale from less than 0.5 ml to several hundred liters and in any column geometry.

VIRUS-LIKE PARTICLES (VLPs) AS A PLATFORM FOR THE DEVELOPMENT OF YELLOW FEVER AND ZIKA VIRUS VACCINE CANDIDATES

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Key Words: virus-like particles, yellow fever virus, zika virus, stable transfection, upstream process optimization.

Flaviviruses are arboviruses that have been posing serious challenges to global health since 2015. Zika virus (ZIKV) emerged in 2015 in Brazil and quickly spread to over 60 countries in Africa, Asia and the Americas, causing Guillain-Barré syndrome in adults and serious congenital malformations in fetuses of infected mothers. Besides mosquito-borne transmission, zika virus can persist for months in sexual fluids and thus poses risk also to non-endemic countries due to sexual transmission of returning travelers. Although the number of cases decreased significantly due to herd immunity in affected countries, development of a vaccine for ZIKV is of great importance to avoid future resurgence of the virus in endemic areas or future spread to currently non-endemic regions.

Yellow fever (YF) is a “historically devastating disease” (Paules and Fauci, 2017), which in past centuries killed approximately 10% of the population of cities like Philadelphia and Barcelona. Although a very effective vaccine exists for YFV, it can cause fatal adverse effects in a small proportion of vaccinees, and recent outbreaks have shown that due to its limited production in embryonated eggs the risk of serious vaccine shortages is high. Fractionating the vaccine dosis (1/5) was the emergency solution introduced by the WHO in 2016 to stop an outbreak in Africa, and is currently being adopted for mass vaccination in Brazil to try to stop the serious outbreak ongoing since 2017. The potential risk of YFV spreading to highly populated areas with no vaccination coverage, where the mosquito vector is present, such as Asia, makes urgent the development of new YFV vaccines.

In this context, virus-like particles (VLPs) can be a promising platform for developing safe and effective vaccines for YFV, ZIKV and other flaviviruses. In this work, we developed stable recombinant cell lines constitutively expressing the structural prM (pre-membrane) and E (envelope) proteins of ZIKV and YFV. Sucrose cushion ultracentrifugation and TEM images have confirmed that VLPs resembling in both size and shape the respective native viruses are formed.

In order to optimize expression, cell transfection protocol was optimized using different transfection reagents, media and host cell lines, including CHO, HEK293, BHK, MDCK and Vero cells. Stable cell lines derived from CHO-K1 and HEK293-3F6 gave the most promising results and were followed for up to 20 weeks post-transfection in the presence and absence of the selection marker, showing that cells grow to high densities with high viabilities and keeping the expression of VLPs.

The use of FACS to sort for high producer cells allowed obtaining enriched cell pools producing significantly higher amounts of VLPs and confirmed the hypothesis that secreted VLPs can be transiently detected on the cell membrane surface. Kinetic studies to evaluate different culture media and cultivation conditions under batch, pseudoperfusion and perfusion mode were carried out with the final aim of increasing productivity and reducing production costs.
Ongoing studies are focusing, on one hand, to purify the ZIKV and YFV VLPs for immunogenicity studies and, on the other hand, to express VLPs of other flaviviruses circulating in the Americas, as preparedness measure for future threats. VLPs of DENV 1, 2, 3 and 4, as well as of Saint Louis encephalitis (SLEV), Ilheus (ILHV) and Cacipacore (CPCV) viruses have already been successfully expressed by transient transfection.

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MANUFACTURING STRATEGIES FOR SUSTAINABLE SUPPLY OF ULTRA-LOW COST VACCINES FOR GLOBAL HEALTH

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Key words: Vaccines, *Pichia pastoris*, manufacturing platform, cost of goods (COG), rotavirus, process economics, optimisation, decisional tools, process intensification, process integration, perfusion, facility design

The ability for governments across the globe to protect infants from diseases caused by viruses such as polio, human papillomavirus and rotavirus through immunization is highly contingent on the development and manufacture of efficacious and cost-effective vaccines. Historically, vaccine manufacturers operate at large, and at times, overstretched capacities to benefit from economies of scale. However, this has led to expensive batch failures that have added to cost and interrupted supply.

This presentation describes a detailed economic analysis of multiple approaches for the manufacture of a *Pichia pastoris*-based vaccine with the aim of proposing strategies to produce a ultra-low cost vaccine against rotavirus with a target COG per dose of ¢15 for the finished drug product. This analysis was carried out using an advanced integrated decisional tool developed *in-house*. The case study assesses the cost-effectiveness of multiple manufacturing flowsheets combining different upstream and downstream techniques (e.g. fed-batch v perfusion, chromatography v crystallisation) as well as different facility designs (e.g. in-house v outsourced reagent production, fully integrated process v segregated process steps), across different geographic locations (Europe v India) and number of manufacturing sites. The key cost drivers across these scenarios were identified through a detailed sensitivity analysis. This allowed for process performance targets for sustainable supply of ultra-low cost rotavirus vaccines to be identified using an optimization algorithm.

The key output of this work is to break the economies of scale model and explore how advances in manufacturing can be integrated into decisional tools models to facilitate the guaranteed, uninterrupted supply for low cost vaccines for Global Health.

A TAILOR-MADE PURIFICATION STRATEGY FOR ONCOLYTIC MEASLES VIRUSES USING MEMBRANE-BASED PROCESSES

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Key Words: virus stability, tangential flow filtration, concentration, diafiltration

Cancer patients can benefit from the Measles virus, since in the early 70s a relation between cancer remission and an infection with Measles was first mentioned (Bluming, Ziegler 1971). Further studies confirmed this oncolytic activity and therefore, the Measles virus became highly interesting for the application in cancer treatment.

However, for the widespread application as a therapeutic agent several bottlenecks have to be overcome in context of quantity and quality. For one therapeutic dose of oncolytic Measles viruses (OMV) at least 10^{11} infectious particles are needed (one vaccination contains ~10^3 $TCID_{50}$) (Russell et al. 2014).
Besides that, the impurities, such as host cell proteins (HCP) and host cell DNA (hcDNA), must be reduced to appropriate limits set by regulatory authorities. The full recovery of OMV infectivity must also addressed. This underlines the need of a tailor-made downstream processing.

After we established a high titer production process, achieving OMV titers of 10^{11} $TCID_{50}$ mL^{-1} (Grein et al. 2017), we are now focused on the downstream processing of OMV. For this purpose we characterized the OMV regarding process parameters used in DSP, such as stability towards ionic strength, osmolality, agglomeration and shear stress. Based on this, a clarification step was conducted, followed by the further purification with tangential flow filtration (TFF). By using polyether sulfone flat sheet membranes in concentration mode, we were able to recover the infectious virus and lowered the impurities by ~70% for hcDNA and ~80% for protein content. In the next purification step, we applied a discontinuous diafiltration and could deplete the impurities by ~95% in total. These results showed that TFF is an appropriate tool for the purification and formulation of OMV.

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FORMULATION CONSIDERATIONS FOR THE DEVELOPMENT OF ADJUVANTED VACCINES

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Key Words: Adjuvants, formulation, emulsion, aluminum salts, E6020.

Adjuvants constitute an important class of products for the development of modern vaccines, especially for vaccines based on highly purified protein antigens. However, there are only a few adjuvants that are used in licensed human vaccines, with aluminum salts (AlOOH and AlPO4) and squalene emulsions (AS03, MF59) being the most widely injected adjuvants in humans.

While simple aluminum salts and emulsion adjuvants are very good at increasing antibody and Th-2 responses, they often lack the ability to induce strong Th-1 type cellular responses. One approach to increase the capacity of these adjuvants to induce Th-1 responses is to combine them with a small molecular weight immunopotentiator, such as a Toll-like receptor (TLR) agonist. We and others found that formulation plays an important role when developing such adjuvant combinations. With the example of a small molecule TLR-4 agonist, E6020, combined respectively to aluminum salt and to a squalene emulsion, this presentation will highlight some key formulation parameters that control the safety, potency and stability of these adjuvant combinations.

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INTRADERMAL ADMINISTRATION OF SYNTHETIC DNA VACCINES INDUCE ROBUST CELLULAR AND HUMORAL IMMUNE RESPONSES

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There has long been an interest by health agencies such as Program for Appropriate Technology in Health (PATH) and the World Health Organization (WHO) to deliver vaccines by the intradermal route (ID). The skin is full of immunological cells, most notably antigen presenting cells. Therefore, ID injection may be an efficient means of inducing an immune response to vaccines. Inovio has developed ID injection followed by a shallow *in vivo* electroporation (ID-EP) for synthetic DNA vaccine delivery. We have tested a number of our vaccines in clinical trials using the ID-EP delivery system including vaccines targeted against HIV, ZIKA virus and EBOLA virus. The success of the ID method takes on particular importance for a vaccine that will be used in an emergency setting such as during a possible Ebola virus outbreak. Such a vaccine will need to quickly induce robust, protective immune responses. INO-4201, our DNA based *Zaire Ebolavirus* (EBOV) vaccine, was analyzed following ID delivery. Immune responses generated by INO-4201 after the 2mg intradermal administration using the Cellectra in vivo electroporation device in volunteers revealed the induction of robust Ebola virus GP-specific antibodies, CD4+ as well as CD8+ T cell responses. Specifically, 100% seroconversion, as gauged by binding ELISA, was detected after two doses of INO-4201. The reciprocal geometric mean endpoint titer at that time was 39,664.20 and was boosted by administration of the third dose to 46,968.00. Examination of the EBOLA virus GP specific T cell response as assessed by Interferon gamma (IFNγ) ELISpot revealed a mean peak response magnitude of 295.3 SFU per 10^6 PBMCs. Importantly, the induction of robust immune response by ID-EP was mirrored in the ZIKA virus and HIV DNA vaccine clinical trials. These results indicate that ID methods to deliver DNA-based vaccines are important for further clinical development.

THERMOSTABILIZATION OF ADENOVIRUS-VECTORED VACCINES, REMOVING THE NEED FOR CONTINUAL COLD-CHAIN STORAGE

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Key Words: Thermostability, simian adenovirus, cold-chain, formulation, one-health

Challenges around affordable and reliable supply of vaccines that need to be transported and maintained in the cold-chain to remain effective are a hindrance to realizing their full potential. We will describe preparation for GMP manufacture and Phase I clinical trial of a new technology for vaccine thermostabilisation. We will also describe application of the same technology to a novel veterinary vaccine which is entering advanced development.

The sugar-matrix thermostabilisation (SMT) technology involves application of vaccine in a simple disaccharide-based buffer to a non-woven matrix, similar to a pad of filter paper. This is followed by drying at ambient temperature and pressure (i.e. without a freezing step, enhancing suitability for freeze-sensitive products). The materials and process are simple and cheap.

