VACCIPHARMA 2012

2th International Congress on Pharmacology of Vaccines

Cuban Society of Pharmacology
Latin American Association of Immunology

June 16-20, 2012

Over Vieu

The Cuban Society of Pharmacology and Latin American Association of Immunology are organizing the Second International Congress on Pharmacology of Vaccines (**VacciPharma 2012**), scheduled for June **16t^{h} to 20th, 2012** at Cayo Santa María, Villa Clara, Cuba.

This Congress will be formed by three different Workshops, running in parallel:

- Prophylactic vaccines
- New technologies in prophylactic vaccine development
- Therapeutic vaccines

A Symposium on **Veterinary vaccines** and a **Regional Meeting on DTP/HB/Hib/IPV Combined Vaccine** will be also held during the Congress.

The key objectives of the congress are:

- To provide a progressive and high-quality state-of-the-art report for scientists, manufacturers, governmental authorities and healthcare workers who need to be updated about the latest scientific developments for vaccines (Human and veterinary), including basic science discovery, product development, market introduction, adoption into immunization programs and surveillance.
- To promote the scientific collaboration among experts and institutions through the experience exchange, the presentation of results and the discussion on the Conference topics.
- To accelerate progress in the development of vaccines and the acceptance and the introduction of related new concepts, trends, methods and technologies.
- To encourage not only a prophylactic approach to healthcare by using vaccines, but also the vaccine treatment of chronic and neoplastic diseases.

Congress Venue

Barceló Cayo Santa María Hotel, Cayo Santa María, Villa Clara, Cuba

Organized by:

Cuban Society of Pharmacology (SCF)
Latin American Association of Immunology (ALAI)
Latin American Association of Pharmacology

Co-organized by:

Finlay Institute
Centre of Molecular Immunology (CIM)
Centre for Genetic Engineering and Biotechnology (CIGB)
Tropical Medicine Institute “Pedro Kourí” (IPK)
State Centre for the Quality Control of Drugs (CECMED)
National Centre for Animal and Plant Health (CENSA)
Pan-American Health Organization (PAHO)
Brazilian Society for Neuroscience and Behaviour (SBNeC)

Sponsored by:

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BDC
New England Anti-Vivisection Society (NEAVS)
UNICEF, Cuba
PAHO, Cuba
ELEA-Chemo
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Pre-congress activities

Monolith School Cuba 2012: Practical Course on Novel IgG Purification Process and PAT Application (June 14-15, 2012)**.** The program will be designed for experienced scientists in the use and application of monolith bio-chromatography. The price for the training class is 45 Euros. Venue: Centre for Genetic Engineering and Biotechnology. Contact us by email: Dra. Miladys Limonta (miladys.limonta@cigb.edu.cu)

Updating on Surveillance Systems for Immunopreventive Diseases *(June 15, 2012)***.** The price for the course is 30 Euros. Venue: Tropical Medicine Institute "Pedro Kourí". Contact us by email: Dra. Sonia Resik, PhD. (sresik@ipk.sld.cu)

CONGRESS GENERAL SCHEDULE

Day / Session	ACTIVITIES
16 / (12:00-16:00)	Congress Registration
16 / (18:00–20:00)	Opening Lectures
16 / 20:30-	Welcome dinner and Cocktail
17-19 / (9:00-11:00)	Workshop and Symposium Sessions
17-19 / (11:00–11:20)	Coffee break
17-19 / (11:20-13:30)	Workshop and Symposium Sessions
17-19 / (15:00-17:00)	Workshop and Symposium Sessions
17 and 18 / (20:30-21:30)	Poster Sessions / Collateral scientific activities, Trade Exposition and Commercial Presentations
19 / 21:00-22:00	Closing Ceremony
19 / 22:00-	Farewell activity
20 / (9:00 – 12:00)	Collateral activities

PROPHYLACTIC VACCINES WORKSHOP

General Schedule (Room I)

	Sunday, June 17th	Monday, June 18th	Tuesday, June 19th
9:00-11:00 am	Quality Control and Assurance / Vaccine certification	Current & Novel strategies in Development	Preclinical and clinical studies for prophylactic Vaccines
11:00-11:20 am	**Coffee Break**		
11:20 am-1:30 pm	Alternative Methods for Potency and Toxicity of vaccines	Conjugated and plain polysaccharide vaccines	Current Experience in Regulatory issues of Vaccines
1:30-2:30 pm	**Lunch**		
3:00-5:00 pm	Symposium Veterinary Vaccines	Symposium Tuberculosis Vaccines	Meeting for the constitution of the Regional Network for 3Rs Alternative Methods
7:00-8:30 pm	**Dinner**		
8:30-9:30 pm	Poster Session / Trading Exhibition and Commercial Presentations	Poster Session / Trading Exhibition and Commercial Presentations	

THERAPEUTICS VACCINES WORKSHOP

General Schedule (Room II)

	June 17th	June 18th	June 19th
9:00-11:00 am	Vaccines Design and Process Development	Experimental and Clinical Evaluation of Cancer Therapeutic Vaccines	Cuban experience: From bench to clinical practice
11:00-11:20 am		**Coffee Break**	
11:20am-1:30 pm	Vaccines Design and Process Development	Experimental and Clinical Evaluation of Cancer Therapeutic Vaccines	Regulatory Landscape for Therapeutic Vaccines
1:30-2:30 pm		**Lunch**	
3:00-5:00 pm	Satellite Meeting Mucosal Vaccines	Experimental and Clinical Evaluation of Therapeutic Vaccines	Regional Meeting on Combined vaccines based on DPT-HB-Hib-IPV
7:00-8:30 pm		**Dinner**	
8:30-9:30 pm	Poster Session / Trading Exhibition and Commercial Presentations	Poster Session / Trading Exhibition and Commercial Presentations	

BIOSEPARATION SESSION

General Schedule (Room III)

	Sunday, June 17th	Monday, June 18th	Tuesday, June 19th
11:00-11:20 am		**Coffee Break**	
11:20am-1:30 pm		**Bioseparation Session**	
1:30-2:30 pm		**Lunch**	

PROPHYLACTIC VACCINES WORKSHOP

ABSTRACTS: ORAL PRESENTATION

SUNDAY 17 (MORNING) ROOM I

• Session: Quality Control and Assurance / Vaccine Certification

Chairmen: Javier Martin (NIBSC, UK) and Sonia Resik (IPK, Cuba)

MOLECULAR CHARACTERISATION OF POLIOVIRUS INACTIVATED WITH FORMALDEHYDE

Martin J

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To gain better understanding on the effect of formaldehyde inactivation on the biology of poliovirus (PV), some properties of inactivated viral particles and viral RNA were analysed. The ability of inactivated PV to bind to the poliovirus receptor (PVR) was analysed in a series of binding assays using expressed soluble PVR and cells susceptible for PV infection. Analysis of PV-PVR interaction using the surface plasmon resonance (BIACORE) technique showed that inactivated PV retained the ability to bind to soluble PVR. Inactivated PV was also able to bind to L20B cells as shown by a FACS flow cytometry method that detected virus particles on the cell surface and a real-time RT-PCR assay that measured the amount of viral RNA associated with cells. In both cases, binding of virus to cells was inhibited by pre-incubation with soluble PVR and/or anti-poliovirus monoclonal antibodies. A series of transfection and RT-PCR assays were used to study changes in the biological activity and integrity of viral RNA during formaldehyde inactivation. The results suggested a rapid alteration of viral RNA molecules during inactivation. The implications for the development of improved inactivated poliovaccines and methods for their quality assessment will be discussed.

SABIN-IPV DEVELOPMENT FOR CLINICAL STUDIES AND TECHNOLOGY TRANSFER TO LOCAL VACCINE MANUFACTURERS

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RIVM developed a production process for a novel inactivated polio vaccine based on attenuated Sabin-strains (Sabin-IPV), and the current large-scale Salk-IPV production technology. The use of attenuated Sabin strains instead of currently used wild-type polio strains may result in reduced containment requirements during vaccine production. Further, it opens the opportunity for process improvements. In this way, a more affordable IPV is strived for. To achieve these goals, a lab-scale process was set-up based on historical Salk-IPV manufacturing data. Currently, both USP (cell and virus culture) and DSP (clarification, concentration, purification and inactivation) unit-operations approximate the large-scale. Using this model, a modified process was developed to generate Sabin-IPV for the currently ongoing phase I clinical trials. Next to that, technology transfer to local vaccine manufacturers has started. An update on these activities will be given. In parallel, a research program is ongoing to further modernize and optimize the process, and reduce the cost per dose. In USP, increased cell densities, were realized. After subsequent virus culture and purification, this resulted in significantly increased product yields.

Currently, the obtained results in both USP and DSP are being confirmed, and the operational ranges are being studied for future technology transfer purposes. Clinical trials, up to phase II, using Sabin-IPV have been conducted by different institutes in several countries. A review of the results, and the current status in these Sabin-IPV clinical studies, will be given. In order to ultimately replace regular IPV, Sabin-IPV should show a comparable or better: (i) safety profile, and (ii) protection against polioviruses.

DISPOSABLE TECHNOLOGY APPLIED TO CONVENCIONAL VACCINE ACTIVE INGREDIENTS PRODUCTION

Cardoso D, Herrera Y, Abreu E, Álvarez E, Barberá R, Rodríguez T, Montes L, Delamatter E, Sánchez Y, Echarte M, Amondaray D, Barzaga E, Díaz JC, Carrillo A, Lafargue Y, Reyes L, Torres LE, Costa W, Disartuar R, Fuentes LN, López J, Campa R, Molina Y, Terry O, Ramírez MA. González F, Carvallo N, Batista D, Cartaya Y, Jiménez G, Landa G, Paneque O, Martínez I, Rumbaut Z, Romero Y, Hevia Y, Hernández Y, Casanova Y, Cintria R, Russell L, Rodríguez A y Mandiarote A.

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Introduction: The biopharmaceutical industry is increasing the application of disposables in technological processes. The industry is always concern about cost, available capacity, cleaning, space and other issues, to introduce new product lines. A number of disposable applications in conventional active ingredient vaccine production were introduced in Finlay Institute. **Methods:** a) Introducción of Palletank® with 3D disposable 500L bags, as reservoir for cetrimide precipitation of bacterial inactivated *Neisseria meningococcal* A and C polysaccharide b) Generalization of the use of 2D disposable bags Tank Line in polysaccharides purification step of alcoholic precipitation, and phenol precipitation c) Introduction of a Palletank® termoregulated with 3D disposable 500L bags, as reservoir to inactivate *Bordetella pertussis* d) Introduction of 3D disposable 100L formulation bags for mixing cell concentrated inactivated of different strain of *B. pertussis*, to obtain API e) The use of 3D disposable of different volumes in supplement media preparation, and 2D tankliner disposable of different volumes in media and buffer preparation. **Results:** The disposable technology results demonstrated reduced operation time and auxiliary service consumption in CIP/SIP, reduced autoclave sterilization runs, reduced risk of contamination and cross-contamination, more productivity, and more safety. **Conclusion:** Performed applications are reliable and economically acceptable; products complain QC specification and all major and minor changes of disposable technology have been approved by national regulatory agency and quality assurance. All these productions have been certified by CECMED and WHO.

• Session: Alternative methods for Potency and Toxicity of vaccines

Chairmen: Coenraad Hendriksen (NVI, The Netherlands) and Klaus Cussler (PEI, Germany)

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM) AND ITS 3R'S ACTIVITIES IN THE FIELD OF VACCINES

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The European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe is an intergovernmental standard setting body dedicated to promoting quality of human and veterinary medicines, including vaccines. The missions of the EDQM include among others, the elaboration of the

European Pharmacopoeia (Ph. Eur.) by its groups of Experts and the coordination of the European network of public control laboratories that perform official control authority batch release of human and veterinary vaccines (OMCL network). Furthermore, the EDQM runs the Biological Standardisation Programme (BSP) through which it elaborates official reference standards and validates new common quality control testing methods for biologicals. The 3R's activities of the EDQM are based on the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes", which was elaborated by the Council of Europe in 1986 and which is the first international legal text in this field. The EDQM is dedicated to strengthening the use of the 3Rs principles in the quality control of medicines and fosters relevant proposals for new alternative methods. It benefits from the unique international environment constituted by the European Pharmacopoeia Convention and the European OMCL network in order to validate alternative approaches through large collaborative studies, to coordinate common efforts and improve harmonisation among official control laboratories, manufacturers and authorities. A review of the EDQM activities in validating and promoting the use of the 3Rs principles will be presented. Current challenges faced during the process of development, validation and acceptance of alternative approaches will be discussed. Future plans for the encouragement, harmonisation and implementation of the use of 3Rs principles by OMCLs and manufacturers will be presented.

FROM MICE AND GUINEA PIGS: THE HISTORY OF GENERAL SAFETY TESTS IN THE QUALITY CONTROL OF BIOLOGICAL PRODUCTS

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Immunological medicines have strict quality control requirements laid down in national pharmacopoeias and guidelines of international regulatory bodies such as the World Health Organization (WHO). Safety tests are required for batch release to be performed by the marketing authorisation holder and the national authorities. The General Safety Test (GST) or Abnormal Toxicity Test (ATT) is an animal-based safety test mentioned in all pharmacopoeias and legal requirements around the world. Discussions about the use of laboratory animals and the 3Rs in the quality control of biological questioned the GST because the purpose of the GST, namely the detection of any possible contamination in the final lot of a product, is vague. The statistics based on two or five animals is very imprecise. When a questionnaire from Europe revealed that more than 4,000 consecutive batches passed the test with no batch rejection, the European Pharmacopoeia Commission decided to delete the ATT as a routine animal safety test already fifteen years ago (Schwanig et al. 1997). However, the GST is still required in other parts of the world and by the WHO. The GST dates back to the 1960s, when fermentation processes were not yet fully controlled. The guinea pig and the mouse test known as GST was first mentioned at that time period in international guidelines. However, historical documents going back to the year 1900 provide evidence that two independent safety test had built the basis of the GST. The test in mice should estimate the preservation of antiserum. The guinea pig test should avoid contamination by tetanus toxin. This historical data provide further evidence that the GST has lost its scientific relevance. It is recommended that WHO and national pharmacopoeias follow the approach of the European Pharmacopoeia to waive the GST unless a scientific rational is provided for specific products.

THE CONSISTENCY APPROACH IN LOT RELEASE TESTING OF VACCINES

Hendriksen C[1], Metz B[2], van der Gun J[1], Kersten G[2]

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Vaccine lot release testing is characterised by extensive use of laboratory animals, particularly to demonstrate product safety and potency. Successes have been achieved in replacing existing animal models by Three R methods ranging from cell-based methods, serological approaches to the implementation of humane endpoints to remove lethality as testparameter. However, progress is tedious, time consuming and costly. A new paradigm in lot release testing of established vaccines (e.g. Tetanus

and Diphtheria toxoid) is the consistency approach. This approach starts from the idea that subsequent lots of vaccine produced can be compared to an earlier (reference) lot (clinical -, historical batch) with a thoroughly tested and well defined profile of safety and efficacy/potency. The concept of consistency for lot release has come within the reach due to improvements in production and control: vaccine starting material is better characterised (quality by design), production processes have been optimised and standardised, a tight protocol for in-process testing has been implemented, quality monitoring systems such as GMP and QA are now state-of-the-art and, pharmacovigilance is used for post-marketing surveillance and last but not least, new physicochemical and immunochemical techniques have become available. Consistency testing may lead to a significant reduction in animal use, since a narrow set of animal tests performed on each final lot, with questionable power to predict vaccine behaviour in the target populations, may be replaced by a battery of meaningful physicochemical-, immunochemical- and eventual *in vitro* functional tests with enhanced capacity to measure equivalence with batches of proven safety and efficacy. The paradigm of consistency is an interesting strategy for vaccine manufacturers as it might also allow for a reduction in testing costs and a shortening of the testing period. The concept of consistency testing was adopted in 2011 by the European Partnership on Alternative approaches to animal testing (EPAA) as a promising strategy to animal reduction. EPAA is a public-private partnership between the European Commission and the industry.This presentation will provide an introduction of the consistency approach and discuss advantages and limitations. The main focus of the presentation, however, will be on the aims. Objectives and activities of EPAA's Vaccine Project on Three Rs and consistency testing.

THE CONSISTENCY APPROACH FOR DIPHTHERIA TOXOID VACCINES

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Tetanus and diphtheria toxoid vaccines are among the safest and most successful vaccines produced over the past decades. According to the leading requirements (PhEur. WHO, USP/FDA) each lot of diphtheria and tetanus vaccine should be tested for potency in an animal model based on measuring the vaccine induced protection by a lethal challenge with toxin or by bleeding the animals followed by an in vitro or in vivo antibody titration. In general the potency of both diphtheria and tetanus vaccine are far above the minimum protective level. Diphtheria and tetanus vaccine are produced worldwide and the annual animal usage for potency testing is extensive. In contrast with the conventional diphtheria and tetanus vaccine, for HiB, HepB and IPV vaccines opportunities exists to release vaccines on the basis of an in vitro characterization/quantification test only. Prerequisite is the demonstration of consistency in production and the predictability with respect to pass or fail the specifications of the in-vitro assay. At the NVI the Consistency Approach was explored for diphtheria vaccine. Based on a risk assessment, in process quality indicators were selected as well as batch release QC results for various lots of routinely produced D vaccines all passing the in vivo potency test. Furthermore, additional testing was performed on the bulk toxoid using a panel of in vitro assays (SDS-PAGE, Moab-ELISA, TNBS and Fluorescence Spectroscopy) to specify the profile of D vaccine. The results show that in a strict GMP setting and after demonstration of consistency in production the combined results during manufacturing (in process controls), release testing and additional testing are good quality indicators and safeguards for product consistency. It is concluded that diphtheria vaccines produced by a manufacturer with a demonstrated consistency in production using a panel of tests to obtain additional quality information, can be released on the basis of in vitro tests only.

IN VITRO ALTERNATIVES TO *IN VIVO* POTENCY AND SAFETY TESTING OF PERTUSSIS VACCINES

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Whole cell and acellular pertussis vaccines have been produced for many years and their use has resulted in a dramatically decrease of whooping cough incidences. The release of both vaccines requires animal based potency and safety tests. The safety tests are primarily performed because of concern of

contamination with lipopolysaccharide and pertussis toxin (PTx), while potency tests are performed to control whether the vaccine induces immunological responses. We are working on the replacement of both types of tests. The development of the alternative tests is performed within the scope of the consistency approach. This approach concerns careful monitoring of the product and production procedures throughout the production processes. Quality control is performed by means of physico-chemical and functional *in vitro* models. This approach results in a holistic picture of the product quality. We aim to develop functional *in vitro* models for quality control of pertussis vaccines. Current safety testing for PTx relies on the histamine sensitisation test, an animal test based on the sensitisation effect of PTx. We studied the *in vivo* function of PTx and the knowledge obtained was used to develop the *in vitro* cAMP-PTx assay. In the cAMP-PTx assay, PTx content is measured by changes of cAMP levels in a rat vascular smooth muscle cell line. The assay indicates of the ability of PTx to ADP-ribosylate inhibitory G-proteins. We demonstrated that the presence of PTx in acellular pertussis vaccines leads to a dose-dependent increase in cAMP levels. We currently focus on optimalisation, standardisation of improvement of the sensitivity. Secondly we aim to set up functional *in vitro* tests which can give a reflection of functional aspects of vaccine quality. Therefore we use (co-) cultures of donor or cell line derived dendritic cells with and without lymphocytes to determine functional characteristics of pertussis vaccine *in vitro* that can reflect vaccine quality. We aim to use these control tests to detect changes in vaccines quality. Overall we aim to develop a panel of functional *in vitro* tests that, in combination with physico-chemical tests, enable *in vitro* characterization of pertussis vaccine safety and potency and a move toward animal free quality control testing.

REPLACEMENT OF THE INTRACEREBRAL CHALLENGE METHOD FOR DETERMING POTENCY OF WHOLE PERTUSSIS VACCINES: APPROACH COMBINING A SEROLOGICAL METHOD WITH AN IMPROVED VACCINE ANALYTICAL CHARACTERIZATION

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Introduction: Traditionally, the Potency of whole-cell pertussis vaccines has been determined by the Kendrick test (MPT). However, this assay has been criticized due to the great variability, the poor reproducibility and the unacceptable pain and suffering incurred by the animals. The Pertussis Serology Potency Test (PSPT) seems to be the most suitable alternative to MPT and a correlation between both methods has been described. Nonetheless, some issues related to the relevance of the antibodies for protection remain rather unclear. The present Paper aims to evaluate the suitability of PSPT to replace the MPT combined to an analytical strategy for a better characterization of our whole cell Pertussis vaccines. **Materials and Methods:** Several whole cell Pertussis vaccine batches were tested in parallel by MPT and PSPT. The functionality of antibodies produced during PSPT was evaluated by using an *in vitro* opsonophagocytosis assay. The complement activating and bactericidal capacities were also evaluated. All batches were characterized by a 2D-electrophoresis procedure and by antigen ELISA using monoclonal antibodies. **Results:** As described before, MPT and PSPT correlated and the serological method was able to discriminate the sub-potent batches in an equivalent way of MPT. Although the complement activating and the bactericidal titres showed poor correlations regarding PSPT titres, a strong correlation against the opsonophagocytosis assay was obtained. 2-D electrophoresis and antigen ELISAs provided relevant information on the antigen profile of our vaccines and the manufacturing consistency, with interesting links to the PSPT results. **Conclusions:** The use of a faster and more robust and reproducible serological method, together with a better characterization of our vaccines, could be combined to replace the old-fashion MPT. Further studies on the relevance of the antibodies produced by PSPT for protection should be performed, but the activation of phagocytosis by antibodies in PSPT is a mechanism to be followed.

USE OF A BIOMIMETIC SYSTEM TO PREDICT SAFETY AND IMMUNOGENICITY OF BIOTHERAPEUTICS IN HUMANS

Luna E, Agrawal P, Mehta R, Swaminathan S, Varghese K, Kamala T, Schanen BC, Nguyen, M, Parkhill RL, Warren WL and Drake III DR

Sanofi Pasteur-Vaxdesign Campus. Orlando, USA.

Introduction: In the past decade, new developments in genetic engineering, biotechnology, synthetic chemistry and molecular biology have allowed the creation of immune modulators for the treatment of different conditions. To evaluate the safety, immunogenicity and biological effects of these compounds, animal models are usually the first option despite some limitations in translating these results from the bench to bedside. To overcome this challenge, we developed a biomimetic of the human immune system, the MIMIC System (Modular Immune In vitro Construct), that comprises a series of modular assays based on quiescent primary human immune cells of principally blood origin placed into tissue-engineered constructs that are functionally and microenviromentally equivalent to select elements of immune system. Then MIMIC system is comprised of three modules: The Peripheral Tissue Equivalent (PTE) module that resembles the innate arm of the immune system, The Lymphoid Tissue Equivalent (LTE) module, which resembles an artificial lymph node, and The Functional assays module to examine the products of adaptive immunity (cells and specific Ab). **Material and Methods:** We present the reproducibility of the PTE module using frozen PBMC at different time-points and the evaluation of reactogenicity and immunogenicity of adjuvants and biotherapeutics like Herceptin and its biosimilars in terms of phenotypic maturation markers from DCs, as well as, cytokine production using Bioplex. We will also present data for the vaccine response in PTE-derived DC and cytokine-derived DC in terms of specific naïve and memory CD4 T cell responses. **Results:** We observe very high reproducibility using the PTE module for DC activation. We also observe enhanced DC activation using biosimilars, such as for Herceptin, as compared to the brand-name pharmaceutical monoclonal antibody. **Conclusion**: The MIMIC system offers a very robust in vitro platform to predict human immune response, which we believe will accelerate the entry of human products into clinical trials with reduced costs and increased safety.

SUNDAY 17 (AFTERNOON)

• Session: Veterinary Vaccines Symposium

Chairmen: José Antonio Aguero and Mario P. Estrada (CENSA, Cuba)

EXPLORING CURRENT TRENDS IN VETERINARY VACCINE DEVELOPMENT

Peters AR[1] and **Ferro VA[2]**

[1]Arpexas Consultancy Ltd., Folkestone, Kent, UK; [2]University of Strathclyde, Glasgow, UK

The global veterinary vaccine market in 2011 was approximately US$5.4 billion and accounts for approximately 25.6% of the total animal health market of $21.10bn. The veterinary vaccine market is dominated (77%) globally by 4 major companies, Merck Sharpe and Dohme, Merial, Pfizer and Boehringer Ingelheim. The vaccine market has been growing by more than 4% compared to the whole industry by 2.5 to 3.0%. This differential growth in demand for vaccines will continue for the following reasons: Increased international animal production due to demand from emerging economies, increased international animal (and food, people) movements and trade, increased demand for preventive measures (prophylaxis), increased regulatory hurdles for pharmaceuticals in food animals e.g. antimicrobials, emerging disease threats e.g. Avian influenza, Bluetongue, Rift Valley fever including role of climate change, advances in technology -Host pathogen interactions, genomics, proteomics, bioinformatics etc-, threat of bioterrorism and reflected in national government, EU and international investment in R&D in infectious disease. The characteristics of an ideal vaccine are the following: multivalency, specific

immunity against disease and infection, protection of neonates in the face of maternal derived immunity, long duration of immunity, no boosters needed, no adverse reactions, needle free delivery, differentiation from infected animals (DIVA), cheap to manufacture and reproducible in quality. Few existing products meet more than 1 or 2 of these criteria. Therefore there is still a lot of scope for vaccine improvement. Thus there will be a continued requirement for vaccine research and development to meet the needs of existing and emerging diseases of veterinary importance.

BIOTECHNOLOGY PLATFORM IN BACTERIA, YEAST AND MAMMALIAN CELLS TO PRODUCE VETERINARY SUBUNIT VACCINES

Estrada MP, Suárez M, Alina R, Prieto Y, Méndez L, Carpio Y, Basabe L, González E, Rodríguez MP, Farnós O, Rodriguez E, Borroto C

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Introduction: The practice of vaccination for the prevention of animal disease has been used for centuries and has proven to be a powerful tool for the alleviation of animal suffering as well as the economic well being of producers of animal products. The associated evolution of new technology in the field of molecular biology and immunology has furthermore had a large impact on the development of new vaccine strategies and the quality of the products that are produced. One of the biggest advantages of subunit vaccines is that they are generally compatible with DIVA (differentiating infected and vaccinated animals) strategies. The Center for Genetic Engineering and Biotechnology of Havana had created a platform to produce different and complex antigens for veterinary vaccines in order to satisfy the necessity of animal health in Cuba. **Materials and Methods:** Antigens produced in *E. coli* (bacteria), *P. pastoris* (yeast) and HEK293 or CHO (mammalian cells) had been probed as veterinary vaccine immunogens. **Results:** The impact in the control of bovine ticks for more than 15 years using an immunogen produced in yeast, as vaccine, is one strong example of the possibilities of this technology. Other three vaccine candidates to control ectoparasite in salmon, classical swine fever and rabbit hemorrhagic disease were produced, in three different expression systems, and it was demonstrated the efficacy and potentiality in each case. These results of subunit vaccines have demonstrated some advantages over live attenuated and inactivated vaccines, including the ability to induce strong humoral and cell-mediated immune response. On the other hand some of our vaccine candidate has been developed with a DIVA diagnostic system in order to use both in a control and eradication program in Cuba. Furthermore they have shown an excellent safety profile. Our experience has shown this may be affected by the gene expression system used. **Conclusion:** The biotechnology platform created is able to produce a wide range of antigens from a huge variety of pathogens with a high possibility to maintain the antigenic epitope in each case to be useful as a subunit veterinary vaccine.

POSITIVE SELECTION PRESSURE ON THE B/C DOMAINS OF THE E2-GENE OF CLASSICAL SWINE FEVER VIRUS IN ENDEMIC AREAS UNDER C-STRAIN VACCINATION

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In Cuba, classical swine fever has become an endemic disease with several outbreaks each year despite of the vaccination program implemented. Interestingly, a trend towards a milder, presentation of the disease has been observed among the animals during the last years. This study aimed to assess positive selection pressure acting on partial E2 gene of CSF viruses to gain insights into the mechanisms governing virulence and the driving forces of classical swine fever virus evolution in swine populations under regular vaccination. Phylogenetic analysis were performed to detect positive selection acting on a particular lineage as well as among sites of the E2-B/C-domain of CSFV nucleotide sequences, reported in a previous study and in the present work, several models, available in the CODEML module of PAML 4.3, were used. In addition, a representative Cuban CSF isolate was assessed in an experimental infection trial for their clinical virulence in order to expand the knowledge regarding CSF viruses circulating in pig populations. The viruses sequenced in this study were grouped in a defined cluster within the genotype 1.2 as has been reported previously for Cuban CSF viruses. The selection pressure along the lineage analysis didn't find evidence of positive selection (dN/dS of > 1) along any branch. The positive selective pressure analysis estimated six new sites under positive selection on E2 partial gene analysed. Besides, the clinical manifestations of the CSF-disease were related mainly with a mild course of the illness. The high number of positively selected sites suggests that these changes could be associated to viral evasion of the host-immune response. These observations highlight a possible association between escape viral variants and the alterations observed in the virulence and pathogenesis of the virus. Therefore, while the vaccination programs have not led to a genotype change, alterations in virulence were suggested to arise.

MYCOPLASMA DETECTION AS PART OF THE QUALITY SYSTEMS IN THE CONTROL OF HUMAN AND VETERINARY VACCINES

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Introduction: Mycoplasmas frequently infect (between 15 and 18%) cell cultures, sera and biopharmaceutical products. The need of strictly control such pollution is based on undesirable effects produced by these microorganisms in the individual receiving these contaminated products, the failure in the interpretation of a diagnostic and in alterations induced by mycoplasmas and cell activities and functions. As example, the European Pharmacopoeia, sections 2.6.7 and 2.6.16 points out mycoplasma test performance to any vaccine and biotech product of medical application. The International Health Organization has within its requirements the detection mycoplasmas as conclusive evidence for the release or not of a certain product. From the development of biotechnology in our country, it is necessary to strictly control such pollutions, specifically those produced by mycoplasmas, as part of the quality systems for the production and release of such products as in the case of vaccine candidates against AIDS, head and neck cancer, vaccine against Hepatitis B and other products, such Heberprot B, monoclonal antibodies and recombinant erythropoietin. **Materials and Methods:** Thus, MYCOLAB (National Reference Laboratory for the Diagnostic of Mycoplasmas) has developed a diagnostic system integrated into a quality management system by NC: ISO/IEC 17025, which has validated trials (such as microbiological culture, nucleic acid hybridization and polymerase chain reaction). Its performance has been tested through interlaboratory studies; such trials fulfill the requirements of technical biosafety management referred by different Pharmacopoeia and international standards. **Results:** All this activity led MYCOLAB to receive the condition of laboratory accredited for the diagnostic of these organisms by the Accreditation Body of the Republic of Cuba (ONARC), attached to ISO. This allows providing a fast, safe and reliable service in detection of these contaminants in vaccines as part of quality systems.

ADVERSE EVENT REPORTS AFTER VACCINATION OF CATTLE IN GERMANY

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A nationwide reporting and monitoring system, known as Pharmacovigilance (PhV), exists in Germany and all other EU member states to oversee the continued safety of veterinary medicines under actual use conditions. PhV data are collected and scientifically evaluated by national competent authorities. The reports are collated in a national database which exchanges the information with the European Database EudraVigilance Veterinary. The Paul-Ehrlich-Institute is the German national authority responsible for PhV of immunological medicinal products for human and veterinary use. This presentation gives an overview about safety aspects focused on bovine vaccines during the last decade. Apart from routine surveillance three major issues will be highlighted: 1-Mass vaccination in ruminants was performed in most parts in Europe to combat the bluetonge virus epidemic in 2008/2009. An enormous increase of PhV reports had to be managed and evaluated; 2-a new disease called Bovine Neonatal Pancytopenia (BNP) emerged in Germany in 2007. The disease was associated with the use of an inactivated BVD vaccine. The current hypothesis is that antibodies in the colostrum of vaccinated dams trigger an immune reaction in some calves, resulting in the development of BNP; 3-anaphylaxis is a major problem in cattle and may be triggered by different product groups and mechanisms. Endotoxin in bacterial vaccines could be the cause, but other mechanisms are also involved.

COMPUTATIONAL IDENTIFICATION OF *Mycoplasma gallisepticum* MEMBRANE-ASSOCIATED PROTEINS USING AN *in house* DEVELOPED PIPELINE

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Introduction: *Mycoplasma gallisepticum* (MG) is a major avian pathogen. Its attachment to bird mucosal epithelium is a prerequisite for infection and is achieved by mycoplasma surface lipoproteins. The experimental identification of adhesins or cytadhesin-related molecules would involve an extensive trial and error system of testing and elimination of candidates. Bioinformatic tools have revolutionized these studies. Signal peptides I and II direct nascent proteins to surface and extracellular locations. Integral membrane proteins come in two structural classes, the alpha-helical (AMPs) and the transmembrane beta-barrels (TMBBs). The goal of this work was to research *in silico*, by properly combining different programs for prediction of the mentioned features, the potential of MG proteins of being surface located. **Materials and Methods:** The deduced proteome from MG R (low) strain was explored. Firstly, lipoproteins were predicted using Lipo P. Positives proteins were included in the final set, whereas those without any predicted lipoprotein signal peptide were subsequently analyzed with SignalP. Positives went to the TMHMM predictor and classified as AMP or, in the negative case, analyzed for TMBBs. The remaining SignalP-positive proteins were included as non-classified surface proteins. SignalP-negative proteins were also analyzed with TMHMM; the positives were included in the final set. All proteins were processed by SPAAN looking for citadhesins. **Results:** From the 763 proteins analysed, 294 were predicted as surface-located, divided as follow: 52 lipoproteins, 51 non-classified surface proteins, 175 AMPs, 16 TMBBs and 80 citadhesins. From proteins previously experimentally described as membrane proteins, the 98.4% was detected as positive and lipoproteins and citadhesins were in a high percent identified as such. **Conclusions:** The implemented Pipeline showed a high prediction capacity of surface exposed proteins for MG and, an important set of proteins, probably exposed in the membrane and therefore suitable targets for vaccine and/or diagnostic methods development, is now available.

RESULTS OF EVALUATION OF A CUBAN INACTIVATED VACCINE FOR NEWCASTLE AND AVIAN INFECTIOUS BRONCHITIS DISEASES

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Newcastle and Avian Infectious Bronchitis diseases cause respiratory, digestive and nervous affectations in the birds. In the egg-laying hens it cause low yields in the production of eggs. The objective of this work was to evaluate an inactivated bivalent vaccine to develop immunity for these agents in the egg-laying hens. Vaccine contains the strain La Sota of the virus of Newcastle disease and the strain H-120 of Avian Infectious Bronchitis and it uses the Montanide Incomplete Seppic adjuvant ISA-70. Safety test was carried out applying double dose in chickens of 21 days of age and in birds of 18 weeks of age. The birds were evaluated clinically and pathological macroscopic and histological studies were carried out. Fragments of organs and tissues were processed by court in paraffin coloured with hematoxilina and eosina. Chickens that had not received live vaccine against these agents and egg-laying hens of 18 weeks of age that received the scheme of vaccination of live vaccines established in the Cuban`s aviculture were vaccinated to evaluate immunogenicity. A foreign registered vaccine was used as positive control. After 28 days of vaccination, levels of inhibitors of the haemagglutination antibodies were determined for both viruses with commercial specific kit. Analysis of variance was used to compare haemagglutination inhibition titres of antibodies. In the safety test, deposit of vaccine and infiltration of lymphocytes in some cases took place. Geometric means of the levels of inhibitors of the haemagglutination antibodies for the Avian Infectious Bronchitis virus was above 6log2. For Newcastle disease virus, the titles of inhibitors of the haemagglutination antibodies were superior at 7log2. Statistically, this result does not differ from media obtained with of foreign inactivated vaccine that uses Cuban`s aviculture nowadays. Inactivated vaccine resulted to be satisfactory for all the carried out tests.
Key words: Avian Infectious Bronchitis, Newcastle, killed vaccine.

MONDAY 18 (MORNING)

• Session: Current & Novel strategies in Prophylactic Vaccines Development

Chairmen: Ole Olesen (EMA, Belgium) and Reinaldo Acevedo (Finlay Institute, Cuba)

NEW OPPORTUNITIES FOR VACCINE DEVELOPMENT AGAINST POVERTY-RELATED AND NEGLECTED INFECTIOUS DISEASES

Olesen O

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The use of prophylactic vaccines is saving millions of lives every year around the globe, and routine vaccinations remain the most cost-effective measure to improve global public health. A number of important diseases continue nevertheless to frustrate attempts to produce effective vaccines against them. This includes many neglected infectious diseases, which are mainly (or only) prevalent in low-income countries, and therefore attract little interest from commercial vaccine manufacturers. In addition to this, many neglected infectious diseases are caused by extraordinary pathogens such as HIV, kinetoplastids and helminths. These pathogens are scientifically challenging to confront with classical vaccine technologies, and it is therefore encouraging that vaccine research has seen a remarkable renaissance during the last decade. This has resulted in important scientific breakthroughs such as the full genome sequencing of several infectious pathogens, refined knowledge about the human immune response system, and better understanding of complex host-pathogen interactions**.** International public organisations such as the European Commission (EC) can play an important role in supporting the translation of new basic research results into novel vaccines, both through financial support and by brokering partnerships between key stakeholders. During the last 5 years, the EC has thus established a number of collaborative research consortia with participants from the public and private sector, and including scientists from both Europe and disease-endemic countries. These initiatives are starting to

generate concrete results, and give new hope that vaccines against diseases such as leishmaniasis, schistosomiasis, tuberculosis and other neglected infections will one day become possible.

TRENDS IN OMV DEVELOPMENT: THE FINLAY EXPERIENCE

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Introduction: OMV (outer membrane vesicle) vaccines were developed more than 20 years ago against *Neisseria meningitidis* serogroup B. Recently, a vaccine against this pathogen was developed using OMV plus recombinant antigens. Likewise, the adjuvant potential of *Neisseria* OMV has already being demonstrated with model and immunological relevant antigens. The aim of this work is to provide examples of OMV candidates obtained at Finlay Institute with vaccine potential. **Methods:** GMP platform developed at production facilities from Finlay Institute was used to obtain OMV from *N. meningitidis* serogroup A (OMV_A), serogroup W135 (OMV_{W135}) and serogroup X (OMVx), but also from *Vibrio cholerae* (OMVc), *Bordetella Pertussis* (OMVp) and *Mycobacterium smegmatis* (OMVsm) and BCG (OMV_{BCG}). The immunogenicity of these structures was evaluated with functional bactericidal and challenge assays in mice models to demonstrate their protective potential. **Results**: Physico chemical characterization demonstrates that OMV from different microorganisms were obtained and some of the must important antigens in their structure were identified. Sera from BALB/c mice immunised with a bivalent formulation of OMV_A and OMV_{W135} or monovalent OMVx was immunogenic and developed bactericidal titers against respective meningococcal production strains. OMVc administered by intranasal route elicited high ELISA specific antibodies against LPS, OmpU, MSHA and other antigens in the vesicles; vibriocidal activity of sera of mice immunised was also demonstrated. OMV form *B. pertussis* was as immunogenic as the commercial DTPw vaccine, with the whole cell pertussis component, and no differences were observe in challenge experiments between both formulations. Finally, the two vesicles obtained from *Mycobacterium*, OMVsm and OMV_{BCG}, contain crossreactive antigens with *M. tuberculosis* and protect in BALB/c mice challenged with the pathogen by intratraqueal route. **Conclusions:** OMV remains an important technology for development of vaccine candidates and adjuvants. The use of OMV must be re-explored by mucosal route.

DEVELOPMENT OF AN OUTER MEMBRANE VESICLE VACCINE AGAINST SEROGROUP A AND W_{135} MENINGOCOCCAL DISEASE FOR AFRICA

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Introduction: Meningococci of serogroups A and W_{135} are the main causes of meningococcal disease epidemics in sub-Saharan Africa. The outbreaks in Africa are clonal and express the same surface proteins (PorA and PorB) over time. In this situation an outer membrane vesicle (OMV) vaccine may be effective. NIPH and Finlay Institute have both long time experience in research and manufacturing of group B OMV vaccines and are now collaborating to bring forward an A+W OMV vaccine to a clinical phase I trial. **Materials and methods:**. The vaccine is produced from representative epidemic serogroup A and W_{135} strains from Africa. Assays to assess identity, purity and potency of the vaccine were tailored to the A+W OMV vaccine. Several pilot batches of OMVs from each serogroup were tested in these assays. Toxicological evaluations were performed in rats. The immunogenicity of the OMV vaccine was compared in mice with commercially available conjugate and plain ACYW polysaccharide vaccines (Menveo®, MenAfriVac ® and Mencevax®). Antibody responses against polysaccharides and OMVs were

measured by ELISA and functional activities were detected by bactericidal (SBA) and opsonophagocytic (OPA) assays. **Results**: The production has successfully been scaled up to industrial manufacturing in a 750L fermentor under GMP-conditions. Quality assurance testing indicated that the vaccine passed the pre-defined standard acceptance criteria for pyrogenicity and endotoxin content for MenB OMV vaccines. The functional antibody titres (SBA and OPA) against the A and W_{135} strains were significantly higher in mice immunised with the OMV vaccine than with those immunised with the conjugate or plain polysaccharides vaccines. **Conclusions:** These results indicate that the A+W OMV vaccine is suitable for testing in humans, and that it has the potential to become an affordable and effective vaccine for prevention of meningococcal disease in Africa.

IMMUNOGENICITY IN MICE INOCULATED WITH DIFFERENT COMBINATIONS OF OUTER MEMBRANE PROTEINS FROM A AND W_{135} SEROGROUPS AND A POLYSACHARIDE

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Introduction: Serogroup A and W_{135} meningococcal disease causes a high burden of disease in many African countries. Plain polysaccharide vaccines are available, but they are poorly effective in young children, and polyvalent conjugate vaccines are considered to be too expensive. Outer membrane vesicle (OMV) vaccines from serogroup B have proven to be safe and immunogenic in various epidemic situations. A bivalent OMV vaccine against A and W_{135} serogroups could be an attractive alternative for "Meningitis Belt" in Africa. **Materials and Methods:** Different combinations of monovalent and bivalent OMV vaccines, with plain and conjugated A polysaccharide, were inoculated in Balb/c mice and compared with commercially available Men Afri Vac conjugated vaccine, in a two doses subcutaneous scheme. ELISA IgG titer and bactericidal titer determination (SBA) were used to measure immune response. **Results:** A high immune response was found in ELISA and SBA in groups inoculated with OMVs and conjugated polysaccharide when the same serogroup was used as coating antigen or target strain. The group who received both OMVs plus plain polysaccharide gave better response than those that received plain A polysaccharide alone (no detectable response), measured against this antigen. The best titers, by ELISA, were achieved in the group inoculated with commercial vaccine, but only directed against homologous serogroup. **Conclusions:** A bivalent OMV vaccine could be more immunogenic than plain polysaccharide vaccine, and could be effective against a broader group of strains. This vaccine could be an interesting alternative to mono and multivalent conjugated vaccines for Africa.

A VACCINE CANDIDATE BASED ON A SPECIFIC RATION OF HCV STRUCTURAL PROTEINS AND NS3 INDUCES BROAD SPECIFIC CELLULAR IMMUNE RESPONSE AND NEUTRALIZING ANTIBODIES IN ANIMAL MODELS

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Introduction: Hepatitis C virus infection is among the leading causes of chronic liver disease, but currently there is no vaccine available. Neutralizing antibodies and robust T cell responses have been shown to be related to the clearance of HCV and have shed lights on the potential success of HCV vaccines. In this work, we perform a surface response study to select the optimal ratio of recombinant HCV structural antigens able to induce functional immune response. The selected protein formulation was combined with NS3 and its immunogenicity was evaluated in mice and African green monkeys. **Materials and methods:** Animals were purchased from CENPALAB (Centro para la Produccion de Animales de Laboratorio, Cuba). Female BALB/c (H-2d) mice, 6–8 weeks old, 18–20 g of weight and African green monkeys (Cercophitecus aethiops sabaeus), 3–7 kg weight and 4–7 years old, were used for immunogenicity studies. Recombinant Co, E1, E2 and NS3 proteins encompassing amino acids 1–120, 192–340, 384–680 and 1192-1457, respectively, of the HCV polyprotein from genotype 1b Cuban isolate were formulated in aluminum hydroxide for animal immunization. **Results:** Specific T-cell proliferative response against HCV structural antigens was induced in BALB/c mice by CoE1E2 vaccination. Moreover, after challenge with HCV recombinant vaccinia virus, all mice controlled the viraemia and 80% were protected. In addition, mice immunized with CoE1E2/NS3 developed potent antibody response against HCV proteins. In fact, IgG purified from CoE1E2/NS3 immunized mice neutralized the infectivity of heterologous cell cultured HCV (HCVcc) of Con1-JFH1 chimera. On the other hand, monkeys vaccinated with CoE1E2/NS3 developed strong humoral response against HCV E2 conserved epitopes in addition to specific CD4+T cell and IFN-g secreting T cell responses against Core, E1, E2 and NS3 proteins. **Conclusion:** These results suggest that CoE1E2/NS3 might constitute an effective immunogen against HCV.

