5th Antibody Validation Workshop
10th EuroMAbNet Meeting

BOOK OF ABSTRACTS

LADY MARGARET HALL
TALBOT HALL, OXFORD
UNITED KINGDOM
27TH - 28TH SEPTEMBER 2018
BOOK OF ABSTRACTS OF THE 10TH EUROMABNET MEETING

VENUE
27-28 September 2018
Talbot Hall • Oxford • United Kingdom

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Welcome to the 10th EuroMabNet meeting  
27-28 September, Talbot Hall, Oxford, United Kingdom

To everyone attending the 10th EuroMabNet Meeting and 5th Antibody Validation Workshop,

I cannot believe that it has been 10 years since a small group of us started the European Monoclonal Antibody Network here in Oxford. It is lovely to celebrate this 10th Anniversary here, where it all began. It has been wonderful to see our community grow and to have more members continue to join our society from across Europe. I look forward to hearing about the exciting work going on in your laboratories; at each meeting I have learned so many new things.

There is now a growing movement to improve Antibody Validation and I like to think that we have played an important part in this. Firstly by publishing our EuroMabNet guidelines and also by offering our Antibody Validation Workshops to train young scientists in the principles of antibody validation. The latter has also given us an opportunity to work more closely with commercial antibody providers and to learn what they are contributing in this field. We are very grateful for the support of all of our sponsors.

Finally I would like to take this opportunity to welcome you all to our beautiful city of Oxford. I look forward to an interesting meeting and I hope that you enjoy your stay here and that you have a chance to explore the city during your visit.

Kind Regards,

Prof. Alison H Banham MA DPhil FRCPath FEur.Acad.Canc.Sci
Head of the Nuffield Division of Clinical Laboratory Sciences
Professor of Haemato-oncology
Vice President of EuroMabNet
**WORKSHOP PROGRAMME**

**10th EUROMABNET MEETING**

**5th ANTIBODY VALIDATION WORKSHOP - THURSDAY, SEPTEMBER 27th**

**MORNING SESSION**

9:00  **Prof. Alison Banham**  ▫ Nuffield Division of Clinical Laboratory Sciences, University of Oxford, UK  
*Opening introduction*

9:10  **Dr. Karen Pulford**  ▫ University of Oxford, UK  
*The problems arising from poor antibody validation*

9:40  **Prof. Friedrich Koch-Nolte**  ▫ Professor of Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany  
*A basic introduction to antibodies - different types, isotypes, recombinant antibodies and methods of production*

**COFFEE BREAK (10:20-10:50)**

10:50  **Prof. Alison Banham**  ▫ Nuffield Division of Clinical Laboratory Sciences, University of Oxford, UK  
*How to approach an antibody project*

11:30  **Dr. Giovanna Roncador**  ▫ Head of the Monoclonal Antibody Unit, CNIO, Madrid, Spain  
*Principles of antibody validation - examples showing approach to using controls, demonstrating reactivity with recombinant and endogenous target antigen, specificity and reproducibility*

12:10  **Dr. Berislav Lisnić**  ▫ Center for Proteomics and Department of Histology and Embryology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia  
*Reproducibility and dissemination of data*

12:30  **Dr. Hanna Dreja**  ▫ Principal Scientist, Abcam  
*Getting accurate and reliable results: from selection to validation*

12:45  **Dr. Anthony Couvillon**  ▫ Scientific Project Manager, Cell Signaling Technology  
*Fact vs Fiction in Antibody Validation*

**LUNCH (13:00-14:00)**

**AFTERNOON SESSION**

14:00  **Dr. Deepa B. Shankar**  ▫ Director of Research and Development for Antibodies and Immunoassays, Thermo Fisher Scientific  
*Nailing it with Thermo Fisher Scientific’s antibody validation toolbox!*

14:20  Interactive Session “Meet the Experts”  ▫ (two sequential sessions, 1 hour each)  
1. Meet the expert in immunohistochemistry  
2. Meet the expert in western blotting  
3. Meet the expert in flow cytometry

15:25  **Interactive Session “Meet the Experts”**  ▫ (two sequential sessions, 1 hour each)

18:30  **EuroMabNet round table**  ▫ (EuroMabNet members only)

**EuroMabNet DINNER in Jerwood Room (20:00)**  
(EuroMabNet members only)
10th EUROMABNET MEETING
SCIENTIFIC SECTION - FRIDAY, SEPTEMBER 28th

MORNING SESSION
Chair: Alison Banham

9:00  Prof. Alison Banham  o  Nuffield Division of Clinical Laboratory Sciences, University of Oxford, UK
Opening and welcome remarks

9:10  Dr. Karen Pulford  o  University of Oxford, UK
David Mason’s LRF Immunodiagnostics Unit, IHC and the birth of EuroMabNet

9:40  Dr. Andrew Chalmers  o  University of Bath, UK
Antibody validation for ICC: Guidelines and checklists

10:10  Rasmus Røge, MD  o  Icheme organizer, Institute of Pathology, Aalborg University Hospital, Aalborg, Denmark
Nordic Quality Control (NordiQC) - External Immunohistochemical Quality Assessment

10:40  Selected student short talk
Heather Bax  o  King’s College London, London, UK
An immunologically relevant rodent model demonstrates efficacy and safety of a tumour-specific IgE therapy

10:55  Selected student short talk
András Ács  o  MS Proteomics Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary & Semmelweis University, Budapest, Hungary
Structural characterisation of immunoglobulin G Glycans

COFFEE BREAK AND POSTER SESSION (11:10-11:40)