We have previously shown that SMT allows for the storage of viral vectored vaccines such as modified vaccinia virus Ankara (MVA) and adenovirus vectors at up to 45^{o}C for several months with minimal losses[1,2]. More recently we have shown the technique can improve stability of various other vaccine types, ranging from virus-like particles through to enveloped RNA viruses. In many cases, the level of thermostability achieved would allow for “last mile” vaccine distribution via the ‘extended controlled temperature chain’ (ECTC), or even allow prolonged storage at uncontrolled ambient temperature. This would decrease distribution-associated costs/ losses and increase vaccination feasibility in hard-to-reach areas.

We have now received funding for GMP manufacture and Phase I clinical trial of an SMT-formulated adenovirus-vectored rabies vaccine, ChAdOx2 RabG. We will describe the production of custom wet-laid non-woven matrices with optimized SMT performance, using processes and materials suitable for use as an input to a GMP process. We will further describe the development of simple apparatus suitable for executing the process for pilot GMP batches, the optimization of the drying process and excipient composition, and the application of frequency modulation spectroscopy for non-destructive analysis of residual moisture content. Finally, we will describe the application of the technology to a formulation of ChAdOx1 RVF, an adenovirus-vectored vaccine against Rift Valley Fever Virus which is being developed for both human and veterinary use. In this case, SMT is applied to an ultra-low-cost drug substance designed for veterinary use (cell lysate which has been clarified and ultrafiltered but not chromatographically purified), emphasizing the suitability of the approach for low-cost and One Health applications.

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SAFETY AND BIODISTRIBUTION OF SULFATED ARCHAEAL GLYCOLIPID ARCHAEOSOMES AS VACCINE ADJUVANTS

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Key Words: Archaeosome, adjuvants, vaccine, safety , biodistribution, Sulfated archaeal glycolipids.

Archaeosomes are liposomes comprised of ether lipids derived from various archaea which, as adjuvants, can induce robust, long-lasting humoral and cell-mediated immune responses to entrapped antigens. Traditional total polar lipid (TPL) archaeosome formulations are relatively complex and semi-synthetic archaeosomes involve many synthetic steps to arrive at the final desired glycolipid composition. We have developed a novel archaeosome formulation comprising a sulfated saccharide group covalently linked to the free sn-1 hydroxyl backbone of an archaeal core lipid (sulfated S-lactosylarchaeol, SLA) mixed with uncharged glycolipid (lactosylarchaeol, LA). This new class of adjuvants can be easily synthesized and retains strong immunostimulatory activity for induction of cell-mediated immunity following systemic immunization. Herein, we demonstrate the safety of SLA/LA archaeosomes following intramuscular injection to mice and evaluate the immunogenicity, in vivo distribution and cellular uptake of antigen (ovalbumin) encapsulated into SLA/LA archaeosomes. Overall, we have found that semi-synthetic sulfated glycolipid archaeosomes are a safe and effective novel class of adjuvants capable of inducing strong antigen-specific immune responses in mice and protection against subsequent B16 melanoma tumor challenge. A key step in their mechanism of action appears to be the recruitment of immune cells to the injection site and the subsequent trafficking of antigen to local draining lymph nodes. A better understanding of the safety and mechanism of action of novel adjuvants such as archaeosomes is a key step in their advancement into clinical use.

RECOMBINANT HEMAGGLUTININ PROTEINS FORMULATED IN A NOVEL PELC/CpG ADJUVANT FOR H7N9 SUBUNIT VACCINE DEVELOPMENT

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Key Words: H7N9, recombinant HA, PELC/CpG

Humans infected with H7N9 avian influenza viruses can result in severe pneumonia and acute respiratory syndrome with an approximately 40% mortality rate, and there is an urgent need to develop an effective vaccine to reduce its pandemic potential. In this study, we used a novel PELC/CpG adjuvant for recombinant H7HA (rH7HA) subunit vaccine development. After immunizing BALB/c mice intramuscularly, rH7HA proteins formulated in this adjuvant instead of an alum adjuvant elicited higher IgG, hemagglutination-inhibition, and virus neutralizing antibodies in sera; induced higher numbers of H7HA-specific IFN-γ-secreting T cells and antibody secreting cells in spleen; and provided improved protection against live virus challenges. Our results indicate that rH7HA proteins formulated in PELC/CpG adjuvant can induce potent anti-H7N9 immunity that may provide useful information for H7N9 subunit vaccine development.

ACCELLERATED PROCESS DEVELOPMENT AND STOCKPILE FOR MERS, LASSA AND NIPAH VIRAL VACCINES

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Key Words: MERS, Lassa, Nipah Vaccine Development

CEPI (the Coalition for Epidemic Preparedness Innovation) was launched in January 2017. The global need for CEPI emerged after the devastating Ebola crisis in 2014/15 that caused over 11,000 deaths and had an economic impact of at least $2.8 billion in the worst-affected countries alone[1]. The collective response to Ebola had fallen short, and it was evident we needed a better system to produce proven vaccines against known epidemic threats. A year ago at Davos, the governments of India and Norway and Guinea; the Bill & Melinda Gates Foundation, Wellcome and the World Economic Forum backed the creation of CEPI, an innovative partnership of public, private, philanthropic and civil society organizations, to provide a global insurance policy to defend against future epidemics. Through a call for proposal to vaccine developers, CEPI launched a portfolio of projects covering the development of MERS, Lassa and Nipah viral vaccines, based on WHO blueprint list of pathogens and in addition, a number of platform technologies are currently evaluated for the development of rapid response against unknown pathogens. In this paper, we introduce the CEPI process development, the vaccine technologies and stockpile strategies for MERS, Lassa and Nipah candidates in the CEPI portfolio. The cell lines, process and scale up portfolio strategies will be reviewed for emergency settings vs conventional vaccine process development. The emergency stockpile development strategy will be presented in this paper. In addition, we also highlight the critical areas of dialogue with regulatory authorities for the enhancement of use of experimental vaccine candidates in emergency settings for efficacy trials.

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RAPID RESPONSE PIPELINE FOR STABILIZED SUBUNIT VACCINES

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Key Words: emerging epidemics, rapid response, subunit vaccines

The Coalition for Epidemic Preparedness Innovations (CEPI) have recently put out a call for proposals aimed at platform technologies that can enable rapid vaccine development for novel or previously unrecognized viruses. We have proposed a streamlined process for the generation of stabilized subunit vaccines. This project brings together unique proprietary recombinant technology for generating stabilized subunit vaccines (the molecular clamp), a highly skilled team from some of Australia's leading scientific organizations and world-class facilities. Molecular clamp is a broadly applicable platform technology that facilitates expression of recombinant viral glycoproteins in subunit form without loss of native antigenicity. The molecular clamp imparts superior stability over alternative trimerization domains, efficiently stabilizing soluble viral fusion proteins in their native trimeric 'pre-fusion' form. This form is equivalent to that expressed on the virion surface and the principle target for a protective neutralizing antibody response. Through stabilization of the pre-fusion form, the molecular clamp promotes the production of highly neutralizing and broadly cross-reactive antibodies. Importantly, the molecular clamp does not required prior knowledge of a proteins quaternary structure.

The goal of this project is to establish a holistic and robust pipeline to rapidly generate novel subunit vaccines purely from sequence information. Within this pipeline, pre-clinical development, including the generation of evidence for safety and immunogenicity in animal models, is to be completed within a 16 week window allowing candidate vaccines to then progress directly into Phase I clinical trials. Phase I trials, including regulatory approval, patient immunization and analysis of safety and immunogenicity will be completed within 10 weeks (week 17-26 of the pipeline). As part of the project, large-scale manufacture of >200,000 vaccine doses will be completed within a further 8 weeks. Vaccine produced through this pipeline will therefore be available for rapid deployment and provide the best possible opportunity to counter emerging viral epidemics.

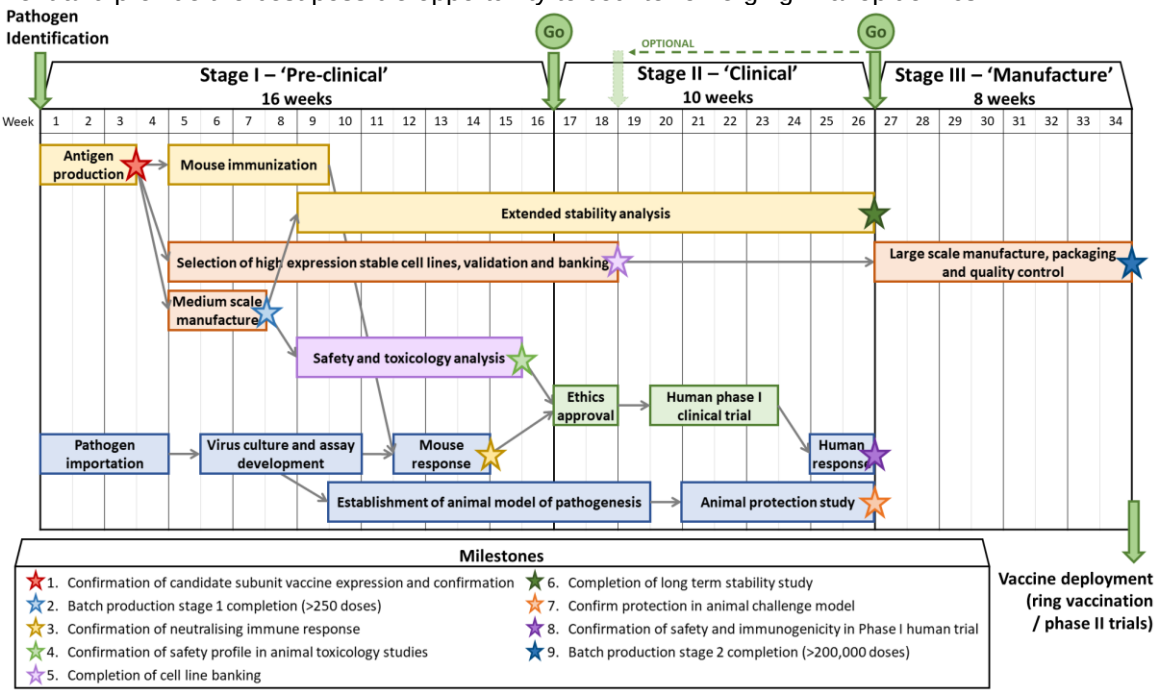

Figure 1. Overview of rapid response pipeline design. Key pipeline components are designated by color. Yellow: Vaccine design and animal immunizations; Red: Vaccine manufacture; Purple: Toxicology analysis; Blue: Immunogenicity and animal protection studies; Green Phase I clinical trials. Note: stage III is able to be optionally triggered at week 18, prior to completion of stage II. Early triggering of stage III at week 18 in the event of a 'worst case scenario' will decrease the total time to deployment to 26 weeks from pathogen identification.