NEW VACCINE APPROACHES FOR HUMAN VIRAL DISEASES: PRECLINICAL STUDIES IN NHP

Le Grand R

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The Division of Immuno-Virology is devising and investigating various innovative approaches based on original viral and non-viral DNA delivery methods, multivalent recombinant DNA vaccines, new adjutants, and recombinant commensal bacteria. Our main goal is to decrease the viral load (therapeutic vaccine) as well as blocking virus entry through the mucous membranes (preventative vaccine). By combining skin electroporation and an original multigenic DNA vaccine, we have recently obtained long-lasting and highly effective systemic SIV-specific immunity in challenged monkeys. Novel avenues based on the use of dendritic cells are also being explored. We are developing our capability to quantify the expansion, activation and migration of various subsets of immune cells in vivo in infected and/or vaccinated primates to evaluate vaccine candidates further as a prerequisite to human clinical trials and to increase our understanding of critical steps in virus entry and replication. This will be made possible by the quite unique capability of the CEA in imaging technologies at high-resolution. These approaches are being extended to emerging infectious diseases, like Chikungunya virus and Dengue infections for which we have recently developed non-human primate models.

• Session: Conjugated and plain polysaccharide vaccines

Chairmen: Vicente Vérez (CQB, Cuba) and Ana María Henao-Restrepo (WHO, Switzerland)

CONJUGATE VACCINES AND THE NASOPHARYNGEAL COLONIZATION OF PNEUMOCOCCI. JUST AN INITIAL STEP FOR A LONG BATTLE?

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**(Dedicated to the Memory of Violeta Fernández Santana deceased 20 November 2011 for her extraordinary contribution to our progress in conjugate vaccine against S. pneumoniae)*

Streptococcus pneumoniae is a leading cause of bacterial infections in children during the first years of life and also in the elderly with clinical syndromes, varying from non-invasive respiratory diseases (pneumonia, otitis media) to invasive pneumococcal diseases (IPD; sepsis, bacteremia and meningitis). An important step in the fight against pneumococcal infections has been the introduction of vaccine based on the capsular polysaccharide. The conjugate vaccine induces an immunity that also reduces nasopharyngeal colonization. However, the existence of more than 90 serotypes of pneumococci can induce the replacement of the removed serotypes after vaccination. The accumulate knowledge on the transmission as well as the force governing this equilibrium allow us to propose a new alternative for the development and introduction of a Cuban pneumococcal conjugate vaccine.

DEVELOPMENT OF POLYSACCHARIDE VACCINE FROM *NEISSERIA MENINGITIDIS* SEROGROUPS ACYW$_{135}$, TO MUSLIM MARKET

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Meningococcal disease continues to be a significant global concern, with an estimated 1.2 million cases and 135000 deaths annually. Polysaccharides vaccines are shown to be efficacious in children and adults, but the efficacy is limited in infants and young children; on the other hand, the conjugate vaccines are too expensive and not reachable by poor countries, been widely used single polysaccharide vaccines to epidemic control in many countries, helping to save lives. Muslim people are an interesting market segment, so vaccines are direct to reach those populations. A technology update, in order to get higher polysaccharide expression in fermentation with shorter purification steps, to obtain a competitive product. The goal of this work is to develop a technology to obtain capsular purified polysaccharide from *Nm*. serogroups ACYW$_{135}$ focus on Muslim vaccines formulation. New strains, with traceable story were evaluated; raw material HALAL or non animal origin certified were used in a concentrated media, with 5 from 9 traditional components and cetrimide precipitation with selective ethanol extraction in purification steps were evaluated. The strains and raw materials get high polysaccharide expression; the polysaccharides purified fulfill the specifications. The new process allows obtaining a polysaccharide according to the Muslim requirements.

IMMUNOGENICITY AND MEMORY ELICITED BY AN HEPTAVALENT CONJUGATE VACCINE AGAINST *STREPTOCOCCUS PNEUMONIAE*

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Introduction: *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia, bacteremia, meningitis, and otitis media, mainly in infants and elderly people. More than 90 serotypes have been identified until now, that is why, it is necessary to develop multivalent vaccines to induce protection. Currently, the Center for Biomolecular Chemistry is developing an heptavalent conjugated vaccine against serotypes 1, 5, 6B, 14, 18C, 19F and 23F. The aim of this research is to demonstrate the immunogenicity and memory of each conjugate in the heptavalent formulation compare with its monovalent formulation, and evaluate the induction of mucosal immunity. **Materials and Methods:** New Zealand rabbits were immunized with three doses of each monovalent conjugate or heptavalent formulation, with AlPO4 as

adjuvant. The dose of each conjugate was 2 µg PS except 4 µg for 6B. The serum and mucosal antibody response and the antibody avidity were evaluated by ELISA. For memory study, a booster with plain PS (25 µg) was immunized 4 months after the first immunization. **Results and Discussion:** The heptavalent vaccine candidate elicited high titers of total IgG against each PS and carrier protein, without significant differences among them after the third dose. No significant difference in IgG antibody response was detected between the conjugate mono and multivalent formulations; that means the heptavalent formulation conserves the immunogenicity of each monovalent conjugate. The titer of IgG anti-TT induced by heptavalent formulation was similar to each monovalent, suggesting no tolerance or suppression immunologic with higher doses of TT. The avidity of antibodies elicited by heptavalent formulation against each PS was high and similar to rabbit reference serum specific by each serotype. Also, immunological memory was demonstrated because the rabbits responded to plain PS inducing high titers of IgG antibodies after the booster dose. In addition, high levels of IgG were detected in saliva and respiratory mucosa from immunized rabbits. **Conclusions:** The heptavalent formulation against Neumococco is immunogenic in rabbits, and induces memory and mucosal immunity. These preclinical evidences are the support of clinical phase for the license of the vaccine.

PREPARATION OF CONJUGATES FROM CAPSULAR POLYSACCHARIDES OF SEROGROUPS A, C, Y AND W135 OF *N. MENINGITIDIS* AND DIPHTHERIA TOXOID

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Introduction: *N. meningitidis* is a leading cause of bacterial meningitis and sepsis throughout the world. There are thirteen different serogroups of this bacterium, which have been identified on the basis of their capsular polysaccharides (PsC). Six of these serogroups (A, B, C, W135, Y and X) are the main cause of meningococcal diseases nowadays. Conventional vaccines based on meningococcal PsC elicit immune response in children and adults; however their efficacy in infants and young children is limited, due to the Thymus-independent nature of the PsC. These T-independent antigens could become T-dependent through conjugation to a carrier protein. The conjugate vaccines have demonstrated to be very effective but also very expensive. Currently, the Center of Biomolecular Chemistry and the Finlay Institute have a join research project with the aim of developing conjugated vaccines against serogroups A, C, W135 and Y of *N. meningitidis*. The aim of this work was to prepare conjugates from capsular polysaccharides of groups A, C, Y and W135 *N. meningitidis* and Diphtheria Toxoid. **Materials and Methods:** General working methodology was as follows: i. Fragmentation of PsC, ii. De-OAcetilatión iii. Activation, iv. Conjugation. We used acid hydrolysis; periodate oxidation and reductive amination respectively and the final products of these reactions were characterized by physicochemical techniques. Conjugates were immunized in Balb/C mice and the immunological response was evaluated by immunoenzymatic and bactericidal assays. **Results:** High recovering in fragmentation and activation reactions were obtained. The conjugates have a broad and controlled carbohydrate to protein ratio and low free protein. The **IgG** antibody titres elicited by the conjugates were high, specific against PsC and bactericidal. **Conclusion:** The methodology of preparation *N. meningitidis* A, C, Y and W135 conjugate is ready for further development at industrial scale.

IMMUNOGENICITY OF *SALMONELLA* TYPHI VI POLYSACCHARIDE-DIPHTHERIA TOXOID (VI-DT) CONJUGATE VACCINE AGAINST TYPHOID FEVER

Fernández S[1], Soubal JP[2], Acevedo R[1], Aranguren Y[1], Santana D[2], Cabrera O[1], Romeu B[1], Cuello M[1], Lastre M[1], Pérez O[1], Blaín K[1], Cabrera R[1], Zayas C[1], Mandiarote A.[1],Díaz D[1], Serrano D[1], Valmaseda T[1], Valdés Y[2], Vérez V[2], García L[1], Cardoso D[1]

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Introduction: Typhoid fever, caused by *Salmonella enterica* serovar Typhi, continues to be a major public health problem with 21 million cases and 600 000 deaths annually according with estimates of World Health Organization. None of the available typhoid vaccines are licensed for children under 2 years old, the age group who reaches the highest mortality. Conjugation of polysaccharides to an immunogenic protein revert the Thymus-independent pattern of polysaccharides to a T-dependent pattern and induce immune response in infants. The aim of this work was to investigate the immune response induce by formulations based on conjugates from Vi polysaccharide of *Salmonella* Typhi and diphtheria toxoid (Vi-DT) as part of the project conducted by Finlay Institute and Biomolecular Chemistry Center. **Materials and Methods:** Experimental lots of Vi-DT conjugates were obtained via a carbodiimide-mediated reaction using adipic acid dihydrazide as linker. Formulations with or without adjuvant (Alum) were evaluated in Balb/C mice in a two doses scheme separated 28 days. Immune response was determined by ELISA method. **Results:** All lots evaluated elicited high titers of anti-PsVi IgG after first dose and remained high after 100 days. Alum formulated conjugates, with a dose ten times lower, induced immune response as high as non adjuvanted formulation. **Conclusions:** These results demonstrated that Vi-DT conjugates are immunogenic and further studies are required to define the vaccine formulation.

MONDAY 18 (AFTERNOON)

• Session: Symposium Tuberculosis

Chairmen: Gregory Hussey (University of Cape Town, South Africa) and Rogelio Hernandez Pando (México)

EFFICIENCY OF ACELLULAR VACCINES ADJUVANTED BY INTERFERON GAMMA BY DIFFERENT ROUTE OF ADMINISTRATION AGAINST EXPERIMENTAL TUBERCULOSIS

Yola Fishman, Avi-Hai Hovav and **Herve Bercovier**

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An effective vaccine is necessary to control world Tuberculosis. If BCG effectiveness varies from 0 to 80 %, this suggests that an effective vaccine can be designed. Numerous new vaccines are being developed, including live auxotrophic mutants of Mtb, improved recombinant BCG, and acellular vaccines. We developed a multivalent vaccines that consists of six Mtb antigens: 85B, 38-kDa, ESAT-6, CFP21, Mtb8.4 and 16-kDa. Immunization of mice with these antigens emulsified in Ribi adjuvant system or in liposomes and supplemented with recombinant IFN-gamma, resulted in strong Th1 immune response and a high protection level that was comparable or better to that of BCG. The intra rectal administration of the acellular vaccines showed promising results and could provide a safe mode of “needle free” delivery of TB vaccine in HIV endemic areas.

THE SOUTH AFRICAN TB VACCINE INITIATIVE’S CONTRIBUTION TO THE DEVELOPMENT OF NEW TB VACCINES

Hussey GD, Mahomed H and Hanekom W

South African TB Vaccine Initiative, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town. South Africa.

Approximately 1% of the South African population develops TB disease every year and over 60% of our TB patients also have HIV infection, placing us at the epicenter of the TB/HIV co-epidemic. Advances in TB diagnosis and treatment are needed to control the epidemic. However, an effective vaccine would constitute the most sustainable intervention. The vision of the South African Tuberculosis Vaccine Initiative (SATVI, www.satvi.uct.ac.za) is "A World Without TB", and our mission is "Innovative And High-Quality TB Vaccine Research in Africa, to Impact the Global Epidemic." Over the last 10 years, our focus has been twofold: first, clinical trials of BCG and of new candidate vaccines, and second, complementary research that addresses critical questions in TB vaccine development. SATVI is now widely regarded as the leading clinical TB vaccine clinical research site worldwide. Our field site is located in a rural area about 110km from Cape Town with a population of 350,000 and our admin offices and state-of-the-art immunology laboratory is located at UCT's Health Science Faculty. Our initial flagship project was a phase IV randomized clinical trial to assess whether route of newborn BCG administration determined efficacy in protecting against childhood TB. The study enrolled 11,680 infants at birth and showed equivalence in TB disease rates following BCG vaccination by an intrademal route and by a percutaneous route. This study was critical for developing infrastructure and capacity to conduct large-scale TB vaccine trials as well as for guiding policy of BCG vaccination. To date, SATVI has conducted clinical trials of 5 new TB vaccine candidates, in 11 different protocols. We are currently conducting early efficacy trials of 2 candidates, MVA85A and Aeras-402, in infants. The results of the trial of MVA85A, involving 2,797 infants are expected to be available at the end of this year.In addition, as a principle, SATVI uses registration-standard vaccine trials to explore other critical areas in vaccine development. These include, for example, novel approaches to informed consent, tools to measure the tuberculin skin test , approaches to diagnosing TB disease in children for late-phase vaccine trials, or detailed studies of the vaccination-induced immune response. In addition, specific clinical studies have been conducted to address additional questions in relation to the epidemiology and dynamics of TB in infants and adolescents. Pivotal results of these studies will be highlighted.

THE USE OF MUTANT MYCOBACTERIA AS NEW VACCINES TO PREVENT TUBERCULOSIS

Hernández Pando R

Experimental Pathology Section. Department of Pathology. National Institute of Medicine Sciences and Nutrtion "Salvador Zubiràn", Mexico.

Given the variable protective efficacy generated by Mycobacterium bovis BCG (Bacillus Calmette–Guerin), there is a concerted effort worldwide to develop better vaccines that could be used to reduce the burden of tuberculosis.Diverse methods have facilitated genetic manipulation of Mycobacterium tuberculosis, which in combination with the availability of the complete sequence of the genome of M. tuberculosis have enabled to study the contribution of individual genes to virulence One of the approaches to identify the genes responsible for pathogenicity has been the development of M. tuberculosis mutants, which are then tested for multiplication in mouse or guinea pig lungs. Several studies using this functional genomic approach have reported the development of several mutants with different attenuation ranges and a potential as vaccine candidates in animal models. These mutant bacilli should be deeply attenuated due that they are administered as live replicating organisms and they must have high immunogenicity, particularly inducing strong cell mediated immunity mediated by Th-1 and CD-8 cells. This is the case of mutants such as phoP, mce-2 or sig E factor in which genes related to metabolic bacterial adaptation (phoP, sigE) or virulence (mce2) have been deleted. The advantage of using attenuated M. tuberculosis strains as vaccine candidates is that these strains have more than 100 genes that have been deleted in BCG. Thus, vaccination with auxotrophic mutant mycobacteria can actually induce a strong and long immune stimulation conferring high levels of protection.

VACCINE CANDIDATES DERIVED FROM NON PATHOGENIC MYCOBACTERIA

Acosta A., Kadir Ramlah, Ahmad Fauzan, Puig A, Borrero R, García MA, Marron R, Tirado Y, Reyes F, Álvarez N, Fernández S, Zayas C, Infante JF, Fariñas M, Mohd Nor N, Pérez-Quiñoy JL, Hernández Pando R, Sarmiento ME

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Proteoliposomes from BCG and *M. smegmatis* and liposomes from *M. smegmatis* were obtained and their humoral and cellular antigenicity was evaluated with samples from tuberculosis patients and healthy controls PPD+ and PPD-. The immunogenicity and induction of cross reactive responses in mice against *M. tuberculosis* were evaluated using different adjuvants. The antigenicity of the different vaccine candidates was demonstrated in humans. All the candidates demonstrated immunogenicity in mice and induced cross reactive responses against *M. tuberculosis* antigens. The profile of the immune responses obtained differed according the combination of experimental vaccine candidate and adjuvant used. Some of the vaccine candidates showed protective capacity in challenge models in mice.

ANTIMICROBIAL PEPTIDES AS NEW AGENTS TO PREVENT AND TREAT PULMONARY TUBERCULOSIS

Hernández Pando R

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Tuberculosis is a growing worldwide health problem due in part to the scarce development of new drugs and the emerging of new strains such as multidrug-resistant and extensively multidrug-resistant that are threatening and impairing the control of this disease. These problems motivate the search for new drugs and therapy strategies. One potential strategy is the use of cationic antimicrobial peptides which have wide antimicrobial activity. Antimicrobial peptides (AMP´s) are gene-encoded, amphipatic, cationic peptides that are produced by several species of mammals, birds, reptiles and amphibians. These peptides inhibit microbial growth through different mechanisms, such as membrane interactions that lead to microbial lysis, inhibiting DNA replication and through the binding of ribosomes. The main groups of AMP´s are cathelicidin, defensins and histatins. BALB/c mice produced low quantities of defensins during late progressive TB, and when both peptides were overproduced by the intratracheal administration of isoleucine (an efficient defensins inductor), these animals efficiently controlled the infection by drug sensible and drug resistant bacilli. The same result was obtained when semi-synthetic peptides (E2, E6, and CP26) or cathelicidins were administered by intratracheal instilation. In murine model of latent infection there was a very high production of beta-defensins and they were strikingly suppressed after disease reactivation induced by corticosteroid treatment, and this was prevented when antimicrobial peptides were administered before glucorticoids administration. Thus, it seems that AMPs have significant participation in the immunopathogenesis of experimental TB and they have some potential as immunotherapeutic agents.

LACTOBACILLI AS LIVE VECTORS FOR ORAL VACCINATION AGAINST TUBERCULOSIS

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Tuberculosis (TB) is one of the world's deadly infectious diseases with more than 9 million people becoming sick worldwide each year and a third of the global population infected. The only licensed vaccine for TB prevention, the BCG (Bacille Calmette-Guérin) protects infants from the more invasive forms of TB, although it fails in protecting adolescents and adults which remain susceptible to *Mycobacterium tuberculosis*. Hence, different strategies have been attempted to create a new generation of vaccines. Since *M. tuberculosis* is a mucosal pathogen, it has been suggested that TB vaccine administration through the mucosal route could be more effective than the parenteral administration to induce an adaptive immune response. Several studies have shown the capacity of recombinant lactobacilli expressing antigens to induce specific humoral and cellular immune-responses when is orally administered. Taking all this in mind, we have considered lactobacilli as good candidates to be used as live vectors for mucosal immunization and a valuable strategy for TB vaccine development. The technology developed in our group to generate food-grade recombinant lactobacilli has been used to construct a *Lactobacillus casei* strain able to produce a multiepitopic chimeric *M. tuberculosis* antigen covalently anchored on the outside of the cell wall. The possibilities and perspectives of this system will be discussed

TUESDAY 19 (MORNING)

• Session: Preclinical and clinical studies for prophylactic vaccines

Chairmen: Deybis Orta (CECMED, Cuba) and Sonia Resik (IPK, Cuba)

NON-CLINICAL ASSAY: REPEATED DOSE TOXICITY STUDY OF THE MENINGOCOCCAL SEROGROUP AW_{135} OUTER MEMBRANE VESICLE VACCINE IN SPRAGUE DAWLEY RATS

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Introduction: The outer membrane vesicles vaccine from A and W_{135} meningococcal serogroups, are the result of scientific collaboration between Finlay Institute and Norwegian Institute of Public Health. **Objective:** To study the toxicological potential of this vaccine, we conducted a repeated dose toxicity study **Materials and methods:** 130 Sprague Dawley rats were inoculated by intramuscular route. It was administered 0.2 mL volume of the vaccine in each dose. Rats were observed daily in search of local and systemic symptoms of toxicity during 12 weeks, together with water and food consumption, corporal weight measurements, and haematological, biochemical and immunological studies. Animals were euthanized and periodically subjected to necropsy. Pathological studies were carried out to identify any adverse effects following immunization with the experimental vaccine. **Results:** Neither toxicity symptoms were observed during the study in animals, nor differences of toxicological concern among the experimental groups in body weight, drinking water and food. Pathological changes in toxicological value, analyzes macroscopic or microscopic, at the inoculation site were not observed whilst macrophage of type granulomatous processes were described. The relative weights of solid organs were within historical averages and reported for the species in both sexes and ages. The animals showed no haematological and biochemical alterations in any of the experimental groups. Moreover, the model used demonstrated relevance to have a good response of antibody titers to both serogroups, after the different doses applied. **Conclusion:** These results revealed that, under the study conditions and according to established criteria,

the vaccine candidate showed no clinical symptoms or, deaths due to product administration, in repeated doses protocol.

EARLY LIFE MURINE IMMUNIZATION AS A TOOL IN THE PRECLINICAL TESTING OF VACCINE ANTIGENS: MENINGOCOCCAL PROTEINS AS MODEL ANTIGENS

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Introduction: The capacity to enhance early life vaccine antibody responses through new adjuvants and antigen delivery systems is critical for neonatal vaccinology, to overcome the immaturity of the immune system at said age. Due to safety and ethical issues linked to human infant studies, infant mice are being used increasingly for the evaluation of vaccine candidates and adjuvant properties in early life. Murine models of early life immunization may reproduce the main known characteristics of neonatal and infant vaccine antibody responses, if immunization is initiated ≥ 7 days of age. **Materials and Methods:** Firstly, we investigated how the route, adjuvant, dose and schedule of immunization influence the immunogenicity and protective ability of meningococcal outer membrane vesicles (OMV) in neonatal Balb/c or OF1 mice. Several adjuvants and schedules were evaluated by parenteral and mucosal route. Later, the immunogenicity of the recombinant meningococcal antigens NMB0928, NMB0606 and NMA0939 was explored in the same model. **Results:** The administration of two parenteral doses of OMV combined with adjuvants, given at 7 and 14 days after birth, induced a significant antibody response, and was highly effective in conferring protection to 21 days-old sensitized mice challenged with meningococci. Even when the antibody response does not differ in mice immunized without adjuvant, the levels of protection are severely affected when the OMV were administered alone. Moreover, the humoral immune response against OMV was significantly increased after the use of two mucosal adjuvants in neonatal immunization. A memory response was induced in mice immunized as neonates. The three recombinant proteins administered with adjuvant were immunogenic in mice sensitized as early as 7 days after birth. **Conclusions:** Preclinical testing of bacterial protein antigens is feasible in this model of early life immunity, where the immunogenicity of said antigens is strongly influenced by the schedule and adjuvant employed.

PATTERN AND DETERMINANTS OF CHILDHOOD IMMUNISATION RESEARCH PRODUCTIVITY IN AFRICA

Wiysonge CS[1], Uthman OA[2], Ndumbe PM[3], **Hussey GD[1]**

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Introduction: Factors that influence the publication of research on immunisation in Africa are not known. We therefore undertook this study to fill this research gap by providing insights into factors associated with childhood immunisation research productivity in the continent. We postulated that research output may influence immunisation coverage. **Methods:** We conducted a bibliometric analysis of childhood immunisation research output from Africa; using research articles indexed in PubMed as a surrogate for total research productivity. We used negative binomial regression models to explore the factors associated with research productivity. **Results:** We identified 1641 articles on childhood immunisation indexed in PubMed between 1974 and 2010 with authors from Africa; which represent only 8.9% of the global output. Five countries (South Africa, Nigeria, Gambia, Egypt and Kenya) contributed 48% of the articles. In univariable analyses, the country's GDP, total health expenditure, private health expenditure, and research and development expenditure had a significant positive association with increased research

productivity Immunisation coverage, adult literacy rate, human development index, and physician density had no significant association. However, in the multivarable model only private health expenditure maintained significant statistical association with number of immunisation articles. Gambia, Guinea-Bissau, Guinea, Sao Tome and Principe, and Zimbabwe had better ratings when the research productivity was adjusted for gross domestic product (GDP). When controlled for total expenditure on health, Gambia, Guinea-Bissau, Guinea, Sao Tome and Principe, and Malawi were the most productive. **Conclusions:** Immunisation research productivity in Africa is highly skewed, with private health expenditure (which may reflect the economic development of a country) having a significant positive association. However, the contribution of authors from Africa to global childhood immunisation research output was minimal. The lack of association between research productivity and immunisation coverage may be an indication of lack of interactive communication between health decision-makers, programme managers, and researchers; to ensure that policies are always informed by the best available evidence.

NO MORE HEPATITIS B (HB) SICK CHILDREN IN CUBA: THE CUBAN HB RECOMBINANT VACCINE HISTORY, DEVELOPMENT, IMPACT AND PERSPECTIVES AS A COMBINED THERAPEUTIC VACCINE CANDIDATE

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The recombinant HB vaccine (recHBvac), second outcome (1980-90's) after interferon of the Cuban biotech-pharmaceutical industry, created and manufactured by CIGB, has been used for over two decades and its impact on Cuban population, particularly children, progressively reached its potential in this century. A small number of new acute HB cases in the general population, and almost none in children, were reported. Since then, and in the last five years, the incidence of HB dropped to zero in children and to a few sporadic cases in general population. This extraordinary achievement (more so for a limited resource developing country) is linked not only to vaccine quality but also vaccination strategy, coverage and logistics provided by the Cuban health system. This experience deserves further analysis to draw lessons to be learned by the scientific community, especially in similar countries likely to benefit from them. Our presentation addresses this issue, focusing first on the unique physicochemical/biological properties of recHBvac and then on how it was applied. Moreover, since patents and market validity of high-tech pharmaceuticals are limited, a more refined and further developed product pipeline must be in progress for their replacement. In our case, monovalent recHBvac, although used for particular applications, was surpassed and replaced by the pentavalent (DTP-Hib-HB) vaccine, now on the market. This is consistent with current trends of vaccine design aimed at concentrating several antigens (Ag) into single shot formulations for a more profitable, comfortable and economic use of patient/health-system contacts. Finally, as immunogenicity-enhancing platform, the most outstanding breakthrough will come if currently ongoing and planned clinical trials (CT) abroad confirm the highly promising results of preliminary CTs with the combined two-Ag nasal/parenteral therapeutic vaccine formulation (NASVAC), which, in addition to HBV nucleocapsid Ag, includes the HB surface Ag of recHBvac. The rationale for such an association is discussed.

VACCINE ADVERSE EVENTS IN CUBAN CHILDREN, 1999–2008

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Introduction: Cuba has successfully implemented its National Immunization Program since 1962. The schedule, administered basically to children, is comprised of 11 vaccines protecting against 13 diseases. In 1999 Cuba launched a national vaccine adverse event surveillance system to monitor and assess the safety of the immunization program, its vaccination procedures and the products administered. **Methods:**

A retrospective descriptive study was conducted of adverse events following vaccination reported from January 1999 through December 2008. Variables used: year, number of adverse events, province, type of vaccine, type and severity of adverse events (common minor, rare, severe), vaccination program errors, number of deaths, and final results of investigations of severe events. Percentages and rates per dose administered were calculated. Adverse event rates were calculated per 100,000 doses administered and percentages of individual effects among events reported. **Results:** A total of 45,237,532 doses of vaccine were administered, and 26,159 investigations of vaccine-associated adverse events were reported (overall rate: 57.8/100,000 doses). The group aged 0–5 years reported the highest rate of vaccine-associated adverse events (82/100,000 doses). The DTwP vaccine exhibited the highest rate of adverse events. Common minor events were: fever (17,538), reactions at injection site (4.470) and systemic side effects (2.422). Rare events reported were: persistent crying (2666), hypotonic-hyporesponsive episodes (3), encephalopathy (2) and febrile seizures (112). Severe events included: anaphylaxis (2), respiratory distress (1), multiple organ failure (1), sudden death (1), vaccine-associated paralytic poliomyelitis (2), toxic shock syndrome (3), and sepsis (1). The 10 deaths and 3 cases of disability were investigated by an expert committee, which concluded that 8 of the 13 severe events were vaccination-related. **Conclusiones:** Low rates of severe vaccine-associated adverse events observed in this study underline the low risk of vaccination relative to its demonstrated benefits in Cuba. Decision-making for the continued success of the National Immunization Program is supported by reliable information from comprehensive national surveillance with standarized reporting, along with multidisciplinary expert analysis of rare and severe adverse events and program errors.

EFFECTS OF ALUM PHOSPHATE ADJUVANT OR A BOOSTER DOSE ON IMMUNOGENICITY DURING CLINICAL TRIALS OF QUIMI-HIB® CONJUGATE VACCINE

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Introduction: *Haemophilus influenzae* type b (Hib) conjugate vaccine named Quimi-Hib® (Heberbiotec S.A, La Habana) differs in its composition from other commercial Hib vaccines, which is based on synthetic oligosaccharides that mimic the natural capsular polysaccharide, conjugated with the tetanus toxoid carrier protein. The vaccine has shown to be immunogenic, safe, and well tolerated, as demonstrated in several clinical trials with children. The present study was carried out to evaluate the safety and immunogenicity of Quimi-Hib® (sPRP-TT) conjugate vaccine in relation to its adsorption to aluminum phosphate ($AlPO_4$) adjuvant. **Materials and Methods:** A clinical phase II, double-blind, randomized, multicenter, controlled study was carried out in 208 healthy infants. Participants were randomized in a 1:1 ratio to receive a unique dose of either 10 µg adjuvant free Quimi-Hib vaccine or Quimi-Hib($AlPO_4$) vaccine. Blood samples for IgG specific anti-polysaccharide (PRP) antibody measurement were collected before vaccination and 1 month after the booster dose. **Results:** Out of 208 subjects enrolled, 178 received the booster dose and were included for safety analysis. Local and systemic reactions occurred with low and similar frequencies in both groups. No serious adverse events were reported. A total of 167 subjects were included in the per protocol immunogenicity analysis. Both vaccines were highly immunogenic and equivalent in terms of percentage of acquisition of long-term protective levels. At 4 weeks after the booster dose, the GMCs of anti-PRP increased significantly (46.9; 49.5 µg/mL) (+4.7 fourfold) in both groups (Quimi-Hib; Quimi-Hib-$AlPO_4$) compared to baseline sera (10.7; 10.6 µg/mL), respectively ($p < 0.01$), suggesting a booster response. More than 70% of the infants in both

groups exhibited a two-fold increase in baseline anti-PRP-specific IgG titer. **Conclusions:** The use of $AlPO_4$ adjuvants in Quimi-Hib® vaccine does not negatively impact the safety profile, nor increase the magnitude of anti-PRP antibody titers during a booster scenario.

NEW CUBAN 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE: FIRST STEP FOR CLINICAL TRIALS

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Introduction: Infections caused by *Streptococcus pneumoniae* are responsible for substantial morbidity and mortality, particularly in the very toddler and elderly. The development of pneumococcal conjugate vaccines, in which each of the selected bacterial capsular polysaccharides (PsC) is coupled with a protein carrier molecule, has been a major advance in the prevention of invasive pneumococcal disease (IPD). In Cuba IPD is a health problem because of the very expensive cost of this type of vaccines. Thus, a Cuban 7-valent pneumococcal conjugate vaccine (VCN7-T) is being developed. The aim of this work is to describe the results derived from the first clinical trial application for this vaccine to the Cuban National Regulatory Authority (NRA) **Materials and Methods:** The strategy for manufacturing, control, non-clinical and clinical evaluations of VCN7-T was based on WHO recommendations. A dossier was elaborated according to NRA regulations. Since the 7 active product ingredients (monovalent bulk conjugate) are chemically different the Quality part of the Dossier was organized depending on the similarities of the manufacturing processes. Non-clinical evaluations were carried out on mice, rats and New Zeland rabbits based on 2 WHO surrogates for licensing conjugated vaccines: anti PsC IgG levels and opsonophagocitic assay (OPA) titers higher than 1/8. **Results:** The Proton Nuclear Magnetic Resonance (H^1-NMR) to demonstrate polysaccharide identity and many others assays (~ 300) were carried out as evidence of the quality of the manufacturing process. Non-clinical evaluations showed immunogenicity based on the anti PsC IgG with opsonophagocytic activity, immunological memory and demonstrated product safety. **Conclusions:** A regulatory dossier containing the manufacturing and nonclinical results of Cuban VCN7-T was elaborated and presented to the NRA for clinical Phase I trial authorization. This is the first step in the clinical way for this product and a challenge both to the manufacturer and the NRA.

• Session: Current Experience in Regulatory isues of Vaccines

Chairmen: Carmen Amelia Rodríguez (WHO, Switzerland) and Olga Lidia Jacobo (CECMED, Cuba)

PATTERN AND DETERMINANTS OF CHILDHOOD IMMUNISATION RESEARCH PRODUCTIVITY IN AFRICA

Wiysonge CS[1], Uthman OA[2], Ndumbe PM[3], **Hussey GD[1]**

[1] Vaccines for Africa Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa; [2] African Public Health Foundation, Abuja, Nigeria; [3] Faculty of Health Sciences, University of Buea, Buea, Cameroon.

Introduction: Factors that influence the publication of research on immunisation in Africa are not known. We therefore undertook this study to fill this research gap by providing insights into factors associated with childhood immunisation research productivity in the continent. We postulated that research output may influence immunisation coverage. **Methods:** We conducted a bibliometric analysis of childhood immunisation research output from Africa; using research articles indexed in PubMed as a surrogate for

total research productivity. We used negative binomial regression models to explore the factors associated with research productivity. **Results:** We identified 1641 articles on childhood immunisation indexed in PubMed between 1974 and 2010 with authors from Africa; which represent only 8.9% of the global output. Five countries (South Africa, Nigeria, Gambia, Egypt and Kenya) contributed 48% of the articles. In univariable analyses, the country's GDP, total health expenditure, private health expenditure, and research and development expenditure had a significant positive association with increased research productivity Immunisation coverage, adult literacy rate, human development index, and physician density had no significant association. However, in the multivarable model only private health expenditure maintained significant statistical association with number of immunisation articles. Gambia, Guinea-Bissau, Guinea, Sao Tome and Principe, and Zimbabwe had better ratings when the research productivity was adjusted for gross domestic product (GDP). When controlled for total expenditure on health, Gambia, Guinea-Bissau, Guinea, Sao Tome and Principe, and Malawi were the most productive. **Conclusions:** Immunisation research productivity in Africa is highly skewed, with private health expenditure (which may reflect the economic development of a country) having a significant positive association. However, the contribution of authors from Africa to global childhood immunisation research output was minimal. The lack of association between research productivity and immunisation coverage may be an indication of lack of interactive communication between health decision-makers, programme managers, and researchers; to ensure that policies are always informed by the best available evidence.

ADDRESSING THE GROWING CONFIDENCE GAP IN PUBLIC ACCEPTANCE OF VACCINES IN THE UNITED STATES

Ellis G

United States of America (USA).

The public health community has gotten markedly better at distributing effective vaccines to the children who need them. But researchers are noticing an increase in mistrust of vaccines around the world, and they're concerned that unfounded suspicions could derail immunization programs essential to saving lives. One in eight American parents has refused at least one vaccine recommended for their children by their family doctor, according to a study published in *Journal of Pediatrics.* Therefore, even if only ten of 100 people refuse vaccines but most of them live in the same neighborhood, the likelihood of outbreaks increases due to local breakdown of herd immunity. Fears and suspicions around vaccine safety have already contributed to a slight decline in vaccination rates in the U.S. Vaccine distrust can evolve out of cultural, religious, ethical or sometimes economic or political reasons. Suspicion and apprehension about vaccination is fairly common, particularly among several specific disenfranchised communities in the United States and internationally. For these communities, the suspicion is best understood in a social and historical context of inequality and mistrust. For example, several studies have found that the legacy of racism in medicine and the Tuskegee Syphilis Study, a clinical trial conducted with African Americans who were denied appropriate treatment opportunities, are key factors underlying African Americans' distrust of medical and public health interventions, including vaccination. Through a historical and cultural overview, the focus of this presentation will be on solutions to building and sustaining trust with those who accept and support vaccines, while working to understand and address the growing confidence gap. Addressing mothers' concerns about immunization is important both from an ethical perspective, in assuring that they are fully informed of the risks and benefits of immunizations, as well as from a practical one, in reducing the possibility that people will decide not to immunize themselves or their child. Changes, particularly, in the childhood immunization process should be made to reduce parental concern about vaccine safety. Some changes that may be considered include improved provider communication about immunizations and additional tailored information about the necessity and safety of vaccines.

ORAL CHOLERA VACCINE (OCV) STOCKPILE

Costa A

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Nowadays, there different types of vaccine stockpiles. Vaccine stockpiles are created to meet specific needs normally associated to an emergency. It constitutes a simply buffer stock to avoid routine vaccine supply shortages; or be more strategic for pandemic/epidemic response (H1N1, meningitis, Yellow Fever), against re-emergence of an eradicated disease (Smallpox, Polio) or be part a national policy on biodefense/bioterrorism (Smallpox, Anthrax). Before building a vaccine stockpile there are many issues which should be taken into consideration: storage of vaccine, regulatory issues, forecasting of number of doses needed, criteria for release, appropriate prioritization and a mechanism to ensure sustainable financing. There is an increasing recognition of cholera as a major global public health problem and combined with a very limited supply of oral cholera vaccines (OCVs) withdraw public health international partners to discuss the role of using vaccines to control cholera. After an outbreak of cholera in 1994 in Rwanda that resulted in more than 50,000 deaths, the public health community started to discuss the potential use of OCVs in large scale to control epidemics. Since then there has been a number of meetings, where not clear recommendation were made. Most recently, the 2010 outbreak in Haiti started a renewed debate with new calls for using OCVs and creation of international stockpile. Today an international OCV stockpile does not exits, there is limited evidence to support its cost-effectiveness in epidemic response, therefore the use of such stockpile remains unclear. This presentation will provide information on a WHO project to create an OCV stockpile to be used for emergency response, consequently create the necessary evidence in relation to the impact of OCVs to control or mitigate cholera epidemics.

REGULATION AND APPLICATION OF INFLUENZA VACCINES, EXPERIENCE IN RUSSIA

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The Russian Federation is one of the most important manufacturers of influenza vaccines in the European region, with the volume of batch release allows to meet the civilian demand for the preventive influenza preparations within the framework of the observance of the program of the National calendar of preventive vaccination. Together with the national vaccines, a number of the preparations of foreign production is licensed and adapts in Russia. «Scientific Center for Expertise of Medical Application Products» of the Ministry of Health and Social Development of the Russian Federation is the state expert organization, which regulates the questions, connected with licensing of newborn preparations and carries out functions on certification. We accumulated significant experience according to the regulation and the extensive data on the application of the national seasonal trivalent inactivated and live attenuated influenza vaccines in the Russian Federation. We conduct the comprehensive analysis of application of influenza vaccines and basic aspects, connected with its licensing. Special attention was concerning on the analysis of investigations and regulation during the preparedness of pandemic influenza monovaccine under the conditions for the circulation of the pandemic strain of the influenza virus A/H1N1/pd in the epidemic season of 2009 - 2010. We examined the results of the most significant clinical studies of safety, immunogenicity and effectiveness of influenza vaccines, conducted during the last few years in the Russian Federation. The analysis demonstrates the latitude of spectrum of the conducted investigations and due to a conclusion about correspondence of the registered preparations to the international requirements of safety, immunogenicity and effectiveness. The developed pandemic plan with respect to the licensing of pandemic influenza monovaccine demonstrated the possibility of the operational reaction of the national regulatory organs and manufactures under the risk of pandemic.

PANEL: Impact of the Cuban Experience in Regulatory Issues of Vaccines

Coordinator: Yanet Hechavarría. CECMED, Cuba

FACILITY LICENSING SYSTEM FOR VACCINES MANUFACTURING IN CUBA: 16 YEARS OF EXPERIENCE

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Introduction: One of the basic functions of the National Regulatory Authorities is the granting of licenses. Because of it, each country establishes the requirements to present and evaluate each request. This activity had its origins in Cuba in the early nineties when the licenses for the production of the vaccines VAMENGOC-BC® and Heberbiovac HB® were issued in 1993. The first regulatory document associated with the process was published in 1996. The work had, as its main objective, to evaluate the development and evolution of the license system of pharmaceutical operations in Cuba from the very beginning up to December, 2011. It also included the experience of its application to vaccine manufacturers. **Materials and Methods:** It was necessary not only a detailed revision and analysis of the regulatory bases related to the theme, but also the licenses emitted in the selected establishment during the chosen period. **Results:** It was identified that in the nineties some preliminary documents were published which established the process of license concession of production operations. In 2000 the system of obligatory fulfillment arose. It was completed with Rules two years later which stated the guide of execution and fulfillment and was improved in 2007. In 1996 just 20 % of the manufactures had acquired the license. In the late 2011, 100 % of them had already owned the authorization to carry on their pharmaceutical operations. **Conclusion:** After the work was concluded, it made clear that the process of license concession has progressed during the 16 years of evaluation, as from the point of view of the associated regulatory documents as by the adherence of the vaccine manufacturers to current systems.

A REGULATORY REVIEW ON THE USE OF THIOMERSAL IN CUBAN VACCINES

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Introduction: Thiomersal is an organo-mercurial compound used as preservative in pharmaceutical products since 1930, mostly in vaccines. Since 1999 the concerns about its use has been increased, mainly in paediatric vaccines, based on the hypothesis that high levels of this preservative, exceeding the recommended limits, could be the cause of neurological dysfunctions in children smaller than 18 months. Cuba is a manufacturer of vaccines and most of them contain thiomersal as preservative. The aim of this paper was to evaluate, from a regulatory point of view, the needs of thiomersal as preservative, as well as the quantity to be used in vaccines produced in Cuba. **Material and Methods:** A comparative analysis was conducted between Cuban vaccines and other equivalent vaccines available in the international market regarding the inclusion or not of thiomersal and the concentration used, also, a review of the potentialities of the Cuban industry for eliminating or reducing the quantities of thiomersal in vaccines and a proposal of strategies to reach this purpose were enunciated. **Results:** The conditions of Cuban industry to assume the challenge of eliminating/reducing thiomersal showed that it is possible and feasible to reduce the global content of thiomersal in vaccines in 87.27% for monodose and 36.4% for multidose

presentations. A guideline on how to proceed for licensing a vaccine with a reduced content of thiomersal was proposed. **Conclusion:** The concentration of thiomersal in Cuban vaccines could be reduced under a science-base and regulatory approach.

REGULATORY CAPACITY FACING INFLUENZA PANDEMIC AND THE GLOBAL ACTION PLAN: CUBAN NRA EXPERIENCE

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Introduction: Influenza viruses cause significant morbidity and mortality. Annually, 3 to 5 million people get severe illnesses and 0.25 to 0.5 million deaths occur worldwide. Vaccination is the key for prevention, but seasonal vaccines only had an efficacy of 30-50% in older adults and candidate pandemic vaccines are poorly immunogenic, so it´s expected that Flu viruses still provoke pandemic and ongoing sporadic outbreaks. H1N1 FLU Pandemic raised a global alert for National Health Systems. The present work describes the Cuban Plan to face the Influenza Pandemics and the actions taken to increase our regulatory capacity in correspondence with WHO Global Action Plan (GAP-I) for influenza Vaccines. **Materials and methods:** CECMED, together with policymakers, developed a National Pandemic Plan. Different Agencies/WHO recommendations were applied taking into account the H1N1 pandemic scenario. Regional workshops were performed to strengthen the National Regulatory Agency (NRA) capacity in evaluating the quality, safety and efficacy of vaccine candidates as well as for establishing requirements for an effective post-marketing surveillance. **Results and conclusions:** Pandemic vaccine was introduced in Cuba through the WHO Deployment Plan. This vaccine was donated due to its high cost. National Plan was adequate and covered different areas to guarantee the effective control of the disease: risk groups to be immunized, distribution table, vaccine characteristic, doses and the use and media/public information. Even when Marketing Authorization was not required, other regulatory actions (close monitoring of lots arrival, lot release of the vaccine and appropriate design for active post-marketing surveillance) were carried out. Cuban experience was share among other NRA's helping to identify the main challenges during the implementation of activities, leading the generation of a new Global Action Plan for Influenza Vaccines (GAP II), that remarkably contributes to refine the objectives and a follow-up for the activities described in GAP I, as well as a roadmap for technical implementation, sustainable approach to pandemic preparedness and affordability and effective distribution of pandemic vaccines by countries with low/none access to them.