11:40  Dr. Sophia Karagiannis  o  King’s College London, UK
Monoclonal antibody therapeutic approaches for triple-negative breast cancer

12:10  Prof. Vladka Čurin  o  Center for the Production of Diagnostic Reagents and for Research, Blood Transfusion Centre of Slovenia, Slovenia, Ljubljana
From selective monoclonal antibody to a new biomarker in blood

12:40  Selected student short talk
Sandra Omejec  o  Blood Transfusion Centre of Slovenia, Department for production of diagnostic reagents and research, Slovenia, Ljubljana
Optimization of method for generating human monoclonal antibodies from single B cell isolated from vaccinated donors against Hepatitis B

12:55  Selected student short talk
Julia Hambach  o  Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
Biparatopic human IgG1 heavy chain antibodies carrying two distinct CD38-specific nano-bodies display potent complement-dependent cytotoxicity

LUNCH (13:10-14:00)
AFTERNOON SESSION
Chair: Giovanna Roncador

14:00 KEYNOTE LECTURE
Professor Hermann Waldmann a University of Oxford, UK
“Reprogramming the immune system” with monoclonal antibodies

15:00 Dr. Pierre Martineau a Pierre Martineau, IRCM, Inserm UT194, UM, ICM, Montpellier, France
Harnessing advantages of phage display and monoclonal antibodies with a single vector

COFFEE BREAK AND POSTER SESSION (15:30-16:00)

16:00 Prof. José Ignacio Rodríguez Barbosa a Section of Transplantation Immunobiology, Leon, Spain
Targeting CD160 receptor in immunotherapy

16:30 Dr. Vanda Juranić Lisnić a Center for Proteomics and Department of Histology and Embryology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia
Solving complex immune interactions by custom antibodies – the story of MCMV’s evasion of Ly49 receptors

NEW MEMBER SESSION

17:00 Jakub Z Kaczmarek a SANOVU BIOTECH A/S, Odense, Denmark
Antibody-driven inhibition of PPAD – from assay construction to extensive validation

END OF MEETING (17:30)
Reprogramming the Immune System with Monoclonal Antibodies

Hermann Waldmann
Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

One of the major goals in improving immunosuppressive therapy is to be able to minimise drug usage by harnessing the natural mechanisms of "self-tolerance". I will discuss studies using short-term treatment with monoclonal antibodies to achieve long-term acceptance of allografts and reversal of autoimmunity in murine models, and what we know of the mechanisms involved. In addition I will discuss ways to minimise the immunogenicity of therapeutic monoclonal antibodies.
ORAL PRESENTATIONS
The advent of monoclonal antibody technology started a revolution in clinical diagnostics. David Mason established the Leukaemia Research Fund (LRF) Immunodiagnostics Unit in 1979 with the idea of producing monoclonal antibodies that could be used on tissue sections from tumour biopsies to improve the diagnosis of haematological malignancies. The Unit has, over the course of 38 years, produced in excess of 80 fully validated monoclonal antibodies, of which more than 70 are commercially licensed. Hand in hand with antibody production, the LRF Unit also played an instrumental role in the development of immunohistochemistry (IHC). The work of the Unit, combining monoclonal antibody technology with IHC methodology, has resulted in the LRF Unit playing major roles in leukaemia and lymphoma classification as well as producing antibodies that provide prognostic and/or predictive information or identify potential therapeutic targets. The LRF Unit has also been involved in scores of international collaborations; such as the Human Leucocyte Differentiation Workshops, and importantly, was in at the birth of EuroMAbNet. The talk provides an overview of the work of the LRF Immunodiagnostics Unit in Oxford over the decades.
Antibody validation for ICC: Guidelines and checklists

Andrew Chalmers
CiteAb and the University of Bath, United Kingdom

The Global Biological Standards Institute (GBSI) had a mission to improve the quality and reproducibility of biomedical research and in 2017 set out to produce application specific research antibody validation guidelines. Their vision was to build on the foundations provided by the International Working Group for Antibody Validation (Uhlen et al 2016) and publish extended application specific guidelines. They also proposed to use the guidelines to underpin a scoring system to rate the validation of individual antibodies. This initiative involved representatives from academia, industry and the suppliers and I chaired the ICC working group. Here I will present some of the findings from the ICC panel, in particular our guidelines on the approaches that can be used to validate antibodies for ICC, advantages and concerns with the idea of a scoring system and an alternative proposal for a validation checklist. I hope to stimulate debate about the approaches that can be used to validate antibodies for ICC and whether completing and publishing such application specific guidelines would benefit the research community and improve experimental reproducibility.
Nordic Quality Control (NordiQC) - External Immunohistochemical Quality Assessment

Rasmus Røge
Icheme organizer, Institute of Pathology, Aalborg University Hospital, P.O.Box 561, DK-9100 Aalborg, Denmark

Despite extensive use of immunohistochemistry (IHC), lack of standardization remains a major problem in diagnostic pathology. Nordic Immunohistochemical Quality Control (NordiQC) is an international academic proficiency testing program primarily aimed at assessing the analytical phases of the laboratory IHC quality. About 500 laboratories from 60 countries are currently participating. More than 40,000 IHC slides have been evaluated during 2003–2017. Overall, about 20 % of the staining results in the breast cancer IHC module and about 30 % in the general module have been assessed as insufficient for diagnostic use. The most common causes for insufficient results are less successful antibodies (less robust antibodies, poorly calibrated ready-to-use products and stainer platform-dependent antibodies), insufficiently calibrated antibody dilutions, insufficient epitope retrieval and less sensitive visualization systems. Individually tailored recommendations for protocol optimization and identification of best tissue controls have for many markers improved IHC staining. The overall data generated by NordiQC during 15 years indicates that continuous quality control is valuable and necessary. Detailed description of the results of the NordiQC programme is available on www.nordiqc.org.
An immunologically relevant rodent model demonstrates efficacy and safety of a tumour-specific IgE therapy