USING THE AdVac® VACCINE MANUFACTURING PLATFORM FOR RAPID RESPONSE TO INFECTIOUS DISEASE OUTBREAKS

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Key Words: emergency response, adenovirus, manufacturing platform

In the last decade, we have seen several examples of new or existing infectious diseases that rapidly developed into epidemics with devastating effects and sometimes high mortality. In order to combat these threats and to be prepared for future epidemics, it is essential to develop and produce vaccines against these pathogens. A lesson that can be learned from the recent Ebola epidemic in Western Afrika is that speed at which vaccines are developed is essential. A flexible and robust manufacturing platform can play a vital role in meeting the pressing timelines. At Janssen vaccines, we have developed a drug substance manufacturing platform based on AdVac® and PerC6® technology. This platform is able to handle significant variations that are associated with the production of different vaccine candidates while maintaining a consistent quality of the end product. Furthermore, the platform process is fast, low-footprint, high yield and cost-effective. The platform nature allows for quick incorporation of new vaccine candidates into our manufacturing pipeline with minimal time required for development and with fast and smooth technology transfers between development and GMP manufacturing, since experience, equipment, documentation, analytical methods can be efficiently leveraged between candidates. In this presentation, we will demonstrate the value of the AdVac® manufacturing platform for the prevention and combating of rapidly emerging epidemics of infectious diseases.

PRECLINICAL DEVELOPMENT OF FILOVIRUS AND FLAVIVIRUS VACCINES BASED ON RECOMBINANT INSECT CELL EXPRESSED SUBUNITS

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Key Words: Filovirus, Zika Virus, recombinant, subunit, preclinical

Ebola Virus Disease (EVD) is the most prominent example of filovirus disease but despite being characterized as a Category A Priority Pathogen by NIH/NIAID over a decade ago, it lacked public and private research resources due to the absence of a commercial market. Transmission from wild animals into the human population typically causes outbreaks of limited scale in 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 when a *Zaire Ebolavirus* (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. Despite significant progress with the clinical development of several EBOV vaccine candidates during and after the West African outbreak, no EBOV specific therapeutics and vaccines have yet received regulatory approval. Additional research is needed in particular on understanding the mechanism of protection and defining immune correlates of protection for Ebola and other filovirus vaccines.

For our multivalent filovirus vaccine candidate, we have produced soluble recombinant filovirus glycoproteins (GP) from EBOV, *Marburg marburgvirus* (MARV) and *Sudan ebolavirus* (SUDV) using the Drosophila S2 cell expression system. The immunogenicity of highly purified recombinant subunits and admixtures formulated with or without clinically relevant adjuvants was 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 successfully for protection in the mouse model of EBOV. Further studies allowed us to demonstrate that both humoral and cell-mediated immunity are elicited and can mediate protection. Additional 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 demonstrate that vaccination with formulations of recombinant EBOV subunits and an emulsion-based adjuvant consistently produces high anti-EBOV IgG and virus neutralizing titers. Such vaccination 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. In addition to updates on efficacy testing against EBOV and MARV, we will discuss current formulation optimization efforts in our laboratory including thermostabilization of recombinant subunits as well as defining correlates of protection. These are prerequisites to enable efficient clinical development of a monovalent vaccine candidate for protection against EVD and a multivalent, recombinant subunit vaccine with protective efficacy against EBOV, SUDV and MARV infection.
Recently we have also demonstrated the applicability of our vaccine platform for the rapid development of vaccines against emerging diseases with a focus on Zika virus (ZIKV), a flavivirus, where we were able to demonstrate efficacy in mice and cynomolgus macaques within approximately 13 months from designing the synthetic gene for antigen expression. While a completely different disease from EVD, the recent outbreak of ZIKV in the Americas provided a similar challenge as no vaccine development efforts have been conducted prior to 2016 and an increasing body of evidence suggests that rather than causing a typical, mild form of disease as previously reported, ZIKV infections can cause neurological sequelae as well as fetal and infant malformations.

These results demonstrate that the insect cell expression system can be used to rapidly and efficiently produce recombinant viral subunits from a variety of pathogenic viruses that are highly immunogenic in multiple animal species and are capable of providing effective vaccine protection against live virus challenge.

DEVELOPMENT OF PAN-FILOVIRUS VACCINE AGAINST EBOLA AND MARBURG VIRUS CHALLENGES

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Filoviruses such as Ebola (EBOV) and Marburg (MARV) viruses cause deadly viral hemorrhagic fever in humans with high case fatality rates. To date, no licenced therapeutic or vaccine has been clinically approved to prevent infection. Several vaccine candidates are under development against the few most common filoviruses targeting the virus glycoprotein (GP). However, protective antibodies induced by such GP vaccines are usually limited to the same species. In contrast, T-cell vaccines offer an opportunity to design a single pan-filovirus vaccine protecting against all members of the Filoviridae family. In this study FILOcepX vaccines were constructed targeting the four most conserved regions among the viral proteomes with the aim to induce protective T-cell responses against different filoviruses. BALB/c mice were immunized with FILOcep 1 and 2 vaccines vectored by non-replicating engineered simian adenovirus and poxvirus MVA. Groups of 20 BALB/c mice were primed and boosted with either the FILOcep1 and FILOcep2 vaccines or control ChAdOx1- and MVA-vectored vaccines. Four animals in each group were sacrificed after 1 week of boosting to detect T-cell response for the FILOcepX antigen. High frequency T cells specific responses were detected in mice receiving the test vaccines by IFN-γ ELISPOT kits. Of the remaining 16 animals in each group, 8 were challenged with mouse-adapted EBOV and 8 were challenged with mouse adapted MARV in Containment Level 4 laboratory. All the mice in the control group either died or had to be euthanized between 4 and 6 days post challenge. On the other hand all the FILOcepX vaccinated mice maintained their normal body mass and survived till the end of the scheduled protocol on day 29 post challenge. These FILOcepX vaccines provided 100% protection against the lethal challenges with filoviruses of two different genera. Further evaluation the efficacy of this vaccine in non-human primates (NHPs) is warranted.

HIGH-TITER RAPID RESPONSE PLATFORM FOR EPIDEMIC PREPAREDNESS

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Key Words: Epidemic preparedness, vaccine availability, stockpiling.

Recent epidemics & pandemics have exposed serious flaws in global response to infectious disease outbreaks, as no vaccine were available to stop the diseases, due to limited market incentive.
New initiatives, such as the CEPI “Coalition for Epidemic Preparedness Innovations” are focusing on accelerating vaccine development for priority disease and ensuring their global availability.
In this light, Univercells is dedicated to designing novel biomanufacturing technologies to accommodate new products at short notice.

Univercells innovative manufacturing concept is designed to enable the rapid generation of potent vaccine candidates against a broad spectrum of targets, with the capacity to rapidly scale-up and deploy vaccines at low cost for a rapid response vaccine countermeasure to epidemic threats.

Based on intensification technologies, the platform achieves high productivity on a very low footprint (over 40m doses per year, on 2mx3m), for a cost-effective production of vaccines with a low investment cost.
Such footprint reduction enables the platform to be integrated into isolators for increased safety, facilitating compliance with biosafety levels.

By designing a flexible process that can easily be adapted to any viral vaccine without hardware modification, Univercells wishes to reduce the burden of developing adapted manufacturing processes for newly developed vaccines, facilitating their way towards commercialization.

These low-footprint and highly-contained production unit based on single-use technologies are easily and rapidly deployable, offering several production strategies for epidemics preparedness and response:
- Generate rotating stockpile of pandemic vaccines at low cost
- Quick-reaction units to be deployed in the field during emerging diseases
- Install as mothball extended manufacturing capacity at low CAPEX, for a rapid activation

VACCINES FOR POVERTY ASSOCIATED INFECTIOUS DISEASES: ACCESSING INNOVATION

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There are very few exceptions to the rule that vaccines have been developed in high income countries (HIC) and have been extended over time to low and middle income countries (LMIC). For the diseases of childhood, and now for human papilloma virus, these innovative vaccines have resulted in dramatic reductions in death and improvement in socioeconomic indicators. However, there has often been a delay between the introduction of life-saving (or morbidity reducing) vaccines in HIC and their subsequent introduction in LMIC. Recently a dengue vaccine targeting middle income countries and a typhoid conjugate vaccine whose primary target population is LMIC have been developed. However, accessing innovative vaccines remains a key problem, particularly in countries that are not Gavi eligible or for diseases that are not covered by Gavi. The latter point can be further developed by noting that full implementation of existing vaccines from 2011-2020 will save an estimated 2.5 million lives per year. However, infectious diseases for which no vaccines exist will kill nearly 5 million per year. These diseases include the usual suspects -- HIV, TB and malaria – but also infectious diseases associated with poverty: invasive non-typhoidal Salmonella (up to 650,000 deaths annually), Group A Streptococcus (~450,000 deaths annually), Shigella (~200,000 deaths annually), hepatitis E, among others. In general, the latter group is characterized by burden in LMIC, imprecise epidemiology, and lack of incentivization (for major vaccine companies). Unlike the CEPI outbreak diseases, there is no global fund to accelerate clinical development. While economic development will result in the gradual subsidence of these infectious diseases, vaccines could and should be viewed as a cost-effective alternative and mechanisms to fund the development and deployment of vaccines against poverty associated infectious diseases would have a high rate of return on investment.

FUTURE PROSPECTS AND APPLICATION FOR THE DEVELOPMENT OF ADENOVIRUS-BASED VACCINE

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Key Words: Adenovirus, Ebola.

Ebola viruses (EBOVs) are enveloped, non-segmented, negative-strand RNA viruses belonging to the family Filoviridae. They are known to cause lethal hemorrhagic fever in humans with a mortality rate up to 90%. As an emergency response to the largest Ebola disease epidemic in 2014, several countries had accelerated the process of developing effective vaccines against Ebola.

The vaccine described here, unlike other Ebola vaccine candidates that are under clinical evaluation, is the first Ebola vaccine based on the 2014 Zaire Guinea epidemic strain. This vaccine, Ad5-EBOV, is a replication-defective recombinant human type 5 adenovirus expressing Zaire (Makona, 2014) Ebola virus envelope glycoprotein. A manufacturing process, up to 200L scale, has been developed and optimized. The purified Ad5-EBOV was formulated with the addition of proper stabilizers and lyophilized. The product can be stored at 2 – 8^{o}C for at least one year without losing any infectivity.

The Ad5-EBOV vaccine was tested in guinea pig and non-human primate models. In both cases, it was showed to elicit specific B- and T-cell immunity and conferred 100% protection when animals were challenged with the Ebola virus. Following two successful phase I trials in China, a phase II trial of Ad5-EBOV was conducted in Sierra Leone in 2015 with 500 participants. The results of the trials demonstrated that Ad5-EBOV is safe and highly immunogenic.