IMPACT OF THE NATIONAL CLINICAL TRIALS INSPECTION PROGRAM DURING THE LAST 10 YEARS

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Introduction: The National Inspection Program for Clinical Trials (NIPCT) emerged in 2001 to assess compliance with GCP. Biological products, particularly prophylactic vaccines, demanded more rigorous requirements for the clinical evaluation considering their indication (prophylaxis) and target population (children). The aim of this Paper was to evaluate the results of applying NIPCT and to go in-depth with vaccines in the last 10 years. **Materials and Methods**: The Inspection activity for Clinical Trials was analyzed qualitatively and quantitatively in order to describe the major deficiencies found in inspections carried out during the last 10 years. At the same time, it was evaluated the compliance of GCP and described the measures taken as a result of the inspection. **Results:** In the reviewed period 94

inspections of clinical trials (CT) were conducted, 27.5% (26) of which corresponded to prophylactic vaccines. Inspected studies corresponded to Phase I, 32% (30), Phase II, 52.1% (49), Phase III, 15.9% (15) and 58.4% (54) were trials involving many centers, which are more complex because more researchers and clinical sites are involved. A 86.1% of the inspections were carried out during the study and also 13 follow-up inspections. The major deficiencies related to the promoter, the investigator and the monitor were identified during the process. It was demonstrated compliance with GCP in a 58.5% (55), partial compliance in 25.5% (24) and noncompliance in 16% (15).The most important actions taken as a result of inspections were quantified and described. **Conclusions:** The results obtained showed that the accelerated development of the biopharmaceutical industry, has brought an increase in clinical studies and the implementation and compliance with GCP, that together with the implementation of the NIPCT conducted by the CECMED since 2001, requires assessing and analyzing of the need for the Certification of Good Clinical Practice Clinical Sites and Services involved in clinical trials.

TUESDAY 19. AFTERNOON

• Meeting for the constitution of the Regional Network for 3Rs Alternative Methods

Coordinator: Dr. Mario Landys (Finlay Institute, Cuba) and Idania Rodeiro (CEBIMAR, Cuba)

RE-USE OF RABBITS IN PYROGEN TEST (RPT) OF ANTIMENINGITIDIS C VACCINE REDUCES THE NUMBER OF ANIMALS USED

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Introduction: Rabbits are still used for determining pyrogenicity of samples in the quality control of injectable products, since up to now, there is not a method for replacing this method. In case of biological products it is not recommended to re-use rabbits, due to possible cross-reactions. The National Institute of Quality Control in Health (INCQS) analyses near 300 injectable products and medical devices per year and around 50% are hyperimmune sera and vaccines. For assaying these products, 1200 rabbits are used per year. **Material and Methods:** New Zealand rabbits were injected iv and rectal temperature monitored by Pyromon® System (Ellab). For this study, 5EU/kg LPS-spiked and non-spiked Anti-Meningitidis C vaccine was assayed. Five rabbits per group were used, following the 48 hours-interval administration schedule: I – only spiked sample; II – one non-spiked and one spiked; III – two non-spiked and one spiked; and IV – three non-spiked and one spiked. The result of the last injection of each group (spiked sample) was compared to response of the group I in order to verify if there was any influence of previous non-spiked injections. **Results:** There was no statistical difference when comparing the four spiked responses for any of the products assayed what showed there was not influence of previous injections. **Conclusions:** It is possible to re-use a rabbit that received non-pyrogenic hyperimmune sera or vaccine up to four times in a one-week period without prejudice of animal response. By re-using rabbits, it is possible to reduce in 70% the amount of animals used in Rabbit Pyrogen Test.

INCENTIVE POLICIES AND PANORAMA OF ALTERNATIVE METHODS TO ANIMAL USE IN BRAZIL

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Research on alternative methods to animal experimentation is relatively new in Life Sciences. Although the criticism on animal use is as old as the scientific history, the importance of alternatives was systematized since the publication of The Principles of Humane Experimental Technique by Russel and Burch at 1959. This work introduces the 3Rs concept: refinement, reduction and replacement of animal use. Globally there are some publications and events dedicated to Alternative Methods; however they are concentrated in developed countries. Nevertheless, important scientific movements on this theme are occurring in Latin-America. In 2011, in Brazil, there was a governmental compromise to the implementation of the Brazilian Centre for Validation of Alternative Method (BraCVAM), proposed by important researchers in recent years. Moreover, the Ministry of Science, Technology and Innovation (MCTI) invited the National Institute of Metrology, Quality and Technology (Inmetro) and the BraCVAM proponents to organize the National Network for Alternative Methods (RENAMA). These two important organizations were conceived to encourage and implement new alternative methodologies and to coordinate validation studies. In this context, RENAMA will implement in Brazil, at a short time, six methods: cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests, skin corrosion and irritation, Bovine Corneal Opacity Permeability (BCOP) test and phototoxicity from OECD; and the Monocyte Activation Test (MAT) from European Pharmacopoeia. Inmetro, collaborating with the Globally Harmonized System Classification and Labelling of Chemicals (GHS), will implement *In Vitro* methods from OECD contemplated in RENAMA as well as the Skin Permeability test (TG428/OECD). All activities resulted in cooperation between BraCVAM, Inmetro and the Post-Graduate Program of Sciences and Biotechnology (Fluminense Federal University) to organize the 1th Latin-American Congress on Alternative Methods to Animal Use in the Education, Research and Industry (COLAMA 2012), creating a strong foundation for groups in this new scientific field.

NEW WAY FORWARD ALTERNATIVE METHODS IN BRAZIL: THE CREATION OF BraCVAM AND RENAMA

Presgrave O

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Introduction: Brazilian Law 11,794/2008 rules the animal use and created the Animal Experimentation Control Council (CONCEA) which possesses, among many other activities, the obligation of introducing and monitoring the use of alternative methods. The idea of creating the Brazilian Center for Validation of Alternative Methods (BraCVAM) came up in 2008 and in September 2011 cooperation between Oswaldo Cruz Foundation (FIOCRUZ) and the National Agency for Health Surveillance (ANVISA) plan its creation. By the same time, the Ministry of Science, Technology and Innovation (MCTI) created the National Network of Alternative Methods (RENAMA), which is coordinated by BraCVAM and the National Institute of Metrology, Normalization and Industrial Quality (INMETRO). **Objectives:** BraCVAM is to act by coordinating collaborative studies, looking up for funding and to recommend validated methods to be accepted by Brazilian authorities, following the Organization for Economic Co-operation and Development (OECD) Guideline 34. RENAMA objective is to join all laboratories interested in participating in the validation process, perform Good Laboratory Practice (GLP) accreditation and keep the network available for new collaborative studies. **Results:** The first result of this enterprise is a financial support from National Council of Technological and Scientific Development (CNPq). This funding will help the first activities of RENAMA on starting validation process. It will also be used for starting the process of laboratories

accreditation in GLP. **Discussion:** Creation of BraCVAM and RENAMA will lead Brazil to join countries that search for alternative methods either for experimental and educational purposes. By this way, it will possible to participate on international studies for validation of methods. Internally, BraCVAM and RENAMA will contribute to join institutions that work on alternative methods and it will be possible to develop its own validation strategy of new ingredients or products, especially those ones derived from nature.

CURRENT STATUS AND PERSPECTIVES IN ALTERNATIVES TO PYROGEN TEST.

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Pyrogens are any substance capable to induce a fever reaction when enter the blood stream. Regulators claim for Pyrogenicity tests as prerequisite for registration and commercialization of pharmaceuticals, biological, and biotechnological parenteral products. Pyrogen test has been performed since 1942 (USP 12) by the *in vivo* Rabbit Pyrogen Test (RPT). However, in the last 15 years this test has been increasingly replaced by the LAL assay in most pharmacopeial monographs. The LAL assay is an *in vitro* method able to detect endotoxins or LPS from Gram negative bacteria, and therefore is also known as Bacterial Endotoxin Test (BET). The LAL reagent is produced mainly in US and is solely obtained from the hemolymph of horseshoe crabs, which are threatened species according to IUCN. Mainly due to ethical, conservationist and economical reasons, several efforts are underway in the search for alternatives to the RPT and BET. The more advanced approaches are the Recombinant Factor C (rFC) assay and the Monocyte Activation Test (MAT). Our studies on MAT have revealed that Monocyte Activation Test (MAT) correlates with RPT for pyrogenicity test in Human Serum Albumin and probably other therapeutic proteins, providing higher safety level than LAL. We also have found that MAT could be used as LAL for validation of depyrogenation process. Finally, it will be presented our initial proposal of the Lobster Hemocyte Lysate (LHL) assay. This assay is based on the prophenoloxidase activating system of lobsters, and is able to detect endotoxins, (1,3)-β-glucans and peptidoglycans. The LHL assay seems a promising alternative pyrogen test that is able to detect main pyrogenic compounds, while the source of raw material is not threatened for the time being, but readily available.

THERAPEUTICS VACCINES WORKSHOP

SUNDAY 17 (MORNING) ROOM II

- **Session: Vaccines Design and Process Development**

  *Chairman: Circe Mesa (CIM, Cuba) and Bernd H.A. Rehm (*Massey University, New Zeland)

GANGLIOSIDE CONTAINING NANO-PROTEOLIPOSOMES: AN EFFECTIVE ADJUVANT FOR CYTOTOXIC T LYMPHOCYTE RESPONSE STIMULATION ON IMMUNE-COMPROMISED SCENARIOS THROUGH THE INHIBITION OF MYELOID-DERIVED SUPPRESSOR CELL FUNCTION

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Introduction: Vaccine design for cancer immunotherapy has to deal with immune-compromised patients mainly due to the presence of the tumor itself and the leukopenic state produced by chemotherapy. In this

sense a rational and unconventional design of adjuvants and immune-potentiators needs to be performed. The CIM developed a nano-proteoliposome, called VSSP, incorporating NAcGM3 gangliosides into the outer membrane complex of *Neisseriameningitides* that specifically promotes antigen specific CD8 T cells activation. In this work we assessed its capacity to protect CTL and to deal with the induced Myeloid Derived Suppressor cells (MDSC), in both immune-compromisedscenarios. **Materials and Methods**: Numbers, phenotype and function of CD8 T cells, MDSC and their interaction, were measured in two different established experimental settings: comparing groups of healthy or tumor bearing mice treated or not with VSSP and similarly evaluating the adjuvant effect on leukopenic mice (induced by the administration of the chemotherapeutic agent cyclophosphamide (CY)). Phenotypes of studied cells were evaluated by FACS and functions of CD8 and MDSC were evaluated, by CFSE proliferation, ELISPOT, ^{51}Cr released assay, adoptive transfer experiments and in vivo CTL assays. **Results**: In this work, we demonstrated that the ability of VSSP to induce CTL response is not affected neither in leukopenic nor on tumor bearing mice. VSSP induced not only a faster recovery of immune populations, but also protected the antigen-specific CTL response in CY treated mice, either alone or combined with other immunostimulatory molecules. In both, tumor and leukopenic systems, this effect seems to be due to the capacity of VSSP to impair the immunosuppressive capacity of MDSC. **Conclusions**: The use of VSSP as a cancer vaccine adjuvant might thus improve antitumor efficacy, not only by stimulating a potent immune response against tumor antigens, but also reducing the immunosuppressionprevailing in this kind of patients.

DEVELOPMENT OF EPITOPIC AND SUBUNIT HCV VACCINES: ROLE OF ADJUVANTS AND ENHNCER SEQUENCES

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Developing vaccines against HCV, the main viral cause of liver cirrhosis and cancer, is a global priority. Phase I trials on candidate subunit (E1E2/MF59, core-ISCOMATRIX) or $CD8^+$CTL-epitopic-peptide HCV vaccines (IC41 and core 35-44) showed that superior formulations (adjuvant/carrier or peptide combinations) is needed to augment their efficacies. We evaluated the immunogenicity of HCVcp, ARFP and $CD8^+$CTL-epitopic/polytopic-peptides from different HCV Ags formulated in various human compatible adjuvants/modalities in murine model. Escherichia coli-derived HCVcp, ARFP and synthethic (matrix-based-designed) H-2d and HLA-A2-restricted $CD8^+$CTL-epitopic peptides of C, E1, E2 and N, their polytopic peptides in tandems of CE1E2N, CE2NE1, NE1E2C or their mixture (C+E1+E2+N) were formulated in various adjuvants including: CpG(1826), M720, M206, M1312 (Montanides), Pluronic acid, Imiquimod or their cocktails (e.g:M720+CpG) with/without addition of PADRE. Alternatively, the DNA minigens corresponding to the polytopic-peptides were constructed in pCDNA vectors intact or fused to either ERss or HBsAg and used as immunogens. Formulation of HCVcp and ARFP in montanides developed the highest titers of IgG1, 2a, 2b, and that of IFN-γ and IL-4 cytokines (balanced and strong Th1/Th2 responses) with long-lived CD8+CTLs. Induction of IFN-γ by CTL-peptide immunizations was higher than multiepitope peptides. However Immune-dominancy of CTL epitopes on each other was less profound in Immunization by a single polytopic-peptide vaccine than mixture of CTL-peptides. Inclusion of Immune-enhancers (PADRE/ERss/HBsAg) and combination of CpG in other adjuvants generally had a synergistic enhancing effect on immune responses. DNA-prime/peptide-boosting immunization regimen augmented the Th1 and CTL responses towards partial tumor protection. Subunit and CD8+CTL-peptide HCV vaccines are safe and cost-effective but their efficacy to elicit strong immune responses should be optimized through application of novel immunopotentiators/adjuvants and/or enhancers. Precise recipes for mosaic linkage of the isolated epitopes will wait for deciphering the mechanisms governing the process of the isolated-epitopes in the context of polytope by immune system.

A NEW VERSATILE PLATFORM TECHNOLOGY TOWARDS THE RECOMBINANT PRODUCTION OF PARTICULATE VACCINES

Rehm B

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The formulation of antigens as particles in a size range mimicking infectious viruses and bacteria offers advantages over soluble antigens such as facilitated uptake by antigen-presenting cells (APCs), depot formation as well as co-delivery of antigens and immunomodulatory compounds to the same APC potentially controlling the type of immune response. A variety of bacteria are able to intracellularly produce polyester (polyhydroxybutyrate=PHB) inclusions which serve as carbon and energy reserve. Bioengineering of this natural polyester bead production process enabled the design and production of novel particulate vaccine delivery systems. A cost-effective, scalable and versatile particulate vaccine production process could be developed. This new technology offers an unprecedented design space accompanied with accelerated prototype development. *Escherichia coli* and the food-grade endotoxin-free bacterium *Lactococcus lactis* were engineered to produce spherical (PHB) inclusions which abundantly displayed the hepatitis C virus core (HCc) antigen or the two TB antigens, ESAT6 and Ag85A, respectively. In mice, the immune response induced by this antigen delivery system was compared to that induced by vaccination with only the soluble antigen. Vaccination site lesions were minimal in all mice vaccinated with PHB beads. Antigen displaying PHB beads stimulated an antigen-specific type 1 and 2 immune response. Moreover, a protective immunity was obtained in mice vaccinated against TB. Overall, this novel bead technology offers a safe and efficient vaccine delivery platform suitable for vaccination against viral infections and intracellular pathogens.

DEVELOPMENT AND SCALE UP OF THERAPEUTIC VACCINES AT THE CENTER OF MOLECULAR IMMUNOLOGY

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Introduction: Formulation and drug development Department within EPOVAC is in charge of leading the main transitions from lab to commercial scale of cytoquines and vaccines. Development and scale up of protein and protein conjugates with activity against tumors is one of the main objectives of the Center of molecular Immunology. Several vaccines are in the process of development such as: anti-idiotypemabRacotumumab using alum as adyuvant, ganglioside vaccines $NGCGM_3$/VSSP &$NACGM_3$/VSSP, and recombinant proteins like rP64K and rEGFlinked via glutaraldehyde are the most advanced project with good clinical results in lung and other types of cancer. **Results and Discussion**: As part of the transitions in the production platform, cell culture has been changed from spinners to stirred tank fermenters allowing a significant increase in the production capacity and a reduction in the overall cost. Other change that has been evaluated in trying to set up a robust platform is the transition from dialysis to ultra and nanofiltrationwhich enables the quicker transit through different scales and increased capacity to fulfill GMP and meet regulatory requirements.Clinical trials with products with all this technologies has shown aceptable consistency, which in principle allows to draw a broad design space of the different molecules. **Conclusions:** These product are in differente stages of clinical development: CIMAVAX is a marketed product against lung cáncer, and is widening its marketing spaces in other countries including clinical trials in ICH countries. CIMAbid is the second vaccine in having MAA and is being reviewed in the national regulatory agency as the time of this submission. Ganglioside vaccines are in Phase 2/3 clinical trials to show safety and efficacy.

QUALITY ATTRIBUTES, PROCESS PARAMETERS AND ITS IMPLICATION IN PROCESS CHANGE AND SCALE UP. A CASE STUDY FOR VSSP ADJUVANT/VACCINE

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Introduction: Nanomedicine is an emerging field which involves nanoparticle formulations for diagnosing and treating of complex disease. However, the Implementation of QbD concepts such as: Design Space, Critical Process Parameters (CPPs) and the Critical Quality Attributes (CQAs) have been more difficult in this area because the nanodrugs complexity. The Center of Molecular Immunology has developed a nano-therapeutic product (NAcGM3/VSSP) which is generated by hydrophobic incorporation of NAcGM3 ganglioside into the outer membrane protein complex (OMPC) of *Neisseria meningitidis* bacteria. Nowadays, this nano-formulation is assessed in clinical trials. However the increased demand of the product requires scale up and changes in the process of manufacturing, achieving not only the expected demand but also keeping the critical quality attributes. **Materials and Methods:** In this study, we evaluated the physicochemical properties (protein/ganglioside ratio, protein profile, size distribution, and zeta potential) and biological activity of NacGM3/VSSP vaccine obtained from different process conditions. **Results:** The results showed no difference between the physicochemical properties and biological activity for this vaccine obtained either by dialysis or ultrafiltration/diafiltration technologies. However the detergents relation used in the process formulation showed influence over both physicochemical properties (size distribution, and zeta potential) and antitumoral activity of NacGM3/VSSP vaccine; this fact classify it as a critical process parameter. **Conclusions:** The results obtained in this work allowed us to find both Critical Process Parameters and Critical Quality Attributes for this Nano-therapeutic vaccine and also suggest that the ultrafiltration/diafiltration technology could be used as VSSPs process formulation; which meets the industrial and regulatory/scientific requirements.

UPSTREAM PROCESS DEVELOPMENT TO PRODUCE A pDNA FOR A THERAPEUTIC VACCINE AGAINST HEPATITIS C VIRUS

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Introduction: The gene therapy is a promising process for the prevention treatment and cure of several diseases that is why has gained considerable interest during the last decade. This process requires considerable amounts of plasmid DNA that should be homogeneous with respect to structural form and DNA sequence. The plasmid DNA is an intracellular product, so productivity is proportional to the final cell-density and the specific yeast. For production of large quantities of plasmid DNA an efficient fermentation process needs to be established. Although the processes of production for many gene-therapy vectors have been developed for pharmaceutical companies, the information is scarce and usually not available to the scientific community. In this work, the fermentation process in high-cell-density culture for the production of pIDKE2 plasmid in a recombinant *E.coli* DH10B system was studied. **Material and Methods:** The recombinant host *Escherichia coli* DH10B bearing the plasmid pIDKE2 was grown in Fed batch conditions, the effects of different medium components on plasmid yield and cell mass were evaluated at 50L fermentor scale, with complex medium. **Results:** Scale-up of plasmid DNA production from 5L fermentor to 50L pilot scale fermentor was carried out successfully. The recombinant host *Escherichia coli* DH10B bearing pIDKE2, a plasmid for DNA immunization expressing the first 650 aa of the HCV poyiproteins from the 1b-Cuban isolate genotype, was grown in Fed batch conditions. Final

biomass concentration and specific plasmid DNA yield were increased 3 times in comparison with cultures grown on a standard laboratory medium (TB) on batch mode. The productivity of this process is much higher than the previous works which may be suitable for large scale production of DNA vaccine. **Conclusions:** The advantages of the procedure described over existing technology to produce pharmaceutical grade plasmid DNA for gene therapy include a high cell density culture, improved plasmid productivity and the elimination of undesirable process additives. By employing this simple, scalable and applicable approach we concluded successfully clinical trial (phase I) and currently is in Phase II using the pIDKE2 plasmid; which is the principal component of a candidate therapeutic vaccine against Hepatitis C virus.

STABILITY EVALUATIONS OF CIGB 247 NOMINAL ANTIGEN RECONSTITUTED SOLUTION

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Introduction: CIGB 247 antigen is a fusion protein comprising a mutated variant of Vascular Endothelium Growth Factor (VEGF), fused to a 47 aa fragment of P64k protein. The molecule is used in combination with the aqueous adjuvant Very Small Size Proteoliposomes (VSSP) in order to trigger an immune response vs VEGF, a molecule over-produced by more than 90% of human tumors. A formulation of lyophilized antigen was successfully developed at two antigen strengths (0.1 mg/ vial and 0.4 mg/ vial) showing good stabilities both in shelf conditions (5 ± 3^0C) and under accelerated conditions (25 ± 2^0C). Generally the stability of lyophilized products is affected after reconstitution. Herein we addressed this issue in the case of CIGB 247 antigen. **Materials and Methods:** Reconstituted solution stability was evaluated in terms of protein concentration (microcoomassie), integrity (SDS-PAGE) and aggregation profile (HPLC SE) in samples of six batches (three batch of each strength) stored 0, 24, 48 and 72 hours under two temperature conditions (5 ± 3^0C and 25 ± 2^0C). **Results and discussions:** All batches showed similar stability profiles at 5 ± 3^0C for 72 hours. Nevertheless, when stored at 25 ± 2^0C evaluated parameters were more affected for 0.1 mg strength than for 0.4 mg, suggesting a self stabilizing effect of increase protein concentration. This effect was visible as early as 48 hours indicating a reduction of protein stability at 25 ± 2^0C. **Conclusions:** Reconstituted solutions of antigen CIGB 247 should not to be stored at 5 ± 3^0C for more than 72 hours and can not be stored at 25 ± 2^0C.

SUNDAY 17 (AFTERNOON)

• Satellite Meeting: Mucosal Vaccines

Chairman: Oliver Pérez (Finlay Institute, Cuba) and Alí Harandi (University of Gothenburg, Sweden)

VACCINE ADJUVANTS FOR MUCOSAL IMMUNITY: HOP OR HYPE

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Most pathogens hijack the mucosal tissues to entry the body and/or establish infections in the mucosal tissues. Hence, development of vaccines and other preventive measures such as microbicides to counter mucosally transmitted infections represents a top priority. Mucosal immunization has recently attracted much interest as a means of engendering protection against mucosal pathogens. Nevertheless, only a handful of licensed human vaccines are currently given via mucosal routes. Development of a broad range of mucosal vaccines will require the development of safe and effective mucosal adjuvants and delivery systems. Recently, a number of immunomodulatory agents, including toxin based adjuvants, toll like receptor (TLR) agonists and non TLR-targeting immunostimulators as well as delivery systems have shown promise for mucosal immunization in experimental animals, and some have reached clinical trials. However, there is presently no mucosal adjuvant included in licensed human vaccines. An overview of mucosal vaccines with special emphasis on vaccine adjuvants that are in or close to clinic will be presented.

FINLAY ADJUVANTS FOR MUCOSAL VACCINES

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Background. Mucosal immunization is an attractive route of vaccination for its compliance, ease of administration, reduced infection transmission risk, inducing systemic and mucosal immune e response, and applicability to mass vaccination. Nevertheless, this is a challenge for the absence of mucosal adjuvants. Therefore, AFCo1 have demonstrated be a potent mucosal adjuvant. In addition, most vaccines require several doses which difficult the early full protection and subject loss for subsequent vaccination required. **Aims.** To determine the efficiency of mucosal immunization using model and vaccine antigens with Finlay Adjuvants and to present result of Unitemporal Vaccination (SinTimVaS) as a possibility to increase vaccine coverage. **Results.** AFCo1 function with model and vaccine antigens by mucosal and also by parenteral route with Th1 polarization, CTL response, and memory response. SinTimVaS (Single Time Vaccination Strategy) of 2 priming strategy (one mucosal and another parenteral). One Intranasal dose of AFCo1 and one Intramuscular dose of AFPL1 administrated in SinTimVaS, induce specific systemic and mucosal immune response against all antigens tested. SinTimVaS is not restricted to the combination of intranasal and intramuscular routes. It also functions by combinations of intragastric or sublingual, and subcutaneous routes. In addition, SinTimVaS also functions with other mucosal adjuvant like Cholera Toxin. Lastly, SinTimVaS induces regional and distal mucosal response. **Conclusions.** AFCo1 is a promissory mucosal adjuvant and SinTimVaS was effective against all antigens tested which could increases vaccination coverage, compliance, inducing systemic and mucosal immune responses.

GENETICALLY MODIFIED LACTOBACILLI AS MUCOSAL DELIVERY VECTORS

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Mucosal delivered vaccines could offer unquestionable advantages over traditional systemic vaccines. However, in order to design oral vaccines, a delivery system is needed to avoid the degradation of antigens in the stomach and to skew the immune response towards an adaptive immune response rather than the induction of oral tolerance. Lactic Acid Bacteria have been broadly used in the production of fermented foods and beverages (cheese, yogurt, wine…) and have been consumed in large amounts for thousand of years without causing health problems. Notable among them, is the group of mesophilic lactobacilli, since some strains colonize the human mucosal surfaces and contribute to their protection

against pathogen invasion. Also, some strains have shown intrinsic immune-stimulatory properties. Therefore, these probiotic bacteria are ideal candidates for mucosal delivery of therapeutic and prophylactic molecules. However, it is very important to realize that the construction of genetically engineered lactobacilli as mucosal delivery vehicles must ensure the safety for human consumption and the stability of the cloned gene without selective pressure. Our group has developed a technology to generate food-grade recombinant lactobacilli that express very efficiently heterologous genes.Furthermore, a biological containment mechanism based in the deletion of the *thyA* gene has been developed. The resulting lactobacilli strains are unable to grow in the absence of thymidine, thus preventing their spread into the environment.The construction of a *Lactobacillus casei* strain that produces camelid antibodies against rotavirus will be presented.

NASVAC, A NOVEL THERAPEUTIC VACCINE CANDIDATE AGAINST CHRONIC HEPATITIS B: FROM THE LABORATORY TO THE CLINICAL TRIALS

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Introduction: Chronic hepatitis B is a major health problem, with more than 350 million people infected worldwide. Available therapies have limited efficacy and require long-term continuous and expensive treatments, which often lead to the selection of resistant viral variants and rarely eliminate the virus. Immunotherapies have been investigated as a promising approach. Several vaccine formulations have been clinically tested in chronic patients, none of which have clearly demonstrated their efficacy so far. **Materials and Methods:** The Nasvac therapeutic vaccine candidate comprised two recombinant HBV antigens: HBsAg and HBcAg, both constitutes virus like particles. The preclinical evaluations were done on rodents, in which was explored the intranasal administration and also the simultaneous intranasal-systemic co-administration strategy. Several toxicological studies were done, including immuno-toxicological evaluations using HBsAg transgenic mice and adoptive transfer immunity experiments. Until now three clinical trials were concluded and a phase III was on going in Bangladesh. **Results:** Nasvac was very immunogenic in mice after intranasal or systemic administrations; the immune response elicited includes higher antibody titers, potent proliferative responses and the secretion of gamma interferon by spleen cells. Nasvac, have also demonstrated its safety in several toxicological studies. The clinical trials carried out in Cuba and Bangladesh demonstrate that Nasvac was safe an immunogenic in healthy humans and hepatitis B chronic patients. **Conclusions:** The results obtained with Nasvac in different clinical trials provide preliminary data about its efficacy in hepatitis B chronic patients.

CHALLENGES FACED IN COMMERCIALISING MUCOSAL VACCINE TECHNOLOGIES

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Introduction: Most current vaccines are administered by parenteral injection and only a few are commercially available for mucosal route administration; emphasizing the hurdles encountered in targeting the appropriate sites to induce protective immunity at both mucosal and systemic levels. Understanding how these responses are induced can help rational design of mucosal vaccine formulations, not only for

protection against pathogens, but also in non-infectious applications such as malignancies and reproductive conditions. In order to attract commercial interest to develop the technologies beyond the research bench, different strategies have been employed. **Methods:** In this presentation we will describe the development pathway of two vaccine delivery systems, designed for delivery via two mucosal routes of administration. The first is based on lipids and developed for oral delivery, while the second is constructed from metal nanoparticles for potential use in vaginal delivery. For the oral delivery system, improvements were made in terms of scale-up, reduction in manufacturing time, use of "green-synthesis" methods. Assessment of these modifications on murine *in vivo* responses was then carried out. Different microscopy imaging techniques were used to try and understand the interaction of the delivery system with immune sites in the digestive tract. For the vaginal delivery system, microscopy was also used to examine the physical properties of the nanoparticles and *in vitro* and *ex vivo* studies were carried out to assess their potential for application in mucosal vaccination. **Results and conclusions:** Implementation of simple physical and chemical modifications, that were considered necessary to enable translation of the research from the bench to the market, had significant impact on biological responses. Quality by design will lead to improved vaccines with better efficacy and reproducibility.

ORAL CHOLERA VACCINE: THE CUBAN EXPERIENCE (15 MIN)

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Introduction: World Health Organization (WHO) published alarming data on the re-emergence of cholera in many African an Asian countries, changing the earlier thinking that doubted the value of cholera vaccines, and stating that Oral Cholera Vaccines may become effective partners in rolling back the ever-more-aggressive *Vibrio cholerae* bacterium. In this context, promising results from a Cuban vaccines candidate Phase I/II trial are shown. The Peruvian epidemic became the basis for the attenuated, genetically modified 638 strain, the active ingredient for the new Cuban vaccine candidate. The assessment of safety and reactogenicity was particularly important, since live attenuated vaccines like ours tend to cause more adverse reactions. To assess immunogenicity and the potential protective efficacy of the attenuated strain was as important as to assess safety, reactogenicity and immunogenicity of the lyophilized vaccine candidate in subsequent stages. **Materials and Methods:** Since there is not an animal model that reproduces the pathology of cholerae, 14 double-blind, placebo-controlled clinical trials in healthy adult volunteers in Cuba with fresh culture of 638 strain, including a challenge study with a virulent strain were carried out at Tropical Medicine Institute Pedro Kourí, Havana, with fresh culture of 638 strain. Furthemore, other two studies were similarly designed and carried out with the formulated vaccine candidate in Phase I/II trials in Cuba and Mozambique. **Results:** During all clinical trials only a few amount of adverse event were reported, while vibriocidal antibodies seroconversión reach more than 97% in healthy adults volunteers in Cuba as in apparently healthy volunteers in Mozambique, where cholera is present together with very different nutritional, epidemiological, and environmental conditions. **Conclusions**: The cholera vaccine candidate 638 evaluated in non-endemic areas of cholera was safe, well tolerated and immunogenic in adults volunteers.

MONDAY 18 (MORNING)

• Session: Experimental and Clinical Evaluation of Cancer Therapeutic Vaccines

Chairman: Kalet Léon (CIM, Cuba) and Lutz Gissman (GCC, Germany

USING CONVENTIONAL CHEMOTHERAPY TO SUPPORT THERAPEUTIC VACCINES FOR CANCER

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Recognition of the first therapeutic cancer vaccine (Dendreon) renewed interest in use of cancer vaccine therapy. Literature is full of good phase II studies that have been unsuccessful in multi centre phase III studies. There are numerous reasons for this. A major issue is patient selection, with patients less likely to respond, having other co-morbidities. Several groups, including our own, noted these patients have higher inflammatory markers, raising the question of the role of anti inflammatories prior and during vaccination. We previously published that IMiDs are able to enhance the efficacy of the therapeutic vaccine based on the CT26 model, it has been reported that patients on lenalidomide respond better to Prevnar. Lenalidomide was selected for its anti-TNF activity, being anti inflammatory and having COX-2 inhibition, is anti-angiogenic, and co stimulatory. This led us to look at other commonly used agents with regards their possible role in enhancing response to vaccines. These have included gemcitabine, oxaliplatin and cyclophosphamide. We reported potentiation of the immune response by common chemotherapeutic agents, which is achieved via a number of routes. These drugs are first, directly capable of restoring tumour visibility to immune effector cells; and second, able to enhance adapative T-cell responses through improvements to DC maturation. We have shown tumour cells to exude a complex soup that is biologically active in supporting tumourogenesis, and have shown that chemotherapy can alter the make-up of this soup leading the a neutralisation of their pro-cancerous effects. These data suggests that combining standard chemotherapies with immunotherapies and vaccine-related modalities would be fruitful clinically.

THERAPEUTIC COMBINATIONS TO POTENTIATE CANCER VACCINES: A BRIEF SUMMARY OF CIM EXPERIENCE

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Introduction: Cancer vaccines are progressively becoming a therapeutic option for oncologist. However, only the rational combination of such vaccines with other oncotherapies is expected to provide a significant impact in the actual clinical settings. At the center of molecular immunology (CIM) several cancer vaccines, immune-modulatory treatments and oncospecific antibodies have been developed over the last 15 years. In particular, CIM vaccines in the EGF-EGFr system and the ganglioside NGcGM3 antigenic system are in relatively advanced stages of clinical developed. In this presentation the result of several preclinical studies of therapeutic combinations, conducted at CIM will be summarized. **Material and Methods:** I this work a broad scope of combinations is studied, using transplantable murine tumors on immune-competent mice. They include: a) Combinations of cancer vaccines (OVA based and EGF based vaccine) with standard chemotherapies (paclitaxel and carboplatin); b) Combinations of cancer vaccines (NGcGM3 based vaccine) with oncospecific therapies based on monoclonal antibodies (7A7-anti-EGFr antibodies); c) Combinations of cancer vaccines (OVA based and cellular based vaccines) with immunomodulatory therapies based on novel interleukin-2 muteins (no-alpha and no-gamma IL2-muteins). **Results:** The data will show how rational combinations can indeed significantly potentiate the therapeutic efficacy of cancer vaccines. We will illustrate with data three main concepts: a) some

chemotherapies like paclitaxel or carboplatin, but not others, like cyclophosphamide, are suitable for concomitant application of cancer vaccines; b) Combination of NGcGM3 based vaccines with anti-EGFr antibodies can have a synergistic anti-tumoral effect in tumors that co-express these two targets; c) Immune-modulation of regulatory T cells, with IL2 mutants, can be successfully used to potentate the activity of cancer vaccines. **Conclusion:** Overall our data illustrate the potentialities of therapeutic combinations. Moreover it illustrates the value of current CIM preclinical strategy for therapeutic combinations as a guiding tool for indentifying clinically appealing combinations.

UPDATE OF HER1 VACCINE FOR TREATMENT OF EPITHELIAL TUMORS: PRIME-BOOSTING STRATEGY TO POTENTIATE THE VACCINE EFFECT

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Introduction: The Epidermal Growth Factor Receptor (HER1) belongs to the erbB family and plays a central role in regulating neoplastic processes. HER1 can be found overexpressed in many human epithelial tumors which have been correlated with disease progression and bad prognosis. Active specific immunotherapy targeting this receptor constitutes a new approach for cancer treatment. A vaccine based on the extracellular domain of HER1 (HER1-ECD) adjuvated in Very Small Sized Proteoliposomes (VSSP), showed antimetastatic effect over a murine model, and is currently being tested in patients. **Materials and Methods:** Gene codifying for HER1 extracellular domain (HER1-ECD) was cloned into pcDNA3 and Lentiviral vector. Sera titters were measured by ELISA and tumor cells recognition by FACS. Viability of tumor cells was evaluated by MTT colorimetric method. CD8+ induced response was evaluated by IFNγ secretion. Antimetastatic effect was measured over 3ll-D122 tumor model. **Results:** Vaccination of mice with HER1/VSSP generates strong immune response in mice and monkeys. Vaccine is currently being tested in Phase I clinical trial in patients with refractory prostate carcinoma without any toxicity. Because antimetastatic effect of the vaccine showed to depend on CD8+ T cells, we evaluated a new strategy to increase the effectiveness of the vaccine which consists in priming with LV-HER1 and boosting with HER1/VSSP. This strategy increased the specific CTL response and antimetastatic effect of the vaccine. **Conclusions:** Our results demonstrated that HER1 vaccine is a good approach to circumvent HER1 tolerance inducing anti-metastatic effect in preclinical studies, and non-toxicity in patients. Besides, prime-boosting strategy by using LV vector resulted superior to HER1 vaccine alone in terms of CTL induction and effectiveness.

ANTI-EGF/EGFR THERAPEUTIC CANCER VACCINES AND THE WOUND HEALING PROCESS

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Introduction: Wound healing is a complex process involving blood clotting, inflammation, tissue formation, and tissue remodeling. It is common to utilize a multi-modal treatment approach towards solid tumors, often including surgical resection, and it has become apparent that some targeted vaccines can impair wound healing or cause increasing risk of peri-operative complications. There are limited data regarding the wound healing process of cancer vaccines blocking the EGF/EGFR system. The aim of these studies is to elucidate the non-clinical and clinical effects of the CIMAvax and the EGFR-based (HER-1 Cuban cancer vaccines) on the skin wound healing process. **Materials and Methods:** In the non-clinical setting, mice were immunized with an EGF-vaccine by intramuscular route. In the second study mice were vaccinated with the extracellular domain (ECD) of autologous EGFR to overcome the tolerance to self-EGFR. Because EGF/EGFR-signaling plays an important role in the inflammation stage of wound

healing; the main objective of these studies were to explore the possible role of the murine EGF and (m) EGFR-ECD vaccines in the croton-oil-induced ear edema and wound healing process in mice mimicking the possible post-surgical wound complications. In the clinical setting for the EGF vaccine we reviewed the reported wound complications in the current literature and an update the Phase I Clinical Trial for HER-1 vaccine in patients with prostatic cancer from epithelial origin. **Results:** Apparently, there were no deleterious effects of the anti-EGF/EGFR vaccines in the wound healing post-operative process. **Conclusions:** Taking into account that treatment with anti-EGF/EGFR vaccines inhibits tumor cell proliferation, and the lack of deleterious effects of these vaccines in the wound healing post-operative process; we suggest that these kinds of drugs could be maintained and their effects tested, with very special surveillance during the post-surgical period.

ADVANCES IN UNDERSTANDING OF PHOTODYNAMIC THERAPY-GENERATED CANCER VACCINES

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Introduction: The development of photodynamic therapy (PDT)-generated cancer vaccines was initiated around ten years ago by the report on the effects of a prophylactic vaccine on a mouse tumor model. This was inspired by the realization that inflicting photoreactive drug-mediated stress in cancer cells makes them a potent source for eliciting a strong sustained adaptive immune response against antigens contained in these cells. Further research resulted in the establishment of PDT-generated therapeutic whole-cell cancer vaccines that are of clinical interest for treating a variety of malignant diseases. **Materials and methods:** Unlike in standard clinical PDT, where the patent is administered a photosensitizing drug followed at the predetermined time interval by a direct exposure of his tumor to light, PDT vaccine protocol calls for treating a fraction of operatively resected tumor tissue by PDT *ex vivo* to produce the vaccine material for injecting into the patient from which the tumor tissue originated (autologous vaccine). Our pre-clinical research on such vaccines was based on mouse model of human head and neck cancer (SCCVII tumors). **Results:** One of the key factors contributing to PDT vaccine efficacy is the expression of PDT-induced molecular/biological changes in vaccine cells, particularly those associated with cell death. Relevant cell death-associated changes include the progression of apoptotic and necrotic death processes with the emergence of death signal molecules on the cell surface and the upregulation of genes in vaccine cells responsible for production of important immune response mediators such as heat shock proteins. **Conclusions:** The process of phagocytic disposal of injected vaccine cells is pivotal for the optimal presentation of offered antigenic repertoire. The potency of PDT-generated autologous whole-cell cancer vaccines depends critically on the expressed cell death-associated molecular patterns (DAMPs) and alternate elements among phagocytic receptors and phagocytic cell types engaged in the process of vaccine cell efferocytosis.

CIGB-247: CANCER THERAPEUTIC VACCINE USING THE VEGF MOLECULE AS TARGET

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Introduction: The vascular endothelial factor (VEGF) and its receptors is the main growth factor system involved in tumor angiogenesis. Cancer immunotherapy targeting VEGF or its receptors is an emerging strategy for controlling tumor growth and progression. Along this research line, our group has developed the cancer vaccine candidate CIGB-247, that comprehends a recombinant modified human VEGF antigen, combined with a clinically tested bacterial adjuvant (very small sized proteoliposomes or VSSP), produced from the outer membrane of *Neisseria meningitidis*. **Materials and Methods:**. The anti-tumor

and anti-metastatic effects of the CIGB-247 vaccine were tested. The inmunogenicity and safety studies were also carried out in New Zeland rabbits, Winstar rats and in *Cercopithecusaethiopssabaeus*non-human primates. These results support the further clinical development of the CIGB-247 therapeutic cancer vaccine and a Phase I clinical trial is running. **Results:** Vaccination with CIGB-247 produces anti-tumor effects in mice injected with B16F10, CT26 and F3II tumor cells, measured as a reduction in tumor engraftment, slower tumor growth kinetics, and/or increased animal survival. CIGB-247 vaccination also significantly reduced the number and size of experimental and/or spontaneous metastatic tumor foci in lungs for the CT26, 3LL-D122 and F3II tumor models. Vaccination produces antibodies that block VEGF–VEGF receptor interaction in mice, rats, rabbits, and non-human primates. Specific T-cell cytotoxic responses against tumor cells that produce VEGF were documented in mice. Positive DTH and specific T-cell cytotoxic responses against autologouds VEGF-charged PBMC were found in non-human primates after vaccination. CIGB-247 and show an excellent safety profile in mice, rats, rabbits, and non-human primates. **Conclusions:** Altogether, these results support the clinical development of the CIGB-247 therapeutic cancer vaccine, and inform on the potential mechanisms involved in its anti-tumor effect

CHARACTERIZATION OF THE CYTOTOXIC ANTIBODY RESPONSE AGAINST NEUGCGM3 GANGLIOSIDE ELICITED IN NON-SMALL CELL LUNG CANCER PATIENTS IMMUNIZED WITH AN ANTI-IDIOTYPIC ANTIBODY

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Introduction: N-glycolylated (NeuGc) gangliosides are tumor related antigens, important for tumor biology and that are not naturally expressed in normal human tissues. 1E10 mAb is an anti-Id murine mAb (Ab2 mAb) specific for an Ab1 mAb that reacts with NeuGc-containing gangliosides. In this study, we report on the immune responses elicited in 20 non-small cell lung cancer patients treated with 1 mg of aluminum hydroxide-precipitated 1E10 mAb. **Materials and Methods:** The anti-1E10 and anti-NeuGcGM3 antibody response elicited in the cancer patients was tested by ELISA and by flow cytometry against tumor cell lines expressing this antigen. The capacity of the sera to induce tumor cell death was assessed by propidium iodide incorporation assay and the morphology of the cells was studied by light, fluorescence and scanning microscopy. **Results:** In the hyperimmune sera from 16 of 20 patients, a strong specific Ab response of both IgM and IgGisotypes against NeuGcGM3 ganglioside was observed. The induced anti-NeuGcGM3 antibodies not only recognized, but also directly killed tumor cells expressing the antigen, by a mechanism independent of complement activation and different from apoptosis. It is a very quick process and involves cytosqueleton reorganization. The antibodies induce cellular swelling and the formation of big membrane lesions that allow the leakage of cytoplasm and the loose of the cell membrane integrity. All these characteristics resemble a process of oncotic necrosis. Significant immunoreactivity and cytotoxicity to NeuGcGM3 was still detected after the complete abrogation of the reactivity against 1E10 mAb by the adsorption of patients´ sera with this Ab. **Conclusions:** To our knowledge, this is the first report of the active induction in cancer patients of NeuGcGM3 specific antibodies able to induce oncotic necrosis to tumor cells. We hypothesize that Id-Ag+ Abs could reflect the activation of an autologous ganglioside related idiotypic cascade into the patients. Patients that developed IgG and/or IgM Abs against NeuGcGM3 showed significantly longer median survival times.

CLINICAL TRIAL IIB/III WITH VACCINE NGCGM3/VSSP IN THE ADJUVANT SETTING OF OPERATED EARLY BREAST CANCER PATIENTS. APPLICATION EXPERIENCE IN THE HAVANA NATIONAL INSTITUTE OF ONCOLOGY AND RADIOBIOLOGY

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Background: Breast cancer is an enormous threat to women's health. It represents the third cause among the malignant diseases and the most common cause in the oncological affections suffered by the women all around the world, and the second cause of death in this population. In Cuba both indicators (incidence and mortality) are growing. Specific, active immunotherapy (vaccination) for cancer aims at generating or enhancing the immune response to cancer cells are under investigation. The rationale of this approach is based on the possibility of exploiting the specificity of the immune system to target cancer cells, with little or no associated toxicity to normal cells. National Institute of Oncology and Radiobiology (INOR) is the national reference institution for Oncology in Cuba and around 500 new cases of Breast Cancer are treated per year. We propose to illustrate our experience about the therapeutic vaccine Nglicolil-GM3/VSSP used with adjuvant intention in a serie of patients with operated early breast cancer in stage II-III and free of disease. **Methods:** The therapeutic vaccine Nglicolil-GM3/VSSP is proved in a clinical trial phase IIb/III. An exploratory analysis is been made to the first 150 included patients that had already concluded the programmed immunization chronogram, being evaluated the basal characteristics, toxicity profile and the appearance of relapse of the disease in this patients group. **Results:** The more frequent adverse events were: injection site pain, injection site eritema and induration, headache, arthralgia and fatigue. Most were classified as mild intensity. Relapse of the disease was presented in 17 of the studied patients with the presence of 12 deceased. **Conclusions:** In an initial evaluation to 150 patients included in the INOR, the use of the vaccine Nglicolil-GM3/VSSP in the adjuvant setting in patients with operated breast cancer, has shown to be safe and well tolerated. Further evaluation involving more cases must be performed to assess efficacy.