Heather J Bax1,2 and Debra H Josephs1,2, Mano Nakamura1, Tihomir S Dodev1, Gareth Muirhead1, Louise Saul1,2, Panagiotis Karagiannis1,2, Kristina M Ilieva1,5, Silvia Crescioli1, Patrycja Gazinska5, Natalie Woodman1, Christopher Selkirk1, Heike Lentfe1, Claire Barton1, Silvana Canevari1, Mariangela Fugini1, Noel Downes10, David Dombrowicz1, Christopher J Corrigan12, Frank O Nestle1,13, Paul S Jones1, Hannah J Gould3, Philip J Blower8, Sophia Tsoka4, James F Spicer1, Sophia N Karagiannis1,5

1St John’s Institute Dermatology, School of Basic & Medical Biosciences, King’s College London, UK
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4Department of Informatics, Faculty of Natural and Mathematical Sciences, King’s College London, UK
5Breast Cancer Now Unit, Comprehensive Cancer Centre, School of Cancer & Pharmaceutical Sciences, King’s College London, UK
6King’s Health Partners Cancer Biobank, Comprehensive Cancer Centre, School of Cancer & Pharmaceutical Sciences, King’s College London, UK
7Biotherapeutics Development Unit, Cancer Research UK, UK
8Centre for Drug Development, Cancer Research UK, UK
9Molecular Therapies Unit, Comprehensive Cancer Centre, School of Cancer & Pharmaceutical Sciences, King’s College London, UK
10Biotherapeutics Development Unit, Cancer Research UK, UK
11St John’s Institute Dermatology, School of Basic & Medical Biosciences, King’s College London, UK
12Division of Asthma, Allergy and Lung Biology, MRC and Asthma UK Centre for Allergic Mechanisms of Asthma, King’s College London, UK
13Immunology and Inflammation Therapeutic Research Area, Sanofi US, Cambridge, Massachusetts, USA
14School of Biomedical Engineering & Imaging Sciences, King’s College London, UK

Nearly all monoclonal antibody cancer therapeutics are of one antibody class, IgG. However, the emerging field of AllergoOncology includes the hypothesis that efficacy may be enhanced by development of tumour-antigen specific IgE therapies, due to the superior tissue bioavailability and higher cognate receptor affinity of this antibody class.

Assessment of such novel agents requires the design of biologically-relevant in vivo model systems that consider class-specific immunological functions and safety. Although the murine system is less relevant to human IgE biology, IgE receptor expression and cellular distribution in rats mirrors that of humans. We therefore developed a surrogate immunocompetent rat model bearing syngeneic rat tumours expressing human folate receptor alpha (FRα; a tumour antigen expressed by up to 90% of epithelial ovarian carcinomas and other tumours) in order to evaluate surrogate rat MOv18 antibodies, engineered to recognise human FRα, and with rat Fc regions to provide immunological insights more likely to recapitulate human IgE-FcεRI interactions.

Significant tumour growth restriction was measured in immunocompetent tumour-bearing rats treated with rodent MOv18 IgE, in the absence of physiological or immunological evidence of cytokine storm or signs of a type I hypersensitivity reaction, nor haematological, biochemical or histopathological signs of moderate or severe toxicities. In spite of no signs of IgE-related toxicities, we observed marked elevation of immune cell infiltration into the tumour lesions of rats treated with rodent MOv18 IgE, as well as gene set enrichment of immunological pathways in tumour-bearing lungs from these animals, and increased serum levels of TNFα, a mediator previously associated with IgE-mediated anti-tumour and anti-parasitic functions.

Our study indicates both efficacy and safety of MOv18 IgE, and supported translation of this novel therapeutic approach to the clinic where our IgE is now the first IgE immunotherapy to be evaluated in man in a first-in-class, first-in-man clinical trial.
Structural characterisation of immunoglobulin G Glycans
András Ács, Eszter Szarka, Gabriella Sármay, Oliver Ozohanics, András Telekes, Károly Vékey, László Drahos, Lilla Turiák
1 MS Proteomics Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary
2 Semmelweis University, Budapest, Hungary
3 Eötvös Loránd University, Department of Immunology, Budapest, Hungary

Standard proteomic analysis is a widely used method in medical research. The latest technological advances facilitate the study of not just the protein composition of a biological matrix, but also post-translational modifications (PTMs). Glycosylation occurs frequently on the human proteome and represents great heterogeneity on different proteins. We established a reliable method to quantify oligosaccharide moieties and one of the most characteristic feature, fucosylation. We applied this technique on antibodies which are N-glycosylated at the constant heavy 2 domain.

A complex purification procedure was established to yield sufficient amount of IgG for glycopeptide analysis. As an initial biological matrix, blood serum was used. Several purification steps were applied, including dialysis and IgG-specific immunochromatographic column. Prior to proteomic analysis, samples were digested with Lys-C/Trypsin. For mass spectrometric analysis we used a nanoLC coupled to a Bruker Maxis II Q-TOF and raw data was evaluated using an in-house developed software called GlycoPattern.