ADJUVANT MANUFACTURING SCALE-UP AND TECHNOLOGY TRANSFER

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Key Words: vaccine adjuvant, oil-in-water emulsion, technology transfer, manufacturing scale-up

Strategies to increase the availability of cGMP adjuvant formulations for emerging and re-emerging infectious diseases comprise an essential component of global pandemic preparedness. We have focused on two approaches to increase global adjuvant supply and build local capacity: (1) scale-up of our cGMP adjuvant manufacturing capacity through equipment and process improvements, and (2) technology transfer of adjuvant manufacturing know-how to developing countries. Regarding manufacturing scale-up, we have increased our cGMP oil-in-water emulsion adjuvant manufacturing capacity from 2K doses/batch to 5M doses/batch by upgrading processing equipment and implementing innovative process efficiency improvements. We demonstrate this new capacity by manufacturing proof-of-concept batches of emulsion at the 5M dose scale and demonstrating acceptable particle size, emulsion component concentrations, pH, osmolality, and visual appearance. Regarding technology transfer, we highlight our local capacity building efforts in India, Romania, and South Africa, resulting in successful local production of adjuvant formulations and, in the case of India, Phase 1 clinical testing of the manufactured material as a component of a malaria vaccine [1-3]. Together, these efforts have enabled enhanced global adjuvant manufacturing capability, facilitating local capacity building and increased pandemic preparedness.

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A VACCINE FOR EBOLA VIRUS – APPROACHES AND RESULTS OF ACCELERATED PROCESS DEVELOPMENT AND CHARACTERIZATION STUDIES

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Key Words: Ebola virus, vaccine, process development, MOI, purification

In 2014, West Africa experienced the worst outbreak of Ebola virus in history with 10 times more cases than in all previous outbreaks combined. In response to this public health emergency, MSD and a global network of partners collaborated to speed the research, development, and deployment of a candidate vaccine that ultimately provided the first evidence of efficacy in human subjects for any Ebola vaccine. While work continues to ultimately license the candidate vaccine, a risk-based approach to process development and characterization was used to accelerate and prioritize the study of parameters. Risk was evaluated by experts familiar with unit operations and parameters in similar licensed live viral vaccines and resulted in an overall plan of study encompassing five major areas – cell expansion, viral infection, purification, formulation, and general robustness. In parallel to batch size scale-up to support commercial production, a scale-down model comparable to commercial scale production was developed and enabled high-throughput experimentation. This approach reduced experiment cycle time from eight weeks to three weeks, reduced process volumes enabling design of experiments, and resulted in high-throughput execution of lab-scale studies. Typically, potency is extremely sensitive to multiplicity of infection (MOI); this vaccine is capable of producing acceptable potencies during viral infection with a 1000x range of MOIs. The most critical parameter during purification is digestion, which results in a ~10-fold increase in product potency. The final tangential flow filtration unit operation is extremely robust with no critical process parameters while still being capable of effectively clearing residual enzyme. Additionally, the implementation of a fully disposable single-use drug substance manufacturing process also helped accelerate process development and characterization activities. Component user requirements and schematic drawings were used to design prototypes which were evaluated using innovative shake-down studies. This approach resulted in a rugged system of end-to-end, single-use disposable components with 42 modular, “plug-and-play” designs available to support >500 single-use assemblies needed in production. Components were delivered to the commercial manufacturing site within 15 months with no required design changes following water-run testing. Taken together these approaches helped accelerate process development and characterization studies that will expedite the licensure of an Ebola virus vaccine.

PRODUCT DEVELOPMENT AND PROGRAMATIC IMPLMENTATION OF TYPBAR TCV® AND ROTAVAC® VACCINES

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TYP TCV® from Bharat Biotech, is the First Typhoid Conjugate Vaccine Prequalified by WHO. It is the first typhoid vaccine, clinically proven to be administered to children from 6 months of age to adults and confers long term protection against salmonella typhi.

International Health Metrics and Evaluation (IHME) estimates that in 2016, there were approximately 12 million cases of typhoid fever resulting in around 130,000 deaths. In most developing countries the cost of a course of treatment for typhoid fever ranges from $50 to $5000 for outpatient and inpatient treatments.

TYPBAR TCV® is a result of dedicated product development at Bharat Biotech since 2001, with the strains provided by Dr. John Robbins, where all aspects of the product profile were studied and evaluated in human clinical trials. With 5 years of follow up data for seroconversion, TYPBAR TCV® at 25µg / dose has proven long term protection for children and adults alike and can be administered to children from 6 months of age and WHO-SAGE recommended the use of typhoid conjugate vaccines for use in infants between 6 and 23 months of age and catch up vaccinations for children between 2 and 15 years of age. This recommendation paves the way for countries to introduce the vaccine into their immunization programs. Oxford University conducted a human challenge study with TYPBARTCV®, where the subjects were challenged with live S. Typhi proving a protective efficacy of 87% against typhoid fever.

Rotavirus is the leading cause of severe diarrhoea and death among children less than five years of age around the world. According to a recent study, 37 percent of the 578,000 childhood diarrheal deaths in 2013 were due to rotavirus.

Vaccinations are an important part of global public health efforts to meet the Sustainable Developmental Goals of UNDP. ROTAVAC® was developed as a result of a multi country - multi partner collaborative model of Team science for over 2 decades and has covered all aspects of product development, licensure, and WHO prequalification, and represents a true reflection of “Clone to Clinic to Global Access”, resulting in a lifesaving product for global populations. The product profile of ROTAVAC® has been optimally designed for the developing world by the developing world to aid ease of administration, reduced training requirements, and with the lowest cold chain footprint for an oral rotavirus vaccine in the world.

The project to develop ROTAVAC® has resulted in more than 15 international publications in peer reviewed journals, including the pivotal phase III publication in Lancet in 2014 proving its comparable efficacy in the developing world. ROTAVAC® has 5 global patents, granted in countries such as India, USA, UK, South Africa, China, Nigeria, among several others. Product registrations for ROTAVAC® are in process in more than 30 countries globally.

A NOVEL VACCINIA VIRUS BACKBONE FOR THE DELIVERY OF IMMUNOTHERAPEUTIC GENES

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Vaccinia virus has a large and still incompletely understood genome although several strains of this virus are already in clinical development for the treatment of cancer. For the most part, clinical candidates have been attenuated from their wild type vaccine strains through deletion of metabolic genes like the viral thymidine kinase gene. We decided to carry out a more in depth understanding of the genetic elements of vaccinia which could be modulated to improve the oncolytic/therapeutic characteristics of the virus. Using a variety of cancer cell lines and primary tumor explants, we performed a fitness assay that compares head to head five wild-type Vaccinia strains to identify the genetic elements that together create an optimal "*oncolytic engine*". Using a transposon insertion strategy and deep sequencing of viral populations we systematically examined vaccinia genes that do or do not play a role in the therapeutic activity of the virus. Our studies allowed us to identify large areas of the vaccinia genome that when deleted, augment the oncolytic activity of a newly created recombinant virus (35 genes deleted). This novel virus was compared to five vaccinia strains currently in the clinic and in a variety of assays, the deleted virus displayed superior therapeutic activity and an enhanced safety profile. Studies in a variety of *in vitro* and *in vivo* models will be presented illustrating the strategy we have used to create this optimized oncolytic virus platform. We are currently carrying out further pre-clinical studies to accelerate the translation of this new virus into the clinic.

HER2 CANCER VACCINE OPTIMIZATION BY COMBINING *DROSOPHILA* S2 INSECT CELL MANUFACTURING WITH A NOVEL VLP-DISPLAY TECHNOLOGY

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Key Words: HER2, VLP-display, breast cancer

Breast cancer is a widespread oncology indication affecting more than 1.3 million people worldwide annually, 20%-30% of which are HER2 positive. HER2 is a tyrosine kinase receptor that is frequently overexpressed in several solid-tumor cancers (incl. breast, prostate, gastric, esophageal and osteosarcoma) where it denotes an aggressive phenotype, high metastatic rate, and poor prognosis. In a human context, passive HER2-targeted immunotherapy using monoclonal antibodies (mAb, e.g. Trastuzumab and Pertuzumab) has proven to be an effective treatment modality, which has dramatically improved clinical outcomes. Unfortunately, mAb therapy is very expensive and the repeated injections of high doses can be associated with severe side-effects that reduce efficacy.

Vaccines are highly cost-effective, but overall progress in development of anti-cancer vaccines based on cancer-associated antigens (e.g. HER2) has been hampered by inherent immune-tolerogenic mechanisms rendering the immune system incapable of reacting against the body's own cells/proteins (i.e. self-antigens). Consequently, many attempts to develop anti-cancer vaccines have failed in clinical trials due to insufficient immunogenicity. To circumvent this central issue, we have developed a proprietary virus-like particle (VLP)-based vaccine delivery platform. Notably, the VLP-platform is currently the only available technology to effectively facilitate multivalent "virus-like" display of large/complex vaccine antigens. This is key to overcome immune-tolerance and enable induction of therapeutically potent antibody responses directed against cancer-associated self-antigens.

In this talk I will discuss the non-viral *Drosophila* S2 insect cell production system and how it was applied to the production of hHer2/neu antigen, including using advanced production methods such as perfusion for clinical material manufacture. Furthermore, I will present our data from a transgenic mouse model for spontaneous breast cancer development, where high-density display of the HER2 extracellular domain on the surface of virus-like particles (VLPs) enables induction of therapeutically potent anti-HER2 responses. Split-protein tag/catcher conjugation was used to facilitate directional covalent attachment of HER2 to the surface of icosahedral bacteriophage-derived VLPs, thereby harnessing the VLP platform to effectively overcome B-cell tolerance. Vaccine efficacy was demonstrated both in prevention and therapy of mammary carcinomas in HER2 transgenic mice. Thus, the HER2-VLP vaccine shows promise as a new strategy for treatment of HER2-positive cancer. The modular VLP system may also represent an effective tool for development of self-antigen based vaccines against other non-communicable diseases.

RAPID DESIGN/DEVELOPMENT AND CLINICAL DEPLOYMENT OF SYNTHETIC DNA VACCINE TECHNOLOGY FOR DIFFICULT IMMUNE TARGETS

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Recent major improvements in synthetic DNA vaccine technology have changed our view of the use of this platform to impact infectious disease. Synthetic DNA has moved from a weak immune performer, to a highly consistent immune potent platform with the ability to generate protective immune responses in a rapid fashion. We present important recent examples of translational development for important targets such as HPV infection, Ebola, and Zika. Finally, the enhancement of this technology now enables an ability to launch DNA encoded MAb's. This opens novel areas and new approaches for difficult and important disease targets.

