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VACCINE SYMPOSIUM

# STRUCTURAL VACCINOLOGY

12 September 2019

Universiteit Utrecht

NWO WG VACCINES

UMC Utrecht

# VACCINE SYMPOSIUM

12 SEPTEMBER 2019

**09.30** **Andrew Ward**, Integrative Structural Biology, Scripps Institute, San Diego
"Using the broad resolving power of the electron microscope to drive vaccine development"

**10.10** **Rogier Sanders**, Amsterdam UMC and Weill Cornell Medical Center, New York
"Structure-based HIV-1 vaccine design"

**10.40** **Berend Jan Bosch**, UU, Faculty of Veterinary Medicine
"Interrogating humoral immune responses to find key protective antibodies against coronaviruses"

11.10 Break

**11.30** **Hans Langedijk**, Janssen, Leiden
"Stabilization of conserved metastable structural elements in class I fusion proteins for optimal vaccine design"

**12.00** **Joost Snijder**, UU, Faculty of Science/Seattle
"Integrating MS and cryoEM to monitor glycosylation of viral antigens and its role in antigen-antibody interactions"

**12.30** **Robert de Vries**, UU, Faculty of Science
"Understanding Influenza A virus receptor specificity is essential for H3N2 vaccine development"

13.00 Lunch

**13.40** **Henderik W. Frijlink**, RUG, Groningen Research Institute of Pharmacy
"Dry vaccines for pulmonary administration"

**SHORT TALKS**

14.10 **Matthijs Raadsen**, EUR Viroscience
"Clinical development of a novel vaccine for MERS coronavirus based on the Modified Vaccinia Ankara (MVA) vector"

14.25 Break

15.00 **Philip Brouwer**, Amsterdam UMC
"A two-component nanoparticle vaccine candidate presenting stabilized Lassavirus glycoproteins"

15.15 **Kwinten Sliepen**, Amsterdam UMC
"Novel hepatitis C virus vaccine candidates based on E1E2 glycoproteins displayed on designed two-component nanoparticles"

## Using the broad resolving power of the electron microscope to drive vaccine development

Andrew Ward

*Department of Integrative Structural and Computational Biology,*
*California Campus, Scrips Research Institute*

Rational immunogen design aims to focus antibody responses to vulnerable antigenic sites on the surface of viruses by engineering protein subunit vaccines. Given the size of these viral antigens there is however potential for eliciting unwanted, off-target responses that compete with more desirable epitopes. My lab use cryoEM and a variety of other assays to provide atomic level information for guiding rational immunogen design and structurally characterizing immune responses to these experimental vaccines in animals. Our pipeline approach drives the iterative improvement of these subunit vaccines and we are currently conducting a variety of studies to focus immune responses on key epitopes on the HIV envelope glycoprotein.

## Structure-based HIV-1 vaccine design

Rogier Sanders

*Amsterdam UMC and Weill Medical College of Cornell University, New York, USA*

Inducing HIV-1 neutralizing antibodies against neutralization-resistant (Tier-2) virus strains by vaccination has been a challenge. While native-like (SOSIP) envelope trimers based on various HIV-1 strains can induce neutralizing antibodies against the autologous (Tier-2) viruses, the induction of broadly neutralizing antibodies (bNAbs), probably a prerequisite for an HIV-1 vaccine, is much more challenging. A critical step in this process is the activation of naïve B cells expressing germline antibody precursors that have the potential to evolve into bNAbs. VRC01-class bNAbs, named after its first member VRC01 and targeting the CD4 binding site, are attractive for germline-targeting because they share distinct genetic features and are found in multiple HIV-1 infected individuals. We have reengineered the native-like BG505 SOSIP trimer to engage germline precursors of VRC01-class bNAbs. The resulting GT1, GT1.1 and GT1.2 trimers (GT for germline targeting) bind multiple VRC01-class bNAb germline precursors in vitro. Crystal structures of GT1 and GT1.2 reveal a native-like conformation and the successful incorporation of design features associated with binding of VRC01-class bNAb germline precursors. Immunization experiments in knock-in mouse models expressing VRC01-class germline precursors show that these trimers activate germline precursor B cells in vivo, resulting in the secretion of specific antibodies into the sera. The Ab response in VRC01-class precursor knock-in mice can be further 'shaped' by the design and selection of next-step 'shaping' immunogens and 'polishing' immunogens. Sequence analysis of the B cell receptors of memory B cells in these mice after receiving a regimen of germline-targeting, 'shaping' and 'polishing' immunogens reveals that such a regimen selects for VRC01-class somatic mutations as well as rare insertions and deletions that are found in VRC01-class bNAbs. VRC01-class MAbs isolated from these mice have the capacity to neutralize heterologous wild-type HIV-1 isolates. Thus, germline-targeting using SOSIP trimers is a promising strategy for the induction of HIV-1 bNAbs.