Sample preparation procedure resulted in adequate amount (50 µg) of IgG for subsequent analysis. Glycosylation pattern of IgG subclasses were determined. The analysis revealed 25 different oligosaccharide structures from all the three major types of N-glycan classes with various number of fucose and sialic acid residues. Detailed characterisation of glycan composition contributes to improve quality control over antibody production and facilitates refined biomarker research.
Monoclonal antibody therapeutic approaches for triple-negative breast cancer

Sophia Karagiannis
St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King’s College London, London, United Kingdom

Highly-aggressive triple negative forms of breast cancer (TNBCs) lack validated targets and effective therapies. We have developed an integrated approach to identify targets for antibody therapy in TNBC, design monoclonal antibodies and interrogate their anti-tumour functions in vitro and in vivo. Employing a combination of genomic and immunohistochemical evaluations, we demonstrate that the tumor-associated antigens Folate Receptor alpha (FRα), highly expressed in ovarian carcinomas, and Chondroitin Sulphate Proteoglycan 4 (CSPG4), expressed in malignant melanomas, are overexpressed in subsets of TNBCs and can be targeted with monoclonal antibodies. Treatment with an anti-CSPG4 antibody significantly restricted orthotopic TNBC growth in vivo. A monoclonal antibody recognizing FRα triggered immune-dependent FRα-expressing breast cancer cell death by healthy volunteer and breast cancer patient effector cells, and significantly restricted orthotopic TNBC line and patient-derived tumor xenograft growth. Our findings indicate that FRα and CSPG4 may present targetable molecules with monoclonal antibody therapy against TNBC, and may lead to new treatment avenues for this patient group who are normally offered few systemic therapeutic options.
Brain disorders are recognized as one of the main economic and social burdens of the modern world. There is a worldwide consent that their impact on the society is unpredictable.

Prion protein (PrP) is a naturally occurring glycoprotein which binds to the cell membrane via a glycosphatidylinositol (GPI) anchor, is abundantly present on the cell surfaces of neurons and is linked to the cell membrane via a GPI-anchor. For unknown reasons, the α-helix-rich three-dimensional structure of the cellular form of PrP (PrPC) can transform into a β-sheet-rich molecule, which is the pathological form of PrP (PrPSc). The accumulation of PrPSc aggregates over time results in transmissible spongiform encephalopathies (TSE), also known as prion diseases. Prion diseases have important clinical and neuropathological features that are similar to those of Alzheimer’s disease (AD).

During normal metabolism, PrPC can undergo different posttranslational cleavages (α, β, γ) and is also the target of shedding. According to data, available in literature and our results we hypothesize that the proteolytic site in the human sequence is located between Tyr226 and Gln227. In our previous studies, we prepared monoclonal antibody V5B2, which selectively recognized the anchorless truncated form of PrP that ended with amino acid residue Tyr226, and we named it PrP226*. We speculate that PrP226* is produced by shedding and may be the only shed PrP, also involved in the pathogenesis of AD.

In this presentation, anti-PrP monoclonal antibody will be described, which selectively recognizes truncated form of PrP, PrP226*, in TSE infected brain. The presence and possible role of this antibody and PrP226* in health and disease will be discussed.
Monoclonal antibody based therapies are increasingly applied to prevent and treat human diseases. While the majority of therapeutic monoclonal antibodies on the market are still chimeric or humanized antibodies, originally produced in rodents, the focus in the last years is on the isolation and development of fully human antibodies. Fresh blood sample of vaccinated healthy donor at the peak of humoral immune response is a valuable source of affinity-maturated B cells (plasmablasts) that secrete antibodies specific to vaccinated antigen. For our research model we chose hepatitis B surface antigen (HBsAg), which is the major viral envelope protein of hepatitis B and is currently used for vaccination. Our goal was to develop method for the efficient isolation of memory B cells from peripheral blood mononuclear cells (PBMCs) from blood of vaccinated donors. The main focus is on the memory B cells and plasmablasts that express surface somatically mutated B cell antigen receptors (BCRs) with high affinities for our chosen antigen. PBMCs from vaccinated healthy donors against hepatitis B were collected 7 days post vaccination and immortalized with Epstein-Barr virus (EBV). We designed and optimized ELISA for the assessment of the supernatants for the presence of immunoglobulins G (IgG), specific for HBsAg. After three weeks of expansion in the culture EBV transformed PBMCs started to lose the ability to secrete HBsAg specific IgGs and after 4 weeks there were no more IgGs specific for HBsAg in the supernatants. The cell cultures with the highest positive results in ELISA were used for further isolation of antigen-specific cells. Custom assembled HBsAg-coated magnetic beads were used for the selection. After robust antigen-specific selection we were able to efficiently expand, freeze and thaw cells with specific binding to HBsAg. Our next goal is to amplify HBsAg specific single B cell by RT-PCR, clone immunoglobulin G genes into expression vectors and transfect into human cell line for production of human monoclonal antibodies.
Biparatopic human IgG1 heavy chain antibodies carrying two distinct CD38-specific nanobodies display potent complement-dependent cytotoxicity

Julia Hambach, Kerstin Schütze, Katharina Petry, William Fumey, Niklas Schuster, Levin Schriewer, Jana Röckendorf, Stephan Menzel, Birte Albrecht, Friedrich Haag, Catelijne Stortelers, Peter Bannas, Friedrich Koch-Nolte