DEVELOPMENT OF AN ANALYTICAL PLATFORM FOR DELIVERY OF RECOMBINANT ONCOLYTIC VIRUSES

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Over the past two decades, a number of modified DNA and RNA viruses have shown great promise in cancer therapy. Their tumor specific cytotoxic activity involves several mechanisms. These include preferential uptake and replication of the virus in cancer cells, followed by infection and lysis of these cells (oncolysis). In addition, oncolytic viruses (OV), especially those engineered with appropriate transgenes, have been shown to induce tumor specific immune responses, thereby opening up an exciting area of cancer immunotherapy.

One OV, containing a recombinant Herpes Simplex Virus (HSV) with a Granulocyte Macrophage Colony Stimulating Factor (GMCSF) transgene insert, was approved in 2015 for treatment of refractory melanoma. While this drug, known as T-Vec or Imlygic, is administered intratumorally, several other OVs are in pre-clinical and clinical development with intravenous (IV) administration as an objective. The IV route potentially offers the advantages of accessing metastatic sites and eliciting systemic immunotherapeutic response. A recombinant form of the Newcastle Disease Virus (rNDV) has been recently developed that also incorporates GMCSF as a transgene. NDV is a single stranded, negative sense, enveloped RNA virus and, in native form, is not a human but an avian pathogen. Genetic engineering was performed to reduce avian pathogenicity and increase immunotherapeutic potential against cancer [1]. In addition to lysis of cancer cells produced by viral infection, rNDV has demonstrated induction of cytokine and chemokine responses, cell infiltration and long - term suppression of tumor growth in animal models.

Production of recombinant viruses requires selection of appropriate host cells, based on considerations of yield and quality of the product. In recent years, regulatory agencies have been open to use of human cell lines as host, including some tumor derived cells, such as HeLa. An advantage offered by HeLa cells is potential incorporation into the virus of host proteins known as regulators of complement activation (RCA). This allows a longer half-life of the virus in circulation. However, impurities derived from host cells as well as process conditions must be kept to the lowest levels possible. A thorough analytical and biophysical evaluation of the purified virus particles includes measurements of (a) infectivity, (b) morphology, (c) size distribution, (c) genetic integrity, and (d) major viral proteins. In addition, levels of contaminants such as residual host cell proteins and DNA must be evaluated, including sizes of residual DNA fragments. Robust and accurate measurement of infectious titer is especially important as this determines doses of an OV to be delivered to patients. It also provides a measure of virus stability and is critical to supporting process development. We have optimized a Fluorescent Focus Assay (FFA) that produces concordant results with Plaque Assay (PA) over several orders of magnitude of infectious titers [2]. FFA provides a faster turn-around in a higher throughput format compared to PA. These and additional structure-function assays were developed to support process development and deliver a well-characterized OV for cancer immunotherapy.

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[1]Includes work performed at Biopharmaceutical Development, MedImmune LLC, Gaithersburg, MD, USA

LEVERAGING VECTORED VACCINE CANDIDATES MANUFACTURING TO GMP COMPATIBLE BIOPROCESSES

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Background

Vectored vaccines are very efficient in the *in vivo* delivery of antigens either in the form of antigen protein and peptides or genetic material. The bioprocess of vectored vaccines poses however several challenge since the viral particles to be effective must maintain their infectivity. Lentiviral and adenoviral vectors are among the particles more used in the treatment of cancer diseases modulating the immune system. Both viral vectors are currently produced in transient upstream process. While the adenoviral vectors are produced at high titers the lentiviral vector upstream process still requires further improvement. The non-lytic nature of lentivirus enables the design of stable cell lines which may improve its yields through perfusion and longer term productions, reducing costs. The application of novel methods for the downstream processing such as continuous purification will contribute to increase the yield and lower the overall cost of the manufacturing processes.

Experimental approach

At the upstream process, many of the challenges lentiviral bioproducts present in its manufacturing are related to the apoptosis-leading cytotoxicity of some of the vector components. Supported on our long track experience and enabling tools developed for gammaretrovirus manufacturing, we undertook the challenge of establishing a constitutive stable lentiviral producer cell line. To address this challenge we proposed to eliminate or reduce the cytotoxicity of the lentiviral vector expression components. At the downstream process lentiviral vectors face the challenges common to retroviridae family of vectors namely short half-lives at room temperature, sensitivity to pH variations and salt concentrations, and shear stress. The purification strategy developed was designed to be based on disposable and easily scalable technologies. A final concentration achieving 10^8 TU mL^{-1} was targeted since the concentration step itself allows to reduce the burden on process and improve the transduction efficiency.

To address the high doses requirements we will report an improved oncolytic adenovirus purification process for phase I and II clinical trials and present a case on the use of Polysorb 20 as a replacement for Triton X-100 during cell lysis. Product recovery, potency, purity and the effect of manufacturing holding points will be discussed.

Results and discussion

A lentiviral producer cell line constitutively producing titers above 10^6 TU.mL^{-1}.day^{-1} was established. The cell line showed to be stable, consistently maintaining vector productivity over one month in the absence of antibiotics. At the bioreaction process it was possible to maintain the cells continuously producing over 10 days. At downstream we implemented scalable protocols for lentiviral and adenoviral vectors that is easy to transfer to GMP environment, combining microfiltration, anion-exchange, and ultrafiltration membranes technologies toward maximization of infectious virus recovery, allowing generation of clinical-grade viral vectors without the need for cleaning validation in a cost-effective manner.

Herein we will present and discuss the challenges on the biomanufacturing of lentiviral as well as adenoviral virus, the strategies and novel technologies to be adopted in order to enable a faster development of novel vectored vaccine candidates focusing on several case studies, supported by process technology innovation.

PURIFICATION OF FLAVIVIRUS VLPs BY A TWO-STEP CHROMATOGRAPHIC PROCESS

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Key Words: virus-like particles (VLPs), yellow fever virus, zika virus, downstream processing, chromatographic purification

Flaviviruses are enveloped viruses with positive-sense, single-stranded RNA, which are most commonly transmitted by infected mosquitoes. Besides for example dengue viruses (DENV), which have been already for decades posing challenges to public health worldwide, zika virus (ZIKV) and yellow fever virus (YFV) are flaviviruses that have caused significant outbreaks in the last few years. Thus, based on our experience of expressing virus-like particles (VLPs) of several different flaviviruses in recombinant mammalian cells, this work focuses on the development of efficient chromatographic purification processes for zika and yellow-fever VLPs. ZIKV has been discovered in 1947, and since 2007 it has caused isolated outbreaks in Pacific Islands. However, in 2015 it was identified for the first time in Brazil and then quickly spread to over 60 countries between 2015 and 2016. Although most zika patients are asymptomatic, in a small proportion of adults ZIKV infection can cause Guillain-Barré syndrome, and in fetuses of infected women it frequently causes serious congenital malformations, especially in the central nervous system. Since it can be transmitted also by the sexual route and can persist for very long periods in body fluids (including sperm), the development of a vaccine is needed to prevent the spread of the virus to non-endemic countries and to prevent outbreaks to periodically occur in regions where the virus is already circulating.

Yellow fever virus is a highly lethal virus, which causes death in about 6-10% of non-vaccinated individuals. In past centuries, before the introduction of the current live-attenuated vaccine, 10% of the population of cities like Philadelphia (USA) and Barcelona (Spain) died in YF outbreaks. The current vaccine is very safe and provides life-long protection from a single dose. However, it can also cause fatal adverse effects in a small proportion of vaccines, and the egg-based production is limited in capacity. This latter fact led to worldwide vaccine shortages during an outbreak in Africa in 2016 and in Brazil in 2017-2018. Although the WHO introduced during the African outbreak in 2016 the use of a fractional (1/5) dose as an emergency measure to control outbreaks, even if using fractional doses of the current vaccine, shortage would be an issue if YF outbreaks spread and especially if it gets to be locally transmitted in Asia, where the mosquito vector is widespread.

In this work, a two-step chromatographic process was developed for the purification of zika and yellow fever VLPs from CHO- and HEK293-derived cell culture supernatant, building on previous experience acquired on the purification of yellow fever whole virus from Vero cell culture (Pato et al., 2014, doi: 10.1016/j.vaccine.2014.02.036). The initial clarification of the cell culture suspension was performed by centrifugation and/or filtration, followed by anion exchange chromatography and then a multimodal chromatographic step. The anion exchanger used was a Q membrane adsorber, due to its easy scalability, simplicity to handle, absence of diffusional limitations, and good performance at high flow rates for the capture of large molecules such as VLPs. This capture step allowed a high degree of concentration and an efficient DNA removal. In order to enhance HCP removal, a CaptoCore 700 multimodal column was used in a flow-through mode, allowing contaminants to be adsorbed while VLPs were excluded by size. Samples from all steps of the process were characterized by immunoassays, total protein determination, SDS-PAGE and Western blot.
The promising results obtained for zika and yellow fever VLPs indicate that this process could be potentially applied also to other flavivirus VLPs that we have been expressing in our lab, such as DENV1-4, SLEV, CPCV and ILHV. Overall, the presented downstream process could potentially represent a simple, robust and economic platform technology for the production of cell culture-derived recombinant flavivirus vaccines.

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FLAVIVIRUS PRODUCTION IN PERFUSION PROCESSES USING THE EB66® CELL LINE

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Key Words: EB66®, flavivirus, perfusion, on-line capacitance, cryo-bag.

The outbreak of mosquito-borne yellow fever virus (YFV) in Angola 2016 rapidly spread to urban regions and other countries. Vaccination campaigns were subsequently intensified, but the increased vaccine demand led to depleted stockpiles. Current yellow fever vaccine manufacturing processes rely on embryonated chicken eggs, which are strongly limited with respect to flexible capacity increase in emergencies. The global vaccine demand is estimated by the WHO to 1.38 billion doses needed to eliminate epidemics. Thus, an urgent need for an improved production platform is needed, ideally transferable to new vaccine developments against emerging flaviviruses, such as Zika virus.

Here we present a cell culture-based YFV 17D and Zika virus (ZIKV) production process using the EB66® cell line. The avian EB66® suspension cell line grew fast and stable in chemically defined medium to cell concentrations of 1.8×10^7 cells/mL in shake flasks and batch mode. Seed virus was prepared from Vero-derived YFV and ZIKV material over five passages in EB66® cells. Thereby, infectious virus titers successfully increased by one log unit and maximum titers of 1.4×10^8 PFU/mL (infectious virions per mL) and 8.0×10^7 PFU/mL were obtained two days post infection for YFV and ZIKV, respectively.

The process was intensified using perfusion bioreactors to increase cell concentrations. Therefore, EB66® cells were cultivated in 1 L benchtop bioreactors equipped with an alternating tangential flow filtration (ATF2) perfusion unit. Perfusion rates were adjusted to maintain glutamine concentrations above 1 mM and cells grew up to 9.5×10^7 cells/mL. A maximum YFV titer of 7.3×10^8 PFU/mL was achieved. The cell-specific virus yield (CSVY) was 8 PFU/cell, similar to shake flask experiments.

