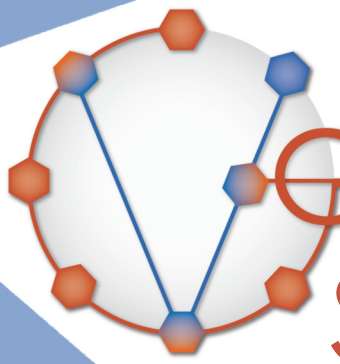

GLYCOVAX SIXTH WORKSHOP

"VACCINE FORMULATION AND NANOTECHNOLOGIES'"

15th-16th May 2019
Consiglio Nazionale delle Ricerche (CNR) - Milan, Italy

PROCEEDINGS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 675671

INTRODUCTION

GLYCOVAX - A Training Network for the Rational Design of the Next Generation of Well-Defined Glycoconjugate Vaccines GLYCOVAX is a European Training Network (ETN) funded in the framework of H2020 Marie Skłodowska - Curie ITN programme. The GLYCOVAX network aims at the education of promising young scientists who will learn how to rationally design well-defined and innovative glycoconjugate vaccines to improve current preventive therapies and tackle unmet medical needs. The project is based on a profound interaction between the academic and industrial sectors, involving 8 academic groups and 2 industrial partners. In this highly multidisciplinary environment 14 Early Stage Researchers (ESRs) are trained in the growing field of glycoscience and vaccinology, enriching their skills and combining different state-of-the-art methodologies for the rational design of innovative glycoconjugates.

For more details and news, visit the website www.glycovax.eu.

WORKSHOPS

Six workshops will be organized during GLYCOVAX. In these workshops, the ESRs will attend seminars from internationally recognized experts in scientific areas related to the network objectives. The topics selected for the workshops aim at covering the state-of-the-art of disciplines strongly connected with the GLYCOVAX program. These forums are intended also to stimulate the establishment of new scientific collaborations inside and outside the network.

In particular, the sixth Workshop **"Vaccine formulation and nanotechnologies"** has been organized by UMIL, CNR Research Area, Milan, Italy.

Lectures dealt with:

- Vaccine formulations
- Why using adjuvants
- Cyclodextrins
- Liponanoparticles
- Gold nanoparticles

Workshop of GLYCOVAX and NanoCarb networks

VACCINE FORMULATION and NANOTECHNOLOGY

13th-16th May 2019 - Consiglio Nazionale delle Ricerche (CNR) - Milan, Italy

WEDNESDAY, 15TH MAY 2019

09.00 - 09.30 **Introduction on Vaccines and Nanotechnology**
Luigi Lay - Università degli Studi di Milano, Italy
Laura Polito - Università degli Studi di Milano, Italy / Consiglio Nazionale delle Ricerche, Italy

09.30 - 10.15 **Vaccines formulation: an overview**
Francesco Berti - GlaxoSmithKline Vaccines S.r.l., Italy

10.15 - 11.00 **Synthetic multivalent ligands for biomacromolecule targeting**
Francesco Sansone - Università degli Studi di Parma, Italy

11.00 - 11.30 *Coffee break*

11.30 - 12.15 **OMV and carriers**
Francesca Micoli - GlaxoSmithKline Vaccines S.r.l., Italy

12.15 – 13.00 **Discovery of new generation of adjuvants and delivery of adjuvanted vaccines**
Barbara Christiane Baudner - GlaxoSmithKline Vaccines S.r.l., Italy

13.00 - 14.00 Lunch

14.00 - 15.30 **Joint meeting GLYCOVAX-NanoCarb ESRs**

JOINT SOCIAL EVENT (GLYCOVAX + NanoCarb networks)

16.15 *Visit* of the 'Last Supper' by Leonardo da Vinci and Santa Maria delle Grazie Church
Followed by *Networking Get-Together* at Colonial Cafè - Corso Magenta, 85 - 20123 Milan

Workshop of GLYCOVAX and NanoCarb networks

VACCINE FORMULATION and NANOTECHNOLOGY

13th-16th May 2019 - Consiglio Nazionale delle Ricerche (CNR) - Milan, Italy

THURSDAY, 16th MAY 2019

09.00 - 09.45 **Cancer treatment: hurdles and hopes from a clinician's perspective**
Luca Toschi - Humanitas Cancer Center, Italy

09.45 - 10.30 **Cancer nanotechnology: hopes and challenges**
Zeljka Kreptic - University of Salford, Uniterd Kingdom