## Interrogating humoral immune responses to find key protective antibodies against coronaviruses

Ivy Widjaja[1], Chunyan Wang[1], Rien van Haperen[2,3], Javier Gutiérrez-Álvarez[4], Brenda van Dieren[1], Nisreen M.A. Okba[2], V. Stalin Raj[2], Wentao Li[1], Raul Fernandez-Delgado[4], Frank Grosveld[2,3], Frank J.M. van Kuppeveld[1], Bart L. Haagmans[2], Luis Enjuanes[4], Dubravka Drabek[2,3] and Berend-Jan Bosch[1]

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[4] *National Center for Biotechnology - Spanish National Research Council, Madrid, Spain*

The Middle-East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic virus that causes severe and often fatal respiratory disease in humans. Efforts to develop antibody-based therapies have mainly focused on neutralizing antibodies that target the receptor binding domain of the viral spike protein thereby blocking receptor binding. We developed a set of human monoclonal antibodies that target functionally distinct domains of the MERS-CoV spike protein. These antibodies belong to six distinct epitope groups and interfere with the three critical entry functions of the MERS-CoV spike protein: sialic acid binding, receptor binding and membrane fusion. Passive immunization with potently as well as with poorly neutralizing antibodies protected mice from lethal MERS-CoV challenge. Collectively, these antibodies offer new ways to gain humoral protection in humans against the emerging MERS-CoV by targeting different spike protein epitopes and functions.

## Stabilization of conserved metastable structural elements in class I fusion proteins for optimal vaccine design

Johannes P.M. Langedijk

*Janssen Vaccines & Prevention B.V., Leiden, The Netherlands*

Traditionally vaccines are made of inactivated, or live-attenuated pathogens, however modern vaccines are often based on a vector that expresses a single viral protein or an isolated, highly purified antigenic protein subunit. For such modern approaches a deep understanding of the protein structure, function and stability is needed to guarantee successful expression of natively folded stable protein multimers which can induce an immune response with strong neutralizing activity against the pathogen.

Advances in structural vaccinology and knowledge on the function of the viral surface proteins has resulted in structure-based designs of vaccine candidates for RSV and HIV. In both fusion proteins we have introduced stabilizing mutations in the metastable hinge loop preceding the base helix. We and others have now applied this to various other class I fusion proteins. Another conserved metastable structural element in these fusion proteins is a metastable intersubunit beta sheet composed of beta strands of the head and the fusion domain. Detachment or ‘‘splaying’’ of the head of the class I fusion proteins is expected ultimately to disrupt the intersubunit sheet and trigger refolding. We found several examples how stabilization of the intersubunit b-sheet may be a general strategy for conformationally fixing the prefusion state. These new insights can potentially be important for the design of future vaccine immunogens.

## Integrating MS and cryoEM to monitor glycosylation of viral antigens and its role in antigen-antibody interactions

Joost Snijder

*Biomolecular Mass Spectrometry and Proteomics group, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands.*

Surface antigens of enveloped viruses are notoriously heavily glycosylated. Besides their role in receptor binding and host cell attachment, the glycans on viral envelope proteins are thought to have a steric shielding effect on interactions with neutralizing antibodies of the host immune system. The glycans of viral envelope proteins represent challenging targets in structural studies, due to their inherent heterogeneity and flexibility. However, recent advances in glycoproteomics and cryo electron microscopy enable a more comprehensive analysis of glycosylation in viral envelope proteins. Glycoproteomics can be used to survey site-specific glycosylation patterns with the aid of new fragmentation techniques, capable of detecting several dozens of unique glycan compositions per glycosylation site from complex mixtures. These surveys of glycan composition complement detailed structural studies of the antigens by cryoEM, aiding model building and providing a better understanding of antigen-antibody interactions. Applications of this integrated structural biology approach will be discussed following examples of human coronaviruses, HIV, and Epstein-Barr Virus. These studies reveal a dual role for viral N-linked glycans in antigen-antibody interactions, as some provide a steric shielding effect, while others form an integral part of the epitope and appear to play a crucial role in neutralization mechanisms.