1 Universitätsklinikum Hamburg-Eppendorf, Germany; 2 Ablynx (Belgium), Belgium

The cell surface ecto-enzyme CD38 is overexpressed on multiple myeloma and other hematological malignancies. Recent approval of the monoclonal antibody daratumumab by the FDA underlines the importance of CD38 as a target in immunotherapy (1). A nanobody corresponds to the isolated VHH variable domain of heavy chain antibodies that naturally occur in camels. Camelid heavy chain antibodies do not contain light chains and consist only of a pair of heavy chains that lack the CH1 domain. The VHH domain is highly soluble and can easily be linked to other VHH domains and/or the hinge-CH2-CH3 domains of any IgG isotype. Because of their smaller size, VHH-containing heavy chain antibodies may provide better tissue penetration than conventional antibodies in vivo (2). From llamas immunized with CD38 we selected nanobodies directed against three distinct epitopes of CD38 (3). By fusing individual nanobodies to the hinge-CH2-CH3 domains of human IgG1, we generated chimeric nanobody-human IgG1 heavy chain antibodies. These showed potent antibody dependent cellular cytotoxicity (ADCC) but little if any complement dependent cytotoxicity (CDC) toward human CD38+ lymphoma cell lines. However, the combination of two heavy chain antibodies recognizing distinct, non-overlapping epitopes on CD38 showed potent CDC. Similarly, combinations of daratumumab with a heavy chain antibody recognizing a non-overlapping epitope on CD38 also showed potent CDC. By genetically fusing two nanobodies directed against distinct epitopes of CD38 to the hinge-CH2-CH3 domains of human IgG1 we then engineered biparatopic heavy chain antibodies. These biparatopic heavy chain antibodies display very high CDC inducing potency.

These results provide a glimpse of the wide variety of antibody constructs that can be generated from soluble VHH domains. Moreover, our study illustrates the therapeutic potential of chimeric nanobody-human IgG heavy chain antibodies. The biparatopic CD38-specific heavy chain antibodies represent alternatives to daratumumab for the treatment of hematological malignancies.
Harnessing advantages of phage display and monoclonal antibodies with a single vector

Pierre Martineau
IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM, Montpellier, France

Display methods are usually restricted to antibody fragments and clone screening based on binding properties. However, the most frequently used molecule is the IgG and binding properties and functional activities cannot be directly extrapolated from the binding activity of the fragment. We developed a new display vector and an engineered companion mammalian cell line that allow both phage display selection and direct generation of monoclonal full IgG secretion. This new system opens the way to large-scale functional screening of IgG molecule (ADCC, CDC, agonist, antagonist, etc.) directly after phage display selection without recloning steps. Because of its flexibility, the system can be easily redesigned for the screening in other IgG formats (other isotypes, bispecifics, fusions, etc.).
CD160 is a protein of the immunoglobulin superfamily with several isoforms resulting from alternative mRNA splicing. CD160 expression is mostly restricted to cytotoxic cells (NK, NKT and memory CD8 T cells) and to all intestinal intraepithelial T lymphocytes. Engagement of CD160 by HVEM enhances the cytotoxic activity of NK cells and inhibits the function of certain T cell subsets. In turn, soluble CD160.Ig and BTLA.Ig can also function as agonistic ligands and costimulate T cells through membrane-bound HVEM.

To gain insight into the functional role of CD160 on NK cell function and unravel their implication in the survival of chronic lymphocytic leukemia cells, in which CD160 expression is constitutive, we adopted a CRISPR/Cas9 genome modification strategy to target exon 2 of CD160 gene, encoding the start codon and the signal peptide. Thus, a deletion of 47 bp was introduced into this exon, which is required for the expression of all spliced variants, leading to a frameshift mutation and subsequent gene inactivation. These mice were then immunized with mouse and human CD160 extracellular region recombinant proteins and mouse cross-reactive monoclonal antibodies of different isotypes were identified against human and mouse CD160 molecules. We are currently testing these reagents in a hematopoietic B cell tumor mouse model and trying to elucidate the role of this molecule on the function of NK cells in the setting of haploidentical bone marrow transplantation and NK cell-mediated hybrid resistance.
Solving complex immune interactions by custom antibodies– the story of MCMV’s evasion of Ly49 receptor

Vanda Juranić Lisnić
Center for Proteomics and Department of Histology and Embryology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia

Cytomegaloviruses downregulate MHC I from the cell surface in order to avoid recognition by T cells. Since this strategy could trigger the NK cell-mediated “missing-self” recognition, murine cytomegalovirus (MCMV) encodes m04/gp34, a protein which brings a portion of MHC I molecules back to the cell surface enabling them to engage inhibitory Ly49 receptors (iLy49s). However, m04 brings only a small portion of MHC I to the surface. We have identified and characterized 11kDa viral protein MAT p1 encoded by the MCMV’s most abundant transcript (MAT) that helps in the m04-mediated MHC I surface rescue and strengthens the interaction between inhibitory Ly49 receptors and their MHC I ligands. Viruses lacking this protein are attenuated in vivo in mice of various MHC I haplotypes in an NK cell-dependent manner. Interestingly, ORF encoding this protein is highly variable among different virus strains which has prompted us to investigate whether it has been under strong selection pressure by the immune system. Differential sensitivity of various mouse strains to MCMV has been linked to the capacity of NK cells to recognize infected cells via activating Ly49 receptors and we and others have previously shown that activating Ly49P receptors require MHC I, m04 and additional virally encoded factor. Using multiple MCMV mutants we show that the unknown virus factor is the same MAT encoded protein involved in the recognition by inhibitory Ly49 receptors. Thus, MATp1 evasion of inhibitory Ly49 receptor has prompted the evolution of activating Ly49 receptors. In the course of this work, several novel monoclonal antibodies have been generated and have been instrumental in solving the complex interactions between NK and CMV-infected cells.
Numerous scientific studies underline that the development and progression of Rheumatoid Arthritis (RA) is clinically associated with periodontal disease (PD). RA affects 0.5–1% of the adult population in industrialized countries and constitutes a global health problem with numerous, serious implications on the society worldwide. The connection between RA and PD is hypothesized to be caused by an oral pathogen – Porphyromonas Gingivalis (Pg) and its citrullination properties. Mechanistically this link may be due to the activity of an enzyme from Pg- termed Peptidyl Arginine Deiminase (PPAD) which preferentially citrullinates C-terminal arginine residues on peptides and proteins. This process creates neo-epitopes on host proteins that can trigger B-cell production of anti-citrullinated protein antibodies (ACPA). Developing an effective inhibitory formulation against PPAD would be an important step in our efforts to dissect the biology of this clinically relevant protein. We have therefore developed chicken polyclonal antibody formulations in a form of affinity purified antibodies against PPAD with an aim of inactivating its enzymatic activity.