10.30 - 11.00 *Coffee break*

11.00 - 11.20 **Gold glyconanoparticles: smallest particles in biomedical use for targeted delivery to tumour and disease sites**
Africa Barrientos - Midatech Pharma España S.L, Spain

11.20 - 11.40 **Lost in translation, nanoparticle characterization in biological fluid**
Marco Monopoli - Royal College of Surgeons in Ireland, Ireland

11.40 – 12.00 **In vitro - in vivo implementation of the preclinical studies in nanomedicine**
Paolo Bigini - Istituto di Ricerche Farmacologiche "Mario Negri", Italy

12.00 - 12.20 **Biological activity of glycans**
Daniel Spencer - Ludger Ltd, United Kingdom

12.20 - 12.50 **The market of drugs: light and shadow**
Silvio Garattini - Istituto di Ricerche Farmacologiche "Mario Negri", Italy

12.50 - 13.00 **Closing remarks**
Luigi Lay - Università degli Studi di Milano, Italy

13.00 - 14.00 *Lunch* and **End of GLYCOVAX meeting**

Meeting venue:
Consiglio Nazionale delle Ricerche (CNR) Research Area
Via Alfonso Corti, 12 - 20133 Milan, Italy

INTRODUCTION ON VACCINES AND NANOTECHNOLOGY

Luigi Lay[1], Laura Polito[2]

1 *Università degli Studi di Milano, Italy*

2 *Istituto di Scienze e Tecnologie Molecolari / Consiglio Nazionale delle Ricerche, Italy*

Notwithstanding the great advances of modern medicine, infectious diseases still have a strong impact on public health, both in industrialized and developing countries, due to their significant health-related costs for clinical treatment. According to the World Health Organization (WHO), vaccination is the most cost-effective strategy for controlling infections caused by pathogenic microorganisms. Actually, vaccines are able to confer long-term protective immunity on the population and have made possible a great revolution in the 20th century, saving millions of lives.[1] The surface of bacterial pathogens is covered with a dense array of complex glycans, such as lipopolysaccharide of Gram-negative bacteria and the polysaccharide coat (capsular polysaccharides, CPS) of encapsulated bacteria that are crucial protective antigens and major virulence factors. A major drawback of polysaccharide-based vaccines, however, is their limited clinical efficacy. They induce T cell-independent immune responses, featured by poor immunogenicity in children under 5 years of age, in elderly and immunocompromised individuals, and fail to generate conventional B cell-mediated immunological memory. Polysaccharide immunogenicity can be strongly enhanced by conjugation to an immunogenic carrier protein, providing T cell-dependent glycoconjugate antigens able to stimulate B cell maturation to memory cells and induce immunoglobulin class switching from IgM to polysaccharide-specific IgG. The introduction of glycoconjugate vaccines represented one of the keys for success of vaccination, especially for infants and young children who are the most affected population by infectious diseases. Hence, the development of cost-effective, glycoconjugate vaccines based on fully synthetic saccharide antigens is gaining growing importance. Synthetic glycans, indeed, possess well-defined compositions, affording highly reproducible biological properties and a better safety profile. This step is crucial for the design of a new generation of improved and safer vaccines obtained either from chemical synthesis or bacterial source. Nevertheless, carbohydrates interact with their ligands in a very weak manner, so much faint that nature found in the multi-presentation (the so called “glycocalix”) a solution to have efficient carbohydrate interactions.[2] Following nature’s design, carbohydrate multivalent systems are, at the present time, the most common strategy used to study weak carbohydrate–carbohydrate or carbohydrate–protein interactions and the resulting biological processes. In the last years, thanks to the cutting-edge level obtained in the manipulation of nanoscopic objects, great attention has been appointed to the development of glyconanoparticles (glycoNPs), which comprises native nanostructures that display on their surface multiple copies of glycans.[2]

In recent years, the huge advances in the nanotechnology field have promised many breakthroughs in countless applications, in such a way that it is widely felt that nanotechnology will represent a new industrial revolution.[2,3] The two main approaches used to synthesize nanomaterials are the so called "bottom-up" or "top-down" protocols. The latter is related to the disruption of bulk materials to the nanometric size by means of physical or mechanical procedures such as laser lithography, etching or mechanical grinding. The "bottom-up" approach instead, is based on the building of the nanoparticles starting from molecular or atomic assembling. This is the most diffuse protocol for the synthesis of colloidal solution, as it can ensure a high quantity of material with a well-defined crystal structure. A number of different type of materials can be easily reduced and manipulated at the nanosize, such as carbon-based NP, inorganic NPs, liposomes etc. When these nanomaterials are designed for biomedical application, we approach the so called "nanomedicine" which can gain many benefits from the use of NPs in early diagnosis and to improve the delivery of drug substances. In the design of a NP for their use in nanomedicine it is crucial to select the optimal core and to properly coat the surface to afford a smart NP, depending on the final application.[4]

[1] Lay et al. Molecules 2018, 23, 1712.