LONG-TERM SURVIVAL OF HIGH-RISK MELANOMA PATIENTS IMMUNIZED WITH AN HYPER-IL-6 MODIFIED ALLOGENEIC WHOLE-CELL VACCINE

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Introduction: Two single-arm, phase II trials (3 and 5) were undertaken to determine the efficacy and toxicity of an adjuvant treatment using Hyper-IL-6-gene modified whole-cell allogeneic melanoma vaccine in patients with stage IIIB-IV resected disease. **Research design and methods:** 97 and 99 patients were enrolled into trial 3 and 5. The primary endpoint was disease free survival (DFS), the secondary overall survival (OS). Vaccine was administered 8 times every 2 weeks (induction), then every month (maintenance) until patient's death. At progression, maintenance was continued or induction was repeated and followed by maintenance. **Results:** Median follow-up was 10.5 and 6.2 years for trials 3 and 5. No grade 3 or 4 toxicities were observed. An extension of DFS and OS was observed, as compared to historical non-treated controls. DFS probability at 5 years for trials 3 and 5 was respectively, 54.8% and 40.6% for stage IIIB, 25.0% and 24.0% for IIIC, and 8.5% and 17.7% for IV. OS probability at 5 years was respectively, 66.7% and 56.3% for IIIB, 43.8% and 39.8% for IIIC, and 26.1% and 41.2% for IV. **Conclusions:** Continuous vaccination, regardless of the disease progression, re-induction and immunization of patients until death, resulted in patients a long-term survival.

MONDAY 18 (AFTERNOON)

- **Session: Experimental and Clinical Evaluation of Therapeutic Vaccines**

  Chairman: Ana María Vázquez (CIM, Cuba) and Alexis Labrada (Biocen, Cuba)

TERAVAC-HIV-1: A MULTIANTIGENIC VACCINE CANDIDATE AGAINST HIV-1

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Introduction: Cell-mediated immune response to HIV-1 has been confirmed as an essential component of the mechanisms for viral replication control during primary and chronic infection. Currently, most of the vaccine candidates in clinical trials were developed to stimulate HIV-specific CD8+ and CD4+ cells. Additionally, the knowledge of the natural infection by HIV-1 emphasizes the importance of the mucosal immunity. Consequently, it is important to develop vaccine candidates for an effective mucosal immunization. We worked on a novel approach to develop a vaccine formulation (TERAVAC) using a recombinant multiepitopic protein (CR3), which comprises CTL and Th epitope rich regions of HIV-1 co-inoculated with the surface (HBsAg) and core (HBcAg) antigens of the hepatitis B virus (HBV) as adjuvant.**Materials and Methods:** Recombinant antigens CR3, HBcAg and HBsAg were produced with more than 95% purity. Humoral responses were tested by ELISA and western blot. The frequency of IFN-γ-secreting cells was quantified with an ELISPOT assay. Proliferation of CR3-specific CD4+ and CD8+ cells was assessed by CFSE staining and flow cytometry. **Results:** According to our studies in mice, the nasal-subcutaneous co-administration of TERAVAC induces a Th1-biased specific response against CR3, CD8+ T cells in spleen and IFN-γ-secreting cells in mesenteric lymph nodes. Cross-reactive p24-specific IFN-γ-secreting cells in spleen were also detected. Moreover, Nef-specific antibodies were elicited in mice sera which might avoid the toxic effects of this antigen. Additionally, we observed anti-HBsAg and anti-HBcAg cellular and humoral responses.**Conclusions:** This study has provided proof of the concept that the co-administration of the soluble recombinant protein CR3 with the main structural Ags of HBV through the nasal and subcutaneous routes results in the stimulation of HIV-specific cellular and humoral responses. A prominent aspect of this strategy is that it considers evolving scenarios in areas where a high incidence of HBV infection is reported.

ANTI-ATHEROSCLEROTIC EFFECT OF ACTIVE IMMUNIZATION WITH AN ANTI-PROTEOGLYCAN ANTIBODY

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Introduction: Atherosclerosis is a major health problem in developed countries and in Cuba. Among the strategies for its treatment, active immunotherapy continues to be an intense area of research and development worldwide. Up to now, therapeutic approaches to atherosclerosis have been largely limited to risk factors, targeting mainly hypercholesterolemia and hypertension. Our therapeutic strategy, not previously explored, is the use of an antibody that recognize glycosaminoglycans (GAGs) and acts as an idiotypic vaccine inducing autologous antibodies against these antigens able to inhibit the retention and oxidation of low-density-lipoproteins (LDL) in the arterial wall and thus preventing the development of atherosclerosis lesions. **Materials and Methods:** chP3R99 mAb reactivity to different GAGs was evaluated by ELISA. Induction of anti-GAGs antibodies by chP3R99 mAb immunization was evaluated in NZW rabbit and Apo E-/- mouse sera by ELISA. Inhibition of LDL-CS binding by chP3R99 mAb, animal sera and IgG from immunized animals was performed by competitive ELISA, and their capacity to prevent LDL oxidation *in vitro* by monitoring malondialdehyde formation. To assess the anti-atherogenic effect of chP3R99 mAb *in vivo*, Lipofundin MCT/LCT 20%-treated NZW rabbits and hypercholesterolemic ApoE-/- mice were subcutaneously immunized with low doses of chP3R99 mAb. Aortic arches were used for histopathology, ultrastructural and redox evaluation. Immunohistochemical studies were performed for detection of macrophages, CD4 and CD8 lymphocytes in aortas. Serum lipid parameters were measured. **Results:** Subcutaneous immunization of NZW rabbits and ApoE-/- mice with chP3R99 mAb prevented atherosclerosis. Histopathological and ultrastructural studies showed no intimal alterations or slight thickening, with preserved junctions between endothelial cells and scarce collagen fibers and glycosaminoglycans. In addition, immunization with chP3R99 mAb suppressed macrophage infiltration in aorta and preserved redox status. The atheroprotective effect was associated with the induction of anti-GAG antibodies capable to block GAG-LDL binding and LDL oxidation**. Conclusions:** These results support anti-sulfated GAG antibody-based immunotherapy as a potential tool to prevent atherosclerosis.

VACCINATION WITH chP3R99 MONOCLONAN ANTIBODY ATTENUATES THE OXIDATIVE STRESS INDUCED BY LIPOFUNDIN IN NZW RABBIT AORTAS

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Introduction: Atherosclerosis is the leading cause of death in the developed countries. Subendothelial retention of proatherogenic lipoproteins by proteoglycans is the key initiating step in atherosclerosis, being these particles more susceptible to oxidation. Atherosclerosis is characterized by a redox balance de regulation in the artery wall.The aim of this study was to analyze how an anti-proteoglycan chimeric antibody influences the behavior of redox biomarkers on lipofundin-induced atherosclerosis in NZW rabbits. **Material and metods:** The redox effect of chP3R99mAb*in vitro* was performed by a LDL Cu^{2+}-oxidation inhibition assay through the formation of malondialdehide. NZW rabbits were subcutaneously immunized with chP3R99 mAb (100 µg, 3 doses at weekly intervals) and atherosclerotic lesions were induced by the administration of Lipofundin (2 mL/kg, 8 days). Redox variables were spectrophotometrically measuredfrom thoracic aorta sections.**Results:** The monoclonal antibody specifically inhibited malondialdehide formation by 80%. Moreover, immunization of rabbits with chP3R99 mAb not only prevented Lipofundin-induced atherosclerosis but also preserved redox status in aortas. Moreover, this effect on redox environment was related to the induction of anti-chondroitin sulfate antibodies in chP3R99-immunized rabbits, also capable of prevent LDL oxidation*in vitro*.
Conclusion: These results demonstrated that immunization with chP3R99 mAb in rabbits preserve redox status.

PROGRESS IN THERAPEUTIC VACCINES FOR ALLERGY IN CUBA: FROM DEVELOPMENT TO INTRODUCTION

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Introduction: Allergen-specific Immunotherapy (ASIT) is the only therapeutic approach capable of modifying the underlying immunological basis of allergic diseases. ASIT has advantages over symptomatic treatments from the point of view of cost-effectiveness and long-lasting effect. However, implementation of wide programs of ASIT is hampered by thelimited availability of registered well-validated allergen vaccines, the duration of the treatment and the risk of systemic reactions. The sublingual routehas the potential to allow the expansion of ASIT, since the possibility of self-administration by the patient and a better safety profile. This work describes the Cuban experience regarding the development of allergen vaccines of three prevalent species of House Dust Mites, and its countrywide introduction into the public healthcare system. **Methods:** Allergen vaccines of *Dermatophagoides pteronyssinus*, *D. siboney* and *Blomiatropicalis*were developed as biopharmaceutical products following European standards. Their efficacy and safety in allergic asthma was assessed in 9 DBPC clinical trials, either by subcutaneous injection or by sublingual route. **Results:** Overall, treatment was effective for 76% of patients. Asthma symptoms and medication declined 60% ($CI_{95\%}$: 51-69) by SCIT or 56% (CI: 43-69%) by SLIT, as compared to placebo. Sublingual route showed no adverse systemic reactions and a lower frequency of local reactions. VALERGEN® vaccines were licensed for therapeutic use, becoming the first registered allergenic products in Cuba. An ongoing Phase IV Pharmacovigilance study in 1719 asthmatic patients in 24 allergy services is confirming the efficacy and safety of these products in the routine clinical practice, particularly, regarding Quality of Life improvement.The number of patients under treatment has been increased over 20 000 in a period of 4 years, achieving an estimated coverage of 5% of the target patients.**Conclusions:**Availability of high quality allergen vaccines becomes a valuable and cost-effective tool for expanding the etiological approach for asthma management.

TUESDAY 19 (MORNING)

- **Session: Cuban experience: From bench to clinical practice**

  Chairman: Beatriz García (CIM; Cuba) and Isis Torrens (CIGB, Cuba)

RACOTUMOMAB: AN ANTI-IDIOTYPE VACCINE RELATED TO N-GLYCOLYL-CONTAINING-GANGLIOSIDES: PRE-CLINICAL AND CLINICAL DATA

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Attractive targets for immunotherapy with antiidiotypemAbs are Neuglycolyl (NeuGc) containing gangliosides, because these glycolipids are not normal components of the cytoplasmatic membrane in humans, but their expression has been demonstrated in several human malignant tumors. Racotumomab is an antiidiotype mAb specific to P3 mAb, an antibody which reacts to NeuGc-containing gangliosides, sulfatides and other antigens expressed in tumors. Preparations containing Racotumomab were able to induce a strong antimetastatic effect in tumor-bearing mice. Different phase I clinical trials have been conducted in patients with advanced melanoma, breast cancer and lung cancer. The results of these clinical trials demonstrated the low toxicity and the high immunogenicity of this vaccine. The induced antibodies recognized and directly killed tumor cells expressing NeuGcGM3. A phase II/III multicenter, controlled, randomized,double blind clinical trial was conducted to evaluate the effect of Racotumomab-Alum in overall survival (OS) in patients with advanced NSCLC who have completed onco-specific treatment and have accomplished partial, complete response or disease stabilization. Patients were randomized to control group (placebo) or vaccine (Racotumomab-Alum). Vaccination consisted of 5 doses, at 14 day intervals (induction period), followed by a maintenance period (1 dose every 28 days) until patient refusal or worsening of ECOG status. Most common adverse events in both groups were injection site reactions and “flu-like” symptoms, grade I/II. Intent to Treat Analysis of OS showed a median OS of 8.27 months in Racotumomab group and 6.27 months in the placebo group (log rank test,p= 0.025), with a Long- term OS rate at 24 months of 17.0% in Racotumomab group versus 7.0%in placebo group.

When the analysis was performed Per protocol (patients that received 5 or more doses of vaccine or placebo) the median OS was 10.90 months in Racotumomab group and 6.90 months in placebo group (log rank test p= 0.0023), and the Long- term OS rate at 24 months was 22% and 8.0 % , respectively. A prospective, randomized, multicenter, open label phase III study to compare OS of patients with recurrent or advanced NSCLC who receive either Racotumomab-Alum and best supportive care or best supportive care alone is now ongoing.

CIMAvax EGF: A PROMISING VACCINE FOR NSCLC PATIENTS

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Epidermal Growth Factor Receptor is over-expressed in many epithelial derived tumors and its role in the development and progression of NSCLC is widely documented. CimaVax-EGF is a therapeutic cancer vaccine composed by human recombinant Epidermal Growth Factor (EGF) conjugated to a carrier protein, p64K from *Neisseria meningitidis*. The vaccine is intended to induce antibodies against self EGF that would block EGF-EGFR interaction. Several pre-clinical studies in mice have been done to proved immunogenicity, toxicity and immunopharmacological determinants. CimaVax-EGF has been evaluated so far in more than 1000 advanced NSCLC patients, as second line therapy. The results of a number of clinical trials in stage IIIb/IV NSCLC and prostate advanced cancer patients demonstrated the low toxicity and the high immunogenicity of this vaccine. The impact of the schedule of treatment in terms of immunogenicity and clinical response has also been studied. Recently, a phase III randomized, multicenter and open label clinical trial was conducted to verify the efficacy of CIMAvax-EGF in advanced NSCLC patients once they respond to the first line of onco-specific treatment. The most frequent adverse events were injection site reactions, fever; headache and vomiting (grade 1 or 2).The efficacy trial confirmed a positive correlation between survival and the magnitude of antibody response generated after immunization. Indeed, vaccinated patients classified as good antibody responders patients; who reached anti-EGF antibody titer higher or equal than 1/4000 during the first 6 months of vaccination; had better survival times. As predictive biomarkers, high baseline circulating EGF concentration was also associated with longer patient survival. Intent to Treat Analysis of overall survival (OS) showed significant longer OS for vaccinated patients. In summary**,** CIMAvax-EGF induced effective specific antibodies involved in the clinical benefit of advanced NSCLC patients. Notably, the baseline of circulating EGF seems to be a predictive biomarker of clinical outcome and the vaccination was able to improve OS in NSCLC patients, after first line of chemotherapy.

THE DEVELOPMENT OF THERAPEUTIC HPV VACCINE CANDIDATE

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Introduction: Human papillomavirus type 16 (HPV 16) infection has been linked to the development of cervical and anal dysplasia and cancer. One hallmark of persistent infection is the synthesis of the viral E7 protein in cervical epithelial cells. The expression of E7 in dysplastic and transformed cells and its recognition by the immune system as a foreign antigen make it an ideal target for immunotherapy. In animal models we have shown that a vaccine consisting of restricted MHC-I HPV16 E7 peptide adjuvated in very small size proteoliposomes (VSSP) is capable of eradicating malignant TC-1 $HPV16^{+}$ tumours in mice. CIGB-228 is a novel therapeutic vaccine consisting of HLA-restricted HPV16 E7 epitope adjuvated

with VSSP. This trial was designed to evaluate the toxicity, safety, immunogenicity, HPV clearance, and lesion regression. **Materials and Methods**: Seven women were entered. All were HLA-A2 positive, had biopsy-proven high-grade CIN, histological positive for HPV16, and persistent post-biopsy lesions visible by digital colposcopy were vaccinated. One weekly injections of CIGB-228 vaccine was given for four weeks. Then, loop electrosurgical excision procedure (LEEP) of the transformation zone was performed. Study subjects were followed for 1 year after LEEP. **Results**: No toxicity beyond grade 1 was observed during and after the four vaccinations. Five of seven women had complete and partial regression. Cellular immune response was seen in all patients. HPV was cleared in three of the patients with complete response. **Conclusions**: CIGB-228 vaccination was well tolerated and capable to induce IFN-γ-associated T-cell response in women with high-grade CIN. In several patients, lesion regression and HPV clearance were observed.

CIGB-230, A VACCINE CANDIDATE AGAINST HEPATITIS C VIRUS INFECTION: RATIONALE, RESULTS IN ANIMAL MODELS AND CLINICAL EVALUATION

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Introduction: Hepatitis C virus (HCV) infection is a worldwide health problem, causing cirrhosis and liver cancer. No vaccine is available against this pathogen and current treatments are still poorly effective for genotype 1 infection. CIGB-230 is a vaccine candidate based on the mixture of a recombinant HCV core protein with a plasmid for DNA immunization expressing the HCV structural antigens. The rationale, results in animal models, as well as in Phase I and II clinical evaluation of CIGB-230, will be presented. **Materials and methods**: Mice, rats and monkeys were used for the evaluation of the immunogenicity and/or toxicity. On the other hand, 15 and 92 patients, with detectable HCV RNA genotype 1b, non-responders or naïve to antiviral therapy, were included in the Phase I and II clinical trials, respectively. In Phase II, all patients received regular interferon (IFN) and ribavirin for 48 weeks. CIGB-230 was intramuscularly injected, with moment of administration and the number of immunizations as variables. **Results:** CIGB-230 elicited relevant humoral and cellular immune responses against HCV structural proteins in animal models. Stability and toxicity studies rendered satisfactory results. Clinical evaluation showed that CIGB-230 was safe. CIGB-230 was able to induce *de novo* specific proliferative response against HCV structural antigens, as well as to modify cross-reactive neutralizing antibody response, even in the unfavorable immunological scenario characterized by significant reduction of leukocytes counts due to IFN based-therapy. Moreover, concomitant administration of CIGB-230, particularly the 9 doses early add-on schedule, conferred an advantage for treatment outcome, increasing sustained virological response, and with a wider biochemical response, showing a better benefit-risk ratio than the control group. Mechanism of action and future developments will be discussed. **Conclusions:** CIGB-230 has demonstrated to be safe, immunogenic, and confers an advantage for therapy outcome when combined with current treatment, with rational bases for improvement.

PRECLINICAL EVALUATION AND CLINICAL OUTCOMES OF PROSTATE CANCER PATIENTS VACCINATED WITH HEBERPROVAC, A NEW GONADOTROPHIN BASED VACCINE

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Introduction: The development and functioning of the prostate gland, as well as its benign and neoplastic growth is dependent of androgen. Previous studies with Gonadotrophin Releasing Hormone (GnRH/LHRH) vaccines have demonstrated the usefulness of immunization against this hormone in prostate cancer. To this purpose, we generated a completely synthetic peptide modified at position 6 and attached to tetanic toxoid (TT) helper T cell epitope. **Methods:** For preclinical studies we emulsifying the mix of GnRHm1-TT peptide with Very Small Size Proteoliposomes (VSSP) and Montanide ISA 51 with mechanical adjuvation. The Phase I clinical trial using this vaccine candidate was carry out in 8 patients diagnosed with stage III/IV prostate cancer. **Results:** In animal experimentation, we demonstrated that rats immunized with the GnRHm1-TT peptide /VSSP/ Montanide emulsion, produced 1:6000 antibody titres against GnRH ($p<0,001$), decreased of testosterone levels to castration values (1,7nmol/L) and there was a three fold reduction of the prostate and testes weight ($p<0,001$). Similar results were obtained in rabbits and monkeys. This vaccine candidate generated a significant tumor growth inhibition of Dunning R3327-H androgen responsive prostate tumor in Copenhagen rats as well as in DD/S mice implanted with the hormone-sensitive Shionogi tumor ($p<0,01$). A clinical trial carried out with this vaccine candidate was completed with 6/8 patients diagnosed with advanced prostate cancer. No serious adverse side effects were detected. All 6 patients who completed the study produced antibodies titres and exhibited a fall in testosterone and prostate specific antigen. Over the ensuing 5 years these patients were healthy and maintained normal PSA. **Conclusions:** we conclude that the good results obtained in preclinical and in clinical settings make Heberprovac, the GnRH based vaccine that combine Tipe 1 and tipe 2 adjuvants, a good alternative for the treatment of advanced prostate cancer patients.

• Session: Regulatory Landscape for Therapeutic Vaccines

Chairman: Antonio Vallín (CIM; Cuba) and Andrzej Mackiewicz (Poland)

PROCESS SCALE UP TO COMMERCIAL: FROM CLINICAL RELEVANT DATA TO PRODUCT REGISTRATION IN RACOTUMOMAB VACCINE

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Introduction: Process and product development is complex, time and resource consuming from the organization. Companies around the world invest hundreds of millions of dollars to take a product to the market making medicines available only to few people due to the high cost of development and marketing. Guideline for design and development of products has not been designed until recently (ICH Q8, Q9) allowing to some extent the development based on risk management and knowledge from the inside and outside of the organization. A case study where knowledge gained by CIM in the development of a novel therapeutic vaccine has allowed rather quick process/product development, following a short cut regulatory pathway with Cuban regulatory authority to bring a product fast to the Cuban population while at the same time fulfilling regulatory and GMP requirements. **Materials and Methods:** State of the art analytical tools for characterization, coupled with process validation background and fast information availability and delivery to CECMED are explained in the details. A pathway known in other countries as "orphan designation and fast track review" will be described. **Results:** 1E10 vaccine has been scale up, validated and license in 4 month, a result that is typical achieved in 2-3 years time. Risk assessment not

only for the sake of “quality” but for the sake of patient availability was followed allowing a new molecule to enter in the arsenal of tools to make cancer a “manageable disease”.

SETTING QUALITY SPECIFICATIONS BASED IN IMMUNOGENICITY DATA FOR CIMAVAX EGF. AN ANALYTICAL POINT OF VIEW

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Introduction: CIMAvax-EGF is a therapeutic lung cancer vaccine. It is composed of a chemical conjugate of EGF and a carrier protein (rP64k) coupled trough glutaraldehyde reaction. Previous studies shown the antigen of this vaccine have two main fractions by SEC-HPLC (HMW and LMW) where each one is a mixture of several molecular species. The LMW are free and polymers of EGF. The HMW are rEGF-rP64k conjugates and its polymers. A new process to purify this antigen was developed using ultrafiltration by 50 kDa membrane. In this study it was evaluated the contribution to immunogenicity (production of Ac against rEGF) of each fractions and the results were used to proposed quality specifications for the product obtained by the new process. **Materials and Methods:** Two HPLC-fractions were separated from the vaccine antigen and three peaks composing the LMW fractions were also separated. The protein concentrations of each preparation were estimated and adjusted to a similar concentration of each one in a normal vaccine antigen batch obtained by the old process (dialysis purification). Groups of 5 NMRI mice were immunized with one dose of each preparation (and some Control groups) emulsified with Montanide ISA 51 and titles of serum were evaluated by ELISA. **Results:** Statistical differences among groups were evaluated by ANOVA. EGF and its polymers (immunized together or separated) did not produce significant antibody response. HMW fractions have higher specific activity than the whole antigen. **Conclusions:** It was establish a new specification for SEC-HPLC vaccine for the new vaccine process where the limit for the area% of rEGF-rP64k fraction have to be higher than 70% of the whole chromatogram area, against the previous 29%.

RELATIONSHIP BETWEEN CLINICAL OUTPUT AND PRODUCT QUALITY CHARACTERISTICS: AN ICH Q8 APPROACH TO 1E10 THERAPEUTIC VACCINE DEVELOPMENT

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Introduction: The design and conduct of pharmaceutical development studies should be consistent with their intended scientific purpose. It should be recognized that the level of knowledge gained, provides the basis for science-based submissions and their regulatory evaluation. The linking process-product-patient allow the implementation of the newly develop concepts of Quality by Design and Pharmaceutical Quality Systems. The Center for Molecular Immunology is currently developing a pipeline of around 20 projects and is seeking to implement these new concepts and regulatory opportunities to decrease the development time and better integrate the scientific knowledge subjacent in the organization. **Materials and Methods:** This work describes an attempt to implement product and process understanding through a matrix of correlation and factorial analysis between in process parameters, analytical release data, other quality attributes and clinical outcome. **Results:** The results showed that IgG title against 1E10 development on the patient could be a surrogate in those clinical trials, which had a positive correlation with % adsorption measured to the batches, and also this retrospective analysis allowed evaluating the criticality of vaccine quality attributes and builds a general rule for implementation as a tool in other development projects.

CANCER VACCINE CHARACTERIZATION: FROM BENCH TO CLINIC TRIAL

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Introduction: The development of safe, effective, and affordable vaccines has become a global effort due to its vast impact on overall world health conditions. A brief overview of vaccine characterization techniques, especially in the area of high-resolution mass spectrometry, is presented. It is highly conceivable that the proper use of advanced technologies such as high-resolution mass spectrometry, along with the appropriate chemical and physical property evaluations, will yield tremendous in-depth scientific understanding for the characterization of vaccines in various stages of vaccine development. This work presents the physicochemical and biological characterization of two cancer vaccines: Racotumomab and Her1-ECD. Racotumomab monoclonal antibody is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested as an anti-idiotypic cancer vaccine, adjuvated in Al(OH)3, in several clinical trials for melanoma, breast, and lung cancer. The Her1-ECD is a vaccine preparation based on the extracellular domain of HER1 and it is being evaluated in Phase I clinical study in patients with refractory prostate cancer. **Materials and Methods:** Racotumomab was obtained from ascites fluid and stirred tank fermentation, Her1-ECD was obtained from fermentation in two different media. The mass spectrometry was used for the amino acids sequence analysis, N- and C-terminal, glycosylation and posttranslational modifications. Also we used the DLS for the size distribution and zeta potential analysis. The biological analyses were performed in mice and chickens. **Results:** We observed differences in glycosylation pattern, charge heterogeneity and structural stability between in vivo-produced Racotumomab and bioreactor-obtained Racotumomab. Very similar results were obtained with the Her1-ECD molecules. Interestingly, these modifications had no significant impact on the immune responses elicited in two different animal models. **Conclusions:** We are demonstrated that this approach could potentially be more efficient and effective for supporting vaccine research and development.

DESIGN OF CLINICAL TRIALS FOR ACTIVE CANCER IMMUNOTHERAPY

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Skepticism for active cancer immunotherapy (ACI) has recently been broken by registration of specific vaccine for castration-resistant prostate cancer and non-specific immunomodulatory agent anti-CTLA-4 antibody. In the past significant number of ACI agents failed to demonstrate the clinical benefit for patients in regulatory trials. Accordingly, scientists are looking for the reasons of these failures, since number of vaccines demonstrated clinical efficacy in early phases of clinical development. One of the reasons discussed is the design of clinical trial protocols. Unfortunately, rules of ACI clinical trials design were adopted from those for cancer chemotherapy trials. Completely different pharmacokinetics, mode of action, safety profile, clinical end points of immunotherapeutic and chemical agents were and still are to certain extend ignored by regulatory authorities and clinical researchers. In general clinical testing in the current form is very expensive, time consuming and poorly designed what may lead to overlooking of products clinically beneficial for patients. Accordingly regulatory authorities and researches including Cancer Vaccine Clinical Trial Working Group proposed three regulatory solutions to facilitate clinical development of cancer vaccines: cost-recovery program, conditional marketing authorization, and a new development paradigm. Paradigm includes a model in which cancer vaccines are investigated in two types of clinical trials: proof-of-principle and efficacy. The proof-of-principle trials objectives are: safety; dose selection and schedule of vaccination; demonstration of proof-of-principle. Efficacy trials are randomized

clinical trials with objectives of demonstrating clinical benefit either directly or through a surrogate. The clinical end points are still under debate. New immune related classification of clinical responses has recently been proposed.

TUESDAY 19 (AFTERNOON)

• Regional Meeting on Combined vaccines based on DPT-HB-Hib-IPV (organized by CIGB, Cuba)

Chairmen: Eduardo Martínez, Néstor Expósito, Mabel Izquierdo, Miladys Limonta (CIGB, Cuba)

DEVELOPMENT OF ECUADORIAN PENTAVALENT LIQUID VACCINE IN A SINGLE VIAL

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Introduction: World Health Organization (WHO) has suggested to investigate and development vaccines with the major quantities of antigens, in order to prevent many pathologies in a single dose. For this, Ecuador and Cuba governments through your science and technology institutions have carried out the stages of a project for development and production an ecuadorian pentavalent vaccine in a single vial. Diphtheria, tetanus and pertussis(w) antigens are from "Instituto Nacional de Higiene" (INH-Ecuador) , superficial antigen of hepatitis B virus and poliribosilribitol phosphate are from "Centro de Ingeniería Genética y Biotecnología" (CIGB-Cuba). Compatibility of these active components was very important to determinate manufacture technology. In order to assure immunogenicity and potential efficacy was very important that pharmaceutical ingredients fulfill WHO quality specifications. **Materials and Methods:** Active pharmaceutical ingredients are from two sources: AgsHB and PRP-T produced in CIGB; tetanus and diphtheria purified antitoxins and bordetella pertussis whole inactivated cells produced in INH with quality parameters approved. Chemical reagents are from competent manufacturers. Pre-formulations studies was carried out according to general experimental methodology in order to select adyuvant, quantities of antigens in a human single dose used to inmunice childhood in Ecuador and adsorption kinetic of each antigen. It made experimental lots in a scale small, which had satisfactory results to biological and physical-chemical assays. Furthermore, it made scale major lots for carry out preclinic and toxicology studies and stability. **Results:** Aluminium phosphate was selected because its compatibility with bordetella Pertussis cells, but adsorption kinetic of diphtheria and tetanus anatoxind showed best results with aluminium phosphate. All of five lots formulated fulfill OMS quality control specifications for toxicology, physical-chemical and biological assays. After one year, ecuadorian pentavalent vaccine maintain these specifications. **Conclusions:** Ecuador has a Pentavalent vaccine liquid in a single vial that fulfill OMS quality requirements, its safety and efficacy has been showed, its stability is good to 6 and 12 months studied; these results allow to continue the follow stage of clinical studies.

ADVANCES IN THE CHARACTERIZATION OF A *BORDETELLA PERTUSSIS* DERIVED PROTEOLIPOSOME AS VACCINE CANDIDATE AGAINST WHOOPING COUGH

Pérez JL, Fernández S, Reyes G, Fernández Y, Fajardo EM, Mandiarote A, Landys M, Año G, Padrón MA, Acosta M, Cabreras R, Riverón L, Fariñas M, Díaz D, García L, Cardoso D, Campa C

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Introduction: *Bordetella pertussis,* the whooping cough causative agent, has increased its incidence worldwide among adolescents and adults in spite of the high vaccine coverage. That is the reason why the development of new vaccine strategies which provide a long lasting effective immunity has been recommended. Finlay Institute studies the properties of a *B. pertussis* derived proteoliposome (PLBp), purified from the outer surface of 165 wild type strain, as vaccine candidate. **Results:** Scanning electronic microscopy showed spherical structures 50-140 nm diameter which was confirmed by photonic correlation spectroscopy. Western blot using monoclonal antibodies revealed the presence of relevant antigens used in acellular vaccines. LAL assay showed inferior endotoxine levels when compared with other proteoliposomes vaccine, while pirogenicity assay was negative to 75 ng/mL. Histopathological studies performed after intranasal administration of 18323 WHO reference strain revealed remarkable differences in the lung tissue damage between PLBp immunized and DT immunized mice. In addition PLBp showed similar protective capacity than Finlay Institute DPT vaccine in challenge models. The administration of the PLBp formulated with tetanic and diphtheric toxoid (PLBp-dt) raised a high IgG response against *B. pertussis* in mice. **Conclusions:** This PLBp has shown its properties as vaccine candidate and its potentiality to prevent whooping cough in adolescents and adults in a PLBp-dt combined vaccine.

MONDAY 18 (MORNING)

BIOSEPARATION SESSION ROOM III

Chairman: Miladys Limonta (CIGB, Cuba) and Lothar Jacob (Merck, Germany)

OVERCOMING BOTTLENECKS DURING THE PRODUCTION OF VACCINES

Jacob L
Merck, Germany

Among therapeutic proteins especially vaccines make up an increasing proportion of new drugs. Their production requires robust and rapid processes with high purity and yield. Recovery and purification operations are the most time-consuming and the development of the individual purification strategy is complex. Although many process improvements have been made, designing a “state-of-the-art” down stream process (dsp) is still a key issue and requires a lot of resources. In contrast to small scale purification where ultra centrifuges can be used, for large scale manufacturing processes alternative strategies have to be developped. In many cases, chromatography plays a crucial role in such purification processes with a main focus on ion exchange chromatography. This technique results in high purities combined with high recoveries while the operational costs are rather moderate. In addition, for ion exchange steps, scale-up can be done efficiently from laboratory to production. As a consequence, the development of any downstream purification processes should include the responsibility of implementing appropriate state of the art chromatography media, techniques, equipment, and qualified raw materials. All purification issues should consider the transfer of technology to the process operating under cGMP conditions. To reduce costs, the binding capacity of the resins should be appropriate and the removal of unwanted substances must be considered as well as the re-usability of resins. Experiences with Fractogel® tentacle ion exchangers are subject of the presentation, as well as comparisons of chromatographic materials including and relevant factors for dsp. Also, technical data regarding scale-up procedures and safety aspects are covered.

VIRUSES USED TO BE TRADITIONALLY PURIFIED BY COMBINATION OF FILTRATIONS AND (ULTRA)CENTRIFUGATIONS

Jarc M
BiaSeparations, Slovenia

These methods and processes although well established, can be difficult to scale up and suffer from low productivity. With present demand for fast production of large quantities of vaccines or production of purified virus; techniques such as ultrafiltration and chromatography are often methods of choice. The presentation will focus on the purification methods for human viruses and bacteriophages with an emphasis on chromatography using CIM® monoliths. Basics of process development, including ligand, buffer and pH selection, method design, experiment sequence will be presented and discussed. Purification processes for different viruses will be presented and compared.

CHROMATOGRAPHY FOR REMOVAL ENDOTOXIN IN DIFFERENT VACCINES

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Introduction: Endotoxins (LPS) are unwanted by products of recombinant proteins, purified from *Escherichia coli*. The inherent toxicity of LPS makes their removal an important step for the proteins' application in several biological assays and for a safe parenteral administration. Affinity chromatography is applied with Ca^{+2}-iminodiacetic acid (IMAC-IDA), to remove most of the LPS contaminants from the end product. In this work, the adsorption of LPS by IDA-Ca^{+2} had been investigated. **Materials and Methods:** For this purpose, the binding behavior of LPS solutions was investigated with metal ion (Ca^{+2}) immobilized on different commercially available IMAC (Convective Interaction media, Tentacles, Membrane and Conventional resin). Adsorption isotherms at 25◦C were determined for the 3^2 experimental design considering pH (4.0, 5.5 and 8.0) and ionic strength - I.S. (0.25, 0.5 and 1.0 mol/L NaCl). **Results:** From the results, the best pH condition and I.S. to obtain the highest adsorption capacity (q*) between LPS-IDA-Ca^{+2} was evaluated in dynamic binding capacity. Vaccines model was evaluated in the different support obtained higher removal of LPS and recovery of protein. **Conclusions:** This process is recommended for the removal of contaminating LPS present in vaccines containing the biomolecules under different conditions of pH and ionic strength. Employing CIM technology, it can be obtained the highest procesativity on the removal of LPS.

ROOM POSTERS

PROPHILACTICS VACCINES WORKSHOP

Sunday 17

- **QUALITY CONTROL AND ASSURANCE / ALTERNATIVE METHODS FOR POTENCY AND TOXICITY OF VACCINES**

QUALITY RISK MANAGEMENT APPLICATION IN THE DPT-HB-HIB VACCINE FORMULATION OF NEW PLANT (PPP3) OF BIOCEN

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Introduction: Quality risk management is very important of the biological and biotechnological manufactures, the current Good Manufacturing Practice regulations and appropriate process quality improvement. The objective of this work is show the effective tools use in the DPT-HB-HIB vaccine formulation of new Plant (PPP3) of BIOCEN. **Materials and methods:** A multidisciplinary specialist's team was created (e.g., quality unit, regulatory affairs, production operations). This group studied the Quality Risk Management methodology according ICH Q9 and it was applied. We made the flow diagram, the process mapping, Cause and Effect Diagrams, and other quality improvement tools. Failure Mode Effects Analysis (FMEA) was employed and the Risk Priority Number, RPN was determinate. Risks were evaluated by a subjective measure of the severity of the effect (S), estimate of the expected probability of its occurrence (O) and means detection (D), and corrective action and preventive action were taken of risks mitigation. **Results:** Quality risk management methodology was applied in BIOCEN, as effective quality tool according to the current Good Manufacturing Practice. At new probability of occurrence (O) table was created according to historical data obtained in the biotechnology process of BIOCEN Risk Priority Numbers were calculated and the corrective action and the preventive action were taken. **Conclusions:** Quality risk management is the effective tool in the quality continuous improvement DPT-HB-HIB vaccine formulation of BIOCEN. **References:** ICH Q9 Guideline "Quality Risk Management " 2005. ICH Q10 Guideline "Pharmaceutical Quality System" 2008. IEC 60812 "Analysis techniques for system reliability – Procedure for failure mode and effects analysis (FMEA) 2006 .Proyecto Regulación: 16 - 2012 Directrices sobre Buenas Prácticas de Fabricación de productos farmacéuticos.

STANDARDIZATION OF AN ELISA FOR THE QUANTIFICATION OF TETANUS ANTITOXIN IN MOUSE SERA

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Introduction: Tetanus is a serious disease considered as the third cause of death among the immunopreventive illnesses. The vaccination with Tetanus Toxoid is the only effective way for preventing the disease. The quality and efficacy of these vaccines could be assured by challenge Potency tests in animals. The *in vivo* seroneutralization test (L+/10/50) is one of the most used assays, but the test has limitations in terms of ethic issues and variability. That's why some 3Rs alternatives have been evaluated in order to replace this test. **Objectives:** The aim of this paper was the standardization of a serological test (ELISA) in mice as an alternative for the L+/10/50 in order to determine the Potency of Tetanus

Toxoid vaccines. **Materials and Methods:** Working standards to be used as calibration curve and coating were characterized by L+ and Flocculation tests, respectively. The lineal range for the standard and samples was established. Besides, a Robustness study was developed. **Results:** The activity of the Standard serum was defined as 116 UI/mL by L+ (golden standard). The optimum concentrations for the coating (2Lf/mL) and the conjugate (1/7000) were established. A lineal range for the standard serum (1/800-1/51200, factor 2) and the samples (1/400-1/1600, factor 2) were also defined. As part of the Robustness study, the need of a blocking solution was dismissed. **Conclusions:** A serological test (ELISA) in mice was standardized as a potential 3Rs replacement for determining Potency of Tetanus Toxoid vaccines.

EVALUATION OF AN INTERNATIONAL STANDARD SERUM TO BE USED IN STABILITY STUDIES IN AN ELISA FOR DETERMINING POTENCY OF TETANUS VACCINES

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Introduction: A serological method (ELISA) in guineas-pigs was developed at Finlay Institute for replacing the traditional in vivo seroneutralization test in order to determine Potency for Tetanus Toxoid vaccines. In order to assure a good performance of this test, well characterized and stable standards must be established. The working standard and positive control sera were calibrated by L+, considered as the "golden standard", but an international guinea-pig tetanus antitoxin standard (98/572) for ELISA is also available. The aim of this Paper was to evaluate the suitability of the international standard in order to monitor by ELISA the activity of the working standard sera in shelf life stability studies. **Materials and methods:** The working standard sera were evaluated against the International Guinea Pig tetanus antitoxin serum in a stability scheme comprising an ELISA evaluation (9 vials per test) per year during 3 years. All samples were kept at the shelf life temperature (-70 ^{o}C) during all the study. The maintenance of the serum activities obtained during the characterization was monitored. **Results:** The shelf life studies showed an activity increasing regarding the certified value by L+. The overestimation of the activity seems to be due to the low titre of the international guinea-pig standard, which becomes in a disadvantage to evaluate samples with high Tetanus antitoxin titres as obtained in our in house-ELISA. **Conclusions:** It was demonstrated that the International guinea-pig tetanus antitoxin standard (98/572) is not appropriate to be used to monitor the stability of working standards by means of the serological guinea pig method (ELISA) developed at Finlay Institute for determining Potency of Tetanus vaccines. Some other tests (protein content) should be evaluated to be used in shelf stability studies.

OPTIMIZATION AND VALIDATION OF A MODIFIED WESTERN BLOT FOR DETERMINING IDENTITY OF MENINGOCOCCAL BC VACCINE

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Introduction: Identity tests for vaccines are part of the official requirements for lot release of bulks and finished products, as well as for licensing and commercialization. Traditionally, immunochemical tests like Western blot are used for determining Identity. Its application for determining Identity for Meningococcal B Outer Membrane Vesicles (OMV) allows identifying in a specific way the main antigenic proteins present in the intermediate and final products. However, the current Western used is longer than expected (2 days) for an Identity test. That's why the aim of this Paper was to optimize the test for one single day demonstrating that we are able to get the same results and validating the method under the new working conditions. **Materials and methods:** In general, the method was carried out as usual. However, times for transference, blocking, incubation of anti-P1, P3 and 70 K monoclonal antibodies and Conjugate at 37 °C

were reduced to one, a half, two and one hour, respectively. Under these new conditions, OMV and VA MENGOC BC vaccine samples were tested and the results were compared regarding the old conditions. For validation, it was performed a Specificity study as required for Identity tests. **Results and discussions:** There was no difference in the identification of the antigenic proteins (P1, P3 and 70 K) under the new and the conventional conditions, both for OMV and the finished product. So, it was possible to get information about the presence of protein bands that are relevant for immunogenicity in children in just one day. At the same time, the successful study for specificity revealed that the identification could be performed under the new conditions in a reliable way. **Conclusions:** The modified method allowed to identify the antigenic proteins bands corresponding to the proteins P1,P3 and 70 K in OMV and VA MENGOC BC vaccine in a shorter period of time.

VALIDATION OF CRITICAL SYSTEMS USED IN THE PRODUCTION OF ACTIVE PHARMACEUTICAL INGREDIENTS AND VACCINES AT FINLAY INSTITUTE

Nápoles L, Díaz Y, Hernández M, Fontanet L, López H, Cardoso D, Herrera Y, Heras N, Armona L, Hernández MA, De la Mora C, Toledo Y, Gómez R, Carcache J, Pulido M, Bayolo JV, Núñez N, Rodríguez AR, Mendosa L, Carmona S, Pumarada J, Reyes O, Muchuli O, Ortiz T, Fornells AR, Rodríguez P, Pluma E, Pérez L, Escarp A, Benítez JD, Aguilar S, Cardoso M, Garrido Y, Curvelo Y, García S, Pomares Z, Mandiarote A, Barberá R, Sartorio J, Martínez R and others.
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Introduction: Validation of critical systems is a requirement of current regulations of the pharmaceutical industry because it ensures compliance with good practices and quality of products. **Objective:** To show methodology and results in qualification of critical systems (air clean compressed, pure steam, purified water, water for injection and HVAC/clean rooms), used at Finlay Institute for production of active pharmaceutical ingredients and vaccines. **Material and methods:** Validation included installation qualification (IQ), operation qualification (OQ) and performance qualification (PQ) of each system. IQ and OQ included the reading the critical aspects and parameters. PQ of air compressed and pure steam included daily monitoring for one month, the ends of branches. PQ of water systems was done in 3 phases, differentiated by the frequency of monitoring, with a total length greater than 1 year. PQ of HVAC/clean rooms system covered the dynamic environmental monitoring during (1-3) moths of the amount of particles and micro organisms. **Results:** Systems met the installation criteria, operated properly and are capable of generating and distributing products that met, in general case, their quality specifications: ISO criteria's about dew point, microbiology and oil content in air compressed; USP criteria's about conductivity, total organic carbonmicrobiology and endotoxins content in pharmaceutical waters and pure steam; criteria recommended by EMEA, OMS and ISO about amount of particles and micro organisms in dynamic environmental monitoring to ensure the cleanliness classification areas. **Conclusions:** Critical system used at Finlay Institute for the production is considered valid for use. The results have been considered satisfactory by national and international regulatory agencies.

NEW TRENDS IN ASEPTIC PROCESSING: USE OF DISPOSABLE SYSTEMS FOR THE FORMULATION AND FILLING OF LOW-VOLUME PARENTERAL PRODUCTS

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Introduction: The use of disposable systems minimizes the risks of cross contamination, reduces the efforts to validate the equipment, as well as cleaning and sterilization systems, increasing the reliability and safety of the production process, ideal for multipurpose production facilities. There are two main types of disposable systems, flat bags and three-dimensional mixing (3D). **Materials and Methods:** In this study

we assessed the feasibility of using disposable plastic bags 3D with agitation system incorporated for the formulation and aseptic filling of an alum-adjuvanted vaccine. Consistency of bulk and finished product production process was evaluated by determining pH, thiomersal concentration, sterility of bulk and finished product and the concentration of aluminum as indicator of homogeneity. Validation studies of 3D mixing bags system and connection lines were also conducted by means of a simulation process using culture media .**Results:** The system is feasible for use in the formulation and filling of adjuvanted vaccines. The aluminum concentration, measured at three different points in the bag, meets the specification limits established showing homogeneity and consistency in lots of final product which meets the specified quality requirements. The results of the procedure used for validation studies demonstrate that an aseptic product could be obtained using this new system. An increase and better utilization of installed productive capacity was also reported. **Conclusions:** It was concluded that the use of disposable systems for the formulation and filling of low-volume parenterals may apply competitively among the new trends in aseptic processing.