## Understanding Influenza A virus receptor specificity is essential for H3N2 vaccine development

Frederik Broszeit, Rosanne van Beek, Ivan Gagarinov, Geert-Jan Boons and Robert de Vries

*Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University*

Our current assays to determine the receptor specificity and vaccine efficiency of influenza A virus fail as they do not represent receptors available in the human upper respiratory tract. The lack of these receptors in our laboratory hosts to create vaccines significantly dampen yields, the resulting mismatched vaccines do not afford proper protection and further drive antigenic drift.

The objective of our research is to elucidate the functional receptor of zoonotic and antigenically drifting influenza A viruses. Using these zoonotic and antigenically drifted viruses on our novel glycan arrays, we try to understand how glycan specificity changes due to host switching and immune pressure.

To achieve this goal, we enzymatically synthesize complex glycans including sialic acid and LacNAc modifications that are found on respiratory tract epithelial cells of humans and other IAV hosts. After synthesis, these complex glycans are printed on glass slide and interrogated with influenza A viruses to analyze evolving receptor specificities.

Our results reveal that our glycan array set up is well suited to analyze avian and human-type receptor specificities, NeuAc vs NeuGc binding and demonstrated that H5 viruses from chicken origin, prefer sialylated Lewis X structures. Importantly, human viruses appeared to have changed their receptor binding phenotype to complex N-glycans containing multiple LacNAc repeats.

We conclude that the lack of complex N-glycan receptors in standard laboratory assays is a bottleneck for proper analyses of drifting human viruses

## Dry vaccines for pulmonary administration

Henderik W. Frijlink

*Department of Pharmaceutical Technology and Biopharmacy*
*Groningen Research Institute of Pharmacy, University of Groningen*

Current vaccines suffer from several weaknesses, which may limit their application and reduce their efficacy. The limited stability of many vaccines is a first major problem, the logistics of these instable vaccines require a cold-chain and possibilities for stockpiling (e.g. pandemic preparedness) are limited. A second major problem is the fact that the majority of vaccines are still administered via injection, leading to issues varying from needle phobia to waste disposal. Finally, several vaccines suffer from poor immunogenicity.

For a number of vaccines, innovative formulations and alternative routes of administration may solve several of these problems. Advanced drying technologies such as spray-drying or spray-freeze-drying, combined with the incorporation of the vaccines in stabilizing sugar glasses, allows for the production of stable vaccine containing powders which do no longer require storage at lower temperatures. Such powders may further be processed into special dosage forms suitable for alternative routes of administration. Among these routes the pulmonary route of administration (inhalation) may be suitable for vaccination against airborne diseases.

In this presentation the possibilities of the new vaccine formulations will be illustrated by a range of different examples, including influenza, hepatitis-B and RSV vaccines. Further, results from preclinical in-vivo studies showing for which vaccines inhalation is a suitable route of administration and what the importance of the site of deposition in the lung is on efficacy will be discussed.

## Clinical development of a novel vaccine for MERS coronavirus based on the Modified Vaccinia Ankara (MVA) vector

Matthijs Raadsen, Nisreen Okba, Mart Lamers, Eric van Gorp, Bart Haagmans

*Viroscience lab, Erasmus MC Rotterdam*

Middle East Respiratory Syndrome (MERS) coronavirus is the second highly pathogenic human coronavirus known to date. Since the first human case was identified in 2012, it has caused over 2450 laboratory-confirmed infections, with clinical features ranging from severe pulmonary- and extrapulmonary disease, to mild or asymptomatic cases, with an overall case-fatality rate of ~35 %. MERS has been identified by the World Health Organization (WHO) as an emerging infectious disease requiring urgent research and development efforts for preventative and therapeutic measures. Modified Vaccinia Ankara (MVA) is a vaccinia virus strain that can be made to express exogenous genetic sequences in humans, including viral antigens, thus providing a platform for vaccine development. Due to its abortive replication in mammalian cells, MVA has an excellent safety profile in humans. MVA-MERS-S is an MVA vectored vaccine candidate encoding the full MERS coronavirus spike protein. It is currently being investigated in clinical trials conducted in Hamburg and in our institution in the near future. Early data from a single center phase Ia trial show good tolerability and the induction of both humoral and cellular immune responses in healthy volunteers after 2 injections of MVA-MERS-S. Future trials will seek to verify these results in a broader population, both in Europe and in MERS endemic regions.