[2] Polito et al. Beilstein J. Org. Chem. 2017, 13, 1008–1021.

[3] Polito et al. Int. J. Mol. Sci. 2018, 19, 3385.

[4] Nanomedicine, 2019, 93-126. doi: 10.2217/nnm-2018-0120.

VACCINES FORMULATIONS: AN OVERVIEW

Francesco Berti

GSK Vaccines, Italy

Vaccines for human use has had an enormous impact on the public health. For the development of new vaccines, several technical and clinical tasks have to be fixed. The formulation of vaccines has an important role to optimize the efficacy and safety of vaccines.
An overview on the terminology (Drug Substance, Active Pharmaceutical Ingredient, API; Drug Product; etc.) will be provided. Drug Substance (Antigen) is a pure material which exerts a pharmacological action while Drug Product (Vaccine) is a finished end product which may contain one or more drug substances in combination with excipients and adjuvants meant for use by humans and animals.
In addition a detailed description of the different phases for vaccine development and life-cycle management of the product post licensing will be provided.

SYNTHETIC MULTIVALENT LIGANDS FOR BIOMACROMOLECULE TARGETING

Francesco Sansone

Università degli Studi di Parma, Italy

Many biological processes, both physiologic and pathologic, are characterized by the phenomenon called Multivalency.[1] This is based on the simultaneous occurring of multiple recognition processes between the multiple copies of identical receptor sites and the multiple copies of identical binding units present on two entities interacting one to the other. This particular mode of binding is exploited by Nature to increase strength and selectivity of the reciprocal recognition, especially for those cases where the interaction between a single receptor site and a single substrate unit is rather weak. This is the case of the processes where carbohydrates are involved as substrates, so spread and peculiar that Multivalency is renamed Glycoside Cluster Effect.[2] Some of them are also related with the mechanisms of the immune response.

The relevance of the events for which Multivalency plays a role, in particular when these are associated to severe diseases, boosted in the last decades the design, synthesis and study of artificial multivalent systems with the aim of enhancing the knowledge on this phenomenon and making available potential agents with therapeutic activity.

In this talk, a general presentation of the multivalent effect and its relevance will be done, through some representative examples of biological events where it comes into play.[1-3] The definition of some fundamental parameters[1,4] introduced to evaluate the extent of multivalent effect will be given. Examples of multivalent synthetic systems[5] will be described with the aim of explaining the main features that are in general considered and taken into account to design this kind of ligands.

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[2] a) Y. Lee, R. Lee Acc. Chem. Res. 1995, 28, 321; b) J. J. Lundquist, E. J. Toone Chem. Rev. 2002, 102, 555.

[3] a) R. Haag et al. Med. Chem. Commun. 2014, 5, 862; b) K. Doores et al. PNAS 2010, 107, 13800; c) Section 11.4, Lectins Are Specific Carbohydrate-Binding Proteins, Biochemistry. 5th edition. J. M. Berg, J. L. Tymoczko, L. Stryer; New York: W. H. Freeman; 2002.

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[5] a) P. I. Kitov et al Nature 2000, 403, 669; b) S. André, F. Sansone, H. Kaltner, A. Casnati, J. Kopitz, H.-J. Gabius, R. Ungaro Chem BioChem 2008, 9, 1649; c) M.-P. Dubois et al., Chem. Commun. 2005, 4318; d) O. Renaudet et al., ChemMedChem 2008, 3, 737; e) J. Wang et al., Org. Biomol. Chem. 2007, 5, 1529; f) J. M. de la Fuente et al., Angew. Chem. Int. Ed. 2001, 40, 2257; g) Chem. Commun. 2014, 50, 11029; h) Flavio Manea et al., Adv. Mater. 2008, 20, 4348; i) N. Jayaraman et al., Chem. Soc. Rev. 2013, 42, 4640; j) Virgil Percec et al., J. Am. Chem. Soc. 2013, 135, 9055; k) M. Lampropoulou, K. Yannakopoulou, J. Incl. Phenom. Macrocyclic Chem. 2011, 70, 345; l) J. Voskuhl et al., Chem.–Eur. J. 2010, 16, 2790; m) J. M. Benito et al., J. Am. Chem. Soc. 2004, 126, 10355.