VALIDATION OF THE STERILIZATION EQUIPMENTS FOR SATURATED STEAM (AUTOCLAVES) USED IN THE ANTIBACTERIAL VACCINES PRODUCTION AT FINLAY INSTITUTE

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Introduction: The validation of the sterilization processes that take place in the autoclaves is an international requirement of Good Manufactured Practices to the antibacterial vaccines production; its impact in the reduction or elimination of the microbiologic contamination risks and in the quality and security of the products, people and the environment. The objective of this work was to show the effectiveness of the sterilization processes that are carried out in some autoclaves used in the production and quality control at Finlay Institute. **Materials and Methods:** eight autoclaves were qualified. The validation process of each autoclave included 3 stages: Installation Qualification. It was verified: documentation, critical spare parts, substances for operation and maintenance, instruments calibration, components systems installation according to plans, makers' recommendations and devices of security. Operational Qualification. It was verified: the personnel's qualification, normalized procedures, control systems, integrity of filters, air tightness of the camera and it were carried out the temperature distribution with empty camera. Performance Qualification: validation of method to evaluate the quality of the biological indicators, temperature distribution with loaded camera, penetration of the heat with loaded camera and microbiologic challenge. **Results and Discussion:** In the Installation and Operation qualifications were obtained that the equipments are agree with the international validation approaches. In the studies of temperature carried out during the qualification of the operation and the performance (distribution and penetration) was obtained 100% of execution of the validation requirements. **Conclusions:** The autoclaves evaluated, are installed in an acceptable form, they operate correctly, so all of them are considered validated for the sterilization operation / decontamination during the productive processes to obtain vaccines.

VALIDATION OF A CHROMATOGRAPHIC METHOD FOR THE VALUATION OF THE MOLECULAR SIZE (Kd) OF THE PHARMACEUTICAL ACTIVE INGREDIENT OF THE CUBAN ANTITYPHOIDIC VACCINE

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Introduction: The quality control of bacterial origin polysaccharides, like Polysaccharide Vi purified from *Salmonella typhi,* (pharmaceutical active ingredient of the Cuban antityphoidic vaccine) includes the

determination of the molecular size (Kd), that is related with their immunogenic properties. This determination is carried out in terms of % of Polysaccharide that elute with smaller Kd to the Kd limit value (0.25) recommended by WHO. **Materials and Methods:** The test uses a chromatographic column so was necessary to carry out a calibration test of it. Also, due to the complexity of the method, described in the European Pharmacopeia, to evaluate the molecular size of the Polysaccharide C of *Neisseria meningitidis*, the parameters evaluated were the following ones: Precision (intermediate precision), Specificity and Robustness. **Results and Discussion:** It was verified that the operation of the chromatographic system is very good because % RSD obtained for all the studied parameters was similar to 0. The studied method demonstrated: a good precision, (% RSD = 4.9), acceptable for a physical-chemical method that involves two test methods: the chromatographic and the one used for the evaluation; to be specific, as well as robust before small variations carried out under the conditions test. **Conclusions:** For all the previously exposed the studied method was satisfactory for its employment in the laboratory, because all the analyzed parameters fulfill that settled down for a physical-chemical method of evaluation of biological samples.

QUANTIFICATION OF *NEISSERIA MENINGITIDIS* SEROGROUP X POLYSACCHARIDE BY PHOSPHOROUS NUCLEAR MAGNETIC RESONANCE

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Neisseria meningitidis constitutes the main responsible of meningococcal disease in infants. Serogroups A, B, C, W135, Y, and X have the higher incidence in young children and teenagers. Recently, serogroup X (PsX) has been found to be responsible of different outbreaks of meningococcal diseases, mainly in "Meningitis Belt" of Africa. In the last decade, the application of quantitative nuclear magnetic resonance had an increasing impact on the pharmaceutical industry. In this study one method by quantitative phosphorous nuclear magnetic resonance (qPNMR) is reported for the PsX quantification. Sample of PsX was supplied by the Department of Technological Development from the Finlay Institute, Havana, Cuba. Three set of samples were prepared dissolving different quantities (5–22 mg) of capsular polysaccharide in 0.6 mL of deuterium oxide. For phosphorous spectra, one capillary with di-sodium hydrogen phosphate (Na_2HPO_4) as reference was inserted into the 5 mm NMR tube. NMR analyses were carried out on a Bruker Avance DPX 250-MHz instrument operating with a 5 mm QNP z-axis gradient probe. HSQC experiment was optimized for $^{3}J_{H\text{-}P}$ = 7 Hz (^{1}H-^{31}P). An inversion–recovery experiment was run for the relaxation times calculation. For quantitative purposes the optimized qPNMR experiment was set with a total recycled time of 12 s, a pulse duration P1 was adjusted at 49.8°. The spectral data were apodizated by an exponential function (LB 2.0 Hz). The evaluated assay has been shown to be linear (proportional bias less than 1%), accurate and precise for intermediate precision conditions (relative standard deviation 4.6% for analyst-to-analyst variations) and can be used for purity evaluation of the polysaccharide of *Neisseria meningitidis* serogroup X..

DESIGN OF A METHODOLOGY FOR QUALITY RISK MANAGEMENT OF THE ASSAYS IN THE QUALITY CONTROL LABORATORIES FOR VACCINES, IN NATIONAL CENTER OF BIOPREPARADOS (BIOCEN)

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Introduction: The National Center of Bioproducts (BioCen), has implanted a Quality System (QS), certificate according to NC ISO 9001:2008. The commitment and object of the Quality Control Laboratory is to determine the quality and effectiveness of the vaccines, supported in indicators of efficiency that provide confidence and stability to the results. The continuous improvement of the performance of the QC laboratories should be a permanent concern, for that, it is required the integration of the Quality Risk Management system (QRM) to the Quality System (QS) of the laboratory, maintaining the trust, for its internal and external clients on the quality of laboratories testing for vaccines, the goal of this work was to design a methodology that allows to determine the factors of risk in the laboratory testing. **Materials and Methods:** A characterization of the current situation of the Quality Control Laboratory focused on the risk administration (RM) was carried out, following the recommendation of the International Conference of Harmonization (ICH Q9). In the diagnosis it was established that it is poor the directive staff, specialists and workers knowledge and training in the topic of RM, regulatory aspects related with this thematic should be included in the QS of the laboratory to asses the performance of the processes of Quality Control for the vaccines production. **Results:** As a result of our investigation it was elaborated and applied a methodology for the RM assessment for the testing of Quality Control laboratory for the vaccines.

QUANTITATIVE DETERMINATION OF SACCHARIDE IN DIFFERENT COMBINATION OF CUBAN SYNTETHIC *Haemophilus influenzae type b* BY USE OF HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSE AMPEROMETRIC DETECTION

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Introduction: The quantification of polyribosil ribitol phosphate (PRP) in vaccines containing Hib is one of the most relevant parameter to be tested. The values of PRP play an important role in the effectiveness of the vaccine. European Pharmacopoeia (2008) has established Bial Method for measurement of Total PRP content together with high performance anionic exchange chromatography couple with pulse amperometric detector (HPAEC-PAD). The aim of this work is shown that total PRP in different combination of vaccine with synthetic Hib can be quantified using two methods of hydrolysis by HPAEC-PAD, obtaining the expect values according to the specifications. **Materials and Methods:** Different type of samples were evaluated in the study: Vaccine QuimiHib concentrated, vaccine Hib adsorbed; Active Pharmaceutical Ingredient of Hib (API); PRP not conjugate to Tetanus Toxoid (PRP w/TT) and International Standard PRP. Samples of API, PRP w/TT and PRP international were prepared at concentration of 20 µg/mL. A HPAEC-PAD ICS3000 Dionex was used with Carbopac PA10, precolumn and aminotrap column for basic hydrolysis and Carbopac MA1, precolumn and aminotrap column for acid hydrolysis. The basic hydrolysis were evaluated with 2 conditions; 0.1 M NaOH and 0.3M NaOH, overnight at room temperature; acid hydrolysis was performed with 0.3 M HCl for 2 hours at 95°C, and then neutralized with 0.3M NaOH. Standard of PRP natural and Ribitol were used. **Results:** The values obtained for the samples were: PRP international used as control in the three conditions 0,1M;0.3M NaOH and 0.3M HCl (21.1-21.8-21.9 µg/mL), QuimiHib (36.2-38.4-42.5 µg/mL); Hib adsorbed (15.0-16.1-17.0 µg/mL) API Hib (n.e;16.1-21.3 µg/mL) and PRPw/TT (n.e in basic conditions-20.2 µg/mL). **Conclusions:** Samples of PRP international given similar values in all conditions, but synthetic Hib showed better results with acid hydrolysis and with 0.3M NaOH. We founded the conditions for evaluated monovalent vaccine with synthetic Hib by HPAEC-PAD.

DEVELOPMENT OF A METHODOLOGY FOR CLEANING VALIDATION AT FINLAY INSTITUTE

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Introduction: Cleaning validation is a very important element in production of pharmaceuticals and biologics, and an essential part of quality assurance and manufacturing. The key to an effective cleaning validation is to determine how clean is "clean enough". It is therefore that validation specialist people from Finlay Institute defined and implemented a strategy for the validation of cleaning processes as fermenters, tangential filtration system and other production equipment. **Materials and Methods:** It was established rinsing, swabbing, and visual inspection as sampling methods and the determination of Total Organic carbon (TOC) as the main tool for detecting residues of the previous product. The protocols to be applied were selected from an analysis of criticality and use (for example, if the dedicated or not) in each case. **Results:** Acceptable residue levels were determined to each equipment and cleaning process. The TOC technique proved to be simple, effective and easily applicable to cleaning validation purposes and sanitation procedures were adequate to reduce consistently the waste from the previous process and cleaning agents used. **Conclusions:** The developed methodology has already been implemented in all production facilities at Finlay Institute and it was approved by regulatory authorities from Cuba and abroad.

SCALE UP OF ACID PRECIPITATION STEP ON THE MANUFACTURING OF ACTIVE PHARMACEUTICAL INGREDIENT (API) FOR HEPATITIS B VACCINE

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Introduction: The changes in manufacturing process of an active pharmaceutical ingredient (API) for vaccines formulation involve a long and laborious procedure, because regulatory requirements are becoming stricter and great amount of analysis and results are needed to demonstrate that the modification does not affect final and intermediate products quality, as well as cleaning requirements for the equipments. In this paper the scale up of acid precipitation step for the API production of Hepatitis B vaccine is used as a case study in order to demonstrate the successful introduction of a process change in the production. **Materials and methods:** The scale up was carried out with scale factor of 20 using stirrer tank. The recovery of the stages were calculated by ratio of the total protein concentration determined by Bradford method and hepatitis B surface antigen (HBsAg) concentration estimated by Enzyme-Linked Immunosorbent Assay (ELISA). Characterization of peptide mapping of N and C terminal, transmission electronic microscopy and retention time in RP-HPLC of the HBsAg were used as complementary studies. **Results:** The comparative studies showed in both scales no significant statistically differences for inspection and control points of the production process, also in all case they met the established acceptance criteria. The characterization studies demonstrated the complete integrity and conformation of the HBsAg particle structure, and the similarity of the final product. **Conclusions:** The modification introduced into the manufacturing process has enhanced the consistency of the primary purification stage without affecting final product quality.

INFLUENCE OF THE LENGTH OF THE POLYSACCHARIDE, SPACER AND CARRIER PROTEIN ON CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE CONJUGATES OF VI POLYSACCHARIDE

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Introduction: Typhoid fever is a disease caused by *Salmonella enterica* serovar Typhi. It is estimated that annually sickens 21 million people, of those between 1 and 4 percent die. None of the existing vaccines against this bacterium covers the preschool population. However, it is showed that conjugating the capsular polysaccharide of the bacterium (Vi polysaccharide) to an immunogenic protein, is possible to achieve a high efficiency vaccine in young children. Finlay Institute and Biomolecular Chemistry Center are conducting the project "Production of a conjugate vaccine against Salmonella typhi". In this work we studied the influence of the factors polysaccharide length, spacer and carrier protein in the chemical and physical characteristics of the Vi polysaccharide conjugates. **Materials and Methods:** The length reduced polysaccharide was obtained by sonication. This kept the OAc content and continued been antigenic. The proteins used were tetanus and diphtheria toxoids. These were modified with adipic acid dihydrazide or hydrazine, with no changes in the distribution of molecular size and antigenicity. In all the conjugates obtained OAc content in polysaccharide was maintained and carbohydrate/protein ratio remained around 1/1. With the reduced length polysaccharide the obtained conjugates were smaller, with more unreacted protein. **Results:** No difference was found between adipic acid dihydrazide or hydrazine used, but when unmodified protein was used, conjugates were smaller. **Conclusions:** The size of the polysaccharide had an impact on the chemical and physical characteristics of the conjugates, while carrier protein and the spacer had a more moderate influence.

STABILITY STUDY OF *NEISSERIA MENINGITIDIS* POLYSACCHARIDE SEROGRUPS A AND C

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Introduction: Meningococcal polysaccharide vaccine is an active agent for immunization in order to prevent the infection caused by certain groups of meningococcal bacteria. Serogroups A and C cause approximately the half of the cases of meningitis produced by *Neisseria meningitidis*. The infection produces severe illnesses such as meningococcal meningitis and meningococcemia. Purified polysaccharides are used as active pharmaceutical ingredients in vaccines. The aim of this Paper was to establish the shelf-life for each polysaccharide by a real-time stability study, according to the international and national stability requirements. **Materials and Methods:** For that, 3 industrial scale batches of each polysaccharide were stored at the shelf-life conditions (-20 °C temperature for 24 months) and also under accelerated (2-8 °C for 12 months) and stress (37 °C for a month) conditions. Relevant stability-indicating methods for each Polysaccharide were used for the time intervals previously defined. **Results:** It was demonstrated that each Polysaccharide is stable at -20 °C during 24 months. This study showed a complete fulfilment of the test specifications, with no significant statistical differences ($p>0.05$) among the different time intervals for C Polysaccharide. On the other hand, we found significant statistical differences for the humidity, molecular integrity and O-acetil test results in the case of A Polysaccharide. Under accelerated conditions, the product humidity was affected starting from 6 and 12 months for A and C Polysaccharide, respectively. Under stress conditions the molecular integrity was affected for A polysaccharide while there was no change for C polysaccharide **Conclusions:** The real-time stability study allowed to establish a shelf-life of 24 months at -20 °C for both meningococcal polysaccharides.

ISOLATOR TECHNOLOGY APPLICATION IN THE STERILITY TEST OF THE VACCINE PRODUCTS IN BIOCEN

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Introduction: The sterility test is an important specification of vaccine products. The current GMP regulations specify that no retest is allowed and have the recommendation to use isolator technology as improvement to reduce false positive results. According to that, BIOCEN has taken the decision to design, validate and use the isolator technology for sterility test in its Quality Assurance microbiological laboratory. **Materials and methods:** A project team was created to define the URS of an isolator to perform the sterility test in BIOCEN's conditions. Different isolator's design and manufacturers were evaluated and the most appropriate design to fulfill BIOCEN's requirements was the isolator presented by the Italian manufacturer: COMECER. The isolator FAT test was carried out in Italy and the SATs test in BIOCEN, Cuba. After that, the IQ, OQ and PQ were performed according to the validation protocol. **Results:** An isolator design according to BIOCEN's URS was defined and installed in BIOCEN for the Quality Control of vaccines and other biotechnological products. The IQ, OQ and PQ were performed according to the previously defined validation protocol obtaining the expected results. **Conclusions:** The deigned isolator guarantees a safe environmental condition for the sterility test and make possible to fulfill the requirements of the GMP and current international regulations (EUPh, WHO).

ESTABLISHMENT AND MONITORING OF A AND C POLYSACCHARIDES STABILITY AS INTERNAL CONTROL FOR THE EVALUATION OF vax-MEN-AC® AND VA- MENGOC-BC® VACCINES

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Introduction: Meningococcal Disease constitutes a serious health problem for many countries around the world, affecting children and adolescents mostly, causing a big amount of deaths and in other cases leaving serious sequels disabling the patients affected. The efforts dedicated to the prevention of this disease are big, being vaccination the most efficient way for its control, hence that Finlay Institute has within the main productive lines the vax-MEN-AC® and VA-MENGOC-BC® vaccines. The concentration determination of O-Acetyl, sialic acid and phosphorus is conducted within the indispensable tests for the quality control and release of these vaccines. The present paper has as objective to formulate, characterize and evaluate the stability of a lot of A polysaccharide of *Neisseria meningitidis*, for the control of O-Acetyl and phosphorus and another lot of C polysaccharide of *Neisseria meningitidis* for the control of O-Acetyl and sialic acid. **Materials and Methods:** These lots were prepared at a concentration of 10 mg/mL of polysaccharide; the homogeneity tests, characterization, and stability were conducted by Svennerholm method, O-acetyl and phosphorus. **Results and Discussion:** Both lots turned out to be sufficiently homogeneous, the characterization values were 8,0601 mg/sialic acid and 14,911 µmol/mL of O-acetyl groups for poly C. The phosphorus concentrations for Poly A were 76,3709 µg of P/mg and 19,880 µmol/mL of O-Acetyl groups respectively. Both lots have kept the stability during two years in use. **Conclusions:** The lots of polysaccharides A and C of *purified N.m* comply with the requirements established and they keep their stability in the studied time, to be used as sample of internal control in the Biochemical and physicochemical laboratories for the release of the polysaccharide lots that are produced in the vaccine production plant III.

CONCURRENT VALIDATION OF THE PROCESS FOR OBTAINING THE SEROGROUP C *NEISSERIA MENINGITIDIS*' DRY POLYSACCHARIDE

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Introduction: Concurrent validation of the manufacturing process for production of the active pharmaceutical ingredient (API) is becoming a conditioning requirement for obtaining manufacturing licenses as well as sanitary registrations from regulatory authorities. This study was intended to assess the consistency, reproducibility and quality requirements fulfillment of the process for obtaining the serogroup C *Neisseria meningitidis* dry polysaccharide. **Materials and methods:** Before conducting this study, calibration of measurement instruments of all equipment involved in the process, qualification of critical equipment according to the manufacturing facilities' Master Validation Plan, and the validated state of critical systems (i.e. purified water, injection water, pure steam, and compressed and clean air) was checked. Based on the manufacturing process' production flux, records were designed for registering all critical variables of each process' step. Records were filled for three consecutive sub-processes simultaneously to the occurrence of individual steps. Recorded data was processed. **Results:** Process' reproducibility, consistency, as well as compliance of all quality requirements, were demonstrated. **Conclusions:** Process for obtaining the serogroup C *Neisseria meningitidis* dry polysaccharide is in a validated state.

NEW PROCESS EVALUATION AT LARGE SCALE TO OBTAIN CAPSULAR POLYSACCHARIDE PURIFIED FROM *NEISSERIA MENINGITIDIS* SEROGROUP W_{135}

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Introduction: Although two centuries have passed since Vieusseux described epidemic meningococcal disease, *Neisseria meningitidis (Nm)* remains a leading cause of meningitis and sepsis. There are substantial cyclical fluctuations in meningococcal disease incidence and the occurrence of outbreaks and epidemics. Thus, efforts to control the disease have focused on vaccination. Purified Capsular polysaccharides (PCP) are used in the production of vaccines against these bacterial. W_{135} is one of the five serogroups (A, B, C, W_{135} and Y)causing meningococcal disease and has been of increased interest after an outbreak among Hajj pilgrims in 2000 and an epidemic in Burkina Faso in 2002. The purpose of this work was to obtain PCP from *Nm* serogroup W_{135} by a new process at large scale. **Materials and Methods:** The new process was compared with the current procedure, using the same strain but different media composition and a purification procedure without phenol and ultracentrifugation steps. **Results:** The new process time was reduce by half, and the yield increased more than 7.7 folds, with large impact in cost reduction and environmental. **Conclusion:** The PCP obtained fulfills the WHO requirements and was used in a Men ACW vaccine with successful results in Clinical trial phase I/II.

BIOSAFETY MANAGEMENT SYSTEM DURING VACCINE PRODUCTION AT THE FINLAY INSTITUTE

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Introduction: In any organization, the different part of the system management should be integrated in only one system in order to provide the planification, resources and objectives to evaluate efficacy. The biosafety management is well defined in each step of the Finlay's management model and satisfactory fulfillment of the biological safety legislation in Cuba. A vaccine is a biological that provides immunity against a particular disease. It is usually made of dead or weakened microrganisms which cause the particular disease. Bio safety means the safe handling of infectious biological materials which can cause diseases. **Objective:** This work discusses how to comply with biosafety requirements during vaccine production in an integrated system. **Results:** A new management concept that combines biological risk assessment and mitigation of risks and their integration to quality systems as well as the implementing biosafety in laboratory procedures. **Conclusions:** It was implemented a security manual describing the biosafety aspects of the production process and the quality control activities with particular reference to infectious materials in various Biological Safety Rules.

PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF THE SURFACE ANTIGEN OF THE HEPATITIS B VIRUS (HBAgS) OBTAINED BY A NEW PRODUCTION PROCESS. COMPARABILITY

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Introduction: Current regulations establish that any manufacturing process of biological product where changes have been made, it is required to demonstrate that the drug product before and after changes is equivalent. This paper presents the comparability exercise results performed by the physical-chemical and biological characterization of the HBsAgr manufactured by a new technology, based on significant changes to the traditional manufacturing process of the vaccine against HBV, in order to increase productivity and decrease costs. **Materials and methods:** The AgsHB particle size distribution was performed by a sucrose gradient, transmission electron microscopic (TEM) and SCE- HPLC analysis. The identification of it was carrying out through mass analysis (MALDI) and Western Blotting test. The quantification of specific protein was by ELISA and purity by electrophoresis. The accelerated stability was evaluated using indicating assays (SDS-PAGE, SEC, Western blotting and ELISA). The biological properties of antigen were evaluated by the testing of: immunogenicity, antigenicity and cellular response. **Results:** The peptide maps and the N and C-terminal sequence of the studied batches were consistent with the profile of HBsAg obtained by the manufactured process traditional. The HBsAg particles had an average diameter between 25 and 27 nm, corresponds to that previously reported for HBsAg produced in P. pastoris. The AgsHB was stably for three months at 37 ºC, similarly to traditional antigen. The evaluation of the biological properties of the vaccine based on this antigen also showed similar performance to traditional vaccines. **Conclusions:** The AgsHBr obtained by the new process preserves the physico-chemical and biological properties of antigen produced traditionally in the IGBC, making it feasible to use in the production of vaccine against HBV.

RISK MANAGEMENT FOR THE PRODUCTION OF HBsAG, THE ACTIVE PHARMACEUTICAL INGREDIENT OF CUBAN VACCINE Heberbiovac HB

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Introduction: The production of recombinant surface antigen of hepatitis B (HBsAg), the active pharmaceutical ingredient of Cuban vaccine Heberbiovac HB, was established at Center for Genetic Engineering and Biotechnology since 1991. Some efforts have been conducted to systemic control of the process in order to avoid inconsistencies and product reject. In that sense risk management has come to help in the identification, organization and control of tasks and activities that give more robustness and security to the process. **Materials and Methods:** FMEA study was conducted and directed to four main risks: microbial and pyrogen contamination, purity and recovery/yield. Seventeen risks scenarios were preliminary established for each main risk identified in order to concentrate the attention of specialists, but with the possibility to introduce others. An administrator (manager) was selected and time schedule was defined. Specialists were selected from the more experience people from each stage and others that had worked in the process. Quantification of risk was figured out by risk priority number as product of probability, severity and detectability in an scale of 1 to 5 for each event. Evaluations of specialists were processed by statistical software and averages values were obtained for each risk scenario. A risk scoring criteria was predefined and a value of 27 or less was established as a remote impact. **Results:** Risk assessment allowed defining the principal risk and risking scenarios for the main steps of the process. Corrective and preventive actions were proposed to each identified risk and periodic check of their introduction has been done through annual product review and other quality assessment mechanisms. Recent check of the study has demonstrated that more that 90% of the action proposed has been implemented gradually in the process through change (variations) control system. **Conclusions:** After the application of all this system of procedures we can conclude that a quality risk management program has been established for this production process with successful results in quality and consistency of the product. Risk management should be a tool for the continuous improvement of the process.

APPLICATION OF A RISK ANALYSIS METHOD TO ASSESS DIFFERENT TECHNOLOGIES FOR PRODUCING A MONOCLONAL ANTIBODY EMPLOYED IN HEPATITIS B VACCINE MANUFACTURING

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Introduction: CB.Hep-1 monoclonal antibody (mAb) is used for the immunopurification of recombinant Hepatitis B surface antigen (rHBsAg), which is included in a worldwide vaccination program against the Hepatitis B. The use of CB.Hep-1 mAb as an immunoligand has been addressed into the most efficient step of rHBsAg purification. The analysis of the advantages and disadvantages of different antibody-production technologies open an interrogation about how can we choose the best alternative. Quality Risk Management (QRM) provides an excellent framework for the risk management use in pharmaceutical manufacturing and quality decision-making applications. **Materials and Methods:** This study sought applying a prospective risk analysis methodology Failure Mode Effects Analysis (FMEA) as QRM tool for analyzing four CB.Hep-1 mAb manufacturing technologies: ascites, *In vitro* technology, transgenic plants and transgenic animal process. **Results:** The severity and occurrence of risks analysis evidenced that the percentage of very high severe risks ranged 31.0-38.7% of all risks and the huge majority of risks have a very low occurrence level (61.9-83.3%) in all assessed technologies. The analyses of the overall impact of the criticability factor allowed corroborating that ascites and transgenic plant technologies have a significantly increased risk for the patients safety immunize with this vaccine by the isolation of the mAb from a biological source with high potentiality to be contaminated with pathogenic agents and glycosylation pattern different to mAb produced by ascites that could induce immunogenic and allergenic reactions in humans, respectively. Finally, additive Risk Priority Number was descending ordered as follow: transgenic plants (2636), ascites (2577), transgenic animals (2046) and hollow fiber bioreactors (1654), which also corroborated that *in vitro* technology, should be the technology of choice for CB.Hep-1 mAb manufacturing in terms of risks and mAb molecule quality. **Conclusions:** FMEA was successfully used to assess risks associated with potential problems in CB.Hep-1 mAb manufacturing processes.

DOT BLOT FOR IDENTITY OF POLYSACCHARIDES IN MULTIVALENT VACCINES AGAINST *NEISSERIA MENINGITIDIS*

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From the initial stages in the research and development of biotechnological products is important the application of the guides of recommendations elaborated by the Regulatory Organizations, related with the use of analytic methods necessary to demonstrate the identity, quality and purity of these products. *N. meningitidis* is a gram negative bacterium that has been classified in 13 serogroups. The serogroups A, B, C, Y and W_{135} are the causing of infectious invasive diseases. When the vaccines are compound by a mixture of different polysaccharides (multivalent vaccines), the analytic methods are complex for the interference between those polysaccharides or by the low concentration of those in the vaccine. Several methods have been used in the determination of the identity of polysaccharide in multivalent vaccines. The Dot Blot allows the detection of an antigen through their recognition for a specific antibody. The objective of this work was to determine the identity of the polysaccharides of *N. meningitidis* serogroups A, C, W_{135}, Y and X; the cross reaction among the different serogroups, as well as, the identity of them in the multivalent vaccines obtained in the vice-presidency of R+D from Finlay Institute. 2, 10 and 20 ug of each polysaccharide were applied in nitrocellulose paper and the optima concentration of the commercial serum (Remel) was determined. As result was obtained that all polysaccharides of *N. meningitidis* in samples of purified polysaccharides or in the tetravalent or pentavalent polysaccharide vaccines could be identified only with 2 ug of each polysaccharide and was not observed cross-reaction among polysaccharides in study. In conclusion, was established the conditions to determine the identity of polysaccharides of *N. meningitidis*, in samples of purified polysaccharides or in multivalents vaccines by a Dot Blot.

STABILITY OF A TETANUS WORKING STANDARD VACCINE FOR LETHAL CHALLENG METHOD

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Introduction: Tetanus toxoid is one the most extensively used antigens in vaccinations, both as monovalent vaccine as a component of combined vaccines. The efficacy of tetanus vaccine strongly depends on its potency. WHO requirements set up a challenge method for the determination of the potency by comparison the vaccine batches against a well characterized reference vaccine. The aim of this work was to evaluate the stability of the working reference vaccine, VDTR(1)/08, produced at Finlay Institute to be used in the WHO challenge method for determining Potency of Tetanus vaccines. **Materials and methods:** The working standard vaccine was previously characterised by the challenge lethal test. The in house standard was calibrated against the Third International Standard for Tetanus Toxoid, adsorbed and the activity assigned in Balb/c mice was 232 UI/ml. This batch was stored at 2-8°C. The stability was calculated by comparison with the International Standard for Tetanus Toxoid, absorbed. Four-dilutions of both vaccines were prepared. All animals were challeng with 50 DL50 Tetanus Toxin by subcutaneous injection. **Results:** All assays showed linearity and parallelism and the effective dose protecting 50% of animals (DE_{50}) ranges between the smallest and largest doses used. The toxin solution used to challenge was 36 – 50DL_{50}/ animal in all assays. **Conclusions:** The working reference vaccine, VDTR(1)/08 kept its biological activity.

PERFORMANCE EVALUATION OF PYROGEN TEST TO POLYSACCHARIDES A, C, W-135 AND Vax- MEN AC, Vax-TyVi VACCINES

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Introduction: The polysaccharide vaccine used as antigens, are widely used today and in our facilities that vaccines are made from active pharmaceutical ingredient (API) of purified polysaccharides of *Neisseria meningitidis* dry and *Salmonella tiphy* strain, which are extracted and purified in the production process. USP regulation provides the Pyrogen testing for these products. In order to ensure the quality of API, was performed performance Pyrogen test for polysaccharides A, C, W-135 and Vax-MEN AC, Vax-TyVi vaccines. This assay is based on ownership of certain substances called pyrogenic, to provoke, among other reactions, elevated body temperature of man and some species of laboratory animals such as rabbits, when its are administered intravenously. Consists in determining the elevation of body temperature in rabbits after intravenous inoculation of the test product. **Materials and Methods**: The Pyrogen assay was performed in two stages (sensitization of the colony and evaluation of the samples) following the protocol validation and criteria specified in USP. We used the reference endotoxin LAL assay, which was prepared in two dilutions, the first (2 mg / mL = 20 EU / mL) was used as positive control and the second (0.025 ug / mL = 0.25 EU / mL) to evaluate the sensitivity of the colony of animals and also was used the first as a positive control of the assay. Both steps were performed three times, by different analysts and batches of animals. The animals used were rabbits NZW strain, certificates, all from the CENPALAB, weighing 1,5 to 1,8 kg. **Results and Discussion:** The results obtained demonstrated in first place that animal colony currently used, is sensitive to the presence of pyrogens and the assay method showed consistency in all three times were evaluated, the results were satisfactory, complying with the acceptance criteria established for the test. **Conclusions:** The method we can use with reliable results.

MONDAY 18

• Current & Novel strategies in Profilactics Vaccines Development

OBTAINING AN ANIMAL FREE WORKING SEED FROM *NEISSERIA MENINGITIDIS* SEROGROUP X

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Introduction: The transmission of *Neisseria meningitidis* can be facilitated by overcrowding and the large regional population displacements due to pilgrimages to Mecca. This combination of factors explains the large epidemics. Serogroup X is growing cause of disease that is not covered by existing vaccination programs. Because only there are vaccines against meningitis caused by serogroups $ACYW_{135}$. The Finlay Institute is working in the pharmaceutical development of an alternative vaccine that includes serogroup X, so the main objective of this study was to evaluate and select a vaccine strain with high

growth potential in culture medium without components of animal origin and obtaining of cultivations for the production of working seed lot. **Materials and methods:** Two strains of serogroup X were evaluated (Bufa 7 / 97 and Bufa 2 / 97) in: Growth on free solid medium of animal ingredients, viability, and effectiveness of the medium culture to promote cell growth. The kinetics of growth in liquid medium was monitored by turbidimetry at intervals of 1 hour and viability the end of the culture. **Results:** The strain Bufa 2/97 had a greater adaptability to the culture medium with exponential growth kinetics from the very beginning of inoculum culture sizes as the expression of growth similar to those traditionally found in other serogroups of *Neisseria meningitidis.* The viability was in the logarithmic order of 10^8 CFU. **Conclusion:** The working seed of lot serogroup X *Neisseria menigitidis* was obtained with satisfactory quality parameters and high growth potential in culture media free of animal origin. Suitable performance biomass was achieved as part of drug development for the production of capsular polysaccharides and outer membrane vesicle vaccine purposes.

IMMUNOGENICITY OF NS4B DENGUE 3 VIRUS MIMOTOPE IDENTIFIED FROM A PHAGE DISPLAY PEPTIDE LIBRARY

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Introduction: The identification of dengue B-cell epitopes can provide important information for the development of safe and effective vaccines and contribute to the understanding of the pathogenesis and immunological response in dengue infection. Phage display has proven to be a powerful and economic technique for epitope identification and has been used in epitope mapping in flavivirus. Recently we identified B-cell epitopes specific to dengue non-structural (NS) proteins NS3 and NS4B, that is a small hydrophobic protein that has been associated with the virus replication complex and it is an antagonist of interferon. However has been poorly characterized. In the present work, we study the immunogenicity of a NS4B protein mimotope from dengue virus 3 to determine its possible role in the immune response to dengue infection. **Materials and methods:** A four arms multiple antigenic peptide system (MAP) was synthesized in the Synthetic Peptide laboratory at the Center for Genetic Engineering and Biotechnology. The MAP was synthesized according to NS4b mimotope sequence exposed in phages. BALB/c mice were immunized with the MAP and their sera were evaluated for the presence of anti-dengue specific antibodies by ELISA. The isotype distribution of mouse sera were assessed by the anti-peptide ELISA, but using the corresponding goat antimouse isotyping antibody. **Results:** Of the 10 immunized mice, three (R4, R7 and R8) showed positive immunoreactivity and the seven remaining did not induce significant IgG levels. The peptide-mimotope of NS4b did not induce antiviral antibodies. We found that the IgG2a levels were higher than the IgG1 levels suggesting a Th1 pattern. The highest IgG2a/IgG1 ratio was detected for mouse R7 followed by R8 and R4. **Conclusions:** NS4b MAP was immunogenic in mice and showed a polarization of the immune response toward a Th1 pattern.

HUMAN MONOCLONAL ANTIBODIES AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP B POLYSACCHARIDE. IS THERE ANY CHANCE?

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Introduction: *Neisseria meningitidis* (*Nm*) as epidemic cerebroespinal meningitis causal agent produces an increased morbidity and mortality. This bacterium has a capsular polysaccharide which particularly in serogroup B shows chemical and physical structural homologies with human Sialic Acid and a poor immunogenicity which make difficult the development of vaccines and monoclonal and polyclonal antibodies against this antigen. Recently, it has been created a variety of artificial libraries of human antibodies via the expression and selection in phages employing Molecular Biology technics. Those antibodies can be used as recognizing molecules capable to join to almost any existent target and as alternative to the conventional monoclonal antibodies, with the advantage of being obtained in less time and without animal immunization. Taking into consideration these antecedents, the use of human antibody libraries in phage is an attractive alternative for the production of monoclonal antibodies against "difficult" antigens as *Nm* serogroup B polysaccharide. **Materials and methods:** Our research was a basic experimental, prospective and descriptive piece of work, using a human scFv phage library (Tomlinson J) in order to identify ligands with the capacity to recognize *Nm* serogroup B polysaccharide. **Results and Conclusions:** The results obtained through Polyclonal ELISA screening against serogroup B and C *Nm* capsular polysaccharides suggest the existence of linear fractions of human antibodies (scFv) expressed in phages which recognized the antigen of interest. Preliminary phage monoclonal screening suggested the presence of several clones showing specific recognition a result that should be confirmed in future studies.

IMMUNOGENICITY OF HEPATITIS A VIRUS MIMOTOPES IDENTIFIED FROM A PHAGE DISPLAY PEPTIDE LIBRARY PRESENTED TO THE IMMUNE SYSTEM AS KEYHOLE LIMPET HEMOCYANIN CONJUGATED PEPTIDES AND MULTIPLE ANTIGENIC PEPTIDE SYSTEM

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Introduction: Costs and feasibility are the major barriers to implementing hepatitis A vaccination programs in developing countries. We have previously identified four HAV phage-mimotopes that induced specific anti-HAV antibodies in immunized mice. In the present study, the immunogenicity of two forms of presentations to the immune system of these mimotopes was evaluated, as an alternative source of antigen to develop cheaper hepatitis A virus (HAV) vaccines. **Materials and methods:** Four peptides containing the identified HAV mimotope amino-acid sequences were commercially synthezised (Pierce) and conjugated to Keyhole limpet hemocyanin (KLH) and a four-arms Multiple antigenic peptide system (MAPs) compose by two of these mimotopes was synthesized in the Synthetic Peptide Laboratory at the Center for Genetic Engineering and Biotechnology. BALB/c mice were immunized with the conjugated peptides and MAPs and their sera were evaluated for the presence of anti-peptide and anti-HAV antibodies by ELISA assay. **Results:** The four KLH conjugated peptides and the MAPs were able to induce anti-peptide antibodies. Anti-peptide antibodies at 42 days were only induced by 100 μg of the KLH conjugated peptides. The two dosis (10 and 100 μg) of conjugated peptides induced anti-peptide antibodies at 56 day. The higher anti-peptide titres were obtained for 100μg of antigen at 56 day. In the case of MAPs $_{46\text{-}56}$ only 100 μg of antigen induced significant anti-peptide response at 42 and 56 days. The four conjugated peptides induced anti-HAV antibodies. Anti-HAV antibodies were not detected when the MAPs was used as immunogen. **Conclusions:** The capacity of the peptide mimetics of HAV under study to induce specific anti-HAV antibodies depends on the molecular context in which they are presented being the KLH conjugated peptides more immunogenic than MAPs.

PROTEOLIPOSOMES FROM *MYCOBACTERIUM SMEGMATIS* INDUCE IMMUNE CROSS-REACTIVITY AGAINST *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS IN MICE

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Mycobacterium smegmatis is a nonpathogenic mycobacteria of rapid growth, which shares many characteristics with *Mycobacterium tuberculosis*, the major causative agent of tuberculosis. *M. tuberculosis* has several cell wall glycolipids in common with *M. smegmatis*, which play an important role in the pathogenesis of tuberculosis and the induction of a protective immune response against *M. tuberculosis* infection in some animal models. In this study, was evaluated the humoral immune response and cross reactivity against *M. tuberculosis* in mice of liposomes containing a mix of cell wall glycolipids of *M. smegmatis* and commercial lipids or liposomes containing only lipids from M. smegmatis. BALB/c mice were immunized with the liposomes and the specific antibody response and cross reactivity against *M. tuberculosis* were tested by ELISA. All the candidates induced cross reactive responses against Mtb. The formulations were recognized by the sera from pulmonary Tb patients suggesting the expression of these antigens during the active infection in humans. The results obtained argue in favor of the evaluation of liposomes containing lipids from Ms as TB vaccine candidate.

NEW FACILITY FOR VACCINE PRODUCTION.RESULTS IN THE PRODUCTION OF HEBERPENTA®-L

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Introduction: Current trends in the manufacture of vaccines are very stringent in their requirements. Based on this approach, BioCen designed, built and put into operation a new installation to formulation and filling of low-volume parenterals which was named "*Parenteral Products Plant number 3*" (PPP3). Different operations over different biopharmaceuticals are made in this plant, including the combined vaccine Heberpenta®-L that contains 5 antigenic fractions to protect against tetanus, whooping cough, diphtheria, hepatitis B and influenza type b. **Materials and Methods:** This work shows the details of design and construction. The Environmental Monitoring Program (EMP) was implemented in both resting and operation conditions on the premises of formulation and aseptic filling. Monitoring of critical support systems program was applied. Also, all operations related to simulations with Tryptone Soy Broth before starting manufacture, were carried out. In this case, plastic bags as batch final container were used. **Results:** Three consecutive Heberpenta batches were consistent with requirements. All parameters behave in accordance with expectations. Simulation with culture medium met acceptance criteria. **Conclusion:** The new *Parenteral Product Plant* in BIOCEN complies with GMP requirements to manufacturing low-volume parenterals.

HUMAN SECRETORY IMMUNOGLOBULIN-A EXERTS PROPHYLACTIC EFFECT AGAINST INTRATRACHEAL INFECTION WITH *MYCOBACTERIUM TUBERCULOSIS* IN AN EXPERIMENTAL MOUSE MODEL

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Introduction: Contrary to what was thought a few years ago on the dichotomy between cellular and antibody mediated immune response, at present it is considered that both types of response function as a whole, protecting the host against several pathogens. IgA antibodies can inhibit the adherence of bacteria and other antigens to mucosal surfaces by the properties conferred by the secretory component present in its structure, thus promoting the elimination of immune complexes through the respiratory cilia movement and intestinal peristalsis. The main objective of the present study was to evaluate the protective capacity of hsIgA against infection with *M. tuberculosis*. **Materials and Methods:** hsIgA was purified from human colostrum by a combination of purification methods and it was evaluated its reactivity against mycobacterial antigens and its biodistribution in several fluids of Balb/c mice after intranasal administration. Also, it was evaluated the protective capacity of hsIgA in a murine model against the infection with virulent strain of *M. tuberculosis*. **Results:** hsIgA showed a high recognition against mycobacterial antigens and its levels were detected during 5 and 3 hours, after intranasal inoculation in saliva and trachea-bronchial lavage. It was demonstrated the protective capacity of hsIgA administered by intranasal route against progressive infection by intratracheal route with *Mycobacterium tuberculosis*. Besides, *M. tuberculosis* preincubated with hsIgA induced a significant reduction of the bacterial load in lung and pneumonic area, with increasing in the number of granulomas. **Conclusions:** It was demonstrated, for first time, the prophylactic effect of the passive administration by intranasal route of hsIgA against *M. tuberculosis* infection. Thus, the previous incubation of the micobacteria with this antibody can inhibit the infective potential of the causal agent of tuberculosis, related to increased expression of iNOS in lung. These results could be applied to the design of a new vaccine against tuberculosis, which would induce a high production of hsIgA, able to interact with the micobacteria at the mucosal surfaces.

INFLUENCE OF THE ADJUVANT IN THE IMMUNE RESPONSE TO PROTEOLIPOSOMES AND LIPOSOMES FROM NON-PATHOGENIC MYCOBACTERIA IN MICE

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Tuberculosis is one of the leading causes of mortality due to infectious diseases. A new vaccine, more efficacious than BCG, the current vaccine in use, is urgently needed. The use of proteoliposomes and liposomes from mycobacteria as new experimental vaccines against tuberculosis is an attractive

approach. Proteoliposomes from BCG and *M. smegmatis* and liposomes from *M. smegmatis* were obtained and their humoral and cellular antigenicity was evaluated with samples from tuberculosis patients and healthy controls PPD+ and PPD-. The immunogenicity and induction of cross reactive responses in mice against *M. tuberculosis* were evaluated using different adjuvants. The antigenicity of the different vaccine candidates was demonstrated in humans. All the candidates demonstrated immunogenicity in mice and induced cross reactive responses against *M. tuberculosis* antigens. The profile of the immune responses obtained differed according the combination of experimental vaccine candidate and adjuvant used.