# A two-component nanoparticle vaccine candidate presenting stabilized Lassavirus glycoproteins

Philip J.M. Brouwer[1], Tom P.L. Bijl[1], Aleksandar Antanasijevic[2], Yasunori Watanabe[3], Thijs Steijaert[1], Brooke Nickerson[4], Rashmi Ravichandran[4], Judith A. Burger[1], Max Crispin[3], Andrew B. Ward[2], Neil P.King[4], Rogier W. Sanders[1]

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[4] *University of Washington, Seattle, USA*

The high Lassa fever mortality rates of the recent outbreaks in several endemic West-African countries stress the need for an effective Lassavirus (LASV) vaccine. Among the several vaccine strategies that are currently being pursued, a subunit vaccine that is able to elicit broad and potent neutralizing antibodies would provide a safe and effective option to induce protective immunity. However, the LASV glycoprotein (GP) trimer is intrinsically unstable and falls apart into monomers when it is made as a soluble protein. As a result, recombinant soluble GP formulations usually only induce non-neutralizing antibodies that target the interior of LASV-GP, which is hidden on infectious pre-fusion LASV GP. Here, we have used two-component protein nanoparticles (I53-50NPs) to multivalently present twenty stabilized pre-fusion LASV-GP trimers. Pre-fusion LASV-GPs are genetically linked to the trimeric nanoparticle component to create LASV-GP-I53-50A fusion proteins (LASV-GP-I53-50A). Negative-stain electron microscopy, monoclonal antibody binding and site-specific glycan analysis indicate that the recombinant LASV-GP-trimers on I53-50A closely resemble the viral pre-fusion LASV GP conformation. When mixed with the second nanoparticle component, LASV-GP-I53-50A assembled into monodisperse, well-ordered icosahedral nanoparticles. The immunogenicity of these nanoparticles is currently being tested in rabbits of which the preliminary results will be presented at the meeting.

# Novel hepatitis C virus vaccine candidates based on E1E2 glycoproteins displayed on designed two-component nanoparticles

Kwinten Sliepen[1], Laura Radić[1], Yasunori Watanabe[2], Wilma Lee[3], Sylvie Koekkoek[1], Iván del Moral-Sanchez[1], Janke Schinkel[1], Gabriel Ozorowski[3], Neil P. King[4,5], Andrew B. Ward[3], Max Crispin[2], Rogier W. Sanders[1]

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Hepatitis C virus (HCV) infection is an increasingly larger threat to global health and currently infects approximately 71 million people and causes almost 400,000 deaths each year. The diversity of HCV surpasses that of HIV-1 and this provides a major roadblock for the development of an effective vaccine. Consequently, an HCV vaccine should probably induce broadly neutralizing antibodies (bNAbs) that are capable of targeting and neutralizing most circulating HCV strains.

The E1E2 glycoprotein complex, which is located on the outside of HCV, is the only target for bNAbs. However, soluble E1E2 molecules are difficult to produce and most recombinant HCV glycoproteins vaccines only include the E2 subunit. Furthermore, most HCV glycoprotein vaccines lack any form of multivalent display that could increase B cell activation for improved immunogenicity.

Here, we have developed a recombinant soluble E1E2 (sE1E2) immunogen that could be presented on a computationally designed two-component nanoparticle to enhance its immunogenicity. Conformational anti-E2 bNAbs engaged sE1E2 as efficiently as soluble E2, while sE1E2 also interacted with anti-E1 bNAbs. Furthermore, site-specific glycan analysis revealed that virtually all potential N-glycosylation sites (PNGS) on sE1E2 were occupied by glycans, while E2 monomers contained PNGS that were under-occupied. Nanoparticles were generated by mixing sE1E2 with a second component in vitro and this mix efficiently self-assembled as homogeneous ~40 nm sE1E2-presenting nanoparticles. The sE1E2-nanoparticles exhibited improved binding to several HCV bNAbs. To assess their ability to induce neutralizing antibodies, we immunized rabbits with E2, sE1E2 and sE1E2-nanoparticles and the first results of this immunogenicity study will be presented at the meeting.

This novel sE1E2 immunogen and the two-component nanoparticle platform provide new research avenues for exploring immunization strategies aimed at inducing HCV bNAbs.