OUTER MEMBRANE VESICLES AND CARRIERS

Francesca Micoli

GSK Vaccines Institute for Global Health, Italy

Gram-negative bacteria naturally shed outer membrane vesicles (OMV) as part of a blebbing process. This normally happens at levels too low to be useful for vaccine production. GMMA (Generalized Modules for Membrane Antigens) derive from bacteria engineered to enhance native OMV formation. Additional mutations are introduced to reduce GMMA reactogenicity. Importantly, the GMMA surface is a representation of the outside of the bacteria, with multiple membrane antigens presented to the immune system in their native conformation and correct orientation. GMMA have optimal size for immune stimulation and self-adjuvanting properties.

Large body of preclinical data shows that GMMA are highly immunogenic and elicit substantially higher antibody responses against key vaccine candidate antigens, whether these are polysaccharide or protein moieties, compared with corresponding purified antigens delivered as glycoconjugate vaccines or recombinant formulations.

GMMA are produced by bacterial fermentation with simple filtration-based downstream processing. Such process has been already scaled-up for producing GMP-quality GMMA at large scale for *Shigella sonnei* and nontyphoidal *Salmonella*, pathogens of particular relevance for LMICs. The most advanced GMMA-based vaccine is *S. sonnei*, already tested in clinical trials, resulting to be well tolerated and immunogenic in European and Kenyan adults, and able to induce a strong amnestic response after boosting.

High immunogenicity, low cost, and simplicity of manufacture, make GMMA platform particularly appealing for production of affordable vaccines necessary for immunization campaigns in LMICs.

In addition, we have recently shown that GMMA are amenable to manipulation for presentation of heterologous antigens from other organisms. No immune interference has been verified by presenting multiple polysaccharides on the same GMMA particle. These findings have been extended to different antigens and to GMMA from different sources, supporting the use of GMMA as an attractive tool for the development of effective multivalent vaccines covering multiple diseases with one only vaccine component.

DISCOVERY OF NEW GENERATION OF ADJUVANTS AND DELIVERY OF ADJUVANTED VACCINES

Barbara Baudner

GSK Vaccines, Italy

New generation vaccines will increasingly comprise highly purified recombinant proteins. Unfortunately, these antigens are often poorly immunogenic. Therefore, adjuvants will be required to enable these proteins to become effective vaccines. Principles for the rational optimization of small-molecule immune potentiators (SMIPs) targeting Toll-like receptor 7 as adjuvants will be presented combining medicinal chemistry, formulation science, and quantitative immunopharmacology approaches; highlighting specific safety and efficacy requirements of vaccines.

In addition, conjugation of adjuvants to model antigen as well as the use of various adjuvants for alternative routes of immunization - sublingual and intradermal vaccine delivery - will be introduced. Proof of principal *in vivo* evaluation for selected model antigens using the mouse model will be presented showing that needle free vaccines could be more convenient and effective eliciting both systemic and mucosal immune responses.

CANCER TREATMENT: HURDLES AND HOPES FROM A CLINICIAN'S PERSPECTIVE

Luca Toschi

Humanitas Cancer Center, Italy

Diagnostic and treatment algorithms in human malignancies are evolving at a never-before-seen pace. This revolution has characterized lung cancer more than other neoplasms. Histological subtyping to maximise treatment efficacy and avoid toxicity has marked the beginning of the revolution observed in the last decade, opening the way to molecular characterization to guide genomically-driven treatments with targeted agents, led by Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK) inhibitors. More recently, agents against the Program Death 1 receptor (PD-1) and ligand 1 (PD-L1) have entered the clinical arena, offering new hope to NSCLC patients.

Despite all the advances, with some of them being driven by a fair amount of serendipity, several uncertainties remain to be elucidated. In fact, particularly in field of immunoncology, patient selection remains an unresolved issue, with real life data that often fail to match results of clinical trials, highlighting some relevant gaps between research and clinical practice. In addition, increasing use of new classes of therapeutic agents is unavoidably associated with emergence of toxicities rarely seen before, addressing the need for ad hoc education of all clinicians involved with cancer patients.

Ultimately, effective patient selection should also be pursued for cost-related reasons, due to the skyrocketing prices of new agents, which are much likely to be unbearable in the long term. To this regard, upfront identification of drug-sensitive and drug-resistant patients should be sought with more refined strategies.

GOLD GLYCONANOPARTICLES: SMALLEST PARTICLES IN BIOMEDICAL USE FOR TARGETED DELIVERY TO TUMOUR AND DISEASE SITES.