In order to screen for the inhibitory potential of anti-PPAD antibodies we have developed a novel, in-vitro methodology where the activity of the enzyme can be assayed and afterwards validated with two independent techniques. The assay consists of an initial separation and quantitation with HPLC followed by validation by MALDI TOF/TOF and LC-MS. It is simple, homogeneous and robust, thus providing a reliable measure for testing the potential inhibitory molecules against PPAD’s enzymatic activity. After testing the developed anti-PPAD antibodies on the enzymes activity, our results show that, at an antibody-enzyme ratio of 1:1000, a 70% inhibition effect is observed when compared to the positive control reaction. Due to the reproducibility of observed results and validation supported by two independent techniques we can conclude that the tested, affinity purified antibody preparation has an inhibitory effect on the PPADs enzymatic activity in-vitro. Both the activity assay and the newly developed antibody represent very useful tools that can be further utilized in order to study the biology of PPAD and its relevance in the development of auto-immune diseases.
Development of pH-dependent antibodies for cancer immunotherapy

Tristan Mangeat, Pierre Martineau and Bruno Robert
IRCM-INSERM U1194, University Montpellier, Montpellier, France

Immunotherapy based on antibodies has revolutionized cancer treatment by increasing the life expectancy of patients and in some cases inducing a complete cure. However, these therapeutic antibodies can also target healthy cells expressing the antigen, resulting in unwanted side effects. The challenge relies on the development of antibodies able to bind specifically to the target expressed by tumor cells. Therefore, a promising strategy is the development of pH-dependent antibodies. Indeed, it is well known that cancer cells exhibit high rates of glucose uptake and lactic acid production even in presence of oxygen which leads to an acidic pH of the tumor microenvironment. So, we could imagine developing pH-dependent antibodies against tumor cells in order to avoid the binding of this antibody on healthy cells at a physiological pH. This would decrease the side effects due to the expression of targets on healthy tissue. A first proof of concept has been provided by the company Halozyme®, which has developed a mutated anti-EGFR antibody (Cetuximab) with an altered binding for EGFR at physiological pH and a strong binding at acidic pH.

Therefore, two strategies are performed in parallel in order to identify pH-dependent anti-RTK antibodies. First, the determination of the exact epitope/paratope complex (in collaboration with Centre de Biochimie Structurale, Montpellier) for the RTK receptor extracellular domain/human anti-RTK antibody in order to help us to discriminate specific amino acid mutations in the paratope of a preexisting antibody (like Halozyme®). Second, a combinatorial approach will allow us to directly select for pH-dependent antibodies using libraries of scFvs available in our team and a phage display screening at acidic pH. If successful, these strategies may be applied to other targets like immune checkpoints (e.g. anti-PD1, anti-PDL1 or anti-CTLA4).
Melanoma-associated antigen shedding and its role in immunotherapy
Silvia Mele, Balraj Singh Sandhar, Carl Hobbs, Giulia Chiaruttini, Iwan Williams, Elise French, Kristina Ilieva, Silvia Crescioli, Belul Shifa, Sara Lombardi, Jenny Geh, Ciaran Healy, Katie Lacy, James Spicer, Sophia Karagiannis
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Several cancer-specific antigens identified as targets for antibody-based immunotherapy are shed from the cell membrane. How this process affects the tumour response to antigen-targeted immunotherapy is still unclear. We focused on the melanoma-associated antigen chondroitin sulphate proteoglycan 4 (CSPG4), a potential candidate for antibody-based immunotherapy largely expressed in melanomas.

We analysed the levels of CSPG4 expression via IHC in microarrays containing malignant melanoma tissues (n=428 cores), and normal tissues from various anatomical locations (n=389 cores). Levels of soluble CSPG4 in human serum were analysed via ELISA in a cohort of melanoma patients (n=29) and healthy volunteers (n=20). The presence of shed CSPG4 was also tested in the conditioned media of melanoma cell lines, both before and after binding to chimeric anti-CSPG4 monoclonal antibodies in the presence or absence of a matrix metalloprotease (MMP) inhibitor.

CSPG4 expression was observed in 63% of the malignant melanoma tissues analysed, while low to moderate expression was observed in most normal tissues. Soluble CSPG4 was detected in the serum of both melanoma patients and healthy volunteers, with higher levels detected in the former (33.93±4.67pg/ml vs 21.08±3.69pg/ml; p<0.05). Detectable levels of soluble CSPG4 were also observed in the conditioned media of melanoma cell lines (A2058, A375). Incubation with anti-CSPG4 antibodies increased the shedding of its ectodomain, a process that was partly inhibited by the broad spectrum MMP inhibitor Marimastat.