For ZIKV production, another approach aimed at the use of on-line capacitance sensors to control cell-specific perfusion rates (CSPRs) based on cell concentrations. This automated system was set to a CSPR of 0.017 and 0.034 nL/cell/day leading to maximum cell concentrations of 8.9×10^7 cells/mL and 1.6×10^8 cells/mL. ZIKV titers peaked after three to four days post infection with 2.6×10^9 PFU/mL and 1.0×10^{10} PFU/mL, respectively. CSVYs increased from 5 PFU/cell (shake flask experiments) to 30 PFU/cell and even above 64 PFU/cell in this set-up. The increased CSPR resulted in an improved volumetric productivity by factor three compared to the lower CSPR.

Further process intensification was achieved by direct cell inoculation to the ZIKV production bioreactor. A 15 mL cryo-bag was thawed with 8.5×10^8 cells and cell viabilities of 90% after inoculation quickly increased over the cultivation period.

Taken together, EB66® suspension cells can be grown to concentrations exceeding 1.5×10^8 cells/mL in perfusion bioreactors, and cells are highly permissive for YFV and ZIKV. YFV production using perfusion systems generated virus material equivalent to 10 Mio vaccine doses (4.7 log infectious units per dose) in less than two weeks operation time. With the use of on-line sensors to adjust CSPRs meeting cellular nutrient demands, ZIKV titers exceeding 1.0×10^{10} PFU/mL were obtained for the first time. Direct cryo-bag inoculation shortened the seed train phase, and virus production was initiated with full flexibility. This is a powerful demonstration on how next generation flavivirus vaccine production can be realized.

PRODUCTION AND CHARACTERIZATION OF HER2-DISPLAYING BUDDED VIRUS-LIKE PARTICLES AND THEIR POTENTIAL AS CANCER VACCINES

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Key Words: Human epidermal growth factor receptor 2 (HER2), baculovirus-insect cell expression vector system (BEVS), virus-like particles (VLPs), cancer vaccine.

Cancer biomarkers are often glycosylated membrane receptor proteins present on the cellular surface. In order to utilize such receptor proteins in designing specific and sensitive diagnostic tools or as immunogens for vaccination based treatments, they need to be expressed in their native conformation. However, membrane receptor proteins are notoriously difficult to produce due to their hydrophobic nature and complex structure. The human epidermal growth factor receptor 2 (HER2) is known to be up regulated in a number of cancers including breast cancer, lung cancer, gastric cancer and glioblastoma multiform and was therefore chosen as tumor antigen in our studies. Here we used the baculovirus-insect cell expression vector system (BEVS) to produce budded virus-like particles (VLPs) serving as a display platform for the antigen. VLPs displaying HER2 were produced in *Spodoptera frugiperda* (*Sf*9*)* insect cells and were purified by sucrose gradient ultracentrifugation. The number of secreted particles was quantified by nanoparticle tracking analysis. To confirm the presence and functionality of displayed HER2, VLPs were labeled with gold-conjugated antibodies, were analyzed by transmission electron microscopy and the ability to present native epitopes was tested through enzyme-linked immunosorbent assay (ELISA). Trastuzumab, an anti-HER2 antibody, showed significant binding to antigen displaying VLPs, which demonstrates the potential of this platform to display cell surface biomarkers in their authentic conformation. In the second part of the study, the efficacy of the aforementioned characterized VLPs as a cancer vaccine was investigated. BALB/c mice were injected intramuscularly with control VLPS and HER2-displaying VLPs in combination with two different adjuvants in a prime-boost regimen. As verified by ELISA, HER2-displaying VLP vaccines induced strong antibody responses when tested against recombinant HER2, with variability observed amongst the different adjuvant groups. For further characterization the antibody-dependent cell-mediated cytotoxicity (ADCC) potential of the induced antibodies will be investigated and vaccinated mice will be challenged with HER2 expressing tumors to test the potential of antigen-displaying VLPs as a cancer vaccine. Overall, using our strategy, many other membrane proteins including tumor antigens, immune cell markers and immune receptors could be expressed. These tools could further be instrumental in cancer vaccine design and diagnostics, as well as antibody selection and engineering.

VIRUS-LIKE PARTICLES: A FLEXIBLE PLATFORM FOR UNIVERSAL INFLUENZA VACCINE DEVELOPMENT

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Key Words: Virus-like particles, *Pichia pastoris*, Tandem core, Hepatitis B core protein (HBcAg)

Human influenza remains a global public health threat, namely due to its evolutionary adaptability, which hinders effective prevention. Vaccination is currently the predominant tool in the prevention of infectious disease. However, current production methods for influenza vaccines are not only logistically inadequate in the face of a pandemic, but also rely on targeting two surface proteins on the influenza virus, which are prone to antigenic drift. As a consequence, a new vaccine needs to be developed for each new seasonal epidemic. Additionally, the vaccine strain needs to be selected around eight months prior to administration and can often be mismatched leaving the population unprotected. A 'universal' vaccine, effective irrespective of the surface proteins, would be desirable to offer cross-protectivity across strains.

Tandem core virus-like particles (VLPs), expressed in methylotrophic yeast *Pichia pastoris,* are an exciting alternative to current manufacturing methods. VLPs, due to their inherent safety profile and advances in genetic engineering, have excellent potential both as standalone vaccines for the virus from which they are derived, or as platforms for the display of foreign antigens. The hepatitis B core antigen (HBcAg) is able to spontaneously self-assemble, forming icosahedral particles that are inherently immunogenic. Moreover, the HBcAg is capable of carrying antigen inserts in the major insertion region (MIR) which are displayed on the particle surface.
In order for VLPs to be considered a viable alternative, their bioprocessing must be optimized. Currently, various issues are at play including problems with formation, solubility and immunogenicity, often clone dependent. In this work, two genetically linked HBcAg monomers, carrying different inserts in the MIR, were used to study the effects on fermentation efficiencies using two different induction strategies. Rationalizing an induction strategy would enable the development of an efficient process to produce and purify VLPs. Results indicate that increased biomass is not always synonymous with increased protein expression. Moreover, protein expression and solubility appear to be linked with the complexity of the inserts displayed on the VLP surface.
The aim of this work is to improve the bioprocessing of VLPs in a microbial expression system, using tandem core technology. This proposed method is cheap and rapidly scalable, reduces the cost per dose and eliminates the long production timelines associated with current manufacturing. The very nature of VLPs and the comparable ease of production would enable this to be promoted as a platform process, for a myriad of disease targets.

RAPID VIRUS TITRATION USING FLOW CYTOMETRY

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Key Words: Flow cytometry, virus, titration, granularity, bioprocess development .

Rapid high throughput virus titration methods are essential for facilitating continuous process monitoring and rapid decision making in viral bioprocess development. In spite of repeated efforts to address this need, the industry continues to reply on well understood and trusted plaque assays and end point dilution assays, or variations thereof. Together with the University of Waterloo, we have developed a flow cytometry based assay that is able to give infectious virus titers in a fraction of the time as compared to conventional virus titration assays.

The developed method utilizes the phenomenon of increased granularity in cells after virus infection, with the increase in granularity proportional to the multiplicity of infection of the virus. The assay has been adapted to a 96 well plate format which, in combination with the use of a flow cytometer with an automated sampler, results in a high throughput assay with much reduced operator effort as compared to traditional assays.

Two different cell and virus systems have been examined using this assay. Assay variations in both systems were measured to be ~20%, and assay accuracy was highly comparable to traditional "gold standard" assays such as the plaque assay. Assay analysis was found to be simple and amenable to automation through the use of R scripts. Operator effort was reduced by approximately half per sample, and the assay time was reduced by 75%, when compared to traditional assays. In addition, the simplicity of the assay greatly reduces operator training time.

Studies by other groups provide confidence that the phenomenon of increased cell granularity with virus infection is present in several virus-cell systems. Therefore, the developed method has great potential to be used as a routine high throughput screening technique for a wide range of viruses.

DEFINING THE MULTIPLICITY AND TYPE OF INFECTION FOR THE PRODUCTION OF ZAIRE EBOLA VIRUS-LIKE PARTICLES IN THE INSECT CELL BACULOVIRUS EXPRESSION SYSTEM

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Key Words: Ebola virus, ZEBOV-VLP's, coinfection, hemorrhagic fever, immunogenic response.

Ebola virus hemorrhagic fever affects thousands of people worldwide with high mortality rates. The Ebola virus has a short incubation time between 2-21 days and death usually occurs within 4-10 days[1]. Ebola virus disease is characterized by a sudden onset of fever, weakness, headache, diarrhea and vomiting, internal and external bleeding[2]. In the *Filovirus* family, Zaire Ebola virus (ZEBOV) is the most aggressive and virulent species, its fatality rates have been reported to be up to 90%[3]. Even when important advances in vaccine development have occurred, the need of safe and effective vaccines persists[4]. An alternative is the production of virus-like particles, which are formed by the recombinant virus structural proteins that self-assemble into highly immunogenic structures[5]. The ZEBOV contains three main structural proteins: the glycoprotein (GP), the viral matrix protein 40 (VP40) and the nucleoprotein (NP). GP induces humoral and cellular responses by itself but when VP40 is co-expressed, the immune response increases in a mouse model[6]. NP determines the structure of the resulting VLP. To our knowledge, there is no information about the production conditions that result in coexpression and assembly of ZEBOV recombinant proteins. In this work, a multifactorial experimental design was used to evaluate 32 different conditions for the production of the ZEBOV structural proteins utilizing the insect cell-baculovirus expression system technology (BEST). Multiplicity (MOI = 0.1 or 5 ufp/cell) and consecutive times of infection (0 or 6 hours after the first infection) were the principal factors, and the production of each recombinant protein and assembly of VLP were the evaluated responses. We observed that multiplicity of infection had an impact over expression of the recombinant proteins, higher multiplicities increased yield and VLP assembly. In contrast, later times of infection reduced the production of each protein. The initial presence of VP40 resulted in a higher concentration of NP. The conditions where the simultaneous expression of the three structural proteins and where VLP were detected were identified. The highest MOIs for bacVP40 and bacGP were needed. bacNP should be added during the initial infection with an MOI of 0.1, or at 6 hpi at MOI of 5. The obtained ZEBOV-VLPs were similar to native virus. The obtained VLP are a candidate vaccine under evaluation.