IMMUNOGENICITY ELICITED IN MICE BY A TETRAVALENT CAPSULAR POLYSACCHARIDE–TD CONJUGATED VACCINE AGAINST A, C, W135 AND Y SEROGROUPS OF *NEISSERIA MENINGITIDIS*

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Introduction: Meningococcal diseases are serious threats to global health. Five serogroups -A, B, C, W135 and Y- are responsible for the majority of cases. Vaccination as a means to prevent meningococcal disease caused by *Neisseria meningitidis* is critical given the abrupt onset and rapid progression of this disease. Vaccines utilizing the polysaccharide capsule are poorly immunogenic in young children. The conjugation of polysaccharides to protein carriers (nontoxic diphtheria mutant toxin [CRM197], diphteric toxoid or tetanus toxoid) alters the nature of the antipolysaccharide response to a T dependent response. The aim of this study was to investigate the immunogenicity of tetravalent conjugated vaccine against serogroups A, C, Y and W135 conjugated to diphteric toxoid (TD) in mice. **Materials and methods:** The general methodology for the conjugation was based in the reductive amination method. Alumine phosphate was used as adjuvant. The immunization schedule consisted on 3 doses (4 µg of each capsular polysacharide-TD conjugated) every 14 days in Balb/C mice. Characterization of the immune response was done by ELISA and bactericidal assay. **Results:** The immunization with the tetravalent formulation elicited high titers of anti-CPS IgG after the second and third doses. The avidity of anti-CPS IgG was enhanced from the second dose. The dominant IgG subclass for most of the conjugates was orderly IgG1, IgG3 and IgG2a. The serum antibodies have bactericidal effects against bacteria. **Conclusions:** These results demonstrate tetravalent vaccine immunogenicity. Induced antibodies were avid, specific and functional. Therefore, they are part of the preclinical support for the future development of Cuban conjugated vaccine against *N. meningitidis.*

METHODS TO ASSESS HEBERPENTA-L STABILITY IN DIFFERENT CONDITIONS

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Introduction: Heberpenta-L vaccine protects against diphtheria, tetanus, whooping cough, Hepatitis B and *Haemophilus influenza* type B. This vaccine contains the D, T and P natural antigens, the recombinant hepatitis B surface antigen and the PRP conjugated to tetanus toxoide obtained by chemical synthesis. For licensing a combined vaccine each vaccine component should be tested after combination. The shelf-life must be based on the shortest shelf-life component and should be supported on real-time

stability study. In practice, stability data of the final product should include the data generated on the intermediates of different ages used in the final. **Material and Methods:** Four batches of Heberpenta-L containing different batches of every antigen were submitted to stability study. The antigens storage time prior to formulation varied between 10 and 80% of its respective shelf-life. Orcinol method was used in real-time and accelerated stability studies, and also in stress condition, from 25 to 70 ºC, for quantifying free PRP as indicator of the Hib component integrity because it is the shortest shelf-life antigen. Vaccine degradation was confirmed with in vitro hepatitis B potency test showing the relevance of these two stability-indicating methods. **Results:** Heberpenta-L is stable in refrigeration and also for 14 days at 37 ºC and 90 days at 25 ºC. These stability results support the approved expiry date and they also lead to propose the VVM14 Medium Stability in the vaccine certification process by WHO. Studies showed that Orcinol and in vitro potency tests can be used to assess the HB and Hib components stability respectively saving considerable time and resources. They can be used as confirmatory test if vaccine degradation is suspected, for example in accidental cold chain breaking. **Conclusions:** Stability of Heberpenta-L specifications are guaranteed during the whole shelf-life irrespective of the age of the intermediates when they are used in the production.

EVALUATION OF THE PERFORMANCE OF THE POTENCY ASSAY, IN COMBINED VACCINES, TO DETERMINE THE BIOLOGICAL ACTIVITY OF w *B. PERTUSSIS* COMPONENT

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Introduction: Historically, the vaccination has been one of the interventions of public health of more success, cost and effectiveness. It has allowed reducing the world wide incidence, the mobility and the mortality for diphtheria, tetanus, whooping cough and others. The whooping cough is a contagious illness caused by *B. pertussis* that produces episodes of cough. Among the controls carried out to combined vaccines with *B. pertussis* component, is the Kendricks potency test the “golden standard” test. This assay has been criticized by reasons related with the animals well fear, technical difficulties, poor reproducibility and the use of a challenge way that it is not related with the natural way of infection, nevertheless, it is the current official test in absence of validated alternative for wP and still, it is the only one that has shown a correlation with the protection in children. The purpose of the present work was to evaluate the performance of the challenge intracerebral potency test to determine the biological activity of the component *B. pertussis*, establishing parameters of internal control and evaluating the feasibility of being used for the monitoring system to demonstrate the consistency of production of lots of wP combined vaccines manufactured in BioCen. **Materials and Methods:** WHO Requirements for whole-cell pertussis component was used for 50 lots of Trivac-HB and 5 lots of Pentavalent (DPT-HB-Hib) vaccine. ED 50 were analyzed, FCU, through control graphics and was considered the consistency of the lots, keeping in mind, the obtained potency values. **Results:** The estimation of the consistency show good results giving a measure of security in the vaccine production and testing. **Conclusions:** It was evaluated of satisfactory the performance of the rehearsal of Power of the component *B. pertussis* under the conditions of BioCen Quality Control laboratories.

NEW APPROACH AND TRENDS IN THE DEVELOPMENT OF PREVENTIVE AND THERAPEUTIC VACCINES

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Introduction: Currently, there is an exchange in the international research on vaccine development. Although the vaccine market is dominated by products for infectious diseases, this relationship is reversed in the stages of preclinical and clinical trials on everything related to therapeutic vaccines (chronic non-infectious approach) in which cancer vaccines dominate over infectious. The new impacts will be produced by the therapeutic vaccines. **Materials and Methods**: This works includes the analysis of the main approaches and trends in research and development in the field of vaccines (therapeutic and preventive) and the new routes of administration. The methods used were Bibliometric Analysis and processing of information in scientific in database, ProCite (version 5 for Windows), Bibliolink and ToolInfo. **Results:** These studies allowed the identification of: 1. - There is a change in trends of development of vaccines against infectious diseases to non-infectious. 2. - The great vaccine market is approaching a high level of consolidation and low competition (HI * = 0.18), given that a small number of companies dominate over 81% of the world. 3. - New lines of research projects, use of technologies and the possible trend in the field of vaccines. **Conclusions:** The study vaccine development is evolving into a vibrant segment against chronic non-transmissible diseases such as cancer, diabetes, allergies, addictions, Alzheimer's, obesity, hypertension, asthma, multiple sclerosis, arthritis, among others. However, still continue to develop strategies for infectious diseases such as HIV-AIDS, Malaria, Rotavirus, and Tuberculosis. This paper discusses also their impact on the main lines implemented in the Research and Development Division at the Finlay Institute.

QUALITY CONTROL OF COMBINED VACCINES: A CHALLENGE?

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Introduction: The quality control of vaccines is based on three components: raw material, control, process control and control of the final product, to ensure the safety and efficacy of the product. These elements become more complex when are applied to the control of combined vaccines. During the process of vaccine development is essential to establish the characteristics of the product and critical analytical methods that allow the release of vaccines; this is a key element in the case of novel antigens on the market. This paper will discuss some strategies for the application of analytical methods for the control of novel vaccines and combination vaccines containing antigen of *Haemophilus influenzae* type b obtained by chemical synthesis. **Materials and Methods:** We used lots of Hib vaccine, combined vaccines including Hib antigen for the establishment of quality characteristics depending on the nature of the product. Since the QuimiHi vaccine contains a novel Hib synthetic Ag adequacy of analytical methods were required such as HPLC-GF for molecular size distribution, control of residual solvents by proton NMR. Taking into account the interference due to other components of combined vaccines, fitness orcinol method was required for the quantification of free PRP vaccine. **Results:** It was established the specification of molecular size distribution for Haemophilus influenza type b antigen obtained by chemical synthesis. Control of the solvent used during every phase of chemical reactions was ensured by NMR-H analysis reaching the detection limits specified on international regulations. In addition free PRP was determined in combined vaccine by orcinol. **Conclusions:** There were established quality assurance strategies of combined vaccines taking into account the characteristics of the product and international requirements. These analytical tools along with all the control that takes place during manufacture process ensures the competitiveness of Cuban combination vaccines on the international market.

HISTOPATHOLOGICAL ASSESSMENT OF THE CANDIDATE HEPTAVALENT VACCINE AGAINST *STREPTOCOCCUS PNEUMONIAE*

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Introduction: Vaccine strategies to generate immunological memory against serotypes most prevalent worldwide have been based on obtaining conjugates of bacterial capsule polysaccharides to carrier proteins. However, these vaccines are not an option for developing countries, because of their high prices, making them unavailable to the third world. Biomolecular Chemistry Centre is working on a research project aimed at obtaining a conjugate vaccine, made from fragments of capsular polysaccharides to seven serotypes of *S. pneumoniae*. **Objective:** To evaluate the behavior of body weight, the effect produced on the site of injection and the pathological changes of the organs and tissues during an immunization schedule, using as model Sprague Dawley rats. **Materials and Methods:** Young adult animals were used, divided into 3 experimental groups: A control (placebo) and two treated (vaccine conjugate and plain polysaccharide mixture). The treated groups received a dose level of 500 µL. The immunization schedule was 35 days. The animals were vaccinated and their body weight was taken weekly. The blood draws were performed every 14 days. At the end of the study the necropsy to all animals were made. **Results:** The test showed an increase of body weight per week in all study groups, no deaths related to vaccine administration. Nodules were not detected at site of application and the locomotor system was not affected. The inflammatory reaction chronic and regenerative was related to the nature of the immunogen and adjuvant, shown to be a benign response. The reactivity of the immune system was identified as an increase of lymphoid cells of the spleen, mesenteric and popliteal lymphs, such as the distribution of plasma cells in the medullary cords of lymph. **Conclusion:** This allowed us to test a rating prior to the repeated dose study.

DEVELOPMENT OF A MIXED TETANUS AND DIPHTHERIA ANTITOXIN STANDARD FOR IMMUNOGENICITY STUDIES OF PROPHYLACTIC VACCINES

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Introduction: The clinical evaluation of tetanus antitoxin (TAT) and diphtheria antitoxin (DAT) induced by prophylactic vaccines with tetanus and diphtheria toxoid in their composition requires of standard sera and standardized and validated immunoenzymatic assays. The aim of this work was to prepare a mixed IgG human standard serum (TAT-DAT) to improve the performance of immunogenicity studies in vaccine clinical trials. **Materials and Methods:** The concentration of TAT and DAT in a pool of sera was obtained by means of ELISAs that correlate with in vivo neutralization tests. Human serum albumin was used to fit concentrations, which were verified by means of regression analysis between different dilutions of the new standard serum, respecting to single reference standards. Two hundred eighty eight copies were made during three consecutive days, calculating the regression equations and verifying the linear adjustment. Mean value was calculated for each antitoxin in the standard serum, as well as the standard deviation and the coefficient of variation (CV). **Results:** An average concentration of 0.96 TAT was obtained, similar to that of the reference standard. The CV between plates was 3.644. The concentration of DAT was 0.39, slightly higher than the reference. In addition, an excellent CV of 1.180 was achieved. The coefficients of determination for both TAT and DAT standard curves were higher than 0.99 which show a well-fitting linear regression model. **Conclusions:** For the first time, a mixed standard, useful for the quantification of TAT and DAT in monovalent or multivalent vaccines is described.

APPLICABILITY OF MONOCYTE ACTIVATION TEST TO EVALUATE THE SAFETY OF A DIPHTHERIA-TETANUS-PERTUSSIS COMBINED (DPT) VACCINE AND *NEISSERIA MENINGITIDIS* VACCINE

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Introduction: Quality control tests for vaccines must be able to reliably predict toxic effects in vivo. The presence of endotoxins and other pyrogenic and immunomodulating components in vaccines is a real threat. Endotoxin contamination is often assessed by the Limulus assay which has been widely accepted as an alternative method to the Rabbit Pyrogen Test for pyrogenicity. However, LAL test does not mimic the immunomodulating ability of the product in humans and is endotoxin specific. Thus, we considered the Monocyte Activation Test (MAT) to evaluate both the endotoxin contamination and the proinflammatory and immunomodulating potential of a diphtheria-tetanus-pertussis combined vaccine and a Groups BC *Neisseria meningitidis* vaccine. **Materials and methods:** Cryopreserved human blood from various donors was used as monocytes source. The releasing of Interleukins IL-6 and IL-1β determined by ELISA was used as readout to assess the immunomodulating and pyrogenic effect. Interference-free dilutions were determined by serial dilutions of the sample that were artificially spiked with a known concentration of endotoxin. Endotoxin equivalent concentrations were calculated from endotoxin standard curve and the results obtained with both readouts were compared. **Results:** For both vaccines, higher dilutions of samples were required for the assay using IL-6 as readout, due to a saturating IL-6 response. In DPT vaccines, there is a trend to obtain higher results with IL-6 as readout than with IL-1β, probably because the antigens from gram positive bacteria induce more secretion of IL-6 than IL-1β. In *Neisseria* vaccine, endotoxin concentrations were similar with both readouts since lipopolysaccharides are the only pyrogenic components in the formulation which were estimated from the endotoxin standard curve. **Conclusions:** MAT is a suitable method to evaluate the pyrogenic response of DPT and *Neisseria* vaccines, hence this method can be considered as an alternative in accordance with the 3Rs principle.

OBTAINING PURIFIED OUTER MEMBRANE VESICLES FROM *NEISSERIA MENINGITIDIS* SEROGROUP A AND W_{135} UNDER GOOD MANUFACTURING PRACTICES ENVIRONMENT

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Introduction: Meningococcal disease still is a serious health problem in many geographical areas of the world. Out of the 12 defined meningococcal serogroups, only A, B, C, Y, W_{135} serogroups have been associated with meningococcal disease, and more recently, X serogroup. There are commercially available vaccines based on serogroups A, C, Y and W135 capsular polysaccharides. The outer membrane proteins have been the best vaccine alternative against serogroup B. As part of a scientific collaboration between the Norwegian Institute of Public Health and the Finlay Institute, a vaccine candidate has been produced and evaluated against serogroups A and W135, based on outer membrane vesicles (OMV). The stability study of pilot batches and pre-clinical toxicity evaluation of this vaccine candidate have been completed. The present work aims at obtaining purified OMV out from these serogroups in Good Manufacturing Practices facilities. **Materials and Methods:** Three processes were carried out for each serogroup to a 460 L fermentation scale. The purified OMV were characterized taking into account the quality specifications established for this product. **Results:** In the SDS PAGE made to the

purified OMV, protein profiles were observed with presence of all characteristic bands for these serogroups. In all batches made, pollutant values (nucleic acids and residual polysaccharide) were lower than the established quality specifications. The average yield was 1,62 mg of purified protein per liter of culture for serogroup A and of 9,23 milligram per liter of culture for serogroup W135.**Conclusions:** Serogroup A and W135 purified OMV were obtained in terms of GMP which also met the quality specifications established at Finlay Institute.

STANDARDIZATION OF ELISA METHOD TO MEASURE IMMUNE RESPONSE INDUCED FOR A BIVALENT OUTER MEMBRANE VESICLES VACCINE AGAINST *NEISSERIA MENINGITIDES,* SEROGROUPs A AND W_{135}

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Introduction: Invasive meningococcal disease constitutes a worldwide health problem. Gram negative bacteria, *Neisseria meningitidis* is the causal agent of this disease. The Finlay and Norwegian Institute for Public Health have a collaboration project for the development of a vaccine based on outer membrane vesicles (OMV) against serogroups A and W_{135}. Among quality control analyses of a vaccine candidate, quantification of IgG units/mL is crucial for product release. **Materials and Methods:** Separated single serogroup OMV was used to produce hiperimmune sera for each one and to construct calibration curves. Serogroup A and W_{135} purified OMVs, constituted ELISA plates coating substance. Immunogenicity of the vaccine was evaluated after two doses, 2 µg of protein each, in Balb/C mice with 21 days of interval. The animals were bled 15 days after second immunization. **Results and Discussion:** Reference materials needed for this assay were prepared. The lineal range of the calibration curve and the parallelism between sera obtained after immunization were determined. Coating protein concentration was standardized. The specificity and inter and between assays precision of ELISA method were calculated. The dilutions to use in the assay and the court values settled down to consider animal as a good responder for each serogroup were fixed. **Conclusions:** The complete standardization of this method has allowed the reliable evaluation of mice serum samples from several lots produced at pilot plant scale, as well as the conduction of stability studies of this product. The vaccine candidate evaluated is immunogenic in mice with reliable titers during period of time evaluated.

PEG COATED IRON OXIDE NANOPARTICLES FOR VACCINE APPLICATIONS

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Introduction: Magnetic nanoparticles are attracting widespread attention because of their possible medical applications. In order to avoid opsonisation and the cellular recognition as well as to improve the biocompatibility and pharmacokinetics, some strategies have been implemented, among them the immobilization of polyethylene glycol (PEG) on the surface of the nanoparticles. Nanoparticles containing entrapped or adsorbed antigen and ligand are being investigated as vaccines adjuvants alternatives. The aim of this paper was to evaluate a new approach for the synthesis of magnetic nanoparticles coated with PEG derivate molecules in order to develop a potentially new vaccine adjuvant. **Materials and methods:** Internalization and biocompatibility of this molecule was tested *in vitro* test by using the carcinoma cell line

HeLa. Cells preincubated with nanoparticles, were stained with Prussian blue and cell viability was demonstrated using a standard MTT assay. Magnetite nanoparticles of 12 nm were obtained via thermal decomposition for assuring the nanoparticle homogeneity in terms of size and shape. These particles were first coated with dimercaptosuccinic acid (DMSA) by a ligand exchange method and later three different PEG based polymers were covalently bounded to the nanoparticle surface via an EDC mechanism for amydation. **Results:** Colloidal suspension with hydrodynamic sizes below 100 nm and a low surface charge were obtained in all cases demonstrating the positive PEG effect on the aggregation and the steric stabilization of the magnetic nanoparticles. Cells were visualized outside the membrane without associated toxicity. PEG conjugation increased the half time in blood so it might be possible to decrease the frequency of immunizations and to enhance the bioavailability of the nanoparticles. **Conclusions:** These results suggest that PEGylation of magnetic nanoparticles becomes an interesting and promising alternative for the design of future vaccine and adjuvant candidates.

MULTIPLE ANTIGEN PEPTIDES CONTAINING A PROTECTIVE EPITOPE DERIVED FROM PORA OF *N. MENINGITIDIS* AS SYNTHETIC VACCINE

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Introduction: Peptide vaccines can provide an effective vaccine by focusing the immune response on protective epitopes. The porin PorA of *Neisseria meningitidis* has been considered as an important vaccine candidate. In this work, Linear and Multiple Antigen Peptides (MAPs), comprising B cell epitopes from PorA and T cell epitopes derived either from TT or from the P64k meningococcal protein were evaluated, in terms of immunogenicity in mice. **Materials and Methods:** The linear peptide and MAPs contained a B cell epitope derived from the surface loop 4 of PorA from the *Neisseria meningitidis* strain CU385 and refered T-helper epitopes. They were synthesized manually using the Fmoc/tBu chemistry. The immune response was examined in mice immunized with peptide or MAPs emulsified in Freund´s Adjuvant. **Results and Discussion:** Immunization with all MAPs evoked potent anti-peptide titers, even in the absence of a T cell epitope. Moreover, in all groups that received MAP, the anti-peptide sera reacted with meningococcal outer membrane vesicles in Immunoblot, but only sera produced against MAP containing a T-cell epitope significantly reacted with native meningococcal outer membrane vesicles in ELISA. Sera from mice immunized with the MAP containing a P64k-derived T cell epitope showed similar reactivity with whole meningococci than the one produced in response to a MAP containing a toxoide-derived T cell epitope. **Conclusion:** Even when the anti-peptide titers can be significantly increased using a MAP system without T cell epitopes, the presence of such epitopes is highly recommended not only for the quantity but for the quality of the antibody response directed to conformational epitopes.

RODUCTION OF A MONOCLONAL ANTIBODY BY ASCITES, HOLLOW FIBER SYSTEM AND TRANSGENIC PLANTS FOR VACCINE PRODUCTION USING CB.Hep-1 mAb AS A STUDY CASE

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Introduction: Since 1975, an extraordinary career for improving mAb production methods for pharmaceutical purposes (ascites, *in vitro* technologies, transgenic animals, and dicot or monocot transgenic plants (moss, algae) has been carried out. However, since few years, production demands have generated serious discussions in biotechnology circles over the most feasible methods to meet the huge demand of mAbs. **Objective:** In this work, authors illustrate a summary on a study case in which mice; hollow fiber system and tobacco transgenic plants were assessed for the production of a mAb use for vaccine production. **Results and Conclusions:** This illustration shows that (i) All studied molecules have the same specificity and affinity constant. (ii) The ascites method was more productive than HFB and transgenic tobacco plants to produce the HBsAg-specific mAb. (iii) Viral validation studies of the purification method used to purify CB.Hep-1 mAb from ascites demonstrated a very high virus removal and inactivation capacity, thus, there is not risk of contamination with viruses pathogenic to vaccinated people. In that sense, this method could only be critiqued in regard to the massive use of mice (ethic point o view). (iv) HFB was a viable alternative to ascites method, because it showed clear advantages in terms of molecule quality, easy of downstream process, facility space, recovery, purity, easy of process validation and characterization, and production time, within others; however, it was not equal productive to ascites method requiring hard optimization works to extend the cultivation period of time into bioreactors (≥ 3 months). (v) Due to the adjustable capacity to needs of the low cost biomass production using agriculture and the quality of the purified molecule of PHB-01, transgenic plants is a promising emerging technology to replace large scale ascites method, but further optimization works should be done to increase antibody expression level, stability of affinity matrix to purify plantibody and productivity.

DISULFIDE BOND POLYMERIZATION OF A CYCLIC PEPTIDE DERIVED FROM LOOP 4 OF PORA OF *N. MENINGITIDIS* AS SYNTHETIC VACCINE

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Two peptides derived from the surface loop 4 of class 1 Outer Membrane Protein (OMP) of *Neisseria meningitidis* were synthesized on solid phase using the Boc/Bzl strategy: one containing the entire loop 4 cyclized and the other *one* representing the polymerized cyclic loop 4. To evaluate more efficient cyclic peptide presentation, in the present study a strategy was developed to polymerize cyclic peptides. In order to obtain the polymeric cyclic peptide, two protecting groups for cysteine were used – Acm and Mob. The Cys(Acm)-protected cyclic peptide was obtained after removing the Mob group. The polymerization reaction was carried out by simultaneous deprotection/oxidation of *S*-Acm with iodine. Analysis of the polymeric cyclic peptide in Tris-tricine-SDS-PAGE showed different bands with molecular weights higher than expected for the corresponding monomeric cyclic peptide. Both peptides were used in immunization of four different mouse strains. The antisera raised against the peptides were evaluated by ELISA and Western blotting vs. OMP preparation of *N. meningitidis*. The titers raised against the polymerized cyclic peptide were higher than the ones raised against the cyclic peptide. The antisera elicited against the polymeric cyclic peptide in the CBA/J mouse strain showed opsonic activity.

PREPARATION AND CHARACTERIZATION OF OUTER MEMBRANE VESICLES DERIVED FROM NEISSERIA MENINGITIDIS SEROGROUP X

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Introduction: Meningococcal disease is caused mainly by serogroups A, B, C, Y, W_{135} *N. meningitidis*. However, in several African countries have been reported numerous cases of meningitis caused by *Neisseria meningitidis* serogroup X. Currently there are no licensed vaccines against this pathogen. The Finlay Institute is experienced in obtaining outer membrane vesicle (OMV) derived from *N. meningitidis* serogroup B, which have proven effective in children, youth and adults. The main objective of this work is to obtain and characterize OMV derived from the wild strain Buffa 2/97 *N. meningitidis* serogroup X (OMVx). **Materials and Methods:** Four lots of OMVx were obtained by detergent extraction method with deoxycholate. The size average of OMVx extracted range between 90 and 120 nm. Experimental batches obtained showed a good consistency in the chromatographic (Sephacryl S-300) and electrophoretic profile (SDS-PAGE). High and medium molecular weight antigens were identified by immunoblotting (opcA, rmpM and 70 kDa). The selected lots (102, 103 and 104) were formulated in aluminum hydroxide and immunogenicity was evaluated in BALB/c mice by subcutaneous route. **Results:** OMVx adsorbed in alum induced strong antibody response against antigens present in these vesicles. Elicited antibodies also had bactericidal activity. **Conclusion:** OMV obtained from *N meningitis* serogroup X were immunogenic and constitute a novel vaccine candidate under development.

CHARACTERIZATION OF HOST CELL PROTEINS AND DNA ELIMINATION IN THE PURIFICATION PROCESS OF P64Kr

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Introduction: Analyses of different samples from biotechnology processes are often required in order to demonstrate that residual host cell impurities (HCI) are reduced or eliminated during purification as a marker of process behavior and security product demonstration. The study described herein will describe the characterization of the clearance of HCP and DNA in the entire bioprocessing to purification of recombinant P64K, a novel carrier protein from *Neisseria meningitidis*. **Material and Methods:** During the first step it was characterize the impurity profile (DNA and HCP) at manufacture scale. Subsequently we determined the maximum capacity of the purification process to remove HCP and DNA. It was evaluated in ionic exchange and hydrophobic interaction chromatography considering two load levels: 6 and 8x102 ng/µg of Total protein (TP). **Results:** It was demonstrated on an industrial scale that the process is able to remove DNA from 2,28x106 pg/dose to levels less than 100 pg/dose that represent a cleaning factor of 4.38log. For the HCP, the reduction was from 750 to 0.96 ng/µg (PT), a cleaning factor of 2.89 log. The chromatography step was the main removing operation for both impurities. The load of 600 ng/µg(TP), was the effective limit for the removal of HCP, on the ionic exchange chromatography, reaches a cleaning factor of 1 log and a purity of 77 %. At higher impurity load the chromatography operate inefficiently. The hydrophobic interaction chromatography is able to remove the HCP load, reaching a cleaning factor of 2.4 log. **Conclusions:** It was demonstrated the effectiveness of the process to remove two of the main impurities of the host strain.

IMPLEMENTATION, AND VALIDATION OF THE ANALYTIC TECHNIQUES FOR THE PROCESS CONTROL OF P64kr

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Introduction: The rP64k is a protein with carried property used in vaccine candidates. A production process was designed to meet parenteral product quality specifications according to the use of the product, including an analytical control panel. **Material and Methods:** For productive process control were

established and validated a set of analytics techniques and assays condition according to each processes stages that include methodologies for purity, homogeneity and quantification of the protein. **Results:** Microcoomassie assay showed not interferences with any samples matrix, fulfilled the accuracy and linearity parameters, furthermore repeatability and intermediate precision were according to acceptance criteria (CVs≤5 and ≤10% respectively) and samples were stable for 15 days between 2-8 ^{o}C. The Western blot, for protein identification by monoclonal antibody vs -N and -C terminals were specific for P64k. The detection limits were 0.039µg and 0.0195 µg respectively and there were not chemical interference of samples buffers. SDS-PAGE fulfilled precision parameters. The detection limit was 0.3125µg. CVs intra and inter assays were ≤5 and ≤10% respectively and inter-laboratories ≤15%. **Conclusions:** The validation parameters and practical application showed that these sets of analytical techniques are suitable for process control.

VALIDATION OF THE FLOCCULATION TEST FOR DETERMINING ANTIGEN CONTENT OF DIPHTHERIA AND TETANUS TOXOIDS IN VACCINES

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Introduction: Flocculation assay is part of the quality control requirements for vaccines containing Tetanus and Diphtheria Toxoids, both in monovalent as in combined formulations. The test has been largely used to quantify the antigen content in the Toxoid bulks, but also as an Identity test for finished products. As known, all quality control tests must be validated, but in the case of the Flocculation test it becomes a real necessity taking into account the characteristics of the assay and its inherent high variability. The aim of this Paper was to validate this test for the quantification of antigen content in vaccines containing Diphtheria and Tetanus Toxoids. **Materials and Methods:** Validation was carried out as recommended by the International Conference of Harmonization (ICH) for a quantitative test for active ingredient and final product. The validation parameters evaluated were precision (repeatability, intermediate precision and reproducibility), specificity, accuracy, detection limit and robustness. At the same time, it was evaluated the Kf value. **Results:** All the parameters fulfilled the previously defined criteria under our working conditions. The imprecision of the test was verified, accounted for the high complexity of the biological products present in the samples tested. The variability of the test could be monitored by implementing some internal quality control tools, to be used during the laboratory routine analysis later. **Conclusions:** The test was successfully validated under our operation conditions.

TUESDAY 19)

• PRECLINICAL AND CLINICAL STUDIES FOR PROPHYLACTIC VACCINES

HISTOPATHOLOGICAL STUDY OF CONFRONTATION THROUGH CHALLENGE OF VACCINE CANDIDATES AGAINST *BORDETELLA PERTUSSIS* IN BALB/C MICE

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Introduction: This study had as objective to know the pathological alterations that occur in mice immunized with different vaccine preparations and challenged with the bacteria *Bordetella pertussis.* **Materials and Methods:** An anatomopathological study was carried out in female Balb/c mice supplied by the National Center for Laboratory Animals Breeding (CENPALAB) from Havana, Cuba, divided into four groups composed of three mice each, the administration was performed by subcutaneous route using four

different formulations and challenged with *B. pertussis* inoculated by intranasal route. Were taken samples for histopathological studies from lung fragments, as well as, from any other alterations detected during the macroscopic examination. The samples for histopatological studies were fixed in 10% neutral formaldehyde and processed by the inclusion technique and paraffin cuts, then stained with hematoxiline-eosine and special techniques as Gram and Giemsa. In order to classify the grades and extension of the observed lungs lesions was established one scale. **Results:** During the macroscopic examination were observed congestive processes, at level of the lung, of serious conditions in the groups (I, II, IV), while in the group III the above mentioned processes were classified as mild. The histopathological observations demonstrated serious subacutes bronchopneumonias, except in the group III animals, where only an animal showed a discreet subacute bronchopneumonia. The bronchopneumonic animals at groups (I, II, IV) were characterized for severe broncoectasia, scaly metaplasia of brochial and bronchiolar epithelia, with cilia marked flaking, peribronchials and pervivasculars inflammatory process of mononuclear cells, classified from moderate to serious, alveolar haemorrhagic process, and presence of fibrin and hyaline membranes. Can be observed the presence of coccobacilar formations compatible with *B. pertussis.* **Conclusions:** It was established the presence of subacute bronchopneumony which could be related to the tested products, with a minor affectation in Group III animals.

THE EPIDEMIOLOGY OF ROTAVIRUS DIARRHEA IN CUBA. ANTICIPATING ROTAVIRUS VACCINE

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Background: Rotavirus is the most common cause of severe diarrhea in children to world scale and the surveillance of the virus is needed before and after the introduction of new vaccine to know the prevalence and possible changes in the genotypes circulating in different areas. The knowledge of the natural history of rotavirus infection, provide the groundwork to consider future vaccine strategies in the country. **Objective:** Understand the rotavirus epidemiology in Cuba, in anticipation of the national rotavirus immunization program. **Method:** A total of 415 stool samples from children up to 5 years old with acute diarrhea received in Rotavirus Laboratory in the Tropical Medicine Institute "Pedro Kouri" between 2006 and 2011 were analyzed. The specimens were screened by enzyme linked immunossorbent assay (ELISA), to detect the VP6 antigen and positives samples were studied by polyacrylamide gel electrophoresis (PAGE), RT-PCR to determine G and P genotypes and ten samples selected previously had phylogenetic analysis of VP7 genes. **Results:** The 32.4% of diarrhea were attributable to rotavirus infection. The groups less than 1 year old had been the more affected. Winter seasonality was observed. Among the rotaviruses detected, two long RNA patterns were identified by PAGE (L/A and L/B). The predominant genotype was G1/P[8] (17.5%) and G9/P[8] with 10.5%. The phylogenetic analysis of VP7 genes, showed that G1/P[8] strains were close to those in the Americas and G9/P[8] strains were clustered into the lineage of the emerging G9 strains spreading worldwide. **Conclusion:** The study reinforces the need of a systematic surveillance of the molecular epidemiology of rotavirus with a view to introduce the vaccine in children.

ELIMINATION OF TWO DISEASES PREVENTED BY VACCINATION: MEASLES AND RUBELLA/CRS. CUBA 1988-2011

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Background: The vaccine against measles, rubella and mumps (MMR), came into the routine vaccination programme in Cuba in 1986 with high coverage. Steps were taken for monitoring the progress toward

measles, rubella and Congenital Rubella Syndrome (CRS) elimination and a successfully integrated epidemiologic and laboratory surveillance was implemented. A rapid decrease in the incidence was notified and the last indigenous measles, rubella and CRS cases confirmed by lab were reported in 1993, 1995 and 1989 respectively. **Objective:** To describe the roll of the laboratory surveillance system in measles and rubella/CRS elimination **Methods:** During over 23 years, 21761 and 22024 serum samples from suspected measles and rubella cases were investigated using the hemaglutination inhibition assay (HIA), neutralization assay, Elisa IgM/IgG, viral isolation and RT-PCR. **Results:** Improved laboratory testing algorithms were implemented when new kinds of samples arrive into the laboratory. Laboratory confirmation of decreasing of measles incidence from 16% in 1988 to 0,3% in 1993, demonstrated the impact of the elimination strategies. False positives in ELISA/IgM show the need for additional sample for confirming the infection. Cuban rubella cases occurred by importation in 2004, showing an 88.2% of primary infection by measuring the IgG avidity index. A seroepidemiological survey demonstrated that immunity was slower in the children aged 6-10. **Conclusion:** Laboratory evidence suggested to include in the immunization program a second doses at 6 ages and a campaigned at 12 to 24 years old with MMR vaccine. Cuban experience demonstrated that, if an appropriate vaccination is applied, measles and rubella/CRS can be eliminated.

ASSESSMENT OF SINGLE DOSE AND LOCAL TOLERANCE TOXICITY OF BIVALENT NON CELLULAR VACCINE AGAINST *LEPTOSPIRA SPP*

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Introduction: High antibodies levels and protection against high virulent *Leptospira* strains were obtained to bivalent non cellular vaccine from *Leptospira spp* Canicola and *Leptospira spp* Mozdok. **Objectives:** Male and female Syrian Hamsters were intramuscular inoculated to assess toxicological potential of new vaccine candidate according to the single dose toxicity assay and local tolerance toxicity assay. **Materials and Methods:** 0.2 mL of new vaccine candidate was used in both assay. Once treaties, the animals were observed daily in search of local and systemic symptoms of toxicity. It performed the water and food intake, as well as the corporal weight. At the end of the study the animals were euthanized and subjected to periodically autopsy performed anatomopathological exam to observe possible adverse effects after the immunization with the new vaccine. **Results:** Toxicity symptoms and deaths were not observed during the study. We not observed differences of toxicological interest among the experimental groups in the corporal weight, water and foods intake. In the anatomopathological exam lesions of diagnosis value were not observed, neither lesions of toxicological interest were observed in the inoculation point according to the local tolerance assay. **Conclusions:** It concluded that the vaccine candidate in study does not evidence toxicity in Syrian Hamsters, when being applied in single dose and two doses separated from 21 days in the local tolerance assay. It is recommended to complete the safety study of this vaccine candidate perform the repeat doses toxicity assay.

IMMUNOPROTECTION OF A BIVALENT ACELLULAR VACCINE FORMULATION AGAINST *LEPTOSPIRA SPP* SEROVARS OF EPIDEMIC INTEREST FOR CUBA

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Introduction: The leptospirosis constitutes one of the zoonosis with more impact in the veterinary and human health. The available leptospiral vaccines are fundamentally of inactivated whole cells and most for veterinary use. Although these formulations are effective they have multiple limitations, among those that it not stands out the strait margin of crossed protection against serovars included in the vaccine, low immunologic memory and absence of cellular immune response. **Objectives:** To evaluate the effectiveness of a new bivalent acellular vaccine formulation (*Leptospira spp* Canicola and *Leptospira spp* Mozdok). **Materials and Methods:** Golden/Syrian hamsters were immunized with the new vaccine candidate; schedule of two doses with an interval of three weeks. The homologous and heterologous lethal challenge with 100.000 DL_{50} of the serovars Canicola, Ballum, Mozdok and Cophenageni was evaluated. **Results:** This vaccine candidate protected to 100% of the immunized animals. In all cases the elimination of carrier state was also verified in kidneys, liver and lung. The immunization also generated high levels of IgG after the second dose and in all groups. The animals survived the lethal challenge a year of having applied the immunization schedule. **Conclusions:** These primary results demonstrate the effectiveness of this new vaccine formulation. New and deep researches about the immunologic mechanisms as well as to enlarge the evaluation of heterologous protection with other strains of epidemic interest are recommended.

REACTOGENICITY OF THE A/H1N1 PANDEMIC INFLUENZA VACCINE IN PREGNANT WOMEN IN THE PROVINCE OF MATANZAS

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Introduction: Considering the magnitude and rapidity of the pandemic influenza A (H1N1) spread, vaccination was a high priority, helping to mitigate the negative effects on high risk groups. One of the main groups is pregnant women (PW), so vaccination is considered one of the most effective control and prevention measures. As part of the Cuban National Immunization Program strategy it was used the Glaxo Smith Kline split virion inactivated and adjuvanted vaccine Pandemrix®, donated by WHO and considering the priority of PW. **Methods:** A descriptive study of adverse reactions in immunized PW who live in the province of Matanzas between 1-30 April 2010. Observation during six weeks after the application of the vaccine was undertook through intensive and enhanced real-time adverse reactions surveillance. The registry was established through the survey used by the adverse events national surveillance system. **Results:** There were vaccinated 3470 PW (93.2% vaccination coverage). The overall rate was 41.7 reports per 1000 doses. There were no deaths recorded. Main local adverse reactions were pain; redness and swelling at the injection site (42.7%) which usually disappeared within 72 hours. The most common systemic reactions were headache (12.4%), gastrointestinal symptoms (11.7%), fever (10.3%) and respiratory symptoms (7.5%), rarely malaise (5.5%) and arthralgia (2.0%). **Conclusion:** The vaccine Pandemrix shown to be safe in PW and their application was an exceptional opportunity to determine the safety of adjuvanted vaccine in pregnant women in the region of the Americas.

SPECIFIC ANTIBODIES CONCENTRATION AND SKIN TEST AREA FOR ANTI-HEPATITIS AND TOXOID VACCINES IN ASTHMATIC CHILDREN

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Introduction: Vaccine clinical trials do not include asthmatic people, but atopic children are vaccinated as part of the enhanced plan of immunizations (EPI) even after they have developed bronchial asthma. There is paucity of research about the response of atopic children to conventional vaccines, although there is some evidence of retardation in the maturation of the immune system in the early childhood of those children as compared to normal ones. It is important to assess the quality of the response to vaccination of children with allergic bronchial asthma, because it is clinically relevant and part of the modern concepts of the “second golden age old vaccines”. **Materials and methods:** To evaluate the response, antiHBs was measured in 2 years of children vaccinated with Heberbiovac-HB as part of the EPI (600 asthmatic and 660 non-asthmatic children), the length of the skin test (ST) in 7 years old children (184 asthmatic and 159 non asthmatic) and concentration of antibodies against toxoid vaccines in 2 years old children (36 asthmatic and 32 non asthmatic) at the end of the immunization program. ELISA sandwich and indirect ELISA were used for antibodies. Area of ST in mm^2 was measured. Geometric means and confidence intervals for antibodies concentration and ST were computed and groups were compared by means of a one-tail t-test. **Results:** Lower values in asthmatic children and significant differences were obtained for: concentration of antiHBs (620 vs 1047 IU/L p=0,003), area of the ST (1020.149 vs 1318.149 mm^2 p=0.0173). However, mean values for antitoxins (tetanic 1.72 vs 2.58 IU/ml p=0.4395 and diphteric 0.14 vs 0.32 IU/ml p=0.7195) were not statistically significant. **Conclusions:** The vaccination was effective in asthmatics although humoral and cellular response was lower, especially against anti-hepatitis vaccine. This result may be explained by the slower maturation of the immune system reported by the literature.

COMPARISON OF FOUR RECOMBINANT HEPATITIS B VACCINES APPLIED ON AN ACCELERATED SCHEDULE IN HEALTHY ADULTS

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Introduction: Pre-clinical analyses in mice have demonstrated superior efficacy of *Pichia pastoris* derived recombinant Hepatitis B surface antigen (rHBsAg) compared with other available yeast-derived rHBsAg (*Saccharomices cerevisiae* or *Hansenula polymorpha)* made using the *adw2* HBV subtype. *Pichia pastoris*-derived rHBsAg is the main active pharmaceutical ingredient for *Heberbiovac HB*® vaccine (Heber Biotec, S.A., Havana, Cuba), one of the two Latin American vaccines qualified by the WHO with more than 20 y of clinical use proved to be safe, efficacious and immunogenic. **Materials and Methods:** A post-marketing, double blind, randomized, controlled clinical trial to assess the immunogenicity and safety profiles of 4 commercially available recombinant hepatitis B vaccines was performed. The vaccines included in this study were Heberbiovac-HB® (Heber Biotec S.A., Havana, Cuba), Euvax-B® (LG Chemical, Korea), Hepavax-Gene® (Greencross, Korea) and Engerix-B® (GSK, Belgium). Vaccines were administered intramuscularly to healthy adults in three 20 µg doses at monthly intervals (0–1–2 mo). 400 volunteers aged 18 to 45 y (average age, 35 y) non-reactive for serological markers of hepatitis B virus infection were vaccinated. Volunteers were randomly assigned (ratio 1:1:1:1) to one of the four treatment groups. The antibody response (anti-HBs) was assessed at days 60, 90 and 365 post-vaccination using a commercial kit. **Results:** The four vaccines showed to be safe and highly immunogenic. Similar seroprotection rates (anti-HBs ≥10 IU/L) about one month after application of the 2nd and 3rd dose were obtained for Engerix-B®, Hepavax-Gene®, Euvax-B® and Heberbiovac-HB® vaccines 96.7%, 96.6%,

100%, 100% and 98.8%, 89.5%, 100%, 100%, respectively. Heberbiovac-HB® vaccine achieved significantly higher geometric mean antibody titers (GMT) and rate of good and hyper-responders at all time-points post-vaccination. The GMT on day 365 after full vaccination was significantly reduced in all groups compared with day 90, although Heberbiovac-HB® showed the highest anti-HBs GMT and good-responders rate. **Conclusions:** The four vaccines were well tolerated and poorly reactogenic. No serious adverse events were observed. This study confirms an overall good immune response and rapid priming for the four vaccines in the course of an accelerated schedule, with higher anti-HBs geometric mean concentrations and better responses for Heberbiovac-HB®.

- **Regulatory Issues**

QUALITY RISK MANAGEMENT: A NEW APPROACH TO COMPLIANCE GOOD MANUFACTURING PRACTICES IN CUBA

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Quality Risk Management (QRM) is a systematic process for the assessment, control, communication and review of risks to the quality of the drug (medicinal) product across the product lifecycle. Within the group of injectable, vaccines are highlighted by its highest level of demands; this is due to its nature, in addition to go to the widespread use in healthy populations, mostly children. Those risks can be attributed to the design, production process, storage, transportation and use, as well as factors involved in these processes, as personnel, equipment, facilities and documentation. The application of the demanding regulatory doctrine of Good Practice documents and the increasing development of the pharmaceutical industry requires a greater understanding of the products and processes, as well as the efficient and timely use of resources; implementation of the QRM can a great contribution in this regard. This work aimed to strengthen the regulatory approach of Good Manufacturing Practices for Pharmaceuticals Products (GMPPP) of Center for State Control of Medicines, Equipments and Medical Devices (CECMED) with QRM criteria. In first time was performed a diagnosis about the understanding and implementation of this issue in the national industry by application a questionnaire. From the analysis it was found that the existing level was low, looking better in the biological production centers in relation to drug producers. On the other hand, prepared the draft update of the Regulation No. 16-2006 incorporating risk criteria in 64% of its sections, as well as the draft Guide of Quality Risk Management, which has 9 Chapters highlighting the issues to be considered in establishing a QRM System as part of the Quality Management System, as well as its methodology and tools.

CUBAN DIAGNOSIS FOR IMPLEMENTATION OF HARMONIZED TECHNICAL REQUIREMENTS OF THE AMERICAS REGION FOR MARKETING AUTHORIZATION OF VACCINES

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Introduction: Globalization is a phenomenon that has affected the way of seeing the world and the vaccine scenario had not escape from it. It is for this specific reason that the Pan American Network for Drug Regulatory Harmonization (PANDRH) has created a Working Group on Vaccine. This group principal goal is to promote harmonization for the regulation of vaccines in the Region, ensuring the quality, safety and efficacy of these products, generating more efficient and harmonized mechanisms and also contributing to improve the availability of these products for the American countries. Several activities

were performed to achieve this goal and as result a vaccine harmonized requirements document was developed. **Materials and methods:** The specific technical requirements for vaccine marketing applications, elaborated and issued by PANDRH members, were compared with the regulation applied in Cuba in terms of format and more relevant, comparison of the information requested to applicants for products license. **Results:** It was observed some important differences that influence directly on the goal of harmonization, considering the structure and details level of information included in our documents that differ from the one proposed by PANDRH, spite of this, in both cases, the same information is requested to endorse the registration of a vaccine. The impact on the current procedures and other regulatory documents due to the differences detected between the two documents was then analyzed. **Conclusions:** The outcomes of the present work were reflected in the need of working on the review and modification of the National documents, in order to have a final updated document which harmonize with the requirements that will be adopted by the other countries within the region without excluding the most important parameter for regulation and reach a general understanding on the document by regulators but also manufacturers or applicants.