The higher shedding rate of CSPG4 upon mAb binding could represent an immune escape mechanism able to affect the response to antigen-targeted immunotherapy. Combinatorial therapy may be required to maximise the therapeutic response.
Discovering the immune profile of a novel anti-folate receptor alpha IgE antibody associated with monocyte-mediated anti-tumor functions

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Introduction: Monoclonal antibodies of the IgG class are an established modality for the clinical management of many cancers. Previous and ongoing studies have demonstrated that tumour antigen-specific IgE class antibodies may engender superior anti-tumour effector functions to those triggered by IgG. Monocytes are one of the key immune cells in anti-tumour immunity that can be recruited and activated by IgE. However, the mechanisms by which monocytes engender IgE-mediated effector functions against cancer are insufficiently characterised. Hence, we sought to investigate the immune profile signatures triggered by the engagement of tumor antigen-specific IgE with Fc-receptors on the surface of monocytes.

Methods: Antibody dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP) were measured by flow cytometry. Cell stimulation studies with cytokines or by cross-linking of IgE monoclonal antibodies on the surface of monocytes were conducted. Expression and secretion of immune mediators were assessed using qPCR and ELISA. The effects of cytokines on immune and tumor cells were investigated by cell viability (MTS) assays.

Results: Tumor antigen-specific IgE-dependent monocyte-mediated tumour cell killing correlated with increased TNFα, MCP-1 and IL-10 levels. Cross-linking of IgE on monocytes triggered up-regulation of TNFα. TNFα stimulation of monocytes and tumor cells induced MCP-1 upregulation by both cell types. IL-10 expression was induced only by monocytes stimulated by TNFα and MCP-1 in combination, or by IL-10 in an autocrine manner.

Conclusion: IgE-dependent monocyte-mediated tumor cell cytotoxicity triggers TNFα, MCP-1 and IL-10, an axis normally associated with IgE-mediated anti-parasitic clearance functions. The three cytokines did not have direct cytotoxic effects towards immune or tumor cells, however may be responsible for driving anti-tumour functions by effector cell recruitment and activation. Identifying the immune profiles of IgE-mediated immune activation in monocytes will deepen our understanding of IgE-mediated anti-tumour mechanisms, and may support the development of IgE class as a novel immuno-oncology strategy.
Hapten design for antibody production against small molecules

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For the generation of antibodies against small hapten molecules, the hapten is linked with some carrier protein to make it immunogenic. In the design of hapten conjugates, there are very important aspects to consider: the hapten design, the carrier and also the coupling strategy. The functional group of the hapten governs the selection of the conjugation method to be employed. Moreover, there are other important aspect to consider: the conjugate hapten density. The amount of hapten attached to the carrier influences the strength of the immune response and it is also important in the development of immunoassays. Verification of the coupling reaction and determination of the hapten density can be accomplished by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). We will discuss some examples of it.
Brown fat phenotype as a novel pathway associated with prognosis and tumour progression in colorectal cancer

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Background: Colorectal cancer is a common malignancy with a relatively poor survival rate. The expression of brown fat phenotype has been implicated in tumour growth in prostate and breast carcinomas. However, the prevalence and clinical impact of this pathway has not been examined in colorectal cancer.

Methods: Monoclonal antibodies were developed and used to profile the expression of brown fat-associated proteins; uncoupling protein 1 (UCP1) and cell-death-inducing DNA fragment factor 45-like effector A (CIDEA). A range of bioinformatics tools were used to identify specific and antigenic peptides (8-12 amino acids). Successfully generated antibodies were characterised by immunoblotting using overexpression lysate, and by immunohistochemistry on a multi-tissue microarray. Then, the antibodies were used to profile the expression of proteins by immunohistochemistry on a discovery cohort containing 274 colorectal cancers and 50 normal colonic samples and on a validation cohort containing 549 colorectal cancers.

Results: A significant increase in expression of CIDEA (p<0.001) and UCP1 (p<0.001) was observed in colorectal cancer compared to normal colonic mucosa. There were significant relationships between UCP1 and tumour stage, nodal stage and Dukes stage. There were also significant association between CIDEA and nodal stage. The expression of UCP1 was associated with better survival in the discovery cohort ($\chi^2 = 6.119$, $p=0.013$) and in the validation cohort ($\chi^2 = 11.558$, $p=0.001$).
Generation and characterization of mAbs against *Mycobacterium tuberculosis* arabinomannan

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Tuberculosis (TB) is a dreaded infectious disease and is one of the top 10 causes of death worldwide. In 2016, 10.4 million people fell ill with TB, and 1.7 million died from this disease, as reported by the WHO. *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, can establish latent or progressive infection, although a healthy immune system is present. In this regard, strong evidence suggests that the mycobacterial cell envelope is of paramount importance for survival in the host. The mycobacterial envelope consists of three major components: the plasma membrane, the cell wall, and an outermost capsule. The mycobacterial capsule is mainly composed of proteins and polysaccharides, including arabinomannan (AM). AM-specific humoral response has been associated with a reduced bacterial colonization and increased survival in mice by our research group. Therefore, our aim was to generate monoclonal antibodies (mAbs) against AM as a tool for our future studies.