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COMBINING NOVEL AND TRADITIONAL APPROACHES OF VACCINE DEVELOPMENT TO OVERCOME THE CHALLENGES OF FIRST-IN-HUMAN TRIAL FOR GROUP A STREPTOCOCCUS

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Key Words: Vaccine, Group A Streptococcus, pre-clinical

For nearly 30 years, from 1979 to 2006, clinical trials for a Group A Streptococcus (GAS) vaccines were banned in the U.S after a study suggested there may be an increased risk of acute rheumatic fever (ARF) in vaccine recipients. That study, conducted in 1968, used a crude M protein vaccine to immunize children – following administration, two children developed ARF (Massell BF, 1969). More than a decade since the FDA lifted the ban, safety concerns are still high and new vaccine candidates for Group A Strep face additional scrutiny entering first-in-human trials. This presentation focuses on a case-study for the development of a new vaccine for GAS diseases, specifically the various strategies used to overcome the safety concerns and maximize the chances for successful testing in clinical trials. VaxForm's proprietary vaccine "VaxiStrep," is the result of a combination of novel and traditional approaches in vaccine development. The novel approach entails using a novel recombinant fusion protein as the antigen. While most researchers have focused on combinations of M protein serotypes as the antigen(s) (up to a 26-valent vaccine), VaxForm chose to avoid the inevitable design complexity and stability issues that arise when formulating a multivalent vaccine. VaxiStrep's antigen is a recombinant fusion protein of two streptococcal pyrogenic exotoxins (Spe), SpeA and SpeB, that are widely expressed in GAS strains. In addition to the selection of a safe antigen, VaxForm performed toxicity studies *in vivo* and *in vitro* (human PBMCs) to further demonstrate its safety. An ELISA was developed to show the lack of molecular mimicry between SpeAB and the protein responsible for ARF. SpeAB detoxification was shown by comparing polyclonal T-cell activation of SpeAB vs wild type toxins in human mononuclear cells (PBMCs). Another important part of vaccine design and development is the adjuvant selection. VaxForm decided to explore aluminum-based adjuvants options. Aluminum adjuvants are the most commonly used adjuvants – there is extensive evidence that show their safety, they are approved by the FDA and used in licensed products. Adjuvant dose-response and adsorption studies for VaxiStrep were performed to narrow down the optimal dose and adsorption rate of the antigen. These types of studies followed the more traditional approach of vaccine development, giving the vaccine candidate a better chance for first-in-human approval and clinical trial success. Finally, another aspect that VaxForm selected as a priority was vaccine stabilization. Long term stability studies were performed that demonstrated a shelf-life of over two years when stored refrigerated. Animal studies comparing the potency of a two-year old vaccine lot to a fresh lot showed similar immune response. In addition, titers to the vaccine are long lasting as antibody titers remained at high levels 14 weeks after the booster injection. Conclusions: Bringing a new concept to clinical trials is always a long and challenging task. Learning and understanding the historical perspective when developing a new vaccine can be key in selecting the best approach. In this case, designing a GAS vaccine that would cause the least safety concern was prioritized due to the history of vaccine-induced ARF. Additionally, to avoid delays in clinical trials, robust efficacy and long-term stability of the vaccine was demonstrated in pre-clinical studies. We believe these choices will maximize the chances for VaxiStrep to reach first-in-human trial.

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DEVELOPMENT OF SUSPENSION ADAPTED VERO CELL CULTURE PROCESS FOR PRODUCTION OF VIRUSES

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Key Words: Vero cell culture, viral vaccine, cell adaptation, Vero cell media.

Vero cells are considered as the most widely accepted continuous cell line by the regulatory authorities (such as WHO) for the manufacture of viral vaccines for human use. The continuous Vero cell line has been commercially used, after propagation on microcarriers, for the production of rabies, polio, enterovirus 71 and hantaan virus vaccines. Vero cell culture technologies are also explored for productions of many more viral vaccines over the last two decades. The growth of Vero cells is anchorage-dependent, and cells need to be dissociated enzymatically or mechanically for the process of subcultivation. This process is labor intensive and complicated in process scale-up. Adaptation of Vero cells to grow in suspension will simplify subcultivation and process scale-up significantly.

Here we report on the adaptation of adherent Vero cells to grow in suspension using a serum-free and animal component-free medium developed in-house. The maximum cell density and cell doubling time of the suspension adapted Vero cells in batch culture grown in the in-house developed medium were similar to or better than what was observed for the adherent Vero cells grown in commercial media. The growth of suspension adapted Vero culture was successfully scaled up to 3 L bioreactor. The Vero cells from various stages (both adherent and adapted) were tested for their authenticity using a Short Tandem Repeat (STR) analysis. The testing result indicates that all Vero cell samples have 100% concordance with the Vero DNA control sample, indicating the suspension adapted cells maintained their genetic stability.

Productions of vesicular stomatitis virus (VSV) and influenza virus in adherent culture and suspension adapted culture were compared, showing the suspension adapted Vero cell retained similar viral productivity. The volumetric productivity of VSV in the suspension culture was even higher, and was further increased by almost 200 times when culture was infected at higher cell density and with medium replacement before the virus infection. In contrast, the VSV production decreased when the adherent culture was infected at higher cell density. Additional process development revealed that the maximum cell density in batch culture was doubled, reaching $6x10^6$ cells/mL, when the culture medium was replaced during the process of batch culture, which indicates potential for further increases in product titer.

A PRE-FUSION, TRIMERIC SUBUNIT INFLUENZA HA-BASED VACCINE ELICITS CROSS-PROTECTION BETWEEN HIGHLY DIVERGENT INFLUENZA A VIRUSES

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Key Words: influenza, vaccine

Despite our best efforts to vaccinate against influenza viruses they remain a major cause of morbidity and mortality worldwide, resulting in 3-5 million severe infections and more than 250,000 deaths annually. Constant antigenic changes in circulating viruses means current vaccines must be updated and re-administered annually. This approach is time-consuming and expensive, and is often hindered by mismatches between circulating and vaccine strains. Strain mismatch can contribute to insufficient vaccine efficacy, which has ranged from just 10-60% over the last decade. Furthermore, recent sporadic zoonotic outbreaks of novel highly pathogenic viruses from avian species, to which current vaccines provide no immunity, have been observed, with fatality rates around 40%. This raises serious concerns of a global pandemic with the potential to spread rapidly before a vaccine can be manufactured. Novel approaches to influenza vaccination are clearly needed in order to overcome these limitations with “universal” flu vaccines being the holy grail. We have stabilized recombinant influenza haemagglutinin (rHA) in its native, pre-fusion conformation by the addition of a novel “clamp” stabilization motif to enhance subunit vaccine potency and breadth of protection. Immunisation of mice with clamp-stabilized prefusion rHA elicited a potent neutralizing antibody response (~4-fold improvement over current vaccines). Most importantly, antibodies elicited upon immunisation with clamp-stabilised prefusion rHA showed an 80-fold increase in cross-reactivity to rHA derived from a divergent, highly pathogenic avian virus (H5N1) when compared to the current influenza vaccines. We have also shown that vaccination with clamp-stabilisted rHA based on the H3 subtype (group 2) is capable of providing cross-protection to a challenge with a highly-divergent group 1 virus (H1N1). Ultimately, this approach could represent a potential universal influenza vaccine, providing enhanced cross-protection against both group 1 and 2 seasonal influenza virus strains while simultaneously providing an increased cross-reactive humoral immune response to potential zoonotic pandemic strains.

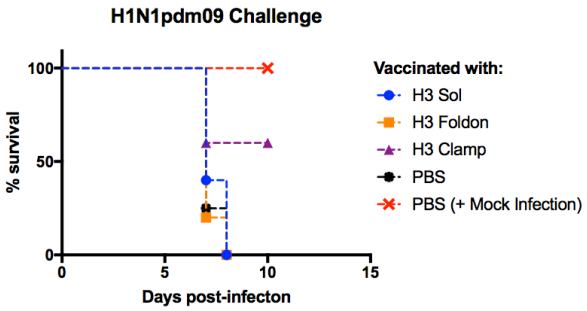

Figure 3 – Mice were vaccinated with rHA with (H3 Clamp) or without (H3 Sol) the molecular clamp trimerisation domain. A previously published influenza vaccine candidate utilizing the foldon trimerisation domain (H3 Foldon) was included as a direct comparison. Mice were challenged with a lethal dose of highly divergent H1N1pdm09 virus. Partial cross-protection was seen only in H3 clamp-vaccinated mice, but not with H3 Sol or H3 Foldon groups.

HER1 THERAPEUTIC CANCER VACCINE: AN ACTIVE IMMUNOTHERAPY TREATMENT FOR PATIENTS WITH TUMORS EXPRESSING THE RECEPTOR OF EPIDERMAL GROWTH FACTOR (EGF-R)

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Key Words: receptor of epidermal growth factor (EGF-R), EGF-R extracellular domain(HER1-ECD), cancer vaccine

Her1 vaccine: It consists of immunizing patients with positive tumors for the epidermal growth factor receptor (EGF-R) with a preparation of EGF-R extracellular domain(HER1-ECD) adjuvanted in VSSP (very small size proteoliposomes) and Montanide ISA51. VSSP adyuvant confers to vaccine the capacity to activate dendritic cells and polarize the immune response towards a TH1 immune pattern, developing TCD8+ cells and antibodies with anti-metastatic effect.

The extracellular domain of the epidermal growth factor receptor was obtained from HEK293 transfectome by using a productive process in a bioreactor in perfusion as mode of fermentation. The protein was purified by immune-affinity chromatography by using specific anti-EGF-R antibody.

Phase I trial, uncontrolled, open and sequential, was conducted in 25 with hormone refractory prostate cancer patients. Five dose levels of HER1-ECD were scaled: 100, 200, 400, 600 and 800 µg in each immunization. The trial showed that the vaccine was safe, not causing severe or very severe related events. The vaccine preparation was shown to be immunogenic. There was a trend towards the survival benefit in some patients.

At the present time another clinical trial is underway in three locations of advanced solid tumors that overexpress the EGF receptor: prostate, colon and head and neck cancer.

PRODUCTION OF BACTERIAL OUTER MEMBRANE VESICLES AS VACCINE PLATFORM

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Key Words: Vaccine platform, Outer Membrane Vesicles, *Neisseria meningitidis*, Lyme disease

Bacterial outer membrane vesicles (OMVs) are non-infectious but highly immunogenic particles. These vesicles are used as vaccines against the disease of the source bacteria. Fascinatingly, the addition of heterologous antigens to these vesicles creates a versatile vaccine platform. Such a platform can be used as an alternative to subunit vaccines, during infectious disease outbreaks or for the development of vaccines against pathogens that require high containment. A unique aspect of this platform is the reusability of the production process for many different vaccines. This in turn could reduce the time to market for new vaccines significantly. We designed a heterologous OMV vaccine concept for Lyme disease based on spontaneous released OMVs from *Neisseria meningitidis* that express the Outer surface protein A (OspA) of *Borrelia burgdorferi* on the surface. The productivity of spontaneously released OMVs was improved by the introduction of oxidative stress to the bacterial culture. Increased dissolved oxygen concentrations during cultivation showed to be an excellent process parameter for enhanced release of OMVs, while the bacterial culture remains viable. This presentation will cover the development of the OMV-based vaccine platform and the impact of changes in the upstream process on the downstream process of the investigational OMV-based Lyme disease vaccine.