CUBAN REGULATORY AUTHORITY. SCOPE AND IMPACT OF THE WHO RECOGNITION OF ITS FUNCTIONALITY FOR VACCIONES

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Introduction: The role of National Regulatory Authorities (NRAs) in the regulation and control of vaccines has been well established by the World Health Organization (WHO). The compromise of NRAs is increasing according to the involvement of each country in the development, manufacturing and exportation of vaccines, with top level for those with national vaccines prequalified by WHO. The aim of our presentation is to characterize and illustrate the meaning and impact of the Certification of Functionality issued by WHO after the assessment of the vaccine regulatory system since the perspective and experience of the Cuban NRA (CECMED). **Materials and Methods:** Data collection was performed from public sources and archival study such as WHO assessments guides, consultations for improving the assessments, and reports to CECMED during the period 2000-2011. Data were organized according to indicators and sub-indicators of WHO current assessment tools, analyzed and summarized. Results from the evaluation of 50 indicators (32 critical) with 178 sub-indicators (89 critical) related to the Vaccine Regulatory System and six regulatory functions were taken into consideration. **Results:** It was found that Cuban NRA fully accomplished all critical items and also had a satisfactory performance for the non critical ones. WHO granted the certification of functionality to CECMED on May 2011, valid until the next inspection in two-five years. Positive results of WHO inspections support the prequalification of the Cuban recombinant Antihepatitis B vaccine and other ongoing processes for including other vaccines. **Conclusions:** This process has contributed to strengthen the CECMED vaccine regulatory capacity to support research, developing, manufacturing and commercialization of vaccines in Cuba, to collaborate with NRAs in capacity building and with WHO in tools routine revision process and developing and harmonizing of a tool for medicines.

ROOM POSTERS

THERAPEUTICS VACCINES WORKSHOP

SUNDAY 17 (NIGHT)

• Poster Session: Vaccines Design and Process Development

Chairman: *Vladimir Peña and Magdalena Plebanski*

METHODOLOGY TO DESIGN AND OPTIMIZE STABLE OILY FORMULATIONS USING MONTANIDE 888 VG

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Introduction: The emulsions are used in biotechnology in the formulation of a large number of immunogens. Efforts have been devoted to study and understand these systems, but the complexity of the emulsifiers, the interaction forces and active ingredients that are used, reduce the accuracy in the prediction of stability. In the present work a study was conducted to determine the influence of phase composition, and ionic strength on the behavior of an emulsion using the Montanide 888 VG as emulsifier. **Materials and Methods:** Using a mixture experimental design mathematical model was adjusted, describing the influence of emulsion composition on the mechanical stability, the thermal stability, viscosity and particle size. A numeric factor for ionic strength was included. Mechanical stability was determined by centrifugation and thermal stability by accelerated test at 37 °C. Viscosity was determined by Ostwald de Weale law using a rotational viscometer. The simulation and optimization models were developed using Matlab 7.0.1 software. **Results:** Emulsifier concentration had a significant influence in the emulsion cost and in it stability. To minimize the emulsion cost, it was observed that employing a composition of 50% aqueous phase, 47.5% mineral oil and 2.5% emulsifier Montanide 888 VG can be obtain a stable emulsion for the formulation of veterinary immunogens. **Conclusions:** Designed methodology allows us design stable emulsions with different aqueous phase composition. Composition of the systems with light paraffin oil and Montanide 888VG as external phase can be optimized to further evaluation of the Active pharmaceutical ingredient.

IMS4112 AND VLP OF HBV AS ADJUVANTS FOR A QUIMERIC PROTEIN OF HIV-1

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Introduction: Nowadays, most vaccine candidates against human immunodeficiency virus (HIV-1) are focused on the induction of an anti-viral Th1-type response. In this regard, we have developed a vaccine candidate formulation named TERAVAC-HIV. It includes three antigens: the recombinant HIV-quimeric protein CR3 comprising Th and CTL epitope rich regions from different viral proteins and the nucleocapsid (C) and surface (S) virus-like particles (VLP) of hepatitis B virus (HBV). The adjuvant effect of HBV VLP

allows the induction of an HIV-specific Th1 cellular response. In the present work, the immunogenicity in mice of TERAVAC-HIV was compared with another formulation based on CR3 adjuvated with the IMS 4112 nanoparticle (Seppic, France). **Materials and Methods:** Intranasal (i.n.), subcutaneous (s.c.) and simultaneous s.c./i.n. immunizations were delivered to 6-8 weeks female Balb/c mice. Animals received 5 μg antigen/route. CR3-specific cellular response was determined by IFN-γ ELISPOT assay and cytokines secretion in culture supernatant fluids was assessed by ELISA. In addition, lymphoproliferation of CR3-specific $CD4^+$ and $CD8^+$ T cells was evaluated after simultaneous s.c./i.n. co-administration. **Results:** The anti-CR3 cellular response was elicited after i.n., s.c. and s.c./i.n. immunization schedules with both formulations. However, the advantage of the HBV VLP over the IMS 4112 was demonstrated in terms of cellular response kinetics after s.c./i.n. co-administration. In this sense, after five doses, only mice immunized with TERAVAC-HIV elicited CR3-specific IFN-γ-secreting cells. This result was accompanied by proliferation of CR3-specific $CD4^+$ and $CD8^+$ T lymphocytes. **Conclusion:** These results in mice suggest that HBV VLP are better adjuvants than the Seppic nanoparticle IMS 4112 to generate an anti-CR3 Th1 cellular response.

SHELF-LIFE STABILITY STUDY OF A NOVEL ADJUVANTED AND ADSORBED HOUSE DUST MITE ALLERGEN VACCINE, PROLINEM-DS

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Introduction: Evaluation of shelf-life stability of pharmaceutical products is required during the pharmaceutical development phase, prior to advancing to clinical trials. The objective of this work was to test the stability of a novel anti-allergic vaccine candidate PROLINEM-DS based on allergens from *Dermatophagoides siboney* and a combination adjuvant containing PL and Alum, known as AFLP1®. **Methods:** The ICH methodology established for stability studies for biological products was followed. Samples of three pilot scale GMP batches were stored at 4 °C and assayed at 0,3,6,9,12,18 and 24 months. Possible desorption from alum gel was monitored, testing the supernatant for allergenic activity (IgE-inhibition ELISA), Der s1 content (MAb-ELISA) and total protein content. Preservation of antigen's integrity was checked by SDS-PAGE and Western-Blotting after forced desorption. Other tests were applied for measuring preservative content, pH stability, and sterility. Acceptance limits matched those used for product release. Since, a potency test is not yet established for this new vaccine, allergen-specific immunogenicity in Balb/C mice was determined at the beginning and end of the study. **Results:** After 24 months no deviations of quality specifications were detected in any parameter. Although a slight tendency toward increasing the allergen activity and Der s 1 content in the supernatant was noted, it was not statistically significant (regression analysis, $p<0.05$). The immunogenicity test showed the expected outcome regarding induction of allergen-specifi c IgG, IgG1 and IgG2a antibodies (the later is dependent of the AFLP1® adjuvant effect), similarly to initial results. **Conclusion:** This study proved the vaccine stability during 24 months as a basis for approval of a reliable expiration period, as part of the pharmaceutical development phase, permit to advance to future clinical trials.

ESTABLISHMENT OF ANALYTICAL SYSTEMS FOR THE CONTROL OF ANTIGEN FOR THERAPEUTIC VACCINE

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Introduction: Immunogenic therapies using as target substances related with cancer progression have been largely studied in recent years. The VEGF (Vascular Endothelium Growth Factor) molecule has been widely related to tumor angiogenesis, and it was obtained by Biomedical Research of CIGB, an antigen which contains VEGF for therapeutic vaccine. A production process was designed to meet parenteral product quality specifications. In order to confirm the quality of the drug a set of analytical techniques was developed, with their appropriate acceptance criteria. **Materials and Methods:** The established techniques included host protein assay, Coomassie protein Assay for measuring protein concentration, inmunoidentification by Western blotting, GF-HPLC and SDS-PAGE. **Results:** The dot blotting technique for the contaminants host protein detection was specific and the detection limit was set at 0.0125 µg. In the Coomassie protein Assay, Intra- and inter-assay variation coefficient was ≤ 6% and ≤ 10%, respectively. The Western blotting using a rabbit polyclonal antibody was specific. The SDS-PAGE assay was established in 12.5% acrilamide gel. The GF-HPLC (Superdex 200) was established to detected aggregate forms. **Conclusions:** The established methods have constituted a valuable tool for the control of the quality of the vaccine and the implementation of the studies of stability.

EVALUATION OF ION-EXCHANGE CHROMATOGRAPHY FOR PURIFICATION OF HBcAg

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The development of a therapeutic vaccine against the Hepatitis B virus is one of the main researching lines of the Centre for Genetic Engineering and Biothecnology (CIGB). In that sense, a vaccine candidate that combines surface antigen (HBsAg) and nucleocapsid protein of hepatitis B virus (HBcAg) has been obtained, and has show encouraging results in recent clinical trials. To obtain in the quantities demanded of this product a technological protocol has been developed for the production of HBcAg, since HBsAg is produced in stable and high quality standards in the CIGB. The most important step in the purification protocol of HBcAg is the anion-exchange chromatography, which can raise the purity of the product up to 60 - 80% approx. In the current process, this chromatographic step has a high variability degree between batches producing result inconsistencies and uncontrolled product losses. Therefore, it was proposed an evaluation of chromatographic step from a full factorial experimental design with two levels and three independent variables, using the statistical package Statgraphics Centurion. **Materials and Methods:** Cell disruption conditions, Anion exchange chromatography process scaling down, Determination of the adsorption capacity in dynamic conditions, Experimental design 2^3 factorial. **Results:** Multiple regression analysis was used to evaluate the relationship between variables with all the data obtained in the experimental runs. The analysis of the graphs of the response surface shows that with the use of the highest pH and low ionic strength the highest adsorption of the protein of interest is obtained. **Conclusions:** The processing of the sample under conditions of pH 8.0 and low ionic strength (0 mol/L NaCl) using the type M matrix favors the purification of HBcAg according to the multiple regression analyses, although these results cannot be taken as definitive and it is necessary to further study the space of the design explored.

DEVELOPMENT AND VALIDATION OF ANALYTICAL TECHNIQUES FOR HCV CORE PROTEIN QUALITY CONTROL IN THE VACCINE CANDIDATE CIGB-230

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Introduction: The HCV core protein is a highly basic, RNA-binding protein that presumably forms the viral nucleocapsid. The biochemical features of this protein have been relatively poorly characterized, and its structure is unknown. A variant of the HCV core protein (HCcAg) was obtained by using *E. coli* expression system. In order to assure the quality of the final product was necessary to establish and validate a set of analytical techniques according to the international standards. **Materials and Methods:** Four analytical techniques were validated: Western blot, dot blot, SDS-Page and Microcoomassie. **Results:** Microcoomassie assay showed not interferences with any samples matrix, fulfilled the accuracy and linearity parameters, furthermore repeatability and intermediate precision were according to acceptance criteria (CVs ≤ 5 and ≤ 10%, respectively) and samples were stable for 15 days at room temperature and between 2-8 ºC. The Western blot, for protein identification by monoclonal antibody was specific for HCV core protein. The detection limit was 1.25 µg and there were not chemical interferences of the samples buffers. SDS-PAGE fulfilled precision parameters. The detection limit was 0.5µg. The repeatability CV assay was ≤1%. CVs intra and inters assays were ≤ 2 %. The dot blot assay showed no interference with the final formulation buffer. The limit of detection was 0.0009 µg. **Conclusions:** The validation parameters and practical application showed that these sets of analytical techniques are suitable for the quality control of HCV core protein.

CORE HCV PROTEIN CHARACTERIZATION FOR VACCINE CANDIDATE CIGB-230

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Introduction: The HCV core protein is a single-stranded RNA positive, belonging to the family Flaviviridae. In the CIGB was obtained a variant of the HCV core protein (HCcAg) from a transformed clone of *E. coli* and designed for the expression of amino-terminal 120 amino acids of the protein virus. The aim of this work is the analytical characterization of HCcAg that would ensure the safety and efficacy of the final product. **Materials and Methods:** To evaluate the characteristics of the final product were monitored parameters such as: Identity, Purity, general safety, pyrogenicity, potential impurities from the host system and product-related impurities. In order to identify product-related impurities was designed a forced degradation study. In addition the analysis of the amino acid sequence of the protein by mass spectrometry was performed. **Results:** It was shown by western blot analysis the identity of monomer in active form of the product and purity greater than 95% by SDS-PAGE. The impurities from the host cells in 10 batches were checked and proved that all meet the limits specified for this product. Using the forced degradation studies were identified degradation and aggregation species. Also, we identified a process-related impurity corresponding to an oxidation of the protein. The protein sequence analysis by mass spectrometry reveal an amino acid change at position 90 of the amino acid sequence **Conclusion:** The protein obtained has characteristics of safety and efficacy in accordance with the characteristics of the product.

DEVELOPMENT ANALYTICAL TECHNIQUES FOR CORE HB PROTEIN QUALITY CONTROL IN NASVAC THERAPEUTIC VACCINE CANDIDATE

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Introduction: Infection with hepatitis B virus (HBV) remains an important global heath concern, despite the availability of multiple prophylactic vaccines. The World Health Organization (WHO) estimates that more than 2 billion persons have been infected with the virus. Hepatitis B core (HBcAg) and surface antigens are the main structural antigens of hepatitis B virus (HBV). In vaccines studies, HBcAg is a potent immunogen even in the absence of adjuvant, and can be used as a carrier molecule for homologous and heterologous epitopes. In our institution was development therapeutic nasal vaccines for chronic hepatitis B infection, they combined the HBcAg and HBsAg antigens. In this work we established and validated some analytical techniques for the quality control of HBcAg antigen. **Materials and Methods:** The analytical techniques established were Dot Blot for host protein contaminant, SDS-PAGE, Immunoidentification by Western Blot, SE-HPLC, total proteins quantification by Lowry, ELISA. **Results:** HBcAg integrity was evaluated using SDS-PAGE, HBcAg protein migrated as a band that corresponded to monomer (Mw ≈ 24 kDa) with a minor fraction at twice this molecular weight representing dimmer (Mw ≈ 48 kDa). Immunogenicity of protein was characterized using two antibodies, polyclonal and monoclonal antibody anti-HBsAg. Dot Blot technique for host contaminant was specific and the detection limit was 0.0078 µg. In ELISA the parameters analyzed were accuracy, precision and linearity, the CVs inter and intra assays were ≤ 10 %, while inter laboratory was ≤ 20%. SE-HPLC was performed in TSK G5000PW column, the signal was detected at 280 nm and flow rate 0.4 mL/min at room temperature. **Conclusions:** Established analytical techniques ensure safe control of HB core protein, active ingredient of NASVAC therapeutic vaccine candidate.

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR EVALUATING THE QUALITY OF CR3 MOLECULE, ACTIVE PHARMACEUTICAL INGREDIENT (API) OF THERAPEUTIC VACCINE AGAINST VIH-1

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Introduction: CR3 recombinant protein does not exist naturally in any host but that is formed by different fragments of proteins present in the Human Immunodeficiency Virus type 1 (HIV-1) as: the cover glycoprotein (gp 160), protein vpr (p66), nef (p27) and gag (p24). This protein has an approximate molecular weight of 52 kDa and an isoelectric point of 9.3, theoretically estimated. Studies have shown that the molecule CR3 alone can not induce a response of Th1 or stimulate CD8 + T cells only when immunized formulated in conjunction with antigens HBsAg and HBcAg are obtained responses from Th1 and stimulation of CD8 + T lymphocytes against HIV. The objective of this work is to establish analytical control and characterization of CR3 API to be used in a therapeutic vaccine. **Materials and Methods:** The analytical techniques established were SDS-PAGE, Dot Blot for host protein contaminant, Immunoidentification by Western Blot, RP-HPLC and total proteins quantification by Abs 280 nm. **Results:** Using Coomassie Blue G-250 staining in SDS-PAGE we evaluated the presence of aggregates and degradation. Immunogenicity of protein was characterized using a monoclonal antibody anti-CR3 who recognizes aggregates and degradation of the protein, work dilution 0.2 µg/mL. Protein from host

contaminant were detected by Dot Blot technique, poly-clonal antibody obtained in rabbit recognizes until 0.0048 µg of contaminants. RP-HPLC was performed in C-4 column, the signal was detected at 226 nm and flow rate 0.8 mL/min at 37 °C, gradient: 25 to 45% B in 36 min, the signal of protein was obtained around 17 min. **Conclusions:** Established analytical methods were appropriated for the analytical control and characterization of CR3 protein, active pharmaceutical ingredient (API) of therapeutic vaccine candidate against VIH-1.

PHARMACEUTICAL DEVELOPMENT AND STABILITY OF A THERAPEUTIC VACCINE WITH CIGB-228 PEPTIDE AGAINST HUMAN PAPILLOMAVIRUS (HPV)

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Introduction: Human papillomavirus (HPV) is the most common sexually transmitted virus worldwide. The CIGB-228 is a synthesized peptide therapeutic vaccine candidate for use in patients with human papillomavirus infection. Preventive vaccination with CIGB-228 adjuvanted on very small sized proteoliposomes (VSSP) have demonstrated antitumoral effect on *in vivo* assay in mice challenged with the cell line TC-1, which expresses the antigen E7 of the HPV 16. **Materials and Methods:** In this work various factors and additives were evaluated for improving the stability of CIGB-228. Factors such as solubility, pH, ionic strength, buffer composition, and excipients were evaluated, as well as the compatibility of the CIGB-228 with the adjuvant VSSP. The stability of peptide at 0,3 mg/mL in buffer acetate at pH 4, 5 and 6 and ionic strength of 10, 50 and 100 mM was assessed. The sucrose and titriplex III were used in the formulation as stabilizers and sucrose was also used like a bulking agent during lyophilization. The stability was followed by the purity of CIGB-228 determined by phase reverse chromatography (RP-HPLC). **Results:** The CIGB-228 peptide was soluble in water between 8 and 500 µg/mL. CIGB-228 aggregation was the most relevant modification under stress conditions. Peptide degradation increased raising the ionic strength and pH. The peptide was more stable in buffer acetate at 10 mM and pH 4. The CIGB-228 formulation demonstrated an adequate physical and chemical stability on this buffer and stabilized with sucrose and titriplex. The chromatography profile of CIGB228 peptide mixed with VSSP was the same evaluated by 24 hours. **Conclusions:** Stability studies in long-term have been demonstrated that CIGB-228 drug product lyophilized is stable by 24 and 6 months storage at 4 and 28 ºC, respectively.

EXPERIMENTAL FACTORIAL DESIGN FOR THE EVALUATION OF ION EXCHANGE CHROMATOGRAPHY IN THE PURIFICATION OF HBcAG

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Introduction: The development of a therapeutic vaccine against the Hepatitis B virus is one of the main researching lines of the Centre for Genetic Engineering and Biotechnology (CIGB). In that sense, a vaccine candidate, that put together hepatitis B virus (HBV) surface (HBsAg) and nucleocapsid (HBcAg) antigens has demonstrated encouraging results in recent clinical studies. To obtain the amounts required of this product a technology has been developed for HBcAg production, since the other component of the vaccine candidate (HBsAg) is stably produced at CIGB with high quality standards. The present work is aimed to explore some possibilities in the improvement of the process, focusing mainly the ion exchange

chromatography step, since it is the most important for the established purification technology. **Materials and methods:** Experimental factorial design at two levels was conducted in order to know the influence of ionic strength, pH and type of chromatographic exchanger in recovery and purity. Statgraphics software was employed to process the results of 16 experimental runs. **Results:** The two type of chromatographic resin studied can be used for this purpose, while at basic pH (8,0) and low salt concentration recovery is improved. Purity is not affected significantly by conditions studied but by the introduction of an additional step of washing with non ionic detergent. **Conclusions:** It is possible to improve recovery in the established chromatographic purification step redefining the design space for the variables ionic strength and pH. Also the introduction of slight variations in washing sequences and composition could increase the purity of the product obtained and cost could be saved by the used of less expensive chromatographic resin.

EFFECT OF DIFFERENT CARBON SOURCES ON THE PRODUCTION OF A RECOMBINANT PROTEIN USED IN CANCER THERAPEUTICS

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Introduction: *E. coli* remains the primary host for the industrial production of recombinant proteins. The primary processing problem in fermentation is the production of organic acids such as acetic acid, which can inhibit both cell growth and recombinant protein production, therefore selection of the carbon source, is one of the most important items during design of a fermentation process. **Materials and Methods:** The microorganism used was the *E.coli* BL 21(DE-3), producer of the recombinant protein P64K-$VEGF_{121}$ (antigen that is part of the CIGB 247 therapeutic vaccine). Cells were cultured with shaking at 180 rpm in 250 mL flasks containing 50 mL minimal medium and in 5 L fermenter at 37°C. Cell density was measured by a spectrophotometer at 620 nm, the expression level was determined by SDS-PAGE and Coomassie blue staining. *ImageJ* software was used to scan the protein band on gel for quantitative analysis and Statgraphics Centurion software was used for statistical design and data analysis. **Results:** Seven carbon source were studied (Glucose, arabinose, fructose, glycerol, sorbitol, sucrose, lactose). A combination of glucose (0.2%) and fructose (1.0 %) allowed to reach high cell density and to produce as insoluble protein in the cytoplasm where it reaches up to 20% of the total number of intracellular proteins expressed. **Conclusions:** The experimental results demonstrated that recombinant *E.coli* BL 21(De-3)/p64K-$hVEGF_{KDR}$.could utilize various carbon sources efficiently as the sole carbon source, but a combination of glucose and fructose had a significant effect on the synthesis of P64K-$hVEGF_{KDR}$. and the growth of cells. Enhanced cell density and antigen productivity were achieved by batch culture in 5 L stirred tank fermenter.

DEVELOPMENT AND VALIDATION OF A QUANTITATIVE REAL-TIME PCR ASSAY FOR THE SPECIFIC DETECTION OF RESIDUAL HOST CELL DNA IN THE ACTIVE PRINCIPLE OF A THERAPEUTIC VACCINE AGAINST HEPATITIS B

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Introduction: Contamination of recombinant vaccines with host genomic DNA (gDNA) has always been a major Quality Assurance concern. A genomic host (*Escherichia coli*) DNA content lower than 10ng/dose is required for the nasal Cuban vaccine, based on the core protein of the Hepatitis B virus. The development

and validation of a real-time PCR assay for detecting host gDNA in final samples of the HBcAg-based recombinant vaccine is presented in this work. **Materials and methods:** gDNA was prepared from *E. coli* strain W3110. Sense (5´ACACGGTCCAGAACTCCTACG-3´) and antisense (5´GCCGGTGCTTCT TCTGCGGGTAACGTCA3´) primers were used to obtain a 181-bp amplicon from the 16S rRNA gene with a hot-start PCR protocol. The obtained amplicons were quantified by monitoring fluorescence upon binding of the DNA-binding dye SYBR Green I. Standard curves (10 ng to 1 pg) were prepared in triplicate by serial dilutions of gDNA stocks, including negative controls with each run. PCR were performed in a Rotor Gene system, using DNA extracted from deproteinized samples. **Results:** This work describes the development and validation, for the first time in Cuba, of a real-time PCR-based protocol for quantitating residual *E. coli* gDNA in recombinant protein samples. The method exhibited a linear response throughout the studied range, with a regression coefficient (r^2) ≥ 0.98 and a slope significantly different from zero (P ≤ 0.01). Repeatability and intermediate precision assays indicated that the method is precise, as both yielded CV ≤ 20%. The method was accurate with the examined samples, producing a t exp < t critical, a global CV < 15% and a regression coefficient (r^2) ≥ 0.98. CI of the slope included 1, and the CI of the intercept included 0. **Conclusion:** In our conditions, qPCR represents a promising technique for developing and establishing quantitative GMP methods to quantify gDNA in pharmaceutical preparations, with high sensitivity and specificity.

PROPERTIES OF LIPOSOMES ENCAPSULATING PURIFIED ALLERGENS FROM *DERMATOPHAGOIDES SIBONEY* MITE

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Introduction: Allergens from mites are considered a major cause of allergic disorders. *Dermatophagoides siboney* specie is endemic of our country and typical from humid tropical regions such as the Caribbean area. Allergen extracts injection is frequently used as allergy immunotherapy, however the occurrence of severe anaphylactic side effect and the necessity to administer a great number of injection prompted the development of safe and efficacious allergen formulations. Lipid-based vesicles (liposomes) have been used as carriers of protein antigens (Ag) for more than 30 years because of their ability to encapsulate Ag and their capacity to stimulate antibody production at low Ag doses in a manner that avoided potential allergic reactions. The liposomal adjuvanticity depends both the antigen and physic-chemical properties of these particles (size and lipid composition). **Materials and methods:** Liposomes composed of dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC) and egg yolk phosphatidylcholine (ePC) containing or not cholesterol (Cho), and encapsulating the major allergens (Ders) from *D. siboney* mite will be obtained by dehydration–rehydration procedures (DRV). DRV of different lipid compositions containing Ders were characterized and allergen-specific immune response induced by these liposomes were studied in Blab/c mice using two doses of 5µg of Ders into liposomes each 14 days by subcutaneous route. The allergen-specific antibody response was assessed determining serum levels of IgE, IgG1, and IgG2a by ELISA. Additionally, cytokine levels were measured in broncho-alveolar lavage (BAL) by ELISA, in mice subjected previously to aerosolized allergen challenge. **Results:** Liposomes/Ders were 3-6 µm, with 35-45% of Ders encapsulated and their retention capacity was 60-80% during 90 days of storage in solution. **Conclusion:** The liposomal lipid composition immunomodulate allergen specific immune response.

CHARACTERIZATION OF THE INTERACTION FORCES AND IMMUNOGENICITY FOR DIFFERENT FORMULATION CONDITIONS OF A CANCER ANTI-IDIOTYPIC VACCINE –ALUMINA

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Introduction: Racotumomab is an anti-idiotypic monoclonal antibody which is in development stage as a vaccine adjuvated in alumina. Influence of buffer pH, ionic strength and phosphate concentration on the adsorption of the antibody to the aluminum gel was investigated. **Materials and Methods:** Charge of the antibody and aluminum gel was measured by electrophoretic light scattering for a better understanding of the interaction between both components. Different formulations modifying adsorption percent and antibody/adjuvant ratio were prepared to evaluate in vivo effects on immunogenicity in Leghorn chickens. **Results:** Size and charge of all the formulations were measured by Dynamic Light Scattering. Interestingly, electrostatic interaction seems to play a minor role in the adsorption of Racotumomab to alum, as the pH and ionic strength had influence on adsorption only in presence of phosphate ions. The major impact on the adsorption mechanism of phosphate suggests that ligand exchange with carboxyl groups of the antibody molecule is responsible for adsorption of the antibody in the formulation. **Conclusions:** No influence of the adsorption and the Racotumumab/Alumina ratio on the immunogenicity of the vaccine was observed.

THERAPEUTIC VACCINES STABILITY STUDIES: 5 YEARS EXPERIENCES AT CIM

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Introduction: Research into cancer vaccines has advanced more slowly than other types of immunotherapy research like monoclonal antibodies or other recombinants proteins. The main reason of this delay is provoked by the complexity of the vaccine; most of them composite by the API and adjuvant. Vaccines are usually of small volume administered subcutaneously or intramuscular. There are not pharmacopeias for therapeutic vaccines neither regulatory requirement as are for the rest of recombinant molecules such as: amount of visible and subvisibles particles, pyrogen or LAL content which make harder the stability evaluation. Biological activity is also of outmost importance for the developmental and stability studies. However biological activity (immunogenicity) is very complex to develop, especially since preclinical data is known to be non-representative of the human mode of actions. Immunogenicity for vaccines is ¨multi targeted¨ and involves the complexity of the hundreds of molecules of the human system. On the other hand, the final formulations are very complex and the identification of their critical quality attributes in the final product is still a challenge. In this context a novel stability approaches are clearly needed. **Materials and Methods:** To analyse the concurrent stability studies and proposal a control strategy of stability based on ICH Q8 and depending on the development phase and time dependent. **Results:** Vaccines have specifications which allow evaluating the quality attributes of the vaccines that are under stability studies. Novel approaches of developing biological activity and specifications are proposed according to newly published guidelines on ICH Q8/Q9.

VERY SMALL SIZE PROTEOLIPOSOMES DERIVED FROM *NEISSERIA MENINGITIDIS*: AN EFFECTIVE ADJUVANT FOR ANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTE RESPONSE STIMULATION UNDER LEUKOPENIC CONDITIONS

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Introduction: Leukopenia is a severe condition resulting from both pathological processes and some treatments, like chemotherapy in cancer patients. However, the activation of the patient immune system is required for the success of immunotherapeutic strategies, as cancer vaccines. In this regard, leukopenia constitutes a major hurdle to overcome, mainly due to the impairment of cytotoxic T lymphocyte (CTL) responses. Adjuvants are basic components of vaccine formulations, which might be useful to stimulate immunity under this immunosuppressed condition. **Materials and Methods:** We tested the capacity of a novel nanoparticulated complex, very small size proteoliposomes (VSSP), to promote CTL even in mice rendered leukopenia by the administration of high doses of the chemotherapeutic agent cyclophosphamide (CY). **Results:** We observed that a VSSP-based OVA vaccine induced a normal antigen-specific CTL response in mice rendered leukopenia, while under the same conditions the OVA antigen formulated in the TLR-3 agonist polyinosinic-polycytidylic acid (P(I:C)) was ineffective. Moreover, an appropriate combination of VSSP with the P(I:C) vaccine was able to restore the $CD8^+$ T cell effector function in leukopenic mice. VSSP induced not only a faster repopulation of immune cells in CY-receiving animals, but also enhanced the recovery of memory T lymphocytes and myeloid dendritic cells (DCs) while simultaneously abrogated the immunosuppressive capacity of myeloid-derived suppressor cells (MDSCs). **Conclusions:** Our results suggest that VSSP could be a particularly suitable immunomodulator to be used in CTL-promoting active immunotherapy strategies operating in severe immune compromised scenarios.

PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF 1E10 ANTI-IDIOTYPE VACCINE

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Introduction: 1E10 monoclonal antibody is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested as an anti-idiotypic cancer vaccine, adjuvated in Al(OH)3, in several clinical trials for melanoma, breast, and lung cancer. During early clinical development this mAb was obtained in vivo from mice ascites fluid. Currently, the production process of 1E10 is being transferred from the in vivo to a bioreactor-based method. **Results:** Here, we present a comprehensive molecular and immunological characterization of 1E10 produced by the two different production processes in order to determine the impact of the manufacturing process in vaccine performance. We observed differences in glycosylation pattern, charge heterogeneity and structural stability between in vivo-produced 1E10 and bioreactor-obtained 1E10. Interestingly, these modifications had no significant impact on the immune responses elicited in two different animal models. **Conclusions:** Changes in 1E10 primary structure like glycosylation; asparagine deamidation and oxidation affected 1E10 structural stability but did not affect the immune response elicited in mice and chickens when compared to 1E10 produced in mice.

INHIBITION OF TUMOR-INDUCED MYELOID-DERIVED SUPPRESSOR CELL FUNCTION BY A NANOPARTICULATED ADJUVANT

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Introduction: The interaction between cancer vaccine adjuvants and myeloid-derived suppressor cells (MDSCs) is currently poorly understood. Very small size proteoliposomes (VSSP) are a nanoparticulated adjuvant under investigation in clinical trials in patients with renal carcinoma, breast cancer, prostate cancer, and cervical intraepithelial neoplasia grade III. **Material and Methods:** VSSP-induced modulation of the percentage and phenotype of splenic and tumor-infiltrating MDSCs was evaluated on tumor-bearing B6 and BALB/c mice, treated with the adjuvant. Also the effect of VSSP on the suppression of antigen-specific and alloreactive CTLs, mediated by tumor-induced MDSCs, was assessed both *in vitro* and *in vivo*. **Results:** We found that VSSP adjuvant produced a significant splenomegaly due to accumulation of $CD11b^{+}Gr\text{-}1^{+}$ cells. However, VSSP-derived MDSCs showed a reduced capacity to suppress both allogeneic and antigen-specific CTL response, compared to tumor-induced MDSCs. Moreover, splenic MDSCs isolated from tumor-bearing mice treated with VSSP were phenotypically more similar to those isolated from VSSP-treated tumor-free mice and greatly less suppressive than tumor-induced MDSCs, both *in vitro* and *in vivo*. Furthermore, different from dendritic cell (DC) vaccination, inoculation of VSSP-based vaccine in EG.7-ovalbumin (OVA) tumor-bearing mice was sufficient to avoid tumor-induced tolerance and stimulate an immune response against OVA antigen, similar to that observed in tumor-free mice. This effect correlated with an accelerated differentiation, promoted by VSSP, of MDSCs into mature antigen-presenting cells (APCs). **Conclusions:** The use of VSSP as a cancer vaccine adjuvant might thus improve antitumor efficacy, not only by stimulating a potent immune response against tumor antigens, but also reducing tumor-induced immunosuppression.

STABILITY EVALUATIONS OF CIGB 247 NOMINAL ANTIGEN RECONSTITUTED SOLUTION

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Introduction: CIGB 247 antigen is a fusion protein comprising a mutated variant of Vascular Endothelium Growth Factor (VEGF), fused to a 47 aa fragment of P64k protein. The molecule is used in combination with the aqueous adjuvant Very Small Size Proteoliposomes (VSSP) in order to trigger an immune response vs VEGF, a molecule over-produced by more than 90% of human tumors. A formulation of lyophilized antigen was successfully developed at two antigen strengths (0.1 mg/ vial and 0.4 mg/ vial) showing good stabilities both in shelf conditions (5 ± 3 ^{0}C) and under accelerated conditions (25 ± 2 ^{0}C). Generally the stability of lyophilized products is affected after reconstitution. Herein we addressed this issue in the case of CIGB 247 antigen. **Materials and Methods:** Reconstituted solution stability was evaluated in terms of protein concentration (microcoomassie), integrity (SDS-PAGE) and aggregation profile (HPLC SE) in samples of six batches (three batch of each strength) stored 0,24, 48 and 72 hours under two temperature conditions (5 ± 3 ^{0}C and 25 ± 2 ^{0}C). **Results and discussions:** All batches showed similar stability profiles at 5 ± 3 ^{0}C for 72 hours. Nevertheless, when stored at 25 ± 2 ^{0}C evaluated parameters were more affected for 0.1 mg strength than for 0.4 mg, suggesting a self stabilizing effect of increase protein concentration. This effect was visible as early as 48 hours indicating a reduction of protein stability at 25 ± 2 ^{0}C. **Conclusions:** Reconstituted solutions of antigen CIGB 247 should not to be stored at 5 ± 3 ^{0}C for more than 72 hours and can not be stored at 25 ± 2 ^{0}C.

EVALUATION OF MOISTURE CONTENT EFFECT ON THE STABILITY OF LYOPHLIZED ANTIGEN FROM CIGB 247 THERAPEUTIC VACCINE

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Introduction: CIGB 247 is the nominal antigen of a new therapeutic vaccine for cancer treatment that targets the Vascular Endothelial Growth Factor (VEGF), a molecule frequently associated with a bad prognosis in cancer. Formulation of lyophilized antigen was successfully developed at two antigen strengths (0.1 mg/ vial and 0.4 mg/vial) showing good stability both in shelf (5 ± 3 ^{0}C) and accelerated (25 ± 2 ^{0}C) conditions. Since stability of lyophilized antigen might be conditioned by moisture content, the present study was undertaken in order to evaluate the influence of this parameter to properly set up end product specifications. **Materials and Methods:** Protein concentration (microcoomassie), integrity (SDS-PAGE) and aggregation profile (HPLC SE) were evaluated under accelerated conditions (37± 2 ^{0}C), in samples of six batches with different moisture content previously measured using Karl Fisher method. Organoleptic appearance of lyophilized and reconstituted product and pH were also evaluated. **Results and discussions:** No differences were found between groups. Protein concentration and organoleptic characteristics were affected in both samples groups with low moisture contents (≤3.5%) and with high moisture content (≥6%). Integrity, aggregation profile and pH were not affected in any group. **Conclusions:** Actual moisture contents specifications for CIGB 247 lyophilized antigen set to less than 5%, doesn't compromise the protein stability.

A NOVEL ANTIGEN-CYTOSOLIC DELIVERY SYSTEM BASED ON STICHOLYSINS ENCAPSULATED INTO LIPOSOMES FOR ENHANCING CELLULAR IMMUNE

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Introduction: Cytosolic delivery strategies of antigenic proteins to antigen-presenting cells (APC) in order to improve the antigen-specific cytotoxic T lymphocyte (CTL) response, are crucial in the development of anti-tumor and anti-intracellular infections vaccines. Liposomes (LP), lipid-vesicles widely used as adjuvants, nowadays are an attractive alternative for antigen delivery into the cytosolic space of APC. With the purpose to improve the antigen delivery from LP, promising strategies have employed the co-encapsulation in these vesicles of bacterial pore-forming toxins with antigens. Sticholysins I and II (Sts, StI/II) are pore-forming toxins produced by the sea anemone *Stichodactyla helianthus* extensively studied in our lab. Due to the functional homology of Sts with bacterial pore-forming toxins, we studied the stimulation of antigen-specific CTL-mediated immune response by LP co-encapsulating Sts with ovalbumin (OVA) as model antigen (LP/OVA+St). **Materials and Methods:** LP were prepared using a lipid composition where Sts do not exhibit pore-forming ability. In vivo assays were carried out using C57BL/6 mice. **Results:** Immunization with LP/OVA+St enhanced significantly an OVA-specific CTL activity keeping the same level of anti-OVA antibody response, in comparison with LP/OVA treatment. Moreover, LP/OVA+St conferred a higher protection to mice challenged with OVA-expressing tumor cells

than LP/OVA. Interestingly, the inclusion into liposomes of a StI mutant forming a reversible-inactive dimmer stabilized by a disulphide bond also induced a similar antigen-specific CTL response, indicating the effectiveness of this safer alternative. Besides, the CTL activity induced by LP/OVA+St was independent of CD4+ T-cells help, while anti-tumor response was affected when the CD8+ T cells were depleted. **Conclusions:** Our results suggest the potentialities of LP encapsulating Sts as a novel and promising tool for priming cellular immune responses.

B-1 CELLS CONTRIBUTION IN THE IMMUNE RESPONSE INDUCED BY ANTIGEN ENCAPSULATED INTO PHOSPHATIDYLCHOLINE-CONTAINING LIPOSOMES

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Introduction: B-1 lymphocytes comprise a unique subset of B cells that differ phenotypically, ontogenetically and functionally from conventional B-2 cells. The most frequent specificities of the antibody repertoire of peritoneal B-1 cells are phosphatidylcholine (PtC) and phosphorylcholine. Liposomes containing phosphatidylcholine have been studied as adjuvants and their interaction with dendritic cells and macrophages has been demonstrated. However, their interaction with B-1 cells has not been explored. **Materials and Methods:** To study the contribution of B-1 cells to the humoral response induced by ovalbumin (OVA) encapsulated into dipalmitoylphosphatidylcholine (DPPC) - and cholesterol (Chol) -containing liposomes and its possible relation to lipid composition; BALB/c and B-1 cells deficient BALB/xid mice were immunized with OVA encapsulated in liposomes of DPPC:Chol/OVA or liposomes of dipalmythoylphosphatidylglycerol and cholesterol (DPPG:Chol/OVA).To determine the contribution of antibodies specificfor DPPC, we evaluated the antibody response induced by DPPC:Chol/OVA in BALB/xid mice reconstituted with antibodies DPPC-specific. The ability of B-1 cells to internalize antigens encapsulated into DPPC:Cholliposomes was also evaluated. **Results:** BALB/*xid*mice showed quantitative and qualitative differences with respect to wild type animals in the anti-OVA antibody response induced with DPPC:Chol/OVA. Transfer of antibodies DPPC-specific to BALB/*xid* mice partially restored the response induced by the liposomal preparation. The OVA-specific immune response was further enhanced in BALB/*xid*mice when reconstituted with B-1 cells. These cells were able to internalize DPPC-containing liposomes and to migrate from the peritoneal cavity to spleen. The adjuvanticity of liposomes was further demonstrated to be dependent on phosphatidylcholine, no enhanced anti-OVA response was induced when the antigen was encapsulated into phosphatidylglycerol and cholesterol-containing liposomes. **Conclusions:** A cognate interaction has been proven between B-1 cells and DPPC-containing liposomes, which modulates the immune response to encapsulated-antigens, providing a novel targeting approach to assess the role of B-1 cells to generate protective immunity to pathogens or pathological processes.

TWO NANO-PARTICULATE ADJUVANT FORMULATIONS FOR IMMUNOTHERAPY OF RESPIRATORY ALLERGY TESTED IN A BIPHASIC PRIMING-BOOSTING MODEL BY SUBCUTANEOUS AND SUBLINGUAL ROUTE IN THE MICE

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Introduction: Novel adjuvants are being increasingly investigated in order to improve conventional allergen-specific immunotherapy, regarding efficacy, shrinking of treatment duration and safety. The aim of this Paper was to evaluate the immune modulating effect of a novel experimental nano-particulate vaccine formulation in a therapeutic murine model of respiratory allergy, using a priming-boosting strategy. **Methods:** Adjuvanted vaccine formulations were prepared by combining purified native allergens of the mite *Dermatophagoides siboney* (Der s 1 and Der s 2) with immune modulating molecules in nano-proteoliposomes (nPL) and nano-cochleates (nCH). C57/Bl6 mice were sensitized administering *D.siboney* allergen by IP route and exposing mice to allergen aerosols, and then, treated with the experimental formulations, first with subcutaneous priming with nPL-Ds and later, with sublingual daily doses during 4 weeks (boosting) with nCH-Ds. Finally, mice were subjected to inhalation allergen challenge. Detailed anatomo-pathological-toxicological studies were performed. **Results:** Subcutaneous priming induced a moderate pro-Th1 allergen-specific response with mixed IgG1 and IgG2a antibodies, moderate amount of IFN-gamma, besides Th2 and Tr1 cytokines. After the boosting intervention by sublingual route, IgG2a values, similar to those of healthy animals, were achieved. In treated mice subjected to allergen challenge, IgE antibodies showed a decrease, together with an increase of the IgG/IgE ratio, increase of IL-10 and CD4+FoxP3+ cells, as well as, decrease of local and systemic eosinophilia and peribronchial inflammatory infiltrate and mucus secretion, as assessed by lung histology. A very adequate safety profile was obtained. **Conclusions:** This novel vaccine formulation, using the priming-boosting strategy by systemic and mucosal routes ,showed promising results by modulating the allergic response first to a Th1 cytokine profile and later to a regulatory and tolerogenic pattern.

MONDAY 18 (NIGHT)

• Poster Session: Experimental and Clinical Evaluation of Therapeutic Vaccines & Regulatory Landscape for Therapeutic Vaccines

Chairman: *Belinda Sánchez and Julianna Lisziewicz*

REPEATED DOSES INTRAMUSCULAR INJECTION OF THE CIMAVAX-EGF VACCINE IN SPRAGUE DAWLEY RATS INDUCES LOCAL AND SYSTEMIC TOXICITY

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Introduction: CIMAvax-EGF consists of a human recombinant Epidermal Growth Factor (EGF), coupled to P64k (recombinant carrier protein from *N.meningitis)*, and Montanide ISA 51 as adjuvant. The vaccine immunization induces a specific antibody production, inhibiting the EGF/EGF-R interaction through EGF deprivation. Our aim was to assess the CIMAvax-EGF toxicity in Sprague Dawley rats after intramuscular administration of repeated doses (6 months) and to determine if rat is a relevant species for studying CIMAvax-EGF vaccine. **Materials and Methods:** Four experimental groups were established: Control, Montanide ISA 51, Treated with 1X and 15X of human total dose of the antigen. Animals were immunized weekly during 9 weeks, plus 9 immunizations every 14 days. Rats were inspected daily for clinical signs. Body weight, food consumption, and rectal temperature were measured. Hematological, biochemical and EGF titles analysis were performed at the beginning, three months and at the end of experimentation.

Gross necropsy and histological examination of tissues were performed on animals at the end of the assay. **Results:** Vaccine provoked the apparition of antibodies against EGF in the rats, demonstrating rat species relevance. Body weight gain, food and water consumption were not affected. CIMAvax-EGF and Montanide ISA 51 produced local damaged at the administration site, showing multiple cysts and granulomas. Both vaccine-treated groups showed neutrophil elevationand AST increase. Rectal temperature was found to be significantly higher in 15X Treated group after immunizations. **Conclusions:**The clinical pathology findings, together with the body temperature results, appears to be caused by the inflammatory reaction at the administration site of the vaccine, mainly mediated by the oil-based adjuvant Montanide ISA 51, probably enhanced by the immunological properties of the antigen. This study showed evidences that intramuscular administration during 26 weeks of CIMAvax-EGF at doses up to 15x human total dose is well tolerated in rats.