C57B/6 mice were immunised with 10 μg of either AM-Ag85b, AM-PA or AM alone, three times in Alum. Splenocytes were fused with myeloma cell line SP2/O-Ag14. After hybridoma selection, 300 discrete colonies were isolated in 96-well plates. Screening against AM and Ag85b was performed using ELISA, and 5 clones were selected as positive producers of AM-specific mAbs. We specifically selected and characterized clone H12, which happened to be an IgG1 suitable for western blot, immunofluorescence and immunolabeling electron microscopy.
Anti-folate receptor alpha–directed antibody therapies restrict the growth of triple negative breast cancer

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Triple negative breast cancer (TNBC) represents a molecularly and clinically diverse disease with cytotoxic chemotherapy the only systemic treatment modality, and no targeted agents approved in adjuvant, neoadjuvant, or metastatic settings. By genomic (N=3414) and immunohistochemical (N=323) analyses, we demonstrate that a significant population of aggressive high-grade TNBCs overexpress the cell surface tumor-associated antigen Folate Receptor alpha (FRα). Importantly, FRα is expressed in post-neoadjuvant chemotherapy residual disease, associated with worse clinical outcomes. RNA interference to deplete FRα decreased Src and ERK signaling and resulted in reduction of cell growth. An anti-FRα antibody (MOv18-IgG1) conjugated with a Src inhibitor significantly restricted TNBC xenograft growth. Moreover, MOv18-IgG1 triggered immune-dependent cancer cell death in vitro by human volunteer and breast cancer patient immune cells, and significantly restricted orthotopic and patient-derived xenograft growth. We show that FRα may present a promising target for therapeutic strategies such as antibody-drug conjugates, or antibody immunotherapy that primes an Fc-mediated anti-tumor immune response in vitro and in vivo in the human patient breast cancer and the patient immune context. Engineering antibodies targeting FRα-expressing breast cancers may provide new strategies to treat patients with poor prognosis who do not adequately benefit from currently-available targeted, and immuno-oncology therapies.
Development and evaluation of T-Zap2, a novel antibody-drug conjugate for the treatment of Her2 positive breast cancer

Ricarda Melina Hoffmann

Background: Breast cancer is the most common type of cancer worldwide and HER2-overexpressing types are associated with poor prognosis due to primary or acquired resistance to targeted therapies such as the monoclonal antibody trastuzumab and the antibody-drug conjugate trastuzumab-emtansine (T-DM1). Antibody-drug conjugates (ADCs) have been developed as a targeted approach to deliver toxins to cancer cells specifically the ADC trastuzumab-emtansine (T-DM1) already in clinical use. A novel ADC T-Zap2 has been developed by linking the antibody trastuzumab to saporin - a ribosome inactivating protein. This study aims to evaluate the novel ADC T-Zap2 as a treatment alternative for HER2 positive breast cancer patients with resistance to T-DM1.

Methods: The plant toxin saporin was linked to the monoclonal antibody trastuzumab via a streptavidin-biotin bridge. Binding kinetics were assessed using flow cytometry, and MTS cell viability assays were performed to compare efficacy of T-Zap2 to T-DM1. Toxicity towards immune cells was investigated by MTS assays using immune cells.

Results: Binding to target cells of T-Zap2 was confirmed. Comparison of T-Zap2 efficacy in breast cancer cell lines with and without resistance against trastuzumab showed a trend for higher efficacy of cell killing by T-Zap2 in trastuzumab resistant cells compared to T-DM1. Toxicity assays revealed no impact of T-Zap2 on cell viability in immune cells.

Conclusions: Cell viability caused by T-Zap2 was more efficient compared to T-DM1 in the trastuzumab resistant cell line HCC1945. This strengthens the hypothesis that T-Zap2 might have potential as new ADC overcoming trastuzumab and T-DM1 resistance. Future work will include in vivo studies and the development of a specific linker to conjugate trastuzumab to saporin to develop an ADC that could prove a novel therapeutic agent for the treatment of HER2 positive breast cancer.
Expression of T Lymphocyte Antigen 9 (Ly9) in human B and T-cell neoplasms

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Background: Ly9, also known as CD229 or SLAMF3, is one of the nine members of the immunoglobulin superfamily (SLAM). It is expressed in all T and B lymphocytes and play an important role in lymphocyte activation and cytotoxicity. Ly9 deficient mice spontaneously develop features of systemic autoimmunity, such as splenomegalic and production of autoantibodies, indicating that the Ly9 cell-surface receptor is involved in the maintenance of immune cell tolerance. No antibodies recognizing this molecule by immunohistochemistry in paraffin tissues have been reported and no extensive study of the expression of Ly9 in normal and neoplastic lymphoid tissue has been performed to date.

Purpose of the study: In the present study, we have investigated Ly9 expression in normal and neoplastic lymphoid tissue using a novel rat monoclonal antibody (PIZCU426A) against Ly9 intracellular domain that recognizes its target molecule in paraffin-embedded tissue sections. A large series of normal tissues and B and T-cell lymphomas was studied, using whole sections and tissue microarrays.

Results: In human reactive tissues, Ly9 was found to be restricted to lymphoid tissue, specifically to mature B and T cells. Ly9 was strongly expressed in all cases of myelomas, marginal zone and MALT lymphomas. Moderate Ly9 expression was also found in the majority of chronic lymphocyte leukemia, follicular, mantle cell and peripheral T cell lymphoma. Ly9 was present in a small proportion of Burkitt, angioimmunoblastic lymphoma and diffuse large B cell lymphoma. Ly9 was not found in Hodgkin lymphoma with the exception of nodular lymphocyte–predominant Hodgkin lymphoma were Ly9 was abundant in the tumour microenvironment.

Conclusions: We describe a new monoclonal antibody that may help diagnostic pathologists in the identification of neoplastic B and T cells in routinely processed tissue samples and may be used to achieve a better understanding of the pathogenic role of Ly9 in inflammatory and malignant diseases.
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