Africa G. Barrientos

Midatech Pharma España S.L, Spain

The MidaCore™ technology platform is based on ultra-small gold glyconanoparticle (GNP) drug conjugates, which at 2nm are among the smallest particles in biomedical use. They are composed of a core of gold atoms decorated with a permutation of therapeutic and targeting molecules. The small size and multi-functional arrangement around the gold core underpin the ability to improve biodistribution, and target tumour and/or immune sites providing a new generation of oncology drugs.
In this presentation, we will review the concept of GNP and its biofunctionalization as well as its production and characterization. Some examples of GNP applied in oncology and immunotherapy areas will be discussed.

LOST IN TRANSLATION, NANOPARTICLE CHARACTERIZATION IN BIOLOGICAL FLUID

Marco Monopoli

Royal College of Surgeons in Ireland, Ireland

Nanotechnology is one of the primary drivers of technology innovation, and it is one of the leading pillars of the six Key Enabling Technologies of H2020. Among the different application scopes of nanotechnology, its use in medicine has attracted considerable attention for its potential advances in healthcare, personalised medicine and to tackle complex issues such as the targeted and programmed delivery of drugs.

Because of their small size, they can directly interact with biomolecules in an entirely different way and their behaviour in biology is still not fully understood. Once in biological fluids, NPs rapidly interact with biomolecules from the environment that firmly and rapidly adsorb to the NP surface forming the long-lived biomolecular corona. [1,2] The biomolecular corona gives a new identity to NP in the biological milieu as it has been shown to interact with cellular receptors directly and can affect the journey as it travels through the body. [3,4] It is now clear that these interactions lead to a dramatic surface changes and a new identity of the NP in biological fluid and the corona can induce unpredictable immunological responses and can hamper their therapeutic efficacy. The protein corona is derived from proteins in biological fluids, many of which are glycosylated. We have now shown that the biomolecular corona has a strong glycosylation component that is biologically active and this class of biomolecules plays a dramatic role in the NP colloidal stability and firmly controls the NP biological fate and, if controlled, can offer new opportunities in nanomedicine. [5]

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[5] Wan S, Kelly PM, Mahon E, Stockmann H, Rudd P, Caruso F, Dawson KA, Yan Y, Monopoli MP*. The "Sweet" Side of the Protein Corona: Effects of Glycosylation

IN VITRO - IN VIVO IMPLEMENTATION OF THE PRECLINICAL STUDIES IN NANOMEDICINE

Paolo Bigini

Istituto di Ricerche Farmacologiche "Mario Negri", Italy

The development of innovative devices to improve the selectivity of drug delivery represents one of the most relevant hopes for the pharmacology of the XXI century. In this context, the exploitation of selected nanomaterials has been even more taken into consideration by scientific community. However, in spite of the great effort and the huge amount of money spent in the last two decades, the results are very poor and somehow controversial. Among the different reasons, the lack of robust assessment about the bio-nano interaction still remains a pivotal point that should be resolved in next years. In particular, parameters such as biodistribution, clearance, accumulation of both nanocarriers and drugs could be easily evaluated by a careful investigation in Preclinical models of human disorders. An overall description of the main points of weakness in preclinical nanomedicine and a brief summary of representative *in vitro* and *in vivo* studies of nanobiology will be described in this talk.

BIOLOGICAL ACTIVITY OF GLYCANS

Daniel Spencer

Ludger Ltd, United Kingdom

Glycans play a key role in the function of many biological processes. In humans glycans are used to signal inflammatory responses, enable hormones such as erythropoietin, FSH and HCG to function with receptors, and to govern the circulatory half-life of a protein. By understanding and controlling the glycosylation forms better biologists and drug manufacturers can understand disease targets better and produce the next generation of bio-better biopharmaceuticals. This presentation touches upon some of the glyco features to be aware of for biomarkers and drug development.

THE MARKET OF DRUGS: LIGHT AND SHADOW

Silvio Garattini

Istituto di Ricerche Farmacologiche "Mario Negri", Italy

Drugs have represented a very important progress in the therapy of diseases,but with the time drugs have become victims of their success. In fact they are becoming consumer goods. The selection of drugs is made on the basis of pre-clinical and clinical studies. Randomized clinical trials are pivotal in the process of drug approval by the regulatory authorities. The results are frequently affected by economical interest making available therefore drugs that are photocopies or even inferior to drugs already available. Efficacy is privileged in respect to the search for toxic effects. There is therefore a need for independent clinical studies to perform comparative studies as well as better trials in the real clinical practice optimising doses and time of treatment.

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