REPEATED DOSE SUBCUTANEOUS ADMINISTRATION OF 1E10 VACCINE IN SPRAGUE DAWLEY RATS

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Introduction: Cancer has become a serious problem for mankind because of its high grade of incidence and mortality. Specific active immunotherapy is a therapeutic approach against this disease, allowing the targeting of immune response against malignant cells with a higher grade of effectiveness. The objective of this work was to determine the possible toxic effects due to the repeated dose subcutaneous administration for 14 days of the therapeutic vaccine 1E10. **Materials and Methods:** It was established three groups of 5 Sprague Dawley rats per sex: Control (Saline), Vehicle (Alumina Gel), and Treated (1E10 vaccine). Animals were daily observed to detect toxicity signs, and rectal temperature was measured before and after the administration of the substances. There were carried out haematological and blood chemistry exams on all animals at the beginning and at the end of the assay, and histopathological examination was performed on day 14th. **Results:** There were not detected any significant variations neither in corporal weight nor rectal temperature or haematology parameters. Blood chemistry analysis showed an increased of bilirubin and creatinine in all groups, not been associated to the vaccine administration. Animals of Vehicle and Treated groups showed multiple white ring-shaped formations in the subcutaneous cellular tissues at the administration site, possible due to the action of the adjuvant (Alumina Gel). **Conclusions:** Obtained results indicated the absence of toxic effects in rats due to the administration of 1E10 vaccine.

CLINICAL EVALUATION AND COMPLIANCE OF GOOD CLINICAL PRACTICES FOR THERAPEUTIC VACCINES IN CUBA

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Introduction: One of the most remarkable achievements of the Biotechnological Industry has been the research and development of therapeutic vaccines for the treatment of several chronic diseases through the induction and or the amplification of the host immune response. The Cuban Regulatory Authority (CECMED), according to the current scientific knowledge and the flexibility and typical characteristics of the clinical development for these biological products, has incorporated new approaches and specific considerations in the regulatory system and it has established the National Inspection Program (PNIEC), a

priority in the assessment of the compliance of Good Clinical Practices in studies to ensure timely access to market of those molecules with quality, safety, efficacy and reliable information for its rational use. The aim of this Paper is to evaluate the results of implementing the clinical evaluation guidelines for therapeutic vaccines against cancer and AIDS. **Materials and methods:** The specific considerations described in national and international regulations for therapeutic vaccines were reviewed and a quantitative-qualitative analysis based on Clinical Trials and marketing requests of therapeutic vaccines was carried out. Also, the documentation issued during the evaluation period 2011-2012 was analyzed, as well as the results derived from the compliance of the PNIEC in the last ten years. **Results:** The implementation of Annex No. 2 of the Regulation No. 27-2000 was officially started in January 2011. Ten clinical trials, seven clinical trials modifications and two marketing authorizations of therapeutic vaccines have been approved according to the considerations included in the current national regulatory guidelines. Besides, the main deficiencies found during the GCP inspections were identified. **Conclusions:** A significant compliance level reached out by promoters, institutions involved and CECMED was shown during the evaluation of the implementation results in the first year, demonstrating an appropriate design for the clinical evaluation strategy.

SAFETY PROFILE IN A FINISHED PHASE III CLINICAL OF THE THERAPEUTIC CANCER VACCINE CIMAVAXEGF USED IN THE TREATMENT OF PATIENTS DIAGNOSED WITH ADVANCED NON-SMALL CELL LUNG CANCER TUMORS

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Introduction: CIMAvax EGF is a therapeutic cancer vaccine, containing the human recombinant Epidermal Growth Factor chemically conjugated to P64k carrier protein from Neisseria meningitidis and emulsified in Montanide ISA 51 VG adjuvant. The clinical development of the vaccine encompass five phase I/II clinical trials, a phase II proof of concept clinical trial and a phase III proof of efficacy clinical trial, 242 patients vaccinated with CIMAvax EGF achieve 3.23 months of survival benefit by Intent to Treat Analysis. With more than 2000 patients treated, CIMAvax EGF recently achieve the sanitary registration approval by the Cuban regulatory agency CECMED to be indicated in the advanced (IIIB/IV) non- small cell lung cancer (NSCLC) setting of adult patients and is also registered in Peru. **Materials and Methods:** Here we present the adverse events (AE) profile associated with the use of CIMAvax EGF in a concluding phase III clinical trial, it was an open, controlled, in wich 400 patients with advanced NSCLC (IIIB/IV) after finish the oncological treatment were randomized in two groups to receive: The first one CIMAvax EGF and other the best conventional treatment. The baseline patient's characteristics, as well as the frequency; intensity and cause relationship of the reported AE were analyzed. **Results**: The more frequently observed AE were: Injection site pain (17.3%), fever (16.0%), headache (11.4%), chills (6.0%), vomiting (6.0%), and arthralgia (5.6%) all them foresaw in the protocol design because were reported in previous

phase I and II trials. Most of the reported AE were of mild to moderate intensity and did not require the suspension of the therapeutic vaccine administration. **Conclusions:** The CIMAvax EGF therapeutic vaccine is a safe option for the treatment of patients at the advanced NSCLC stages and further studies will be held in the post registered open scenario.

PRELIMINARY SAFETY, TOLERANCE AND EFFICACY OF ACTIVE IMMUNOTHERAPY WITH NASVAC® THERAPEUTIC VACCINE CANDIDATE IN REFRACTORY CHRONIC HEPATITIS B PATIENTS

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Introduction: The NASVAC® therapeutic vaccine candidate (CIGB, La Habana), designed for the treatment of chronic hepatitis B, is a bivalent liquid formulation composed of non-covalently linked recombinant surface and core antigens from the Hepatitis B Virus. Nasal inoculation of NASVAC® in the HBsAg transgenic mice model and during a Phase I clinical trial in healthy adults has been safe, strengthening the initial hypothesis associated to its design. **Materials and Methods:** A Phase I open, non-controlled clinical trial was performed to preliminary assess the safety, tolerance and efficacy of an immunotherapy schedule using NASVAC® in 6 patients with refractory chronic hepatitis B (CHB). Patients were positive to HBsAg in serum for ≥6 months and exhibited abnormally high levels of ALAT/ASAT prior inclusion. All subjects received 10 intranasal doses of 100 µg of NASVAC® (1 mL) at weeks 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18. The vaccine was delivered using a nasal spray device (VP7D, Valois, France). Efficacy of vaccination was estimated through evaluation of virological, biochemical and serological parameters in pre-vaccination (week 0) and follow-up (weeks 12, 24, 36, 48, 52, 60, 72) serum samples. **Results:** There were no cases of hepatic, renal or bone marrow dysfunctions during the immunotherapy. No serious adverse events (AE) were recorded. The most frequently recorded AEs were: sneezing (20.5%), malaise (16.1%), headaches (14.7%), asthenia (12.7%) and nasal obstruction (6.8%). Longitudinal scrutiny of the virological, biochemical and serological responses showed HBeAg seroconversion in 1/3 patients (33.3%) at week 24 of follow up. ALAT (or ASAT) levels returned to normal for the 6 patients by week 52 and remained normal till the end of follow up. Sustained significant reduction in viral load was detected in 3 patients (50.0%) since week 52 of follow up. **Conclusions:** NASVAC® was clinically safe and well tolerated in CHB patients. The preliminary efficacy results obtained are encouraging and suggest that rHBsAg and rHBcAg may be important components of an effective therapeutic HBV vaccine and should be considered for additional larger scale testing.

EVALUATION OF A NOVEL THERAPEUTIC ANTI-ALLERGIC VACCINE PROLINEM-DS, IN SENSITIZED MICE

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Introduction: An important trend in allergen-specific immunotherapy is to investigate new adjuvants with immunomodulatory properties. Previous work has shown the pro-Th1 adjuvant effect of *Neisseria meningitidis* proteoliposome (PL). **Objective:** To assess the immunogenicity and non-clinical safety profile of a novel anti-allergic vaccine candidate PROLINEM-DS based on allergens from *Dermatophagoides siboney* and a combination adjuvant containing PL and Alum, known as AFLP1® in sensitized mice. **Methods:** In therapeutic experimental setting C57/Bl6 mice were first sensitized administering *D.siboney* allergen by IP route and exposing mice to allergen aerosols. Later, allergic mice were treated with 3 doses of the AFLP1-adjuvanted vaccine containing 2µg of Der s1 allergen by subcutaneous route. Further, mice were subjected to inhalation allergen challenge. IgE, IgG1, and IgG2a allergen-specific antibody response was measured by ELISA, as well as the specific cellular response (IL-13, IL-10 and INF-γ). Systemic toxicity was assessed measuring body weight and macroscopic and histological examination of several organs and injection site. **Results:** As in the previously tested preventive model, the AFLP1 adjuvanted vaccine was able to induce a mixed IgG1/IgG2a antibody response and moderate levels of IFN-γ, although together with Tr1 (IL-10) and Th2 (Il-13) cytokines. Moreover, after the allergen challenge, it was noted a significant increase ($p<0.05$) of the IgG/IgE ratio, with a decrease in bloods eosinophils and allergic inflammation in lung tissues as compared to allergic controls. No significant differences between body weights and differential leukocyte count in treated mice and negative control group was observed. Normal histology was observed. Local tolerance at the injection site suggests that granulomes in vaccinated subjects are caused by alum. **Conclusions:** The adjuvanted vaccine does not exacerbate the allergic response nor promote Th1 inflammation, supporting a satisfactory safety profile for further clinical trials in humans. This immunomodulatory effect suggests clinical benefits both in cellular and blocking antibody responses.

ADVERSE EVENTS WITH N-GLYCOLILGM3/VSSP MONTANIDE ISA 51 VACCINE IN PATIENTS WITH ADJUVANT BREAST CANCER AND DISEASE-FREE

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Introduction: Breast cancer in Cuba has a high incidence with an annual number of 2000 new cases diagnosed. Treatment with chemotherapy and radiotherapy causes severe adverse reactions. New therapeutic modalities such as ganglioside associated N-GlycolilGM3/VSSP carrier and the adjuvant Montanide ISA 51 are currently assessed in the treatment of this disease. **Objectives:** To assess safety through the reporting of adverse events occurred in patients with this therapy. **Materials and methods:** A review was made of medical records of 23 patients assessed and vaccinated in the oncology department of Camilo Cienfuegos Hospital from 2008 to 2011. Data were related to the type of adverse event, time of occurrence and their classification according to intensity and severity (WHO criteria). The causality relation with the product was evaluated, using the Common Criteria of Adverse Events (CTCAE) version 3.0 of the National Cancer Institute of USA. **Results:** A total of 135 adverse events were reported, of which 123 (91.2%) were classified as mild to moderate, local and systemic type mostly and not related to the vaccine. There were 12 serious adverse events (8.8%) with 5 related to the product (3.7%), of which 1 was evaluated as severe and led to treatment discontinuation. A total of 74 (54.8%) adverse events had a causality relation with the vaccine and were classified as definite, highly probable, probable and possible, and 61 (45.2%) not related. **Conclusions:** The vaccine NGlycolil GM3 was safe in patients treated, most local and systemic adverse events mild or moderate, they did not lead to the discontinuation of therapy.

CIGB-247: VEGF COMBINED WITH VSSP AS CANCER THERAPEUTIC VACCINE

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Introduction: The vascular endothelial factor (VEGF) and its receptors is the main growth factor system involved in tumor angiogenesis. Cancer immunotherapy targeting VEGF or its receptors is an emerging strategy for controlling tumor growth and progression. Along this research line, our group has developed the cancer vaccine candidate CIGB-247, that comprehends a recombinant modified human VEGF antigen, combined with a clinically tested bacterial adjuvant (very small sized proteoliposomes or VSSP), produced from the outer membrane of *Neisseria meningitidis*. **Materials and Methods:** The anti-tumor and anti-metastatic effects of the CIGB-247 vaccine were tested using the B16F10 melanoma, and the CT26 colon, lung 3LL-D122, and breast F3II carcinomas, growing either in C57Bl/6 or BALB/c mice. Tumor and lung metastatic histology, and specific humoral and cellular responses to the vaccine were evaluated. Inmunogenicity and safety studies were also carried out in New Zeland rabbits, Winstar rats and in *Cercopithecus aethiops sabaeus* non human primates. **Results:** Vaccination with CIGB-247 produces anti-tumor effects in mice injected with B16F10, CT26 and F3II tumor cells, measured as a reduction in tumor engraftment, slower tumor growth kinetics, and/or increased animal survival. CIGB-247 vaccination also significantly reduced the number and size of experimental and/or spontaneous metastatic tumor foci in lungs for the CT26, 3LL-D122 and F3II tumor models. Vaccination produces antibodies that block VEGF–VEGF receptor interaction in mice, rats, rabbits, and non human primates. Specific T-cell cytotoxic responses against tumor cells that produce VEGF were documented in mice. Positive DTH and specific T-cell cytotoxic responses against autologous VEGF-charged PBMC were found in non human primates after vaccination. CIGB-247 and show an excellent safety profile in mice, rats, rabbits, and non human primates. **Conclusions:** Altogether, these results support the further clinical development of the CIGB-247 therapeutic cancer vaccine, and inform on the potential mechanisms involved in its anti-tumor effect.

FUSION OF A TUMOR-ASSOCIATED ANTIGEN TO A CELL PENETRATING PEPTIDE IMPROVES PROTEIN-BASED IMMUNOTHERAPY OF CANCER

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Introduction: Infection with human papillomavirus type 16 (HPV16) is strongly associated with a number of disease states, of which cervical and anal cancers represent the most drastic endpoints. Induction of T-cell-mediated immunity, particularly cytotoxic T lymphocytes (CTL), is important in eradication of HPV-induced lesions. Vaccination with proteins or synthetic peptides incorporating CTL epitopes have proven limited due to the failure for exogenous antigens to be presented efficiently to T-cells. Linking of antigens to cell penetrating peptides (CPP) overcomes such obstacles by facilitating cellular uptake, processing and presentation of exogenous antigen for the induction of potent immune responses. In order to test this concept, we designed a novel fusion protein ($LALF_{32-51}$-E7) in which the tumor-associated antigen (E7 from HPV16) is fused to a CPP $LALF_{32-51}$ from *Limulus polyphemus*. **Materials and Methods:** The cell penetrating ability of $LALF_{32-51}$-E7 was evaluated by confocal microscopy, immunofluorescence microscopy and Western Blot. The antitumor response evaluations were conducted in a preclinical model of HPV16-induced cervical carcinoma (TC-1 model). ELISPOT and LDH assays were performed to assess the cell-mediated immune response. **Results:** We demonstrated that $LALF_{32-51}$ penetrates the cell

membrane and delivers E7 into cells. Also we showed in the TC-1 model that vaccination with adjuvant-free $LALF_{32-51}$-E7 fusion protein significantly improves the presentation of E7-derived peptides to T-cells *in vitro* and induces suppression of tumor growth. **Conclusions:** Our results underline the efficacy of this approach at inducing broad immune responses *in vivo*, and offer a new strategy for improving subunit cancer vaccine in a clinical setting.

EGFR-BASED CANCER VACCINE DOES NOT IMPAIR WOUND HEALING AND INFLAMMATION IN MURINE MODELS

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Introduction: Epidermal growth factor receptor (EGFR) is involved in cancer cells distinctive properties. Anti-EGFR therapies have been developed and have been proven effective in clinical trials for a variety of tumor types. Vaccination of mice with the extracellular domain (ECD) of autologous EGFR overcome the tolerance to self-EGFR and has antimetastatic effect on EGFR+ tumor. Because EGFR-signalling is important in wound healing and EGF also plays an important role in the inflammation stage of wound healing; the main objective of this study was to explore the possible role of murine (m) EGFR-ECD vaccine in the croton-oil-induced ear edema and the wound healing in experimental animal models. **Material and Methods:** Mice were immunized four times biweekly with 50 µg of mEGFR-ECD/VSSP/Mont or with PBS/VSSP/Mont by intramuscular way. Seven days after the last immunization, all animals were anesthetized and then, 8 mm diameter, full-thickness skin wound was performed on the back of each animal. **Results:** Immunization induced in mice a strong specific humoral response against the mEGFR-ECD protein and a DTH dose-response curve but interestingly, animals treated with mEGFR-ECD/VSSP/Mont had a similar inflammatory response compared to control ones in the croton oil inflammation model. No differences in healing speed were found for the skin wounds in the mice vaccinated with mEGFR-ECD, with respect to the control animals. Planimetry measurements, histological and morphometrical analysis did not led to significant impairment in tissue repair. **Conclusions:** These data suggest that application of mEGFR-ECD/VSSP vaccine as a therapeutic approach in cancer patients could not elicit a poor healing process after surgery or other invasive procedures.

HEPATITIS C VIRUS SPECIFIC IMMUNE RESPONSE UNDER TRIPLE THERAPY WITH INTERFERON-α PLUS RIBAVIRIN AND A HCV DNA-BASED THERAPEUTIC VACCINE CANDIDATE FOR CHRONIC INFECTION

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Introduction: HCV vaccine candidates have started to be tested with interferon (IFN)-based therapies, aiming at the diversification of T cell responses in the context of reduced viral load. We analyzed HCV-specific immune response in chronically infected patients under therapy with IFN-α plus ribavirin and different number of doses of CIGB-230, a HCV DNA-based therapeutic vaccine candidate. **Materials and**

Methods: Patients were randomized to five groups corresponding to different immunization schedules regarding the start of immunization (simultaneous or on week 12 of antiviral therapy) and a control group. IgG, IFN-γ secretion and lymphoproliferation against structural and non structural antigens were tested at baseline, and at the end of treatment (week 48). **Results:** When patients were analyzed according to virological response, significant differences were not generally observed between responders and non responders in terms of frequency or magnitude of the detected immune responses. IgG was frequent against all tested antigens and showed significant decreases in magnitude after treatment. Cellular responses were scarcely detected at baseline and generally disappeared on week 48. Remarkably, de novo proliferative responses against core antigen were only observed in groups simultaneously receiving CIGB-230. Additionally, significant declines in IFN-γ secretion against all antigens were only observed in the control group. These changes in HCV specific immune responses took place in face of a significant leukopenia induced by IFN treatment in all groups of patients. **Conclusions:** Despite the early virological effect, CIGB-230 was not able to reduce viral relapse, suggesting an incapability to generate substantial T cell memory responses. Therefore, regardless of IFN-based therapy's potential to reduce HCV viral load, its combination with therapeutic vaccination is not an easy task, for the induction of T cell memory, given its antiproliferative properties; and the optimal immunization schedule is still to be defined.

TH2-TH1 SHIFT OF THE HIV-SPECIFIC RESPONSE WITH THE VACCINE CANDIDATE TERAVAC-HIV-1

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Introduction: After HIV infection, a Th1 to Th2 profile shift is observed and it has been related to disease progression. One of the possible benefits of a therapeutic vaccination might be to counterbalance this phenomenon allowing viral replication control by a Th1 immune response. TERAVAC-HIV is a vaccine candidate against HIV-1 that comprises a recombinant protein (CR3) that contains Th and CTL epitopes of HIV and the surface (S) and nucleocapsid (C) antigens of HBV. Previous results showed that both VLP of HBV provide a Th1 adjuvant effect. Immunization of CR3 alone results in a Th2-type response. **Methods:** In the first phase of experimentation to induce a Th2 response, groups of Balb/c mice were immunized three times via SC with: 1-2) Placebo, 3-4) mixture C+S, 5-6) CR3, 7-8) Viral lysate, 9) TERAVAC. In the second phase the capacity of TERAVAC to subvert the Th2 response was studied. The same groups were immunized five times via SC or simultaneously by the SC and IN (IN+SC) routes as follow: 1) C+S (IN+SC), 2) C+S (SC), 3-5-7) CR3+C+S (IN+SC), 4-6-8) CR3+C+S (SC). At the end of each phase of immunization, the IgG subclasses pattern and cytokine secretion after ex vivo stimulation of splenocytes with CR3, were assessed. **Results:** After the first phase of immunization a CR3-specific Th2-type response was verified in animals inoculated with CR3 or viral lysate by induction of IL-4 and IL-10 secretion with negligible levels of IFN-gamma and IgG2a antibodies. However, after TERAVAC immunization the same groups of animals showed high levels of IFN-gamma secretion and production of IgG2a antibodies in serum. **Conclusions:** A successful generation of a Th1-type response was demonstrated under an ongoing-Th2 response after TERAVAC immunization. This suggests a therapeutic benefit of this vaccine candidate in the restoration of the Th1-type HIV-specific cellular response in seropositive patients.

CIGB-230, A DNA THERAPEUTIC VACCINE CANDIDATE, ADDED TO REGULAR INTERFERON PLUS RIBAVIRIN THERAPY IN CHRONIC HEPATITIS C PATIENTS

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Introduction: Hepatitis C virus is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. While current medical therapy is curative only in approximately 50% of patients, there is still a need to develop vaccines to support and improve antiviral therapies. CIGB-230, is a new therapeutic vaccine candidate based on the mixture of a plasmid expressing HCV structural antigens, with a recombinant HCV core protein particles. In the present study, we evaluate the safety, immunogenecity, and the impact of this vaccine candidate, CIGB-230, on plasma viral load and liver histology in HCV genotype 1b patients naive to treatment. **Materials and Methods:** Ninety two voluntaries were randomized in one of five treatment groups. All of them received both: regular interferon (IFN) and ribavirin (RBV) for 48 weeks, additionally they also received one dose of CIGB-230 or placebo each 4 weeks. The moment of CIGB-230 administration and the number of doses were considered as factors in the statistical analysis. **Results:** Local pain, headache, asthenia, fever, general discomfort and arthralgia were the most frequent adverse events. There was 20% superiority about inducing SVR in the early concomitant CIGB-230 group in comparison with later concomitant CIGB-230 group. **Conclusions:** The risk-benefit analysis was favourable to early CIGB-230 nine doses administration. This clinical trial allowed us to obtain relevant information for future studies with a better adjustment of dose and treatment schedule.

NON-CLINICAL IMMUNO-TOXICOLOGICAL EVALUATION OF HER-1 CANCER VACCINE IN NON-HUMAN PRIMATES: A 12-MONTH STUDY

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Introduction: Human epidermal growth factor receptor (HER1) constitutes a tumor associated antigen. It´s overexpression in many epithelial tumors has been associated with bad prognosis and poor survival. Active specific immunotherapy based on the extracellular domain (ECD) of HER1 and adjuvated in VSSP (very small sized proteoliposomes) and Montanide ISA 51-VG is a new and complementary approach for the treatment of epithelial tumors. The present study deals the immunogenicity of this vaccine in non human primates and evaluation of its toxicity during a period of 12 months. **Materials and Methods:**Two groups of 3*Macacafascicularis* monkeys/sex were established: Control (Saline) and Treated (Her-1 vaccine) groups. Immunizations were made on days 0, 14, 28, 42, 56, 84, 112, 140 and 168. Animals were inspected daily for clinical signs. Body weight, rectal temperature, cardiac and respiratory rates were measured during the study before each immunization and monthly later. Hematological, serum biochemical, Delayed Type Hypersensitivity (DTH) and immune response evaluation were performed five times during the study. At the end of the study, skin biopsy was performed in all animals to perform a histopathological analysis. **Results:**Study survival was 100%. Local reactions were observed at the administration site of four treated animals. Clinical pathology parameters were not affected. HER-1 vaccine induced high titles of IgG1 antibodies in the treated animals. HER-1 vaccine induced antibodies recognized Her1+ tumor cell lines, decreased Her1 phosphorylation stimulated by exogenous EGF, and showed anti-proliferative and pro-apoptotic effects in H125 cell line. Delayed type hypersensitivity was not observed. **Conclusions:**In general the present study showed that immunization of *Macacafascicularis* monkeys with HER-1 vaccine was well tolerated, eliciting local reactions at the administration site, and

inducing very high antibodies titers, suggesting HER-1 vaccine could be a safe and successful strategy for epithelial cancer patient's treatment.

LONG TERM SURVIVAL WITH NGCGM3/VSSP VACCINE IN ADVANCED MELANOMA PATIENTS. BRIEF RESULTS AND SOME CASE REPORTS

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Introduction: Active specific immunotherapy is an extensively explored choice in the last decade by the Center of Molecular Immunology, Havana, Cuba, evaluating among others, a cancer vaccine composed by NGcGM3 in a proteoliposome of *Neisseria MeningitidIs* (NGcGM3/VSSP. **Materials and methods:** Fifty-seven patients were included in two clinical trials with NGcGM3/ VSSP with and without Montanide ISA 51 as adjuvant. Immunization schedule was fortnightly for the first five doses and then, monthly up to one year. Main objectives were Immunogenicity, toxicity and tumor response. **Results:** NGcGM3/VSSP toxicity was local pain in the site of injection, flu–like symptoms, consisted of: fever, myalgia, chills, and headache, mainly grades I-II. Pre and post immune antibody titers against NGcGM3 ganglioside were evaluated in all assessable patients. AntiNGM3 IgG and IgM antibody responses were induced in all vaccinated patients. Additionally, IgA specific antibodies were detected. No statistical differences were found among different dose levels of NGGM3 and ganglioside antibody titers. Interestingly, antibody responses against NAcGM2 and NAcGM3 were present. Anti tumor activity with unexpected survival times despite the advanced stage of the patients was shown, mainly associated with the 900 µcg level subcutaneously. Vitiligo as evidence of autoimmunity was observed in some patients mainly in lower dose levels not directly associated with the use of adjuvant. Long term responses mainly long lasting disease stabilization were observed in several patients and hereby reported. **Conclusions:** NGcGM3/VSSP showed immunogenicity and safety with evidences of antitumor activity in advanced melanoma patients, suggesting that NGcGM3 might be a target for the treatment of melanoma. Further studies with this type of formulation are recommended.

IMMUNE RESPONSE TO CANCER VACCINE CAN BE ENHANCED WITH SPATIAL DISTRIBUTION OF ANTIGENS IN WHITE LEGHORN POULTRY

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Introduction: Animal models offer a system that enables a better understanding of basic biological questions. The understanding of cancer vaccine immunopharmacology is rather limited. The novel anti-idiotype vaccine Racotumomab (formerly known as 1E10), was generated from the immunization of BALB/c mice with P3, an idiotypic Ab (Ab1) that recognizes NeuGc-containing gangliosides, sulfated glycolipids, and Ags present in different human tumors. Ab3 antibodies were not detected in the sera of mice treated with anti-idiotype vaccine, but antibodies were detected in the sera of humans and chickens. For this reason, the main objective of this work was to evaluate the effect of several immunopharmacological variables on White Leghorn poultry immune response. **Material and Methods:** We explored the influence of vaccine doses and schedule of administration on the antibody response and morphology of the immune organs in naïve and immunosuppressed chickens. Animals were primed in

one, two or four anatomical sites. Doses from 400 µg (1X), 800 µg (2X) and 1600 µg (4X) equivalent of the Racotumomab-Alum vaccine were administered. Immunosuppression was obtained by Cyclophosphamide administration (600 mg/m^2) intravenously during 5 days. The effects of several combinations of immunotherapy and immunosuppression on the antibody response were also evaluated. Animals injected with normal saline were used as negative control. **Results and discussion:** Priming with four times the Racotumomab-Alum dose increments the specific antibody response demonstrable since 2 weeks after the antigen challenge and 2 weeks later, surprisingly, a dose resulting suboptimal administered at a single site, induced a robust immune response if fractionated to be administered in four sites. These results evidenced that optimizing immunopharmacological determinants contribute to earlier, stronger and prolonged anti-Racotumomab antibody persistence. **Conclusions:** It demonstrates that several determinants influence the anti-Racotumomab antibody response; which would be manipulated contributing to improve the specific immune reactivity.

A DNA-BASED VACCINE CANDIDATE AGAINST HEPATITIS C VIRUS MODIFIED THE NEUTRALIZING ANTIBODY RESPONSE IN HCV-INFECTED PATIENTS

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Introduction: There is no vaccine available against hepatitis C virus (HCV) and therapeutic treatments currently in use have limited efficacy. Some data have revealed that good treatment outcome is associated with higher neutralizing antibody (Nab) titres in patients infected with genotype 1b HCV isolates. CIGB-230 is one of the vaccine candidates for HCV in clinical trials; it is based on the mixture of a plasmid for DNA immunization, expressing HCV structural antigens, with a recombinant HCV core protein. The present work aim to determine NAb response in patients immunized with CIGB-230, during Phase I and Phase II clinical trials. **Material and Methods:** NAb response was determined using HCV pseudoparticles (HCVpp) or HCV obtained from cell culture (HCVcc) both 1a/2a chimeras. **Results:** After final immunization in Phase I clinical trial, NAb response against HCVpp was modified in 9 individuals, including 6 *de novo* responders. Additionally, seven months after the last CIGB-230 dose, the NAb response was maintained and most important appeared "*de novo*" in three patients. In the Phase II clinical trial, 92 chronically infected patients, naïve for antiviral therapy, were immunized with CIGB-230 in four different immunization schedules with IFN+Ribavirin. It was possible to study NAb response in 78 patients at two time points, before immunization (t0) and 1 month after the last dose (t48). It was no difference between the two evaluations in the control group, but two groups receiving CIGB-230 showed significant differences between t0 and t48. It is worth noting that the NAb response determined is cross-reactive since patients were infected by genotype 1b isolates, the vaccine candidate is based also on this genotype, but antibodies were evaluated against genotype 1a HCVcc. **Conclusions:** The immunization of HCV infected patients with CIGB-230, in combination with IFN+Ribavirin therapy, induces changes in the neutralizing antibody response, with potential implications on therapy outcome.

QUALITY OF LIFE OF PROSTATE CANCER PATIENTS TREATED WITH CIMAvaxEGF VACCINE

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Introduction: The evaluation of the quality of life in patients with cancer depends on many variables. In recent years we have developed a series of surveys to measure the quality of life of cancer patients. Monoclonal antibodies are attractive targets in cancer immunotherapy because of the qualitative and quantitative changes that occur during oncogenic transformation, including EGF receptor expression. **Objectives:** To evaluate the influence of CIMAvaxEGF vaccine in improving the quality of life of patients with prostate cancer. **Materials and methods:** We applied transversely two questionnaires: Quality of Life questionnaire EORTC general cancer patients QLQ-C30 and the prostate cancer questionnaire QLQ-PR25 to 101/197 patients treated with CIMAvaxEGF vaccine. Quality of life is an important factor to consider when choosing between different cancer treatments, and should be incorporated in the routine evaluation of these patients. **Results:** We constructed the different levels of quality of life for functional and symptom scales of both questionnaires as required by the Manual of the EORTC. **Conclusions:** The CIMAvaxEGF vaccine is safe and prostate cancer patients treated with this products remains high scale values in functional and global health status and low values in the range of symptoms that represents high quality life.

QUALITY OF LIFE FOR BREAST CANCER PATIENTS TREATED WITH NGLYCOLIL GM3/VSSP VACCINE

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Introduction: The evaluation of the quality of life in patients with cancer depends on many variables. In recent years we have developed a series of surveys to measure the quality of life of cancer patients. Therapeutic vaccines are attractive targets in cancer immunotherapy because of the qualitative and quantitative changes that occur during the oncogenic transformation. **Objectives:** To evaluate the influence of NGlycolil GM3/VSSP vaccine in improving the quality of life of breast cancer patients. **Materials and methods:** We applied transversely two questionnaires: Quality of Life questionnaire EORTC general cancer patients QLQ-C30 and the breast cancer questionnaire QLQ-BR23 to 24 patients treated with NGlycolil GM3/VSSP vaccine carriers of this disease. Quality of life is an important factor to consider when choosing between different alternative cancer treatments acceptable, and should be incorporated as part of the routine evaluation of these patients. **Results:** We constructed the different levels of quality of life for functional and symptom scales both in general and for breast cancer as required by the Manual of the EORTC. **Conclusions:** The NGlycolil GM3/VSSP vaccine is safe and breast cancer patients treated with this vaccine maintains high values in the functional scale and global health status and low values in the range of symptoms that represents high quality life.

ACTIVE SPECIFIC IMMUNOTHERAPY WITH RACOTUMOMAB IN THE TREATMENT OF ADVANCED NON SMALL CELL LUNG CANCER (NSCLC)

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Introduction: Gangliosides are an attractive target for cancer immunotherapy since they are not expressed in normal human tissue but are overexpressed in several solid tumors, including NSCLC and have been implicated in tumor development and progression. Racotumomab is an anti-idiotype murine monoclonal antibody specific to P3 Ab1 mAb, an antibody that reacts with NeuGc-containing gangliosides, sulfatides and other Ags expressed in tumors. Phase I trials conducted in advanced melanoma, breast and lung cancer have demonstrated the low toxicity and high immunogenicity of Racotumomab. **Materials and methods:** A phase II multicentric, randomized, placebo-controlled and double blind clinical trial was developed to evaluate the effect of Racotumomab in Overall Survival (OS) of patients with advanced (IIIB and IV) NSCLC who have completed onco-specific treatment as per the Oncology Therapeutic Guidelines (surgery, chemotherapy, radiotherapy according to initial staging). Patients were randomized 1:1 to receive the vaccine (Racotumomab) or placebo. Treatment consisted in 5 vaccine doses every 2 weeks (induction period) followed by monthly re-immunizations (maintenance period). Anti-NGcGM3 antibody response and its cytotoxic properties were determined in a subset of vaccinated patients. **Results:** The vaccine was safe and immunogenic. The treatment elicited high Abs titers against NeuGcGM3 ganglioside of both isotypes, IgM and IgGin 80% of evaluated patients. Hyperimmune sera were able to specifically recognize and induce cell death to NeuGcGM3 positive cells lines. These properties were abrogated when NGcGM3 negative cell line was used. The cytotoxic capacity of the sera but not antibody titers was significantly associated with longer survival times. The Overall survival intent to treat analysis (ITT) and per protocol population analysis (PPP) showed benefit in vaccinated patients vs. control patients. **Conclusions:** This is the first report in demonstrate that the functional capacity of the antibodies against NeuGcGM3 ganglioside induced by vaccination with Racotumomab has clinical relevance.

ANTI-ATHEROSCLEROTIC EFFECT OF AN ANTIBODY THAT BINDS TO EXTRACELLULAR MATRIX GLYCOSAMINOGLYCANS

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Introduction: Subendothelial retention of proatherogenic lipoproteins by proteoglycans is critical in atherosclerosis. The aim of this study was to characterize the recognition, immunogenicity and anti-atherogenic properties of a chimeric monoclonal antibody (mAb) that reacts with sulphated molecules. **Materials and Methods:** chP3R99 mAb reactivity with proteoglycans and its capacity to inhibit the binding of LDL to proteoglycans was measured by ELISA. Macrophages recognition by chP3R99 and its ability to inhibit the binding and the uptake of oxLDL by these cells was measured by flow cytometry. Inhibition of LDL retention in vivo was measured in the artery wall of rats. Induction of anti-GAGs antibodies by chP3R99 mAb immunization was evaluated in NZW rabbit sera by ELISA. Inhibition of LDL-CS binding by chP3R99 mAb, animal sera and IgG from immunized rabbits was performed by competitive ELISA, and

their capacity to prevent LDL oxidation in vitro by monitoring malondialdehyde formation. To assess the anti-atherogenic effect of chP3R99 mAb in vivo, NZW rabbits were subcutaneously immunized with low doses of chP3R99 mAb prior administration of daily administration of Lipofundin. Aortic arches were used for histopathology, ultrastructural and redox evaluation. Immunohistochemical studies were performed for detection of macrophages, CD4 and CD8 lymphocytes in aortas. Serum lipid parameters were measured. **Results:** chP3R99 mAb recognized GAGs, mainly chondroitin sulfate (CS), by ELISA. This mAb blocked LDL-CS association and LDL oxidation in vitro, and when intravenously injected to Sprague-Dawly rats it inhibited LDL retention and oxidation in the artery wall. Moreover, subcutaneous immunization of NZW rabbits with chP3R99 mAb prevented Lipofundin-induced atherosclerosis. Histopathological and ultrastructural studies showed no intimal alterations or slight thickening, with preserved junctions between endothelial cells and scarce collagen fibers and glycosaminoglycans. In addition, immunization with chP3R99 mAb suppressed macrophage infiltration in aorta and preserved redox status. The atheroprotective effect was associated with the induction of anti-CS antibodies in chP3R99-immunized rabbits, capable to block CS-LDL binding and LDL oxidation. **Conclusions:** These results support anti-sulphated glycosaminoglycans antibody-based immunotherapy as a potential tool to prevent atherosclerosis.

NATURALLY OCCURRING HUMAN ANTIBODIES AGAINST NEUGCGM3: VALIDATION OF AN IMMUNOLOGICALLY RELEVANT GLYCOLIPIDIC TUMOR ANTIGEN

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Introduction: NeuGcGM3 ganglioside is not naturally expressed in normal human tissues but is overexpressed in several tumors and has immunosuppressive capacity, contributing to cancer progression. These features make NeuGcGM3 an attractive candidate for tumor immunotherapy; however, little is known about the natural anti-NeuGcGM3 antibody response in healthy humans. Aims of this study were to analyse and characterize the antibody responses against this ganglioside in healthy donors and cancer patients. **Materials and Methods**: The study comprised 100 healthy donors and 51 age-matched untreated non small cell lung cancer (NSCLC) patients. In all the subjects, specific anti-NeuGcGM3 antibody response was assessed by ELISA. The functionality of these antibodies was tested as their ability to bind and kill tumor cell lines expressing NeuGcGM3. **Results**: 65 healthy donors had antibodies that specifically recognized NeuGcGM3 and kill tumor cells expressing this antigen by a complement mediated mechanism. Interestingly, even after complement inactivation, 17% of the positive sera showed a direct cytotoxic effect on the tumor cells. This cytotoxicity was dependent on the presence of the antigen on the tumor cells and resembles an oncotic kind of cell death. Both, the levels of anti-NeuGcGM3 antibodies in the sera of healthy donors as well as the percentage of donors with this natural immunity decrease with age. Furthermore, we could only detect low reactivity against NeuGcGM3 in the sera of 5 NSCLC patients. **Conclusions**: Healthy human sera contain naturally occurring anti-NeuGcGM3 antibodies with cytotoxic anti-tumor properties, reinforcing the importance of NeuGcGM3 ganglioside as an important target for cancer immunotherapy. Treatments that boosted this anti-NeuGcGM3 antibody response should be considered an important immunotherapeutic regimen against NeuGcGM3 containing tumors as elderly donors and NSCLC patients lacked this natural anti-tumor response.

POTENTIATING NGCGM3 CANCER VACCINE BY ITS COMBINATION WITH ANTI-EGFR MONOCLONAL ANTIBODIES

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Introduction: New clinical trials with NGcGM3/VSSP vaccine in cancer patients are validating the NGcGM3 as therapeutic target. Anti-EGFR has been studied independently in human tumors and there are diverse modalities of monotherapies against of both targets therapeutics. However, the relevance of the bi-therapy against these targets is unknown. Additionally, to design new and effective therapies to confer sensitivity to EGFR therapies for cancer patients, the understanding of tumor biology during tumor progression is of paramount importance. **Material and Methods:** Here we describe the anti-metastatic effect of NGcGM3/VSSP vaccine in combination with passive anti-EGFR therapy in a spontaneous lung metastasis murine model induced by 3LL-D122 Lewis Lung Carcinoma. **Results:** In this study, we show that NGcGM3/VSSP vaccine increases the sensitivity to passive anti-EGFR therapy in animals bearing lung metastases, supported by their synergistic anti-tumoral effect and survival advantage as compared to the monotherapies. Our results showed that NGcGM3/VSSP vaccine increased the phosphorylation of EGFR in lung nodules, suggesting that such therapy render the tumors more sensible to anti-EGFr treatment. The nature of a potential "physical" relationship between EGFr and NGcGM3 on tumor will be discussed, although more studies are required to understand the overall impact of NGcGM3 in EGFR signaling. Other immunological mechanisms potentially involved in the observed success of this combination therapy are also discussed. **Conclusions:** Our results suggest a relationship between N-Glycolyl GM3 and EGFR that may contribute to tumor cell biology. Moreover they suggest that combination of NGcGM3 based vaccines with anti-EGFr antibodies can have a synergistic anti-tumoral effect in tumors that co-express these two targets.

INDUCTION OF ANTI-TUMOR VACCINAL EFFECT BY BLOCKADE OF EGFR ACTIVATION WITH A SPECIFIC ANTIBODY

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Introduction: Despite promising results in the use of anti-EGFR antibodies for cancer therapy, several issues remain to be addressed. An increasing emphasis is being placed on immune effector mechanisms. It has become clear for other antibodies directed to tumor targets that their effects involve the adaptive immunity, mainly by the contribution of Fc region-mediated mechanisms. Given the relevance of EGFR-signaling for tumor biology, we wonder whether the oncogene inhibition could contribute to antibody-induced "vaccine" effect. **Materials and Methods:** Tumor-specific immune response promoted by 7A7 (an anti-murine EGFR neutralizing antibody) or AG1478 (an EGFR tyrosine kinase inhibitor) was measured in D122 lung metastasis-bearing mice using flow cytometry and cytotoxic T lymphocyte assays. Experiments for immunogenic apoptosis evaluation were also conducted. **Results:** In this mice model in which 7A7 and AG1478 displayed potent antimetastatic activities, depletion experiments revealed that only in the case of the antibody, the effect was dependent on CD4+ and CD8+ T cells. Correspondingly, 7A7 administration elicited a remarkable tumor-specific CTL response in hosts. Importantly, experiments using 7A7 F(ab´)2 suggested that in vivo antibody-mediated EGFR blockade may play an important role in the linkage with adaptive immunity. Addressing the possible mechanism involved in this effect, we found quantitative and qualitative differences between 7A7 and AG1478-induced apoptosis. EGFR blocking by 7A7 not only prompted a higher pro-apoptotic effect on tumor metastases compared to AG1478, but also was able to induce apoptosis with immunogenic potential in an Fc-independent manner. As expected, 7A7 but not AG1478, stimulated exposure of danger signals on tumor cells. Subcutaneous injection of 7A7-treated tumor cells induced an antitumor immune response. **Conclusions:** This is the first report of a tumor-specific CTL response generated by antibody-mediated EGFR inhibition, suggesting an important contribution of immunogenic apoptosis to this effect.

ACTIVE IMMUNIZATION AGAINST SULPHATED GLYCOSAMINOGLYCANS REDUCES ATHEROSCLEROSIS IN APOLIPOPROTEIN E-DEFICIENT MICE

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oral

Introduction: The pathogenesis of atherosclerosis is linked to the oxidation of low density lipoproteins which are trapped in the extracellular matrix by interaction with glycosaminoglycan (GAG) side chains of proteoglycans. chP3R99 mAb, which react with sulfated GAGs were tested for their potentially anti-atherogenic properties through the induction of an idiotypic antibody network which may specifically interfere with LDL binding to proteoglycans side chains and foam cell formation. **Materials and Methods:** ApoE-deficient mice fed a high fat high cholesterol diet received 5-6 doses of chP3S98 or chP3R99 antibodies showing low and high reactivity, respectively, against their respective antigens. Reactivity of immune sera against chimeric Abs or GAGs was determined by solid-phase ELISA using chP3R99, chP3S98 or GAG-coated plates. To determine the immunodominance of the anti-idiotypic response against chP3R99 or chP3S98, diluted mouse immune sera were pre-adsorbed with an isotype-matched irrelevant antibody. Adsorbed and non-adsorbed sera were added to chP3R99-, chP3S98- or hR3-coated plates and remnant reactivity was assessed by ELISA. Plasma lipid analysis was assayed using commercially available reagents. ApoE-/- mice were sacrificed at 18 weeks of age and whole aortas were isolated for morphometric analysis. Aortas were opened longitudinally from the heart to the iliac arteries, and the lesions were stained with Oil Red-O and expressed as the percentage of the total aortic surface area covered by lesions. **Results:** Both chimeric antibodies elicited an immunodominant anti-idiotypic response in the absence of adjuvant. Yet, a striking reduction in total lesion areas was observed in mice immunized with chP3R99, but not chP3S98, compared to PBS-treated mice. The anti-atherosclerotic effect was associated with increased mouse sera reactivity against heparin and sulphated GAGs including chondroitin and dermatan sulphate. **Conclusions:** The present study supports use of active immunization and the mounting of an idiotypic antibody network response against GAGs as novel approach to target atherosclerosis.

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isolated for morphometric analysis. Aortas were opened longitudinally from the heart to the iliac arteries, and the lesions were stained with Oil Red-O and expressed as the percentage of the total aortic surface area covered by lesions. **Results:** Both chimeric antibodies elicited an immunodominant anti-idiotypic response in the absence of adjuvant. Yet, a striking reduction in total lesion areas was observed in mice immunized with chP3R99, but not chP3S98, compared to PBS-treated mice. The anti-atherosclerotic effect was associated with increased mouse sera reactivity against heparin and sulphated GAGs including chondroitin and dermatan sulphate. **Conclusions:** The present study supports use of active immunization and the mounting of an idiotypic antibody network response against GAGs as novel approach to target atherosclerosis.