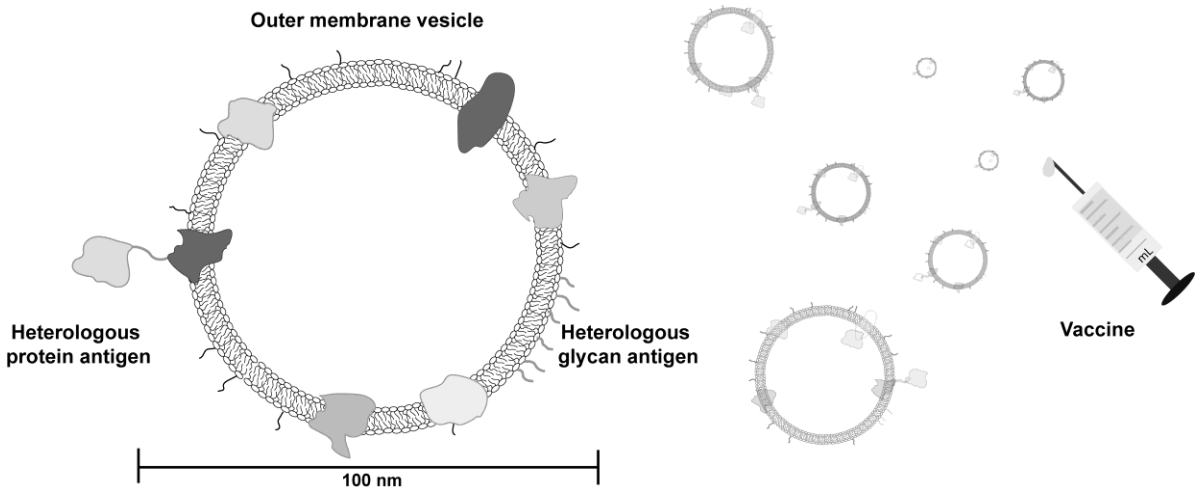

Figure 4 – Heterologous antigens on outer membrane vesicles as vaccine platform

ECTENDED GENE EXPRESSION FOR HIV-1 VLPs AND PRODUCTION ENHANCEMENT USING shRNA AND CHEMICAL ADDITIVES

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Key Words: HIV-1 VLPs, bioreactor perfusion, additives, shRNA, characterization, HEK293

Gag polyprotein from HIV-1 can generate Virus-Like Particles (VLPs) when recombinantly expressed in animal cell platforms. HIV-1 VLP production in HEK293 cells can be improved using different strategies for increasing product titers. One of them is the so-called Extended Gene Expression (EGE), based on repeated medium exchanges and retransfections of the cell culture to prolong the production phase. Another approach to improve transient transfection results is media supplementation with gene expression enhancers such as valproic acid and caffeine, despite their detrimental effect on cell viability. Valproic acid is a histone deacetylase inhibitor while caffeine has a phosphodiesterase inhibition effect. The work presented has three main objectives. First, the combination of the EGE protocol with valproic acid and caffeine supplementation to maximize VLP production; second, the replacement of these chemical additives by shRNA for obtaining the same inhibition action and third the bioreactor scale-up of the process.
The combination of the EGE protocol with caffeine and valproic acid supplementation resulted in a 1.5-fold improvement in HIV-1 VLP production compared with the EGE protocol alone, which is an 18-fold improvement over a conventional batch cultivation. shRNAs encoded in the expression vector were tested to substitute valproic acid and caffeine. This novel strategy enhanced VLP production by 2.5-fold without any detrimental effect on cell viability, which results in obtaining higher quality VLPs. Finally, the combination of shRNA with EGE resulted in more than 14-fold improvement compared with the batch standard protocol traditionally used. This protocol enables the production of high-quality HIV-1 VLPs avoiding toxic effects of the additives but maintaining high product titers.

When EGE process was scaled-up, cell viability was comparable through all processes of the two systems tested; however, the bioreactor allowed for much higher cell densities and specific growth rates. Transfection efficiency was also comparable and successfully achieved in both systems. GagGFP fluorescence quantification revealed similar VLP titres in both shake flasks and bioreactor. A product quality assessment was also carried out to evaluate the two systems. Presence of VLPs in all samples was confirmed by transmission electron microscopy. Nanoparticle tracking analysis showed that the ratio of VLPs/total particles (VLPs and cell vesicles) was higher in the shake flask than in the bioreactor, possibly due to higher cell densities achieved in the bioreactor. Similarly, host cell DNA and host cell protein analyses revealed higher impurity concentrations for the bioreactor compared with shake flasks.
Furthermore, the budding process of VLP in the production process was observed using super resolution confocal microscopy. This technique allowed to quantify the percentage of Gag-GFP that colocalize in the cell membrane and the percentage of the membrane that is occupied by VLPs budding from the cell. This increase in resolution also enabled the direct quantification of VLPs in supernatant samples by confocal microscopy that correlated with the quantification obtained by nanoparticle tracking analysis.

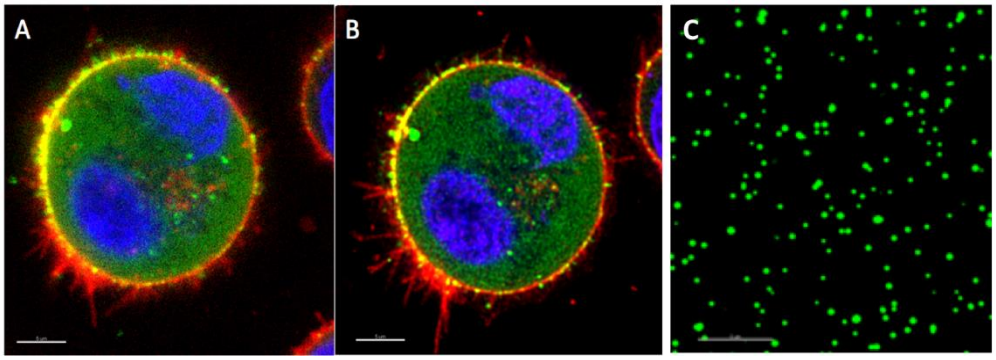

Figure1. Observation of Gag-GFP VLP production in HEK 293 cells by confocal microscopy (A) before Hyvolution processing, (B) after Hyvolution processing (C) visualization of VLPs in the supernatant.

DEVELOPMENT OF SCALABLE MANUFACTURING PROCESS AND GMP-COMPATIBLE FORMULATION FOR A NOVEL RECOMBINANT SCHISTOSOMIASIS VACCINE

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Key Words: Schistosomiasis, vaccine, process development, tangential flow filtration, formulation

Schistosomiasis is a parasitic disease spread by fresh water snails. After malaria, schistosomiasis is the deadliest parasitic disease, plaguing an estimated 200 million people worldwide, and causing up to 280,000 fatalities in Africa. This neglected tropical disease has also emerged as an important co-factor in Africa's HIV/AIDS epidemic, especially among women and adolescent girls. Together with hookworm disease and leishmaniasis, it ranks as the neglected tropical disease with the highest disease burden as defined by disability-adjusted life years (DALYs)[1]. Although treatments exist, such as praziquantel chemotherapy, a vaccine will likely be needed to prevent infection and re-infection, interrupt disease transmission, and ultimately establish long-term control and elimination of the disease. No such vaccine currently exists, but a promising candidate is currently under development at Texas Children's Hospital Center for Vaccine Development (TCH-CVD).

The *Sm*-TSP-2 schistosomiasis vaccine comprises a 9 kDa recombinant protein corresponding to the extracellular domain of a unique *S. mansoni* tetraspanin found in the parasite's tegumental surface. *Sm*-TSP-2 was expressed as a recombinant protein secreted by the yeast *PichiaPink™* and purified in a two-step process, which resulted in a protein recovery yield of 31% and a protein purity of 97%.[2] The developed processes were suitable for production of purified protein for subsequent formulation and Phase 1 clinical studies. However, improvements in process yield and efficiency, as well as transition of the formulation to GMP-compatible materials, are desirable for the advancement of this candidate through subsequent clinical phases and large-scale manufacturing.

TCH-CVD and MilliporeSigma are conducting a collaborative project to optimize the efficiency and scalability of the *Sm*-STP-2 schistosomiasis vaccine manufacturing process. The overall goal of this work is to develop a safe and low-cost process for the purification of the vaccine antigen. This was accomplished by redesigning the original process, which utilized 750kD hollow fiber and 3kD cellulose membrane tangential flow filtration (TFF) devices for the clarification and concentration of the *Pichia pastoris*-based vaccine lysate process feed. The level of solids in the fermentation broth (30%) had required a dilution to enable processing through the hollow fiber device, which led to decreased product yield and increased complexity.

MilliporeSigma assisted TCH-CVD with studies to eliminate the dilution prior to lysate clarification and to also streamline the process to enable Texas Children's to simultaneously clarify and concentrate the yeast lysate. 0.1um, open-channel, stacked plate, membrane sheet TFF devices were successfully used to clarify the undiluted lysate. The TFF operating parameters (specifically the feed and permeate flow rates) were optimized to enable the downstream 3kD concentration process to run concurrently in a cascade TFF. Enhancement of the chromatography operations is currently underway as well. This presentation will detail the optimized clarification and concentration process and highlight the economic and process simplification benefits.

Work was also performed to optimize the vaccine formulation. Extensive studies were previously conducted to identify excipients and conditions to maximize the stability of soluble recombinant *Sm*-TSP-2.[3] Additional components were tested to find alternative GMP-grade reagents that maintained or improved the stability of the vaccine.

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INNOVATIONS IN GLOBAL HEALTH: WHAT HAS BEEN ACCOMPLISHED, WHAT IS ON THE HORIZON AND WHERE ARE MORE INVESTMENTS NEEDED

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The introduction of vaccines ranks as one of the greatest healthcare achievements in modern history. Through the global efforts of the vaccine community, small pox has been eradicated worldwide, as has one of three wild type strains of polio. Other infectious diseases such as measles have been eliminated from large regions of the world. All of these advances have contributed to increases in health around the globe. However, continued success will depend on continued innovation. Recent technical advances include the introduction into clinical studies of new vaccine vectors such as mRNA, new methods to administer vaccine such as microneedle array patches, improvements in the thermostabilization of vaccines and the use of process intensification, continuous processing and modular facility design to improve vaccine manufacturing. Still, significant efforts are needed to see each of these advances through conceptualization and pilot studies into widespread adoption and implementation. In addition, additional investments are needed in novel designs for filling/packaging of vaccines, methods to reduce the number of injections each child must receive and improvements in the speed and reliability of vaccine manufacturing and release. The speaker will touch upon each of these topics during the